1985


Jia-cherng Wu
Louisiana State University and Agricultural & Mechanical College

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_disstheses

Recommended Citation

This Dissertation is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Historical Dissertations and Theses by an authorized administrator of LSU Digital Commons. For more information, please contact gradetd@lsu.edu.
INFORMATION TO USERS

This reproduction was made from a copy of a document sent to us for microfilming. While the most advanced technology has been used to photograph and reproduce this document, the quality of the reproduction is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help clarify markings or notations which may appear on this reproduction.

1. The sign or “target” for pages apparently lacking from the document photographed is “Missing Page(s)”. If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure complete continuity.

2. When an image on the film is obliterated with a round black mark, it is an indication of either blurred copy because of movement during exposure, duplicate copy, or copyrighted materials that should not have been filmed. For blurred pages, a good image of the page can be found in the adjacent frame. If copyrighted materials were deleted, a target note will appear listing the pages in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed, a definite method of “sectioning” the material has been followed. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For illustrations that cannot be satisfactorily reproduced by xerographic means, photographic prints can be purchased at additional cost and inserted into your xerographic copy. These prints are available upon request from the Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases the best available copy has been filmed.
Wu, Jia-Cherng

DEVELOPMENTS OF GAS CHROMATOGRAPHY - ATOMIC ABSORPTION AND H.P.L.C. - ATOMIC ABSORPTION FOR METAL SPECIATION STUDIES

The Louisiana State University and Agricultural and Mechanical Col. Ph.D. 1985

University Microfilms International 300 N. Zeeb Road, Ann Arbor, MI 48106
PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark \(\checkmark\).

1. Glossy photographs or pages _____
2. Colored illustrations, paper or print _____
3. Photographs with dark background _____
4. Illustrations are poor copy _____
5. Pages with black marks, not original copy _____
6. Print shows through as there is text on both sides of page _____
7. Indistinct, broken or small print on several pages \(\checkmark\)
8. Print exceeds margin requirements _____
9. Tightly bound copy with print lost in spine _____
10. Computer printout pages with indistinct print _____
11. Page(s) ________ lacking when material received, and not available from school or author.
12. Page(s) ________ seem to be missing in numbering only as text follows.
13. Two pages numbered __________. Text follows.
14. Curling and wrinkled pages _____
15. Other _________________________________

University Microfilms International

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements of the degree of Doctor of Philosophy

in

The Department of Chemistry

by

Jia-Cherng Wu

B.S. in Chemistry, National Taiwan Normal University, 1977

August 1985
ACKNOWLEDGEMENT

The author wishes to thank his parents and other family members who provided much financial and spiritual support over many years to make this dissertation possible.

Thanks should go to Mr. Les Edelen, Mr. George Sexton, Mr. Rodger Duvall, Mr. Joe Cass, and Mr. George Gascon of the College of Basic Sciences machine and electronic shops for their assistance in construction and repairing much of the equipment used for these studies.

The author gratefully acknowledges the patient guidance and direction of professor J. W. Robinson in the pursuit of the author’s research and during the preparation of this manuscript.

The understanding, patience and assistance of the author’s spouse during this time cannot be sufficiently described. She was more help than she realizes.

The author acknowledges the financial support of the Coates Memorial Fund for the preparation of the this manuscript.
DEDICATION

To my mother and my father
for their belief in me

and

to Liu-Shang for her loving and patience.
FOREWORD

Parts of this dissertation have been presented or published:


Papers in preparation for publication include:

# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENT</td>
</tr>
<tr>
<td>DEDICATION</td>
</tr>
<tr>
<td>FOREWORD</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
</tr>
<tr>
<td>ABSTRACT</td>
</tr>
</tbody>
</table>

## PART ONE

SPECIATION OF MERCURY BY G.C.- A.A.-----------------------------1

### I. CHAPTER 1-INTRODUCTION---------------------------------1

#### A. TOXICITY OF MERCURY-----------------------------------1

#### B. MERCURY IN THE ENVIRONMENT-----------------------------5

#### C. METHYLATION OF MERCURY------------------------------9

#### D. METHODS OF DETERMINATION AND

    SPECIATION OF MERCURY---------------------------------15

#### E. REASONS OF THIS STUDY---------------------------------22

### II. CHAPTER 2-THEORY--------------------------------------25

#### A. GAS CHROMATOGRAPHY-----------------------------------25

    1. HISTORY------------------------------------------25

    2. THEORY-------------------------------------------26

    3. COLUMNS------------------------------------------31

    4. DETECTORS----------------------------------------33

    5. SPECIFIC DETECTORS AND

        COMBINATION TECHNIQUES--------------------------34

#### B. ATOMIC ABSORPTION-------------------------------------35
1. BASIC PRINCIPLES-----------------------------------35
2. LIGHT SOURCES-----------------------------------37
   a. HOLLOW CATHODE LAMP--------------------------37
   b. DEMOUNTABLE HOLLOW CATHODE LAMP--------------39
   c. ELECTRODELESS DISCHARGE LAMP------------------40
3. BACKGROUND CORRECTION IN ATOMIC ABSORPTION------40
4. SIGNAL MODULATION
5. ATOMIZATION PROCESSES IN CARBON ATOMIZER--------46
6. INTERFERENCES IN ATOMIC ABSORPTION---------------47
7. SENSITIVITIES OF ATOMIC ABSORPTION-----------------48

III. CHAPTER 3-EXPERIMENTAL--------------------------50
   A. INSTRUMENTATION AND EQUIPMENT------------------50
      1. EQUIPMENT-G.C.-A.A.--------------------------51
      2. QUARTZ T.A.A.---------------------------------53
      3. T.L.C. AND UV---------------------------------55
   B. CHEMICALS AND REAGENTS--------------------------55
   C. INTERFACING AND DEVELOPMENT OF G.C.-A.A.-------56
      1. INTRODUCTION----------------------------------56
      2. MODIFICATION OF CARBON
ATOMIZER AS A.A. DETECTOR------------------------61

3. MODIFICATION OF ATOMIZER
   DESIGN----------------------------------------62

4. MODIFICATION OF G.C.-A.A.
   INTERFACING SECTION------------------------63

5. SUMMARY---------------------------------------72

D. ANALYSES OF URINE AND PERSPIRATION-----------72

1. INTRODUCTION----------------------------------72

2. COLLECTION OF SAMPLES------------------------75

3. DETERMINATION OF TOTAL
   MERCURY---------------------------------------76
   a. INSTRUMENTATION-THE
      QUARTZ T A.A.-----------------------------77
   b. EXPERIMENTAL PARAMETERS------------------78
   c. EXPERIMENTAL PROCEDURES------------------79
   d. RESULTS AND DISCUSSION-------------------79

4. SPECIATION BY G.C.-A.A.----------------------85
   a. INTRODUCTION-----------------------------85
   b. EXPERIMENTAL PARAMETERS
      G.C.-A.A.---------------------------------86
   c. EXPERIMENTAL PROCEDURES
      G.C.-A.A.---------------------------------86
   d. SEPARATION OF MERCURY
      COMPOUNDS---------------------------------88
   e. STUDY OF URINE AND
      PERSPIRATION BY G.C.-MS-------------------100

viii
IV. CHAPTER 4—SPECIATION OF CHROMIUM BY H.P.L.C—A.A.—133

A. TOXICITY AND BIOLOGICAL IMPORTANCE OF CHROMIUM—133

B. ANALYTICAL DETERMINATION AND SPECIATION OF CHROMIUM—195

C. EXPERIMENTAL—196

1. INTERFACING H.P.L.C.
   AND A.A.—138

2. CHEMICALS AND SOLUTIONS—138

3. DEVELOPING H.P.L.C. SOLVENT SYSTEM BY REVERSED-PHASE T.L.C.—200
   a. EXPERIMENTAL PROCEDURES—200
   b. RESULTS—203

4. SPECIATION OF Cr(III) AND Cr(VI)
   BY H.P.L.C.—A.A.—202
   a. INSTRUMENTATION AND EQUIPMENT—203
   b. EXPERIMENTAL PROCEDURES—206
   c. INSTRUMENTAL PARAMETERS—207
   d. RESULTS—209

5. SPECIATION BY H.P.L.C.—UV—210
   a. INSTRUMENTATION AND EQUIPMENT—210
   b. EXPERIMENTAL PROCEDURES—210
   c. INSTRUMENTAL PARAMETERS—210
   d. RESULTS—214

6. DISCUSSION—214
V. CHAPTER 5 - DIRECT SPECIATION OF ZINC

IN URINE BY H.P.L.C.-A.A.------------------------219

A. INTRODUCTION

1. BIOLOGICAL IMPORTANCE OF ZINC----------219

2. DETERMINATION AND SPECIATION

METHODS OF ZINC-----------------------------221

B. EXPERIMENTAL-------------------------------223

1. INSTRUMENTATION AND CHEMICALS-----------223

2. EXPERIMENTAL PARAMETERS-----------------224

3. EXPERIMENTAL PROCEDURES-----------------224

C. RESULTS AND DISCUSSION-------------------225

VI. CHAPTER 6 - DIRECT SPECIATION OF MAGNESIUM IN URINE

BY H.P.L.C.-A.A.-------------------------------229

A. INTRODUCTION-----------------------------229

1. BIOLOGICAL IMPORTANCE OF MAGNESIUM-----229

2. DETERMINATION AND SPECIATION

METHODS OF MAGNESIUM-----------------------232

B. EXPERIMENTAL-------------------------------234

1. INSTRUMENTATION AND CHEMICALS-----------234

2. EXPERIMENTAL PARAMETERS-----------------234

3. EXPERIMENTAL PROCEDURES-----------------234

C. RESULTS AND DISCUSSION-------------------235

VII. CHAPTER 8 - SUMMARY----------------------233

BIBLIOGRAPHY---------------------------------241

VITA-----------------------------------------260
LIST OF TABLES

1. Sensitivity Improvement Using the New Carbon Atomizer as G.C.-A.A. Detector-------------------73
2. Retention Times of Mercury Compounds Using a 2-Ft DEGC Column-----------------------------99
3. Mercury Contents in Urine Samples--------------------------107
4. Mercury Contents in Sweat Samples------------------------108
5. Mercury Levels in Urine and Sweat from Two Different Subjects-----------------------------109
6. Effect of Ultrasonic Frequency on the Number of Drops and then Volume and Surface Area----------------177
7. Boiling Points, Ultrasonic Drive Frequencies and Sensitivity increases------------------183
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>TITLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mercury Cycle in the Environment</td>
</tr>
<tr>
<td>2.</td>
<td>Proposed Mechanism for Mercury Methylation by Methylcobalamin</td>
</tr>
<tr>
<td>3.</td>
<td>Bond Cleavage of CHg-B 12 for Methylation</td>
</tr>
<tr>
<td>4.</td>
<td>Methylation of Mercury by a Cross-Over mechanism</td>
</tr>
<tr>
<td>5.</td>
<td>A Sample Pretreatment Scheme for the Determination of Mercury by G.C.-E.C.D.</td>
</tr>
<tr>
<td>6.</td>
<td>Hollow Cathode Lamp</td>
</tr>
<tr>
<td>7.</td>
<td>Demountable Hollow Cathode Lamp</td>
</tr>
<tr>
<td>8.</td>
<td>G.C.-A.A. System</td>
</tr>
<tr>
<td>9.</td>
<td>Quartz T A.A. System</td>
</tr>
<tr>
<td>10.</td>
<td>Early G.C.-A.A. Interface</td>
</tr>
<tr>
<td>12.</td>
<td>Last Version of G.C.-A.A. Detector</td>
</tr>
<tr>
<td>13.</td>
<td>Sensitivity of Hg Using Previous G.C.-A.A. Detector</td>
</tr>
<tr>
<td>14.</td>
<td>Carbon T Atomizer with the Enlarged Base</td>
</tr>
<tr>
<td>15.</td>
<td>New Carbon Atomizer for G.C.-A.A.</td>
</tr>
<tr>
<td>16.</td>
<td>Sensitivity of Hg Using the New Carbon Atomizer</td>
</tr>
<tr>
<td>17.</td>
<td>New G.C.-A.A. Interface</td>
</tr>
</tbody>
</table>
18. Signals of Mercury Using Quartz T A.A. ----------------------------------------81
19. Calibration Curve of Total Mercury Using Quartz T A.A. ----------------------83
20. Total Mercury in Urine and Sweat by Quartz T A.A. --------------------------84
21. Separation of Mercury Compounds Using a 5-foot DEGS Column ---------------89
22. Gas Chromatogram of HgCl₂ ------------------------------------------------91
23. Gas Chromatogram of MeHgCl -----------------------------------------------92
24. Gas Chromatogram of a HgCl₂ and MeHgCl Mixture ---------------------------93
25. T.L.C. of the MeHgCl Standard ---------------------------------------------95
26. Gas Chromatogram of a Mixture of HgCl₂, MeHgCl and EtHgCl -------------96
27. Calibration of HgCl₂ Using G.C.-A.A. ---------------------------------------97
29. Mass Spectrum of HgCl₂ ---------------------------------------------------101
30. Mass Spectrum of MeHgCl ---------------------------------------------------102
31. DEGS Column Bleeding by G.C.-MS -----------------------------------------104
32. Gas Chromatogram of Urine and Sweat by G.C.-A.A. --------------------------106
33. Flame A.A. Processes and Flame Profiles -----------------------------------116
34. Perkin Elmer Pneumatic Concentric Nebulizer -----------------------------123
35. Cross-Flow Nebulizer -------------------------------------------------------126
36. Babington Nebulizer --------------------------------------------------------128
58. Chromatogram of Cr(III)-APDC
   Using H.P.L.C.-A.A. -----------------------------211

59. Chromatogram of Cr(VI)-APDC
   Using H.P.L.C.-A.A. -----------------------------212

60. Chromatogram of Cr(III)-APDC and Cr(VI)-APDC
    Mixture Using H.P.L.C.-A.A. -----------------------------213

61. Chromatogram of Cr(III)-APDC
   Using H.P.L.C.-UV ---------------------------------215

62. Chromatogram of Cr(VI)-APDC
   Using H.P.L.C.-UV ---------------------------------216

63. Chromatogram of Cr(III)-APDC and Cr(VI)-APDC
    Mixture Using H.P.L.C.-UV ---------------------------------217

64. Chromatogram of Zinc Ion
    Using H.P.L.C.-A.A. ---------------------------------226

65. Liquid Chromatogram of Urine Using A.A.
    as Zinc Specific Detector -----------------------------227

66. Chromatogram of Magnesium ion
    Using H.P.L.C.-A.A. ---------------------------------236

67. Liquid Chromatogram of Urine Using A.A.
    as Magnesium Specific Detector -----------------------------237
ABSTRACT

PART ONE. SPECIATION OF MERCURY BY G.C.-A.A.

A new graphite atomic absorption (A.A.) detector for gas chromatography (G.C.) has been developed. It was directly interfaced to a gas chromatograph through a pyrex transfer line. The interfaced G.C.-thermal atomizer A.A. system was used to speciate and determine volatile mercury compounds in perspiration and urine.

This interfaced system exhibited the excellent selectivity and high sensitivity to mercury compounds. Because of high sensitivity no sample preparation was necessary, avoiding losses and contamination which often occurs during sample pretreatment.

Total mercury concentrations were determined with a quartz T atomic absorption system by direct injection method without sample pretreatment.

The results indicated inorganic mercury was the only volatile mercury species in the samples. They also indicated the presence of unidentified nonvolatile mercury. However, inorganic mercury seemed to be the major form of mercury excretion from human bodies.

PART TWO. INTERFACING H.P.L.C. AND A.A. FOR METAL SPECIATION STUDIES

A new titanium ultrasonic nebulizer was used for sample introduction and aerosol generation for a flame atomic absorption (A.A.) unit. This new nebulization
system with the help of a desolvation device provided reproducible performance and showed improved sensitivities than typical commercial pneumatical concentric nebulizer.

Extensive evaluations on factors affecting the performance of the A.A. unit were carried out. These factors included droplet desolvation, ultrasonic frequency, element property and gas flow control.

This nebulizer was also used to interfaced flame A.A. with a high performance liquid chromatograph (H.P.L.C.) unit without losing resolution, using a piece of capillary stainless steel tubing.

The interfaced H.P.L.C.-A.A. system was used to speciate Cr (III) and Cr (VI) using reversed-phased technique. It was also utilized to speciate zinc and magnesium in urine using ion chromatographic method with direct injection.
PART ONE
SPECIATION OF MERCURY BY G.C.-A.A.

CHAPTER 1 - INTRODUCTION

A. TOXICITY OF MERCURY

Mercury is the third member of the zinc and cadmium family. Members of this family have comparatively high electronegativity values for metallic elements, and form bonds with nonmetals of significant covalent characters. The covalent properties increase going down the family from zinc to mercury.

Ochiai(1) has divided general toxicity mechanisms for metal ions into the following categories: (a) blocking of the essential biological functional groups of biomolecules (e.g., proteins and enzymes); (b) displacing the essential metal ion in biomolecules; and (c) modifying the active conformation of biomolecules. Toxicity sequences based on Ochiai's categories were summarized by Nieboer and Richardson for different metal ions toward different organisms (2). Metal ions studied include Hg, Cu, Cd, Fe, Cr, Zn, Ni, Co, Mn, Ag, Ca, Pb, Sr, Mg, Li, Al, etc. Mercury ion with Ag, Tl and Cd shows the highest toxic effects among all the metal ions studied.

Mercury is a very toxic, cumulative poison. The toxicity of mercury varies greatly with its chemical form(3). Monovalent mercury is relatively less toxic due to the low solubility of its salts. Calomel, mercurous chloride (Hg₂Cl₂), has been used
medically as a cathartic, diuretic, antiseptic and antisyphilitic. However, tissues and erythrocytes can oxidize monovalent mercury to highly toxic divalent mercury which is more soluble than the monovalent one.

The toxicity of mercury ion is attributed to its high affinity for sulfur-containing compounds and its affinity for other organic ligands.

Acute mercury poisoning can result from inhalation of inorganic mercury at high concentrations. Symptoms are metallic taste, nausea, abdominal pain, vomiting, diarrhea, headache, anuria and salivation. The kidney and intestinal tract are primarily involved. The stomach, gums and salivary glands may become inflamed.

Chronic inorganic mercury poisoning can result from exposure to low concentrations of mercury. Chronic poisoning by inorganic mercury affects primarily the nervous system. Slight anemia, hypothyroidism and increased excitability may result from low occupational exposures.

Both the liquid and vapor form of elemental mercury are toxic due to their lipid solubility, lack of charge and high membrane permeability. Elemental mercury in the vapor form is more hazardous than in the liquid form. Oral ingestion of small quantities of metal is less hazardous since it is relatively inert (e.g., dental amalgams). Liquid elemental mercury has a significant vapor pressure at room temperature and the vapor can have severe physiological effects if inhaled.
Elemental mercury, once inhaled, will penetrate all body tissue and be converted into mercury ions which are partially transported in the blood plasma. It will accumulate in the brain, nervous tissues, salivary glands, liver, kidneys, spleen and bone. Having characteristics intermediate between inorganic and organoalkyl mercury, elemental mercury is somewhat more toxic than the former but less toxic than the organic mercury compounds.

Organomercury compounds are far more toxic than inorganic mercury species. They are lipid soluble and difficult to decompose and be excreted by higher organism. They tend to be concentrated in organic tissues in the food chain. Methyl mercury is extremely toxic and its concentration and fate in the environment are of particular concern. Inorganic mercury can be transformed into methylmercury which can be absorbed and concentrated by aquatic species in aquatic ecosystems. Not only is methylmercury volatile but, if ingested, it produces an insidious destructive lesion of the brain and nervous system which may be largely irreversible.

The most toxic form of mercury, methyl mercury, was preferentially bound to protein once absorbed. Inhibition of neurotransmitter enzymes in rat cerebellum following methylmercury exposure was reported. The results of this study indicate that methyl mercury exposure does produce changes in the activities of several central neurotransmitter
enzymes.

Symptoms resulting from organomercury poisoning, especially alkylmercury, include dryness and irritation in the nasopharynx and mouth, numbness of the hands and feet and damages on the skin. Speech may become slurred and sight and hearing may be impaired. In severe cases, organomercury causes permanent neurological damages. Methylmercury is known to accumulate also in the fetus. Neurological disorders such as mental retardation and convulsive cerebral palsy have occurred in infants whose mothers were exposed to methylmercury during pregnancy (4)(10). Fetal nerve tissue is especially sensitive to methylmercury (13).

There is no specific treatment for poisoning by organomercury compounds. Neither dimercaprol nor penicillamine, both effective in neutralizing poisoning by inorganic mercurials, will either prevent or alleviate the brain damages caused by organomercury species.

Its mobility, retentiveness and preferential concentration in the nervous tissues, especially in the brain, make methyl mercury among the most toxic of mercury compounds.

In summary, all forms of mercury are toxic. Organic mercury species are much more toxic than inorganic mercury compounds due to their effects on nervous system, ease of accumulation, difficulty in excretion and lack of proper treatment after exposure. Elemental mercury is less toxic than organic mercury but more toxic than inorganic mercury due to
its membrane permeability, lipid solubility and ease of transformation into divalent mercury in the tissue.

B. Mercury in the Environment

Mercury is a comparatively rare element, only fifteen are less abundant on the earth. The average proportion of mercury in the earth's crust is about $5 \times 10^{-5}$%; its concentration in seawater is very low, about $3 \times 10^{-5}$% (6).

Despite its rarity, mercury often occurs in concentrated deposits, almost always in form of the sulfide HgS (Cinnabar). Sometimes small droplet of free mercury can be found in Cinnabar mines. The world's largest deposits of cinnabar, at Almaden in Spain, have an average mercury content about 6%. Other important mines with a mercury content around 1% are located in Italy, U.S.A., Canada, Mexico, Brazil, Peru, China, Japan, Russia, Hungary, Yugoslavia and Germany (6).

The production of elemental mercury is achieved by first crushing the ore and then concentrating by selective washing and flotation. It is then roasted in a current of air at about 600-700°C to produce mercury vapor and sulfur dioxide. The vapor is condensed to produce liquid elemental mercury. An alternative method is to roast cinnabar with iron and CaO:

$\text{HgS} + \text{Fe} \rightarrow \text{Hg} + \text{FeS}$

$4 \text{HgS} + 4 \text{CaO} \rightarrow 4 \text{Hg} + 3 \text{CaS} + \text{CaSO}_4$

The crude mercury is then washed with dilute acid or alkaline
solution to remove impurities such as Mg, Al, Cr, Mn, Cd, Ni, Sn, Pb, and Cu.

There is a mercury cycle in the environment due to the natural volatility of elemental mercury. Mercury ore deposits normally produce a considerable mercury vapor pressure that results in a low level, world-wide distribution of mercury. Figure 1 shows a typical mercury cycle in the environment.

The inorganic forms of mercury undergo transformations that mediated by microorganisms (14). Insoluble HgS can be converted to soluble Hg(II) by bacterial oxidation in sediments and soluble Hg(II) can be reduced by some bacterial enzymes to Hg(0). If both Hg(II) and Hg are present together, they will enter into an equilibrium reaction to produce Hg(I) in form of the diatomic ion Hg_{2}^{2+}. This shows a pathway through which mercury can be eliminated from soil and aquatic systems by volatilization.

Mercury can also be transformed into methylmercury through methylation by some bacteria (15)(16)(17) in sediments. The methylation of mercury appears to proceed through transfer of a CH_{3}^{−} group. A small amount of methylmercurium ion can be further methylated to (CH_{3})_{2}Hg in a similar process. The details of the processes and mechanisms of mercury methylation will be discussed in the next section.

The methylmercury ion CH_{3}Hg^{+} produced in sediments is partially extracted into the water and taken up into the food chain. Dimethyl mercury can volatilize into the atmosphere,
Figure 1. Mercury Cycle in the Environment

VOLATILIZATION TO ATMOSPHERE

\[ \text{Hg}^{2+} + \text{Hg}^0 \rightleftharpoons \text{Hg}^{2+} \] (DISPROPORTIONATION EQUILIBRIUM IN SEDIMENTS)

BACTERIA

CH$_3$Hg$^+$ EMISSION INTO WATER EXTRACATION INTO FOOD CHAIN

BACTERIA

(CH$_3$)$_2$Hg VOLATILIZATION TO ATMOSPHERE

PHOTOLYSIS

\[ \text{Hg}^0 + \text{CH}_3^+ \]
where it is photolyzed to elemental mercury and methyl radicals. Dimethyl mercury can also react with mercuric ions to give methylmercurium ion (18)(19).

Industrial input can affect the natural balance of mercury by providing additional inorganic starting material, or by direct input of organomercury compounds.

The environmental hazards of mercury have been well documented especially since the serious and extensive poisoning of the population of Minamata, Japan during the 1960's. The poisoning was caused by the consumption of mercury-contaminated fish which were contaminated by discharges of organomercury by local industry.

The Environmental Protection Agency issued a publication on mercury flow in the environment and regulation offered by various bodies on mercury use (20). Two major bibliographies have been prepared by major chemical producers, Dow Chemical Co.(21) and Imperial Chemical Industries(22). The fate of mercury has been comprehensively discussed by Hartung and Dinman (23).

The major sources of emission of mercury from industry are from mercury production, sulfide ore processes, Coal combustion, oil combustion, chloralkali, paints, electrical apparatus, catalysis, agriculture and pharmaceuticals industries (24). Natural emissions of mercury into air were estimated to be three times greater than man-related discharges. The fate of mercury and its compounds in the air.
is not clear, but the mercury eventually returns to the land and to the water. Natural sources contribute about twice as much mercury to water as do man-related discharges. Most mercury discharged to water appears to be incorporated rapidly into sediments and may be released by biological and mechanical action over a period of times (24).

C. METHYLATION OF MERCURY

Methyl mercury salts were introduced in 1914 in Germany for seed treatments and their uses became widespread. Investigations into methylation of mercury followed the reported human poisoning at Minamata Bay and Niigata in Japan in the late 1950's. These poisonings arose from ingestion of fish and shellfish containing methylmercuric compounds, which were probably derived, at least in part, through methylation of mercuric salts by aquatic organisms (25)(26)(27).

Methylation is an important determinant of metal toxicity particularly for heavy metals such as lead and mercury. Methylation of inorganic mercury was first suggested by Kurland in 1960(28). In 1963, methyl thiomethyl mercury was isolated from shellfish in Minamata Bay, Fujiki suggested that mercury could be methylated by marine life (29). Jensen and Jensen and Jernalov reported that anaerobic microorganisms in sludge (i.e., organisms such as those found in lake-bottom mud) can methylate inorganic mercury and suggested a mechanism for the methylation process (27). In 1968, Wood, Kennedy and Rosen at the University of Illinois published an article confirming
the fact that the methylation reaction can proceed enzymatically by the use of cell extracts of a methanogenic bacterium. The latter was originally isolated from a symbiotic mixed culture from canal mud. Nonenzymatic methylation involving methylcobalamin, a vitamin B12 analog, was also suggested in their report. It should be noted that the richest sources of vitamin B12 include estuarine mud (3 ug/g dry weight) and activated sewage sludge (50 ug/g) (30).

It was explicitly pointed out by Wood and his co-workers the consequences to the food chain of methylation of mercury. Transfer of methyl groups from Co$^{3+}$ to Hg$^{2+}$ in biological systems may also occur as a non-enzymatic reaction. It will be enhanced by anaerobic conditions and by increasing the numbers of bacteria capable of synthesizing methyl cobalamines if the methyl-transfer reaction is significant in biological systems. Pollution of bodies of water with nutrients (i.e., sewage) increases the rate of formation of methylmercury species. The methylmercury produced is then available for incorporation into various organisms in the aquatic environment, and secondarily into terrestrial predators.

After the report by Wood, research has grown extensively on the methylation of mercury and other metals. Reactions involving the vitamin B12 derivative methylcob(III) alamin have been more extensively studied, probably because of the commercial availability of this biogenic reactant and the large number of model cobalt compounds showing strikingly similar
Wood and co-workers proposed a mechanism for methylation of inorganic mercury by methylcobalamin, as shown in Figure 2. In this study, methylation of mercuric acetate was examined and rate constants for each step in the mechanism were determined by UV-visible spectrophotometric techniques (32)(33). Methyl-B12 is capable of transferring methyl entity under different Co-C bond cleavage situations as shown by Figure 3. For mercury, the methyl group is transferred as an anion to the electrophilic mercuric ion. The presence of chloride ion greatly slows the methylation process (34). This is due to the electron-donating ability of the chloride which lowers the electrophilic nature of the metal. Comparison of various anions showed that the rate of methylation varied \( \text{OAC}^- > \text{Cl}^- > \text{SCN}^- > \text{Br}^- > \text{CN}^- \) (35).

Extensive research has also involved individual species of microorganisms or microbial communities as the actual methylators. Several reviews on this topic have been published (15)(16)(17). The indigenous microflora of animal intestines causes methylation of mercury and arsenic (36). This transformation has been reported for such microflora of six fresh water fish species (37). Methylation of mercuric chloride by intestinal flora of rats was also reported by Rowland (38). The rate of methylation increased initially, passed through a maximum, and decreased sharply after sixty hours. Methyl mercury sulfur compound seemed to be involved in the metabolism of mercury in the small intestine (39). In fact, the major
Figure 2. Proposed Mechanism for Mercury Methylation by Methylcobalamin
Bz = 5,6-dimethylbenzimidazole

Figure 3. Bond Cleavage of CH$_3$-B$_{12}$ for Methylation
methyl mercuric compound isolated from toxic Minamata Bay shellfish was the thiomethyl derivative \( \text{CH}_3\text{HgSCH}_3 \) (40). Latter, Craig and Bartlett argued that \( \langle \text{CH}_3\text{Hg}\rangle_2\text{S} \) was the active intermediate in this process, which was then decomposed into \( \langle \text{CH}_3\rangle_2\text{Hg} \) and \( \text{HgS} \) (41).

Photolysis of acetate ion in the presence of \( \text{Hg(II)} \) gives methylmercuric compounds as products (42)(43)(44)(45).

\[
\text{HgX}_2 + \text{CH}_3\text{CO}_2^- \rightarrow \text{CH}_3\text{HgX} + \text{CO}_2 + X^-
\]

The reaction rate is enhanced by the presence of strongly colored solid. Since acetate ion can occur in natural waters, photolysis by sunlight may well be a potential, ubiquitous route to methylmercury compounds. One example is the reported photolysis of some aliphatic amino acids to form methylmercurium ion (46), where \( R = \text{CH}_3, \text{i-C}_3\text{H}_7, \text{i-C}_4\text{H}_9 \) or \( \text{sec-C}_4\text{H}_9 \).

\[
\text{RCH(NH}_2\text{)CO}_2\text{H} + \text{HgCl}_2 \rightarrow \text{CH}_3\text{HgCl}
\]

The presence of the colored \( \text{Cu}^{2+} \) ion will accelerated photomethylation.

Methylation of mercury can also be achieved through a cross-over mechanism proposed by Brinckman, Iverson and co-workers (15)(47). They found that a species of Pseudomonas found in the sediments of Chesapeake Bay would form methyltin compounds; in the presence of both mercury and tin, methylmercuric and methyltin compounds formed. Figure 4 shows
this mechanism. The methylated tin species generated by Pseudomonus reacts environmentally formed Hg(II) to form methylmercuric ion. There has been an increasing use of organotin compounds in recent years in various technological applications (48). This mechanism may indicate another source of mercury methylation in the environment.

Methylation of mercury undoubtedly occurs naturally in the environment. The change in chemical form of mercury due to methylation controls the pathway of entry into human body, the toxicity and retention of mercury and its excretion. The mechanism of formation of methylmercury in the environment is also applicable to other elements, e.g., tin, palladium, platinum, thallium, selenium, and arsenic, all of which form relatively nonpolar metal carbon bonds.

Research efforts into methylation of metals have been intense but irregular. There remains ample area for further research.

D. METHODS OF DETERMINATION AND SPECIATION OF MERCURY

1. DETERMINATION OF TOTAL MERCURY:

Many analytical methods have been used for the determination of mercury. Compounds of mercury generally have very high volatility compared to those of the alkaline earth metals. Collection of mercury compounds is complicated by this high vapor pressure. Frequently, the choice of method of analysis is largely dependent upon what method of sampling is
Figure 4. Methylation of Mercury by a Cross-Over Mechanism
employed. The degree to which the sampling method collects the different forms of mercury provides the basis for the usefulness of the entire procedure.

Sampling mercury compounds from air is more difficult than sampling from water and soil because of the numerous forms in which mercury may exist in the air. The interconvertibility of the various forms of mercury further complicates sampling. Methods concerning sampling mercury from air will be mentioned and different techniques of analysis will be discussed in this section.

Methods of sampling mercury in air can be divided into two general categories, (A) those methods which remove mercury and mercury compounds from air by scrubbing and (B) those methods which collect an air sample. The scrubber type sampling devices include filters, adsorbants, bubblers and amalgamable collectors. These sampling devices collect the mercury samples either discriminately or indiscriminately. However, it is frequently necessary to convert the mercury to an easily determinable form such as Hg (11).

Adsorbents are among the most popular collectors. However, little information is known about their collection efficiencies. Filter papers impregnated with selenium, selenium sulfide and potassium iodide have been used to collect mercury vapor (49). Filter papers made of cellulose fiber, fibrous glass and quartz wool can be used to collect particulates.

Amalgamable collectors can be used to collect elemental mercury
from air. Collectors include gold, platinum and silver in various forms. Again, evaluation of collection efficiencies has not been studied. A sequence of absorbers can be used to sample different forms of mercury selectively before the final analysis by flameless atomic absorption (50).

A filter is used to collect particulates. This is followed by carbon black molecular sieves absorber to collect organomercury vapors. Metallic mercury vapor is trapped in the last absorber.

Collection of a direct air sample is mostly done with dynamic monitoring method in which mercury is drawn directly through a monitoring instrument that can be a remote unit or a portable unit. Frequently, only elemental mercury can be monitored with this method.

The determination of mercury in biological material presents special challenges even at ambient temperature. Difficulties arise from the volatility of reagents and interferences in the sample matrix. For example, there is the additional need for preventing losses of mercury while destroying organic material. Contamination from hydrochloric acid is possible. Hydrochloric acid is made from chlorine that has been produced by electrolysis of sodium chloride with amalgam electrodes (51).

Different procedures and modifications using spectrophotometric techniques have been described for the determination of mercury. Dithizone and mercury reaction is
probably the most popular (52)(53)(54). Monovalent and divalent mercury react with dithizone in acidic solutions to yield yellow and orange complexes, respectively. Interferences can be from other metals such as copper, silver, gold, palladium and platinum. In biological sample analyses, interferences from copper can be serious problem. The mercury-dithizone complex is sensitive to light. Exposure to light will change the colors into greenish tones fairly rapidly. Di-β-napthylthiocarbazone forms a red complex with mercury that is stable in light. This reaction has been utilized to mercury determinations in biological materials (55)(56). Diphosphylcarbazide reacts with mercury to form a blue-violet complex (57). However, interferences from copper, iron and zinc may complicate the procedure when analyzing biological samples (57).

With the advances of science and technology, instrumental methods are now preferred for mercury determination, including atomic absorption, atomic emission and neutron activation methods. The most popular method is the Hatch and Ott method, the cold vapor technique, which involves atomic absorption (58) but includes a tedious sample pretreatment. This method was developed for analyzing water samples although it has been modified for other types of sample. Potassium permanganate is added to the sample contained in a bottle to convert mercury to Hg(II). Hydroxylamine is added to remove excess permanganate. The mercuric ion is reduced by addition of stannous chloride or sulfate. The mercury vapor produced is swept out by a stream of
air into an absorption cell. Determination is carried out by A.A. at 253.7 nm. Introduction of potassium persulfate may be necessary to ensure complete oxidation of some organomercury compounds. Several other modifications were developed based on the cold vapor technique (59)(60).

Flame A.A. for mercury analysis is usually not sensitive enough for many samples. The absorption signal can be enhanced by sodium, potassium or phosphorus. Nevertheless, when the mercury concentration is high in samples, flame A.A. is still a convenient method.

Emission spectroscopy can be used for mercury determinations. However, conventional emission spectroscopy is usually not sufficiently sensitive for the direct measurement of mercury. Methods involving plasma excitation sources, such as DC discharge and ICP, do have the required sensitivity. Emission spectroscopy is less susceptible than atomic absorption to interference from aromatics, since aromatics do not usually emit in the 253.7 nm region. Interference can arise from cobalt in the sample matrix. Cobalt has an emission line at 253.65 nm (61). In the application of emission spectroscopy, the mercury can be reduced to elemental mercury with sodium borohydride solution and allowed to diffuse through a latex or saran wrap membrane into a helium stream. The stream is then swept into the excitation source. This method avoids the interference from cobalt (62).

The total mercury content in fish, common foods and water
samples can be determined by neutron activation analysis (63)(64)(65). However, in each of these analyses, mercury must be separated using either ion exchange or precipitation. Mercury is then isolated and sealed for irradiation. The potential of neutron activation anion exchange technique to distinguish between inorganically bound and organically bound mercury is indicated by the low recovery of the latter (66).

2. SPECIATION OF MERCURY COMPOUNDS

Because of the toxicity differences between the inorganic and organic mercury, there has been a number of methods developed for mercury speciation. Many chromatographic techniques, such as thin-layer chromatography, paper chromatography, ion exchange chromatography, gel filtration, and others, have been applied to the speciation of mercury compounds (67). However, TLC and paper chromatography are more frequently used only for high molecular weight mercury species because of the volatility of smaller mercury compounds (68)(69).

Total mercury and methylmercury in hair have been determined with isotope dilution after digestion (70). Isotope exchange has been used to determine inorganic mercury in the presence of organic mercury in biological samples (71).

Selective adsorption followed by A.A. has been utilized to speciate volatile mercury compounds and elemental mercury vapor in air samples (72).

The cold vapor-A.A. technique have been used for mercury
speciation in solutions \((73)(74)(75)\). These methods involve changes in the reducing conditions to allow sequential reduction of different forms of mercury from solutions before the final determination with A.A.

However, gas chromatography with an electron capture detector (G.C.-E.C.D.) was the most commonly used method for mercury speciation in biological and environmental samples \((76)(77)(78)\). This method does not distinguish mercury from many other compounds and therefore involves a series of extractions followed by the G.C.-E.C.D. determination. A typical sample treatment scheme is shown in figure 5.

These techniques developed for mercury speciation inevitably require elaborate sample pretreatment such as digestion, separation, extraction and concentration. Risks of sample contamination, chemical nature change and sample loss are involved.

E. REASONS OF THIS STUDY:

There are two major sources of error in trace analysis. Sample contamination causes high answers but loss of the analyte causes low answers. The net result is that the range of error is often very wide. Minimal sample handling and chemical pretreatment are important in trace analysis in order to avoid contamination of samples, loss of the analytes and chemical change of the species present. Direct analysis is the best approach for trace analysis, if possible.

Several requirements are necessary before a direct
Sample Containing \( \text{CH}_2\text{HgX} \)

\[ \text{Suspended in } \text{H}_2\text{O} + \text{HCl} \]

\( \text{CH}_2\text{HgCl} \), Sample in Hydrochloric Acid

\[ \text{Extracted with Benzene} \]

\[ \text{Centrifuged} \]

\( \text{CH}_2\text{HgCl} \), Impurities in Benzene

\[ \text{Extracted with Cysteine} \]

\[ \text{Acetate in Water Solution} \]

\[ \text{Centrifuged} \]

\( \text{CH}_2\text{HgSCH}_2\text{CH(NH}_2\text{)}\text{COOH in H}_2\text{O} \)

\[ \text{Acidified with HCl} \]

\( \text{CH}_2\text{HgCl in Hydrochloric Acid} \)

\[ \text{Extracted with Benzene} \]

\[ \text{Dried with Na}_2\text{SO}_4 \]

\( \text{CH}_2\text{HgCl in Benzene, Ready for G. C.- E. C. D. and T. L. C. Analyses} \)

**Figure 5.** A Sample Pretreatment Scheme for the Determination of Mercury by G. C.- E. C. D.
analysis can be actually performed. Sensitivity must be sufficient for the species to be detected. This avoids the necessity for preconcentration of the pertinent species. The method must be capable of detecting all the components of interest but not react to interferences from other components in the sample. Under these conditions, separation or isolation procedures can be eliminated. Data resulting from direct analysis should be more accurate and reliable as a result of the simplified analytical procedures.

After reviewing the literature for mercury speciation and determination, no methods were found for direct analysis for mercury compounds. This study is to develop methodology to perform mercury speciation and determination using direct analysis.

For the determination of total mercury, a specially designed quartz T graphite A.A. was used. Atomic absorption offers a selective method for metal determination. The design of the quartz T A A should permit the elimination of major matrix interferences, as will be discussed later.

Based on the literature, chromatographic techniques are the best separation method. The combination of gas chromatograph and graphite A.A. should provide the basis of a sensitive method for mercury speciation, especially for the volatile mercury compounds.
II CHAPTER 2 - THEORY

A. GAS CHROMATOGRAPHY

1. HISTORY

No other analytical technique has gone through such rapid and intensive development as gas chromatography (G.C.). It actually originated from the selective adsorption technique for determining the light hydrocarbon content of natural gas. In the 1920's the charcoal test was widely used in determining natural gas by adsorption on charcoal followed by removal of the adsorbed compounds by displacement with glycerol or mercury vapors (79). Combination of a temperature control device and mercury vapor were later introduced to get better results by Turner. He also recorded the thermal conductivity of the effluent from the adsorbent (80). The recorder trace is similar to a chromatogram.

Modern gas chromatography using a continuous flow of inert carrier gas was introduced by Hesse and coworkers at the University of Marbury / Lahn, in Germany to separate volatile organic acids. However, the method was termed adsorption distillation that time (81).

Modern gas adsorption chromatography was developed by Cremer, Prior and Muller at the University of Innsbruck. A system consisting of a carrier-gas source sample introduction device, a thermostated separation column and a thermal conductivity detector was developed by them for the analysis of light gases and physico-chemical measurement. Several basic
terms and relationships now universally used were also introduced (82)(83)(84).

The introduction of partition chromatography as the basis of separation by Martin and Synge, in 1941, was a most significant advance in analytical chemistry (85). They were recognized by the 1952 Nobel Prize in Chemistry.

After the introduction of gas-liquid partition chromatography, gas adsorption chromatography was used in practice almost exclusively for the analysis of inorganic gases and C1-C20 hydrocarbons. The idea of partition chromatography was put into practice by Martin himself in 1951 at the National Institute for Medical Research, in London, and associated with A.T. James for volatile fatty acid analysis (86). Due to the success of this technique, more work was followed and published (87)(88)(89). The sensitivity, speed, accuracy and simplicity of this method for the separation, identification and determination of volatile compounds has resulted in a phenomenal growth since then.

2. THEORY

Gas chromatography is a technique for separating volatile substances by flowing a gas stream over a stationary phase. If the stationary phase is a solid, we speak of gas-solid chromatography (G.S.C.). This depends on the adsorptive properties of the column packing to separate samples, primarily gases. If the stationary phase is a liquid, we speak of gas liquid chromatography (G.L.C.). The liquid is spread as a thin
film over an inert solid and the basis for separation is the partitioning of the sample between the mobile phase and the liquid film. The usable temperature range for the liquid phase (substrate) can be as wide as up to 400°C. It is now possible to have chemically bonded liquid phases on the inert solid supports. These make G.L.C. the most versatile and selective form of gas chromatography. It can be utilized to analyze gases, liquids and even solids.

The basis for separation in G.C. is the equilibrium distribution of different compounds between two phases which causes a differential migration rate for different components in the sample. In G.L.C., the net flow rate of a compound through a column is controlled by (a) the fraction of solute in the mobile phase (gas stream) and (b) the partition coefficient of the compound between the mobile and the stationary phase (substrate) which determines the time the compound will spend in the stationary phase.

The resolution of chromatographic peaks is related to two factors: column efficiency and solvent (substrate) efficiency. Column efficiency is concerned with peak broadening of an initially compact sample band as it passes through the column. The degree of broadening results from the column design and operating conditions and can be quantitatively described by the height equivalent of a theoretical plate (HETP), a term borrowed from separation by distillation. The HETP is the length of column necessary to attain the dynamic solute
equilibrium between the mobile phase and the stationary phase.

Solvent (substrate) efficiency results from the solute-solvent interaction which determines the relative position of solute bands on a chromatogram. This is measured by the respective distribution coefficients of the solutes in the solvent at a given temperature.

Column efficiency is measured by the number of theoretical plates. If it is assumed that the entire sample is introduced onto one theoretical plate as a single plug, then the number of theoretical plates is expressed by the empirical formula developed by Martin:

\[ N = 16 \left( \frac{X}{Y} \right)^2 \]

where:  
\( N \) = number of theoretical plates  
\( X \) = distance between the point of injection and the peak maximum.  
\( Y \) = peak width.

To compare columns of different lengths, HETP is preferably used. It is related to \( N \) by:

\[ H = L/N \]

where \( L \) is the length of the chromatographic column, usually in centimeters. The rate theory developed by van Deemter and extended by Glueckauf and other workers is important and useful in optimizing chromatographic performance (90)(91). A basic equation was derived for the height equivalent to a theoretical plate in a G. C. column:

\[ \text{HETP} = A + B / V + CV. \]
Three principal contributions to the broadening of a G. C. band are included:

Term A is the multipath effect and eddy currents, term B is due to the molecular diffusion and C is the resistance to mass transfer. The linear gas velocity is represented by V. Even that V is not always constant over the entire length of the column due to the compressibility of the carrier gas. A plot with HETP against V with a hyperbola shape can normally be obtained. The flow rate with the minimum HETP is the optimum flow rate for column efficiency.

To increase the column efficiency by minimizing the A term, one can use small particles of uniform size consistent with low pressure drop and smaller diameter columns. The method of reducing the B term is to increase the linear flow velocity V and use a higher molecular weight carrier gas since diffusivity of a gas may be reduced by increasing the density of the gas. The density of the gas is determined by the pressure and molecular weight of the gas. To minimize the C term, a thin film of a low viscosity liquid should be used. The flow rate must be low enough and distribution coefficient high enough to favor equilibrium between the liquid and gas phase.

Solvent efficiency depends on the solute-solvent interaction which determines the retention of the solute in the column. The striking feature of G. C. is that a wide range of liquid phases can be used. Substances having the same vapor pressure can be easily separated by appropriate selection of
the liquid phase.

There are four major interaction forces between the stationary phase and the sample which account for G.C. separations (82). These are orientation or Keesom forces, induced dipole or Debye forces, London or non-polar forces and other specific interaction forces. Orientation or Keesom forces result from interaction between two permanent dipoles. Molecules with a permanent dipole can induce dipoles on neighboring molecules and cause interaction. Interactions can also arise from instantaneous dipoles of the two interacting species. This is the only source of attractive energy between two nonpolar substances. Other specific interaction forces includes chemical bonding and complex formation. All these interaction forces determine the solubility and the G.C. separation.

The partition coefficient $K$ represents the combined effects of these forces, where

$$K = \frac{\text{quantity of solute per unit volume of stationary phase}}{\text{quantity of solute per unit volume of mobile phase}}.$$ 

If the partition coefficients of two compounds are dissimilar, separation of them will be possible. The greater the difference in the $K$ values is, the fewer the number of plates required to achieve a separation. Solvent efficiency is measured by the ratio of the partition coefficients of the two compounds to be separated.

It is very important to realize that both and $K$ are
temperature dependent. The partition coefficient, \( K \) decrease with increasing temperature. This also increases the fraction of solute in the gas phase and decrease the retention time. Since it is the liquid phase performing the separation, this in turn gives a decreased separation. To have more interaction with liquid phase, a lower temperature should be used, i.e., more separation and longer analysis time.

3. COLUMNS

Since the actual separation of sample components is achieved in the column, it is the heart of the chromatograph. The choice of column determines the success or failure of a particular separation. Both capillary and packed columns are popular in gas-liquid chromatography. Capillary columns are open tubular columns, ranged from 0.2mm to 0.75mm in i.d., with either a thin film of liquid phase coated or chemically bonded on the wall. A typical analytical packed column is ordinarily 1/16'' to 1/4'' O.D. tubing from 3 to 30 feet in length. It is usually prepared by coating the liquid phase onto certain solid support and then pack the coated solid support into the tubing.

For an efficient separation, the liquid phase in the column should be similar chemically to the components of the mixture. A good understanding about a sample will help in the selection of a suitable substrate for the column. Liquid phases of different nature, such as polarity, can be used for separating components of different chemical classes in a mixture.
A good liquid phase should have the following characteristics: a) be chemically inert toward the solutes of interest and the column temperature, b) be nonvolatile over the operating temperature range, c) be thermally stable and d) be capable of separating sample components.

Solid supports used for supporting the liquid phase should also meet certain requirements including a) large surface area—up to 20 sq. meters per gram, b) uniform pore structure and size, c) chemically inertness and d) good mechanical strength. Usually one must make choice between inertness or efficiency (high surface area).

The amount of liquid phase coated onto the solid support in a column should be enough to coat the particles with a thin uniform layer. Too much liquid phase will collect in pools between particles and decrease the column efficiency. Compounds with low volatility are best run on low-loaded columns—3% or less. Very volatile compounds require higher loading of liquid phase, up to 30%, to achieve enough separation.

The real breakthrough in higher column performance is due to Golay who, in 1956, started to investigated the operation of a GC column from a theoretical basis (93). A packed column was considered as equivalent to a bundle of capillary tubes coated with the liquid phase, with an i.d. of the order of the solid support particle size. This idea is actually the theoretical basis of the open tubular capillary column. In capillary, there is no packing and the liquid phase
is either coated on or chemically bonded to the inside wall of the column tubing which can be glass or fused silica. Today, these columns are used in a large number of laboratories for day-to-day, routine applications.

4. DETECTORS

An automatic titrator was used to detect the fractions emerging from the column by James and Martin in their original work (86). Katharometer (thermal conductivity detector) was introduced by Ray and have been used for many years (94). It remains one of the most popular detectors even today.

Two highly sensitive detectors based on ionization principles were developed in 1958. The flame ionization detector (F.I.D.) was described by Harley, Nell and Pretorius (85). The argon ionization detector was reported by Lovelock and immediately utilized commercially (96)(97)(98). The electron capture detector (E.C.D.) also developed by Lovelock is a highly sensitive and selective detector for halogenated compounds was actually a modification of the original argon detector (99). Today, E.C.D. finds its wide application in pesticide monitoring. The standard source is Ni63.

Other detectors used in G.C. are the coulometric detector, the thermal conductivity detector and the flame photometric detector (100)(101)(102). When choosing a detector, one must consider the selectivity, sensitivity and whether the detector is destructive or nondestructive.
5. SPECIAL DETECTORS and COMBINATION TECHNIQUES

Gas chromatography is one of the most powerful separation techniques and can be interfaced with other analytical systems. This offers even wider range application of G.C. to chemical mixtures.

G.C. interfaced with I.R. is commercially available for years due to the invention of F.T.-I.R. which provides a fast process to obtain I.R. spectra for the components in a mixture. Identification of functional groups or structures can be easily achieved with the help of a microcomputer.

Packed column G.C. can be interfaced with a mass spectrometer using a jet separator. Capillary column can be directly interfaced with the M.S. ionization source without using extra device. Mass spectra of sample components can be obtained immediately after the separation. The selective ion monitoring method enables one to monitor a compound by G.C.-MS at a sensitivity level of $10^{-12}g$. G.C.-MS offers a powerful method for separation and identification. It is used routinely in many laboratories today.

G.C. can be interfaced with tandem M.S. using a triple-quadrupole system. Other inert gas needs to be introduced into the central quadripole for further fragmentation of the molecule fragments. This method enables the further identification of a compound fragment by a second mass spectrometer unit. A typical commercial G.C.-MS-MS can analyze a mass range up to 1000 with median resolution. A
higher resolution can be obtained with the introduction of an extra magnetic mass selector. Today, G.C.-MS-MS is probably the most powerful G.C. system for general-purpose applications.

B. ATOMIC ABSORPTION

1. BASIC PRINCIPLES

Atomic absorption was recognized, in 1953, by Walsh as a potential technique for chemical analyses. In 1955 he published an excellent paper examining the basic principles and the theoretical factors governing the relationship between atomic absorption and atomic concentration (103).

In atomic absorption, molecules or ions are converted into free atoms and then the absorption of radiation by these free atoms is measured. The absorption of radiation by free atoms follows a Beer's law-type relationship in which absorption has a logarithmic relationship to concentration. If the population of free atoms in the light path of an atomizer is homogeneous, concentration is a measurement of the number of free atoms.

The total absorption is proportional to the number of free atoms and the oscillator strength of the absorption line represented as followed equation:

$$
\int_0^\infty K_v \, dv = \left( \frac{\pi e^2}{Mc} \right) N_v \, f
$$

where:

- $K_v$ = absorption coefficient at frequency $v$
e = charge of an electron
m = mass of an electron
c = speed of light

N_v = number of absorbing atoms which can absorb at frequency v

f = oscillator strength of the absorption line, i.e., average number of electrons per atom which can be excited.

The oscillator strength can be defined mathematically as

\[ f = \frac{\lambda_0^2}{8\pi} \frac{g_2}{g_1} A_2^{-1} \]

where:
\( \lambda_0 \) = wavelength of the emission line
\( A_2 \) = Einstein's coefficient of spontaneous emission

in the two states 1 and 2.

The oscillator strength for an element is a constant for a particular resonance line of that element. It is also the limiting factor for the sensitivity of atomic absorption, for a given element at a given line.

The size of the atomic absorption signal is determined by the number of free atoms produced in the atomization process will determine the sensitivity of atomic absorption. This means that atomization efficiency actually controls the sensitivity
2. LIGHT SOURCES

Many different types of light sources are available today for atomic absorption spectrometry. The major requirement of a light source is that it should emit resonance radiation of the element with a half-line width less than the width of the absorption line. The half-width of the line is on the order of 0.001 nm. A good atomic absorption line should be free of significant continuum background. It is also important that no other line enters the spectral bandpass of the light dispersing device since this would not be absorbed and would be a direct interference. A hollow cathode lamp emitting characteristic wavelengths of the element used to construct the lamp is the best A.A. source. Different hollow cathode lamps will be discussed as followed.

a. HOLLOW CATHODE LAMPS

Figure 6 illustrates a schematic representation of a hollow cathode lamp. Hollow cathode lamps, which have been used for many years in spectroscopy are by far the most popular source for atomic absorption.

The cathode consists of a hollow cup made from the same metal as the element to be determined. In some cases, where a solid cathode material cannot be made, the cathode is supported in some other matrix. The anode is a tungsten wire or spherical disk. A glass envelope with quartz window is used to accommodate the two electrodes. Inert gases, neon, argon or
Figure 6. Hollow Cathode Lamp (H.C.L.)
helium, are used as filler gases in the lamps. A voltage about 400 V is applied between the two electrodes with currents ranging from 1 to 50 mA. The inert gas becomes charged at the anode and is attracted at high velocity to the cathode. The collision of the gas onto cathode sputters metal atoms from the cathode cup. Further collisions of the atoms with the filler gas produce excited metal atoms. Light is emitted from the excited atoms when they return to the ground state.

Two major problems involved in hollow cathode lamps are the Doppler effect and self-reversal. The Doppler effect causes line broadening when the cathode is hot. This is detrimental to the successful operation of the lamp, since the 'Wings' of these emission lines cannot be absorbed by the free atoms from the sample.

Self-reversal is due to the reabsorption of the emission lines by a cloud of cold atoms which form when the hollow cathode is operating. They may also be coated on the window of the lamp. This absorption is at the very center of the emission line and can lead to the distortion of calibration curves. Demountable hollow cathodes can be used to alleviate these problems.

b. DEMOUNTABLE HOLLOW CATHODES

A schematic diagram for a demountable hollow cathode is shown in figure 7. In a demountable hollow cathode, the gaseous metal atoms causing self-reversal can be removed by means of a flow of the filler gas. Cooling water is used to maintain low
operational temperature to reduce the Doppler effect. The cathode is changable by opening the housing of the lamp. However, a vacuum system is needed to maintain the low pressure of the lamp.

Due to the removal of the gaseous metal atoms by the stream of filler gas, light intensity from the demountable hollow cathode is usually higher. The only disadvantage of demountable hollow cathode lamps is the longer warm-up time to maintain steady emission.

c. ELECTRODELESS DISCHARGE LAMPS (E.D.L.)

This type of lamp was developed by Dagnall and West (104)(105). The electrodeless discharge lamps (E.D.L.) give stable high intensity emission lines. They also enjoy a longer life time. They are available for most of the elements on the periodic table and especially useful for those volatile elements such as Se, As, Hg, Cd and S.

In an E.D.L., a small amount of metal or metal salt is placed in a small quartz tube. The tube is evacuated before the addition of the filler gas. The tube is then sealed and inductively heated with a radiofrequency field. The ionized filler gas and electrons cause the sputtering of the metal atoms producing the higher intensity lines.

3. BACKGROUND CORRECTION IN ATOMIC ABSORPTION

When organic and inorganic molecules are introduced into a flame atomizer, decomposition may not be complete. Undecomposed fragments absorb over a wide wavelength range. When this
Figure 7. Demountable Hollow Cathode Lamp
absorption includes the atomic absorption wavelength, background correction must be employed to compensate. Several methods for background correction will be discussed in this section.

a. BACKGROUND CORRECTION USING A BLANK SOLUTION

This is only non-instrumental method for background correction in atomic absorption. The blank solution must contain the same matrix as the sample but should exclude the element of interest. The difference in absorption signals between the sample and the blank is assumed to be due to the element being determined. However, in real cases, it is always difficult to have full information on the samples or a reliable blank may not be obtainable. Several other instrumental methods for background correction are more useful.

The general principles of instrumental methods for background correction are based on the utilization of a radiation source that either is not absorbed by the element of interest or if absorbed by the element of interest the absorption is negligible. However, it is absorbed by the background, giving a reliable measurement of background absorption.

b. BACKGROUND CORRECTION USING THE TWO-LINE METHOD

Molecular background can be measured by using an emission line which in wavelength is very close to, but is not the same as the absorption line of the element being determined. The
atomic absorption signal at the resonance line consists of the atomic and background absorption, whereas the absorption at the non-resonance line is purely due to molecular background. The net absorption by the element of interest is the difference between these two absorption signals.

Two measurements of the sample are necessary and they should be carried out simultaneously. If not, it must be assumed that all conditions remain constant between measurements and this is usually an unsafe assumption.

c. BACKGROUND CORRECTION USING CONTINUUM SOURCES

Continuum light sources, usually hydrogen or deuterium lamp can be used for background correction. The spectral line width falling on the detector from the continuum source, about 0.1 nm, is much wider than the absorption line width, about 0.001 nm or 1% of the total light falling on the detector. The contribution of the atomic absorption from the element of interest is negligible using the continuum sources. However, absorption by molecules is across the entire spectral slit width, providing a measurement of the molecular background absorption. If an atomic line is completely absorbed from a waveband of 0.1 nm the total absorption is only 1%. This is a negligible amount and may be ignored. Consequently, the absorption of the continuum sources is a measure of the background absorption.

Many commercial instruments have incorporated a deuterium
lamp into a double beam optical system. Lights from the deuterium lamp and the hollow cathode lamp alternatively pass through the atomizer and are monitored through the detector system. The difference in the absorption signals is manipulated electronically and the net signal is attributed to the element being determined.

d. The ZEEMAN BACKGROUND CORRECTION

Background correction using the Zeeman effect allows one to do background correction using the same source for measuring the sample absorption and the background. With the application of a magnetic field the hollow cathode light source is split into two lines. Their wavelengths are slightly shifted away from the original resonance wavelength. The original unshifted resonance line disappears. The magnetic field to create the Zeeman shift is modulated to provide an oscillating sequence during the analysis time. Modern atomic absorption spectrometer, such as Perkin Elmer Zeeman 5000, places the magnetic field around the atomizer rather than the source. In practice, difficulty was encountered in stabilizing the source when it was placed in the magnetic field. The magnetic field affects the energy states of the element. The wavelength from the hollow cathode will not be absorbed by the element when the magnetic field is on; however it will be absorbed by the molecular background. The wavelength will be absorbed by the element and the background when the magnetic field is off. The difference between the two absorption signals is manipulated
electronically to give the net atomic absorption signal. Although an extra magnetic field is required in Zeeman background correction, it is by far the most convenient and accurate method for atomic absorption background correction.

4. SIGNAL MODULATION

In a flame atomizer free atoms are formed and are distributed between the ground state and the excited state according to the Boltzmann distribution. Some of the excited atoms undergo a transition to the ground state and emit a photon of the same wavelength as that at which they absorb, i.e., the resonance line. These signals can be distinguished using modulation. The major purpose of modulation is to be able to distinguish the light from the source which coming through the atomizer or from the emission of excited atoms in the flame. If the source were not modulated, the extraneous light could even neglect the absorbance.

Source modulation can be achieved by chopping the source with a mechanical chopper and followed by an amplifier tuned to the chopping frequency. Another means of source modulation is to use an AC source with an AC amplifier. Since the light from the flame and the atomic emission is a DC signal, an AC amplification system would only detect the light from the source and eliminate extraneous light interference.

Atomic absorption light sources must be modulated. The modulation of the source is simply a mean of switching it on or off or superimposing another signal overt it.
5. ATOMIZATION PROCESSES IN CARBON ATOMIZERS.

Heated graphite furnaces and carbon rods are major types of flameless process for atomization since the presentation of two important papers at the Sheffield (England) International Conferences on Atomic Absorption Spectroscopy in 1969 by Robinson and by West (108).

It is advantageous to understand the atomization process in carbon atomizer before one can really appreciate various designs of carbon atomizers. A mechanism for the production of free atoms in a graphite furnace was proposed by Campbell and Ottaway based on the reaction:

$$\text{MO (S) + C (S)} \rightarrow \text{CO (g) + M (g)} \quad (110).$$

Most carbon atomizers require three steps for a complete atomization process: 1) drying – evaporation the solvent from the sample, 2) ashing the sample into residue at a higher temperature and 3) atomization at a further higher temperature. Each step in the cycle of dry, ash and atomize is important and the proper time and temperature parameters must be carefully selected. No sample loss is allowed before the final atomization step.

Liquid samples may diffuse into the carbon atomizer. This affects the drying, ashing and atomization cycle since sample must diffuse out of the carbon for a complete detection. Sample diffusion can be minimized by using pyrolytic carbon or
pyrolyzing the atomizer with methane.

Even the atomization cycle remains constant, analytical error may rise from the electrical resistance change due to the aging of carbon atomizer. Care must be taken to compensate this problem.

It is possible to design carbon atomizers which can perform the drying, ashing and atomizing at a different area from the detecting area in the atomizer (108). The atomization area can then be part of the light path. The whole atomizer can be heated with same electrical input. Analysis speed using this type of atomizer is faster due to the elimination of the drying, ashing and atomization cycle. Less matrix interference is encountered since the decomposition and atomization occurs outside of the light path. These types of carbon atomizer are by far the best designed atomizers which will be discussed more in the experimental sections (108).

6. INTERFERENCES IN ATOMIC ABSORPTION

There are essentially three major interferences encountered in atomic spectrometry (108). The excitation interference is due to the energy transfer from one species of excited atoms to the other species of unexcited atoms. This makes the emission intensity from the latter greater due to the increased number of excited atoms. Excitation interferences are much less important in atomic absorption than in atomic emission since it is the unexcited atoms that provide the atomic absorption signals. Spectral interference comes from the
absorption of the same wavelength by two different elements in the sample.

In emission spectroscopy, it is possible that two different elements will emit at very similar wavelengths which are difficult to be resolved by the monochromator. This is radiation interference and is a serious source of error. Spectral interference is not a problem in atomic absorption due to the fact that atomic absorption equipments are modulated to eliminate spectral interference due to emission from the sample or atomizer. In addition, it has never been observed that two different elements absorb at the same wavelength.

Chemical interference is probable the major interference in atomic absorption. It is related to the chemical form of the sample and effects the atomization efficiency, and therefore the total number of free atoms produced from a given sample concentration. A common example is the interference produced by aluminium, silicon and phosphorus in the determination of magnesium, calcium, strontium, barium and many other metals. This is due to the formation of aluminates, silicates, and phosphates which, in many instances, are refractory in the flame. If no measures are taken to correct for this interference, it will result in erroneous analytical data. However, chemical interferences can be controlled by either using a high-temperature atomizing system or by modifying the sample matrix with a suitable complexing agent.

7. SENSITIVITY OF ATOMIC ABSORPTION
The sensitivity of atomic absorption is defined as the quantity of interested element in the sample to give a 1% absorption signal (0.0044 absorbance unit). The quantity of interested element is usually expressed in part per million (the absolute sensitivity). Definition of sensitivity in atomic absorption does not depend on the noise level in the system.

The detection limit can be defined as the quantity of an element which will give the absorbance signal an amount equal to the peak-to-peak noise of the baseline, i.e., the detection limit is defined as the quantity of the element to give a signal-to-noise (S/N) ratio of 2. It is possible for an element to have the sensitivity equal to the detection limit; however, with reduced noise, the detection limit can be much improved with the same sensitivity.

In flame atomic absorption after feeding sample for some time a steady signal is obtained as the indication for the concentration of the element of interest. The signal at the peak atomization temperature is used for determining the concentration in flameless graphite furnace method. The sensitivity of a chromatography atomic absorption combination technique, peak area is actually a more accurate representation of the number of free atoms in the light path.
III. CHAPTER 3- EXPERIMENTAL

A. INSTRUMENTATION AND EQUIPMENT

The purpose of this study is to use a sensitive, selective and direct method for speciation of a metal in complicated matrix. An on-line interfaced G.C.-graphite A.A. should provide an excellent method to achieve this goal.

As described earlier, G.C. is one of the most powerful chromatographic tool for analytical separation, especially for volatile compounds. The majority of the pertinent mercury species, such as methyl mercuric chloride, possesses low point. Graphite furnace atomic absorption gives good sensitivity and a metal specific method for the determination of trace concentration levels of metals. The sensitivity of the graphite atomic absorption method can be further improved by the improvement of the atomizer system or the incorporation of micro-processors.

A typical analysis usually involves a series of steps : 1) Collection of samples, 2) Preparation of samples for analyses, such as digestion, dissolution and preconcentration, 3) Separation of components or isolation from matrix and 4) Final qualitative and quantitative characterization of the pertinent components. An ideal analytical method is a one step analysis. Sample collected can be placed into an instrument and the collected data will give a full characterization of components of interest. There should not be any sample pretreatment procedure before the final determination step to avoid errors
introduced due to handling processes. Sample pretreatment may introduce high answers due to contamination and low answers due to sample loss caused by high volatility of mercury compounds. The net result may create a wide range of error.

An on-line interfaced G.C.-graphite A.A. system combines the powerful G.C. separation technique with a sensitive and highly specific determination tool as its detector. Due to the metal specificity of A.A., one can eliminate many sample pretreatment steps to isolate the metal. Good sensitivity will enable one to avoid preconcentration steps. This combination technique is a big step closer to the ideal analytical method for metal speciation studies.

1. EQUIPMENT - G.C.-A.A.

This section describes the components used in the G.C.-A.A. system. Some components were constructed in our labs for these studies, other components were commercial units taken from other equipment. A schematic diagram for G.C.-A.A. is shown in figure 8.

a> Light sources : Modified from Barnes Glomax Demountable Hollow Cathode Lamp System with Deuterium Lamp.

b> Lamp power supply units

c> lamp Vacuum Pump : Welch Duo-Seal, Model 1404

d> Microtek GC-2000-R gas Chromatograph

e> Mechanical Chopper : Jarrel-Ash mechanical, from 82-360 A.A. Unit.

f> Graphite atomizer : Designed and built in the lab.
DIAGRAM OF GC-AA

1. HOLLOW CATHODE LAMP
2. HCL POWER SUPPLY
3. PRESSURE GAUGE FOR DEMOUNTABLE HCL
4. MICROTEK GC-2000-R GAS CHROMATOGRAPH
5. MECHANICAL CHOPPER
6. GRAPHITE ATOMIZER
7. MONOCHROMATOR
8. PHOTOMULTIPLIER
9. POWER SUPPLY FOR PMT
10. AMPLIFIER
11. RECORDER

Figure 8. G.C.-A.A. System
g) Varian-Techtron type MI (SI-RD-SPEC) monochromator.

h) Hamamatsu Model R-106-UH Photomultiplier.

i) Hewlett Packard Model 6516-A high voltage power supply: used for R-106 photomultiplier

j) Heathkit photometric readout amplifier Model EV-703-31

k) Sargent Model MK recorder with variable range attachment to atomic absorption detector.

l) Hamilton 10 microliter injection syringes

2. QUARTZ T A.A.

This section lists the components of the quartz T A.A. which is a powerful and very sensitive tool for total volatile metal determination (108). In this study, it is used to determine the level of total mercury. The basic principles of the quartz T A.A. will be described in a latter section. Figure 9 shows the schematic of the quartz T A.A. system.

a) Modified Barnes demountable hollow cathode lamp system.

b) Jarrel-Ash mechanical chopper from a model 82-360-AAS

c) Quartz T atomizer made of Quartz Scientific Inc. Clear fused quartz tubing.

d) Jarrel-Ash Model 82-020 monochromator.

e) Hamamatsu R-106-UH Photomultiplier


g) Beckman Model 1005, 10-inch potentiometric strip chart recorder

h) Hewlett Packard Model 6516-A high voltage power supply
DIAGRAM OF QUARTZ T AA

1. HOLLOW CATHODE LAMP 10. PURGING GAS FOR ATOMIZER
2. MECHANICAL CHOPPER 11. CELL PUMP
3. QUARTZ T ATOMIZER 12. LIGHT PATH HEATER
4. MONOCHROMATOR 13. PURGING GAS FOR MONOCHROMATOR
( FOR VACUUM UV LINE )
5. PHOTOMULTIPLIER 14. POWER SUPPLY FOR HCL
6. AMPLIFIER 15. PRESSURE GAUGE FOR HCL
7. RECORDER 16. PRESSURE REGULATOR
8. POWER SUPPLY
9. R. F. COIL

Figure 9. Quartz "T" A.A. System
Lepel high frequency induction heating unit Model T-5-3-KC-E-SW

J> Drummond 1-5 microliter microdispenser

3. T.L.C. AND UV

a> Whatman KC 18 reversed-phase analytical TLC precoated plates

b> Microcaps disposable pipettes

c> Beckman DBG Spectrometer with 10mm quartz cells

B. CHEMICALS AND REAGENTS

Chemicals and reagents were obtained as follows. Stock solutions of methyl mercuric chloride and mercuric chloride were prepared in concentrations of 500 ppm and 1000 ppm respectively. Standard solutions were prepared and diluted to the required concentrations. Diluted solutions of different concentration were prepared daily from the stock solution before use. Distilled deionized water prepared in the lab was used throughout the whole study. Stock solution of 1000 PPM ethylmercuric chloride was prepared from 100 proof ethanol and diluted solutions were prepared from the stock using the 100 proof ethanol. Chemical and reagents used are listed as followed:

a> Mercuric chloride -- MCB, Inc.
b> Methyl mercuric chloride -- Alfa products
c> Ethyl mercuric chloride -- Alfa products
d> Acetonitrile -- J. T. Baker
e> Dithizone -- Fisher Scientific
C. INTERFACING AND DEVELOPMENT OF G.C.-A.A.

1. INTRODUCTION

The development of the G.C.-A.A. combination has gone through many different stages in our group \((\text{111})(\text{112})\). Basically, a graphite furnace atomic absorption was located on the top of a Microtek G.C. 2000-R gas chromatograph and interfaced with the G.C. system (Figure 8). The Microtek G.C.-2000-R had a large exterior frame with vacant spaces inside the instrument which allowed the mounting of the A.A. system and modification of the interface part. The original instrument was a dual column G.C. with both thermal conductivity and flame ionization detectors. The instrument also contained a gas flow control system, a temperature programmer and an electrometer. The electrometer was replaced with the detector read-out system from the A.A. system.
A Pyrex glass transfer line was originally used to interface the G.C. column and the A.A. detector using the stainless coupling union and a piece of Teflon fitting made in the lab. Figure 10 shows the schematic of the interfacing section. Pyrex glass was chosen instead of metal tubing for two major reasons: a) Metal surfaces are known sometimes to have a catalytic effect on the decomposition or isomerization of some compounds and b) Insulation may present a more difficult problem using a metal tubing because of the location of the heating electrodes for the carbon atomizer. This will be described later. The Pyrex glass transfer line was wound with nichrome wires wrapped with asbestos. This was used to heat the transfer line. The temperature of the transfer line was separately controlled with a transformer.

To reduce the possibility of catalytic decomposition, either the Pyrex glass liners or a Teflon liner was used to replace the metal liner in the injection port. The temperature of the injection port was limited to 215°C when the Teflon liner was used.

The graphite A.A. system was a single beam optical system (figure 8). The light source was a demountable hollow cathode system which could be replaced with a deuterium lamp for background determination and correction. A mechanical chopper was used to modulate the light and avoid spectral emission interferences. Two focusing lenses were used for better light collection. The light from the demountable hollow cathode
Figure 10. Early G.C.-A.A. Interface
shone through the carbon atomizer, was dispersed by the
monochromator and detected by the photomultiplier. The signals
were amplified and recorded on the recorder.

A lot of effort was put into the development in the design
of the carbon atomizer. Figure 11 shows different atomizers
designed and used for this G.C.-A.A. system in our group. The
last version with the atomizer housing is shown in figure 12.
In this design, the carbon atomizer was accommodated in a water
cooled housing made of metal. The atomizer was heated with two
water cooled electrodes connected at the top and the bottom of
the housing. The lower electrode was hollow to allow the G.C.
effluent access to the atomizer through the Pyrex glass
transfer line. The G.C. carrier gas also served as the purging
gas for the detector. There were two quartz windows in the
light path on each side of the detector housing. Teflon washers
were used to seal the windows in place and protect them from
their metal supports.

The electrical power used to heat the carbon atomizer was
controlled by a 10:1 stepdown transformer that was connected to
a 135V-15A auto-transformer. An ampere-meter was employed to
monitor the current reading. High current up to 17 A can be
used to heat the atomizer with relatively low voltages.
Atomization temperature can be up to 2100°C with high
electrical input. However, difficulty from insufficient cooling
of the electrodes limited the use of higher atomization
temperature. When a higher temperature was used, water in the
Long Light for Good Sensitivity

Enlarged Carbon Cap to Maintain High Temperature of the Light Path

Restriction for Good Atomization

Figure 11. Early Carbon Atomizer Designs for G.C.-A.A.
cooling system boiled and vibration from the boiled water was transmitted to the whole atomizer housing. It affected the performance of the atomizer and produced excess noise.

2. MODIFICATION OF CARBON ATOMIZER AS A.A. DETECTOR

The carbon T version of the A.A. detector was selected after many developing stages (Fig.11). The design of this atomizer was quite different from a commercial straight tube design. The commercial design often suffers from high molecular background due to insufficient decomposition of the sample matrix. As described earlier, a three stage cycle-drying, ashing and atomizing is required to gain good results. When interfacing G.C. with the graphite furnace was considered, a fast response detector was required since the components eluted from the G.C. column must be detected immediately to assure good resolution from the column. It was not possible to use a three stage heating cycle because this cannot be used on a continuous basis as required of a G.C. detector.

The carbon T design helped to solve these problems to a certain extent. Two pieces of carbon rods were machined to the desired shapes and mechanically fitted into each other. A mixture of furfural alcohol and hydrochloric acid was then used to fix and glue the two pieces together. A drying time of one hour was allowed for the mixture to dry completely.

The sample decomposition and atomization occurs mostly in the vertical portion of the carbon T. The horizontal portion
of the carbon T was the light path; so the detection and measurement were done after the decomposition and atomization. This was a continuous flow process without going through the drying, ashing and atomizing cycle. Molecular background was reduced to the minimum since the sample was decomposed in a different region from the detection region. As shown in figure 12, there was a restriction of the effluent flow at the intersection of the vertical and the horizontal portion by a change of inner diameter of the diameter. This allowed improved contact between the sample and the atomizer carbon surface and improved the atomization efficiency and therefore A.A. sensitivities.

This design was used successfully in determining organolead species in gasoline samples (113). Sensitivities were determined to be $1.5 \times 10^{-9}$ g for lead and $5.0 \times 10^{-8}$ g for mercury and sensitivity decreased with the life-time of the atomizer (114). Figure 13 shows the sensitivity for mercury using the old version atomizer by injecting 2 ul 500PPM methylmercuric chloride into the system. In order to analyze mercury sample directly in biological samples an increase in sensitivity was necessary.

3. MODIFICATION OF ATOMIZER DESIGN

One of the major reasons for poor sensitivity using the earlier carbon T atomizer was caused by losses of analyte at the interface between the carbon atomizer and the Pyrex transfer line (figure 12). There remained a large gap between
CARBON T AA DETECTOR

Figure 12. Last Version of G.C.- A.A. Detector
Figure 13. Sensitivity of Mercury Using Previous G.C.-A.A. Detector
these two parts. It was difficult to reduce the gap without changing the design of the atomizer because of the temperature difference between these two parts. The transfer line was usually heated to the G.C. column temperature, i.e., less than 300°C. However, the atomizer temperature was 1500°C for mercury studies. When the Pyrex transfer line was moved closer to atomizer base, the transfer line melted and sealed. No mercury was detected and the system failed.

The first change in the design of carbon atomizer to make a direct contact between the pyrex transfer line and the atomizer was to introduce a larger carbon piece at the base of the carbon T. This design is shown in figure 14. The large carbon piece was added to reduce the temperature of the base of carbon T. A larger base required a larger electrical power to heat up and no excess power was supplied to gain the required temperature. The temperature is proportional to the electrical resistance. By increasing cross sectional area, the resistance decreases and the temperature of this section drops. This design failed the very first time of operation. The tip of Pyrex transfer line melted and cracked inside this large extension. Temperature of this carbon extension was still too high for direct connection.

The second version of carbon T modification was to further extend the introduced piece with a smaller diameter to fit into the atomizer housing. This design is shown as figure 15. The length of the second extension was studied and finally
Figure 14. Carbon T Atomizer with the Enlarged Base
determined to be 1.5 cm. A shorter extension would not reduced the temperature difference sufficiently and a longer extension could be difficult to machine.

This final design enabled one to make the direct connection between the carbon atomizer and the Pyrex glass transfer line and reduced the sample loss to a minimal. The second advantage from increasing the total length of the carbon atomizer was a better atomization efficiency. The disadvantage of this design was the fact that it was difficult to machine.

When the skill of the author was improved, it took about two hours to make a whole piece of the atomizer. Breakage of carbon pieces during the machining process was common but the process became reasonably efficient with practice.

The analytical sensitivity (1% absorption) for mercury using this design was much improved, up to an average of 3.0 X 10^-10 g. This sensitivity was better than some commercial atomizer even though the G.C.-A.A. included a dispersing column before the atomizer. The sensitivity normally obtained with a standard commercial carbon atomizer is 4.9 X 10^-10g (1% absorption) (114).

The sensitivity of the carbon atomizer decreased with the life-time of the atomizer. The life-time of the atomizer was about 24 hours continuous running time. The carbon piece became porous towards the end of its life time. This phenomenon was especially obvious for the upper portion of the vertical piece. The porosity started from the interior of the atomizer to the
MODIFIED
CARBON TAA DETECTOR

Figure 15. New Carbon Atomizer for G.C.- A.A.
outer surface of the carbon piece, indicating that the upper portion of the vertical piece was responsible for most of the decomposition and atomization processes.

The sensitivity obtained using this new version of atomizer is illustrated in figure 16.

4. MODIFICATION OF THE G.C.-A.A. INTERFACING SECTION

The Pyrex glass transfer line was initially connected to the G.C. column with a coupling union and a Teflon fitting made in the lab (Fig. 10). Difficulty arose from adjusting the tension of the nut used to tighten the Teflon fitting because Teflon expanded during the heating process. The expansion tended to squeeze the G.C. column that used a Teflon tubing. Carrier gas flow rate was very difficult to adjust since it was limited by the Teflon fitting rather than the column packing. When a higher pressure was used to drive the carrier gas, a gas leak was found in the connection area, the teflon fitting and the coupling union. This also affected the sensitivity and gave unrepeatable results.

A small hole was drilled in the base of the capillary Pyrex glass transfer line to accommodate the column end. A 1/4" to 1/8" step-down coupling union with standard commercial fittings was used to connect the transfer line and the column.

Figure 17 illustrates this connection. The tension adjustment and gas leak were prevented by this connection and a better interface was achieved.
Figure 16. Sensitivity of Mercury Using the New Carbon Atomizer

Column: 2-ft 5% DEGS on 80-100 Mesh Chromosorb W. AW.
Carrier Gas: Ar (70 ml/min)
Injection: 210°C
Oven: 170°C

Based on 5 ul Injection of HgCl₂ Solutions

Time (min)
Hole Drilled into the Base of the Transfer Line for Good Connection

Pyrex Glass Transfer Line

Teflon Ferrule for Tight Connection

1/4" to 1/8" Coupling Union with Enlarged Center Passage

Standard 1/8" Ferrule

G.C. Column

Figure 17. New G.C.-A.A. Interface
5. SUMMARY

This section described the processes used in improving the G.C.-A.A. interfacing. A new carbon atomizer was developed to maintain direct interface between the G.C. and the A.A. units. A better method for connecting the G.C. column and the Pyrex glass transfer line was utilized to improve the flow control and prevent carrier gas leakage.

The performance of the G.C.-A.A. was improved. Figure 13 and 16 showed the sensitivity increase for mercury. Table 1 summarizes the comparison of sensitivity for mercury, including that from the commercial equipment.

D. ANALYSES OF URINE AND PERSPIRATION

1. INTRODUCTION

Urine has been used extensively to monitor workers for industrial exposure to mercury. It is easy and convenient to collect samples for routine analysis. Mercury was reported to be accumulated in the kidneys in chronic exposure and eliminated through urine (115). However, it has been found that the excretion of mercury in urine depends upon the chemical form of the exposure. Inorganic Hg compounds are eliminated mainly in the feces and urine (116)(117) and the main route of excretion for methylmercury compounds is via feces (118). These reports indicate that mercury in urine is an unreliable index for individual exposure to mercury, especially to methylmercury, unless the chemical forms of mercury are also reported.
SENSITIVITIES OF DIFFERENT CARBON ATOMIZERS (1% ABS.)

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>COMMERCIAL A.A.</strong></td>
<td></td>
<td>*4.9 x 10^{-10} gm</td>
</tr>
<tr>
<td>(P.E. MODELS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PREVIOUS CARBON ATOMIZER</strong></td>
<td></td>
<td>5.0 x 10^{-8} gm</td>
</tr>
<tr>
<td>(G.C.- A.A.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CURRENT CARBON ATOMIZER</strong></td>
<td></td>
<td>3.0 x 10^{-10} gm</td>
</tr>
<tr>
<td>(G.C.- A.A.)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 1. SENSITIVITY IMPROVEMENT USING THE NEW CARBON ATOMIZER AS G.C.- A.A. DETECTOR**

*This cannot be used as a G.C. detector.*
A number of different analytical methods has been employed in the determination of mercury in urine, including gravimetry (119), polarography (120), X-ray fluorescence (121), atomic emission (122), spectrophotometry (123)(124) and atomic absorption—the cold vapor technique (58)(125). The cold vapor method is the most common method in current use for urine mercury determination. Nevertheless, this method involves many wet chemistry steps for sample preparation and was reported with the problem of incomplete extraction and incomplete destruction of all the organic matrix in the sample (126).

G.C.-E.C.D. was used by Cappon and Smith to separate inorganic and organic mercury after long sample preparation work (127) although the mercury speciation work in urine was very limited.

Sweat has been suggested as a route of excreting non-essential components from human body. Bromine, iodine, silver, mercury, and other pharmacological substances can be discharged from sweat (128).

The secretion by sweat glands is far greater than the secretion of most other glands. Typical sweat glands weighing a total of 40 g are able to secrete 3-10 Kg fluid/day that is even higher than typical saliva glands, which weigh 70 g and secrete 1.5 Kg/day (128). The average amount of perspiration for a 165 lb man at 29°C has been estimated to be 3.0-3.3 Kg/day (128). All these indicate that sweat can be major excretion route for mercury.

A very limited amount of work has been done in
determination of mercury in sweat. The first important report on mercury in sweat was actually published by our research group using the quartz T atomic absorption system (129). An average concentration of 0.7 - 0.8 ppm has been found for mercury in the sweat. No successful data on speciation of mercury in sweat has been reported. This may be due to the difficulty in sweat sample collections.

There is definitely a need to develop a direct method for mercury speciation in both urine and sweat samples. As described earlier, toxicity, retention, excretion and other chemical effects of mercury depend on its chemical forms. All the methods reported for mercury speciation involved elaborate sample treatments to isolate the interested species or avoid matrix effects. Bacterial action in urine samples is well known (130). As mentioned before that bacteria can reduce Hg\(^{2+}\) and Hg\(^{+}\) to Hg\(^{0}\) and methylate mercury. Both methyl mercury and elemental mercury are more volatile than inorganic mercury. Mercury losses can be from storage of samples and the sample pretreatment steps. All these problems can only be solved with a direct speciation method.

2. COLLECTION OF SAMPLES

Since this method was designed to be a direct analysis method, the sample collection step may be the major contribution of contamination if care not taken to avoid it.

All the sample collections were performed by using polyethylene vials. Polyethylene vials were placed into 10%
nitric acid solution overnight and rinsed with distilled deionized water before used for standard solutions.

Urine samples were collected from volunteers who were members of the university population. Subjects studied were not occupationally exposed to mercury except the author himself. Sweat samples were collected under supervision in a sauna at the LSU Field House. Subjects sampled had undergone physical exercise programs and then showered before entering the sauna. Sweat samples were collected only after profuse sweating. Skin contaminations were avoided. Sweat drops were caught from the nose or forehead using the cleaned polyethylene vials; no contact was made between the vial and skin of sampled subjects during sample collection.

Standard solutions were diluted from stock solutions (1000 ppm for mercuric chloride 500 ppm for methymercuric chloride and 1000 ppm for ethylmercuric chloride) on the same day of sample analyses.

3. DETERMINATION OF TOTAL MERCURY

The biggest limitation of G.C.-A.A. is that only volatile compounds can be analyzed. To estimate the content of nonvolatile species in the samples, other methods must be employed to determine the total mercury content.

The quartz T A.A. was developed in our research group to analyze volatile metals in different samples (129)(131)(132). It was employed in this study to determine total mercury content in the urine and sweat samples.
a. INSTRUMENTATION - THE QUARTZ T A.A.

This section describes the techniques and components used in the quartz T A.A. system. A schematic representation of the quartz T A.A. is shown as figure 9. Some components were constructed in our labs, other components were from other equipment.

The light source was a demountable hollow cathode lamp system. The quartz T atomizer was the heart of the quartz T A.A. system. It was constructed from ultrapure quartz tubing in the shape of a 'T' with two quartz discs fused to the ends of the cross piece. Vacuum ports were located approximately one inch from the quartz discs. The length of the cross piece was 35 cm and was wrapped with layers of asbestos chord and tape. These layers of asbestos in conjunction with an electrically heated nichrome wire adequately maintained a light path temperature in excess of 900° C. This temperature was necessary to increase the lifetime of the free atoms in the light path.

The vertical piece with a length about 15 cm was a bed of carbon pieces which was contained in an inner quartz sleeve. This quartz sleeve could be easily inserted and removed from the vertical piece of the 'T'. It served to expedite the loading of the carbon bed and prevented vitrification of the quartz cell wall caused by repeated heating and cooling cycles. This inner sleeve was sealed with an 'O' ring to the top portion of the 'T' to prevent air leakage around the carbon
bed. The whole 'T' was purged with nitrogen to extend the lifetime of the carbon bed. The carbon bed was inductively heated with an R.F. coil. Typical operating temperatures of the bed varied between 1400° C -1500° C measured by the optical pyrometer. This operating temperature was actually limited by the melting point of quartz, i.e., 1650° C.

Samples and standards were introduced directly from the top of the vertical piece of the 'T' using a microdispenser. No pretreatment was necessary for the samples studied. Due to the long carbon bed, it was possible to completely decompose and atomize the sample. Sensitivity was greatly enhanced because of the long light path. This enhancement will be shown later in the results section.

The light passing through the cross piece of the 'T', which was the light path, was refocused by a lens onto the entrance slit of the monochromator and dispersed by the grating inside the monochromator. The absorption line at 253.7 nm was then detected by the R106 photomultiplier (PMT) detector. The R106 had the capability of covering the range from 160.0 to 900.0 nm. The detected signals were amplified by a phase sensitive A.C. amplifier, set to pass only a narrow band of frequencies containing the chopper frequency. A chart recorder was used to record the amplified signals.

b. EXPERIMENTAL PARAMETERS-QUARTZ T A.A.

1) Hollow Cathode Lamp Current : 3 mA, He filler gas.

2) Carbon Bed Temperature : 1450° C
3) Atomizer Purge Gas: purge gas, nitrogen, was supplied to the atomizer at 270 ml/min to provide a positive pressure system and prevent the entrance of ambient air into the atomizer.

4) Light Path Temperature: 900°C

5) Monochromator Slit Width: 25 microns at 253.7 nm line, 0.8A spectra slit width

6) High Voltage on PMT: 500 volts

7) Cell Pumping Rate: 250 ml/min

8) Amplifier: ACVM mode, 200 mv sensitivity, 100 ms time constant.

9) Recorder: 100 mv, linear mode.

c. EXPERIMENTAL PROCEDURES

Urine and sweat samples were collected as previously described. Initially the instrument was warmed up ready for analyses. Samples were analyzed by direct introduction from the top of the quartz T A.A. and required no pretreatment. All the samples were analyzed within two hours of collection. Calibration curves were prepared from 0.1, 0.25, 0.5, 1.0, 2.5 and 5.0 ppm standard mercuric chloride solutions which were prepared on the same day from 1000 ppm stock solution before use. Signal peak heights were used to prepare the calibration curves.

d. RESULTS AND DISCUSSION

About thirty five urine and sweat samples were successfully analyzed during a three-month study period. The
number was limited due to the fact that the author tried to
perform the speciation and total mercury determination at the
same time for samples collected. He had to maintain both
G.C.-A.A. and the quartz T.A.A., which were located at
different rooms, running at the same time. If either one of
the two systems was not functioning properly, data was
collected only for qualitative purpose and no quantitative
information was recorded.

Every four hours of running time the carbon bed of the
quartz T.A.A. had to be replaced due to burning of the carbon
bed. The R.F. could not couple with the carbon when the
porosity was high even though the R.F. power was further
increased.

Difficulty arose from introducing larger quantity of
samples, i.e., more than 2 ul, of liquid into the quartz T.A.A.
system. This was because that sudden expansion of liquid into
gaseous form under high temperature affected the performance of
the system.

The sample injection technique took a lot of practice to
obtain reproducible data. The sample should be introduced onto
the center of the carbon bed. Poor injection caused the sample
to be caught on the wall of the inner sleeve and gave poor
results.

Figure 18 shows a series of signals from the injection of
standard solutions to the quartz T.A.A. system. The signals
were obtained from 0.1, 0.25, 0.5, 1.0, 2.5 and 5.0 ppm
Figure 18. Signals of Mercury Using Quartz T A.A.

Based on 1.0 ul Injections of HgCl₂ Solutions

- 2.50 ppm
- 1.00 ppm
- 0.50 ppm
- 0.25 ppm
- 0.10 ppm
mercuric chloride aqueous solutions. Figure 19 shows a
typical calibration curve for total mercury determination using
the quartz T A.A. system. The sensitivity was estimated to be
about $10^{-10} \text{g (1\% absorption)}$. The 184.9 nm line has a higher
oscillator strength. Although sensitivity can be improved by
using this line in the vacuum U.V. range (131), it was not
tried in this study. When using the 184.9 nm line, the optical
path and the monochromator must be purged to avoid oxygen
absorption which always occurs below 200 nm.

Figure 20 shows the signals obtained by injection of urine
and sweat samples. Table 3 and 4 summarize the urine results
and the sweat results including those from the G.C.-A.A.
studies which will be discussed in the following sections.

No molecular background was found when using the quartz T
A.A. with 253.7 nm line. This means that the carbon bed was
able to decompose the sample completely before the detection
was made. This is one of the advantages from the quartz T A.A.
design.

The results agree reasonably with those obtained by
previous studies performed by one of the author's colleagues
(129)(131) although a different line was used. Data indicated
higher concentration of mercury in urine than in sweat samples.
However, due to the small set of data, no statistical approach
was tried for data handling. Wide concentration ranges were
found for both types of samples. More conclusions can be made
from the combination with the G.C.-A.A. results in the
Figure 19. Calibration Curve of Total Mercury Using Quartz T A.A.
Figure 20. Total Mercury in Urine and Sweat by Quartz T A.A.
following section. Nevertheless, quartz T.A.A. supplied an
excellent method for total mercury determination for those
samples.

4. SPECIATION BY G.C.-A.A.

a. Introduction

A standard practice in trace metal determination is to
determine the total concentration of the metal of interest. As
described earlier in this dissertation, there is an increasing
interest and need in performing speciation studies.

For speciation studies, the great resolving power of G.C.
has been utilized by coupling it with specific detectors.
These detectors include M.S., G.C. electron capture detector,
atomic emission (133) and atomic absorption (134)(135)(136).
However, for mercury speciation, the G.C.-E.C.D. method is the
most popular. This involves extensive wet chemistry isolation
and extraction. The cold vapor A.A. with selective reduction
is another popular method. It also involves a long series of
sample pretreatment.

The G.C.-A.A. combination developed gave better
sensitivity and offered a good means to perform direct
speciation. Although atomic absorption has been coupled with
G.C. for metal speciation works, commercial flame A.A. was
normally utilized without attempts to improve designs for the
necessary sensitivity improvement (135). The design changes
discussed previously enable one to have better sensitivity
than commercial equipment and give many other advantages.
Mercury speciation was performed on this G.C.-A.A. system.

b. Experimental Parameters-G.C.-A.A.

The following parameters were finally used for mercury speciation studies using the G.C.-A.A. system.

a) Column: 1/8" diameter Teflon column 24' long, packed with 5% DEGS on chromosorb W. AW. DMCS.

b) Carrier Gas: Argon (70ml/min)

c) Column Temperature: 170°C

d) Injection Port Temperature: 210°C

e) Pyrex Transfer Line Temperature: 100°C

f) Mercury Demountable Hollow Cathode Lamp Current: 3 mA, He filler gas

g) Voltage on PMT: 350 volts

h) Slit Width: 25 microns at 253.7nm line, 0.05nm spectral slit width.

i) Atomizer Temperature: 1500°C

j) Recorder: 100 mv span

c. EXPERIMENTAL PROCEDURES -G.C.-A.A.

(1). PREPARATION OF COLUMNS

All the columns used were prepared by the author. Teflon tubing was chosen due to its inertness and ease in manipulation. Different column substrates, such as chromsorb 101, Q1-17, QF-1 and DEGS, were selected because of two major reasons: 1) availability and 2) performance in literature.

Column substrates of pre-determined quantities were first dissolved in suitable solvents, such as dichloromethane,
chloroform or acetone, which were then mixed with pre-determined quantities of solid supports. Two hours were usually allowed for sufficient coating to occur. Then the mixture of coated solid supports with solvents were slowly suction dried. Care must be taken to avoid excess disturbance to the coated supports. The coated supports were spread onto a glass pan to assure the total removal of solvents.

A suitable length of Teflon tubing with 1/8'' O.D. and 1/16'' I.D. was obtained. One end of the column was plugged with fine glass wool and connected to a vacuum pump, with a safety glass bottle in between the column and the pump. The coated packing material was slowly poured into the Teflon column using a funnel and the vacuum pump was turned on. A portable electronic vibrator was used to vibrate the column during the packing process to assure tightness of the packing in the column. The packing process was stopped when no more packing could be taken by the column. Another piece of glass wool was used to plug the other end of the column. The column was activated in the oven at the maximum temperature for overnight with carrier gas flow on.

Chromosorb 101 which is a polymeric material retained all mercury compounds when a 24'' column was used. The OV-17 and GF-1 were successfully used by Westoo in the G.C.-E.C.D. methods (76)(77)(78) suffered instability after aqueous sample injection. Nevertheless, the DEGS column gave good stability against water and resonsable separation of mercury compounds.
This column substrate was then used throughout this entire study.

Two different lengths of DEGS columns were tried, the 5-foot column and the 2-foot column. The 5-foot column gave better resolution with long retention times, as shown in figure 21, with more difficulty in adjusting carrier gas flow rate due to the high pressure drop along the column. The 2-foot column which gave better and faster analyses (illustrated in the following section) was chosen for this study.

(2). PROCEDURES FOR SAMPLE ANALYSES

Optimal separation conditions were determined by injecting standard solutions into the G.C.-A.A. system. Calibration curves for both mercuric chloride and methyl mercuric chloride were prepared by injecting 0.1, 0.25, 0.5, 1.0, 2.5 and 5.0 ppm standard solutions of both compounds into the system.

Samples were collected as described earlier in the quartz T.A.A. section and analyzed by direct injection method using Hamiton microliter syringes without any sample pretreatment. Different syringes were used for samples and standards to avoid contamination. Syringes were all pre-washed using 10% nitric acid, distilled deionized water and acetone and vacuum dried.

d. SEPARATION OF MERCURY COMPOUNDS

Before any sample can be analyzed by G.C.-A.A., retention times of the relevant volatile mercury compounds must be determined. Mercuric chloride was chosen to represent
Figure 21. Separation of Mercury Compounds Using a 5-foot DEGS Column

Column: 5-foot, 5% DEGS on 80-100 Mesh Chromosorb W. AW.
Carrier Gas: Argon (70 ml/min)
Injection: 210°C
Oven: 170°C
inorganic devalent mercury and methylmercuric chloride and ethylmercuric chloride were chosen to represent the volatile organic mercury. Chlorides were chosen due to the high salt content of the urine and sweat samples (about 300 mg/100ml)\(\leq 128\). Methylmercuric chloride was chosen due to the fact that methyl mercury was the most important organic mercury species studied. Most of previous speciation work on mercury centered on monitoring methyl mercury species because of its toxicity and environmental fate.

Figure 22 shows the chromatogram of mercuric chloride. The retention time for this compound in the column was extremely short; it was actually the time for a compound to go through the dead volume of the column. This indicated that there was no interaction of mercuric chloride with the column substrate DEGS. Nevertheless, the A.A. detector was able to detect this compound. The same column was hooked up to a gas chromatograph with a flame ionization detector and no signal for mercuric chloride was detected by the F. I. D.

Figure 23 shows the chromatogram for the methymercuric chloride. Figure 24 is the chromatogram for the mixture of mercuric chloride and methyl mercuric chloride. Two distinctive G.C. peaks were recorded for the mixture, when running the methylmercuric chloride alone, a small signal with the same retention time as mercuric chloride was always found. It was first assumed that it was a decomposition product from methylmercuric chloride under the column temperature. However,
FIGURE 22. CHROMATOGRAM OF MERCURIC CHLORIDE BY GC-AA

HgCl₂

1 min
FIGURE 23. CHROMATOGRAM OF METHYLMERCURIC CHLORIDE BY GC-AA
FIGURE 24. CHROMATOGRAM OF MERCURIC CHLORIDE AND METHYLmercuric CHLORIDE BY GC-AA
a increase in column temperature did not increase this small
signal. It was then assumed to be an impurity from the
standard.

Reversed-phase TLC was employed to monitor the purity of
the methylmercuric standard, since this method provides a fast
process to identify impurities under ambient temperature.
Methylmercuric standard solution was spotted on a
reversed-phase C18 plate and developed with a mobile phase
which was a mixture of 55% acetonitrile, 43% 1.0 NaBr aqueous
solution and 2% water. Dithizone in carbon tetrachloride was
used as a detection method. Results showed mercuric chloride
impurity presented in the methylmercuric standard. The
developed T.L.C. plate was shown in figure 25.

Figure 26 shows a chromatogram of mercuric chloride,
methylmercuric chloride and ethyl mercuric chloride.
Ethylmercuric chloride was very insoluble in water. This
chromatogram was collected from a mixture of aqueous mercuric
chloride and methylmercuric chloride solution and ethanolic
ethylmercuric chloride solution. Figure 27 and 28 show the
calibration curves of mercuric chloride and methylmercuric
chloride respectively by injecting standards into the G.C.-A.A.
system.

The separation of these three mercury compounds was rapid.
Table 2 shows the retention times for these compounds. The
separation time was about one minute and signals were
reasonably sharp. Although, as indicated earlier, it was
TLC OF MeHgCl STANDARD

FIGURE 25. TLC OF METHYLMERCURIC CHLORIDE STANDARD
Stationary phase: KC18 reversed-phase coating
Mobile phase: 55% acetonitrile and 43% 1.0M NaBr aqueous solution
Detection: dithizone / CCl₄ solution
FIGURE 26. CHROMATOGRAM OF MERCURIC CHLORIDE, METHYLMERCURIC CHLORIDE AND ETHYLMERCURIC CHLORIDE BY GC-AA
CALIBRATION OF

HgCl₂

(absorbance vs ppm conc.)

FIGURE 27. CALIBRATION OF HgCl₂ USING G.C.-A.A.
FIGURE 28. CALIBRATION OF MeHgCl USING G.C.-A.A.

CALIBRATION OF

MeHgCl

(absorbance vs ppm conc.)
<table>
<thead>
<tr>
<th>MERCURY COMPOUNDS</th>
<th>MERCURIC CHLORIDE</th>
<th>METHYLMERCURIC CHLORIDE</th>
<th>ETHYLMERCURIC CHLORIDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{HgCl}_2 )</td>
<td>( \text{MeHgCl} )</td>
<td>( \text{EtHgCl} )</td>
<td></td>
</tr>
</tbody>
</table>

| RETENTION TIME (sec) | 12 | 42 | 60 |

**TABLE 2. RETENTION TIMES OF MERCURY COMPOUNDS USING A 2-FT DEGS COLUMN**
possible to gain a better separation using a longer column, the author decided to use a shorter column to take the advantage of analysis speed and peak sharpness.

This section illustrates the chromatographic resolution ability of the G.C.-A.A. system for volatile mercury compounds. A deuterium lamp has been used to monitor molecular backgrounds; however, no background was even found.

e. STUDY OF URINE AND PESPIRATION BY G.C.-M.S.

One of the biggest limitations of G.C.-A.A. is its inability to elucidate structures of compounds. Identification of a compound with G.C.-A.A. is purely based on retention times. G.C.-M.S. was used to supply information to compare with results collected by G.C.-A.A. system.

This idea was not successful due to several reasons. Figure 29 and 30 show the mass spectra, obtained from a Hewlett Packard 5985 bench top G.C.-Mass spectrometer for mercuric chloride and methylmercuric chloride without going through the G.C. column. The same G.C. column (DEGS column) was incorporated with the HP-5985 G.C.-M.S. for analyzing mercuric chloride, methylmercuric chloride and mixture of the two compounds, with same G.C. parameters. However, no satisfactory result could be obtained due to the sensitivity, high column bleeding and difficulty in removing water from the G.C.-M.S. interfacing area - the jet separator.

The G.C.-M.S. had difficulty in detecting those compounds due to insufficient sensitivity. High quantities of sample
Figure 29. Mass Spectrum of HgCl$_2$

<table>
<thead>
<tr>
<th>MASS</th>
<th>ABUND</th>
<th>MASS</th>
<th>ABUND</th>
<th>MASS</th>
<th>ABUND</th>
<th>MASS</th>
<th>ABUND</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>22.9</td>
<td>181</td>
<td>8.7</td>
<td>198</td>
<td>32.1</td>
<td>276</td>
<td>14.8</td>
</tr>
<tr>
<td>36</td>
<td>2.6</td>
<td>182</td>
<td>1.9</td>
<td>199</td>
<td>52.2</td>
<td>278</td>
<td>1.6</td>
</tr>
<tr>
<td>37</td>
<td>8.2</td>
<td>185</td>
<td>.2</td>
<td>200</td>
<td>71.5</td>
<td>279</td>
<td>.2</td>
</tr>
<tr>
<td>38</td>
<td>1.3</td>
<td>112</td>
<td>.2</td>
<td>201</td>
<td>42.0</td>
<td>280</td>
<td>.1</td>
</tr>
<tr>
<td>40</td>
<td>1.3</td>
<td>113</td>
<td>.2</td>
<td>202</td>
<td>91.7</td>
<td>281</td>
<td>.2</td>
</tr>
<tr>
<td>41</td>
<td>.3</td>
<td>117</td>
<td>1.0</td>
<td>204</td>
<td>19.5</td>
<td>PAUSE</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>.3</td>
<td>118</td>
<td>1.3</td>
<td>233</td>
<td>2.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>.3</td>
<td>119</td>
<td>.5</td>
<td>234</td>
<td>4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>.4</td>
<td>127</td>
<td>.5</td>
<td>235</td>
<td>6.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>.4</td>
<td>135</td>
<td>5.7</td>
<td>237</td>
<td>10.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>.3</td>
<td>136</td>
<td>7.6</td>
<td>238</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>.3</td>
<td>137</td>
<td>3.9</td>
<td>239</td>
<td>4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>.4</td>
<td>138</td>
<td>1.3</td>
<td>241</td>
<td>.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>71</td>
<td>.4</td>
<td>149</td>
<td>1.6</td>
<td>266</td>
<td>.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>.2</td>
<td>150</td>
<td>.2</td>
<td>268</td>
<td>20.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>77</td>
<td>.2</td>
<td>157</td>
<td>.2</td>
<td>269</td>
<td>36.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>79</td>
<td>.5</td>
<td>158</td>
<td>.2</td>
<td>270</td>
<td>67.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>81</td>
<td>.7</td>
<td>162</td>
<td>.7</td>
<td>271</td>
<td>49.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>83</td>
<td>.2</td>
<td>164</td>
<td>.3</td>
<td>272</td>
<td>100.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>85</td>
<td>.2</td>
<td>167</td>
<td>.4</td>
<td>273</td>
<td>19.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95</td>
<td>.2</td>
<td>196</td>
<td>.5</td>
<td>274</td>
<td>56.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>6.9</td>
<td>196</td>
<td>.5</td>
<td>275</td>
<td>2.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Figure 30. Mass Spectrum of MeHgCl

<table>
<thead>
<tr>
<th>MASS</th>
<th>ABUND</th>
<th>MASS</th>
<th>ABUND</th>
<th>MASS</th>
<th>ABUND</th>
<th>MASS</th>
<th>ABUND</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>3.6</td>
<td>117</td>
<td>.4</td>
<td>215</td>
<td>53.9</td>
<td>277</td>
<td>.5</td>
</tr>
<tr>
<td>36</td>
<td>.6</td>
<td>118</td>
<td>.5</td>
<td>216</td>
<td>40.7</td>
<td>279</td>
<td>1.7</td>
</tr>
<tr>
<td>37</td>
<td>1.5</td>
<td>124</td>
<td>.9</td>
<td>217</td>
<td>55.1</td>
<td>280</td>
<td>1.4</td>
</tr>
<tr>
<td>38</td>
<td>.5</td>
<td>125</td>
<td>1.2</td>
<td>218</td>
<td>3.8</td>
<td>281</td>
<td>2.5</td>
</tr>
<tr>
<td>39</td>
<td>1.8</td>
<td>127</td>
<td>.9</td>
<td>219</td>
<td>12.1</td>
<td>282</td>
<td>.7</td>
</tr>
<tr>
<td>40</td>
<td>3.0</td>
<td>141</td>
<td>.5</td>
<td>233</td>
<td>9.9</td>
<td>285</td>
<td>.4</td>
</tr>
<tr>
<td>41</td>
<td>1.7</td>
<td>142</td>
<td>1.7</td>
<td>234</td>
<td>16.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>2.0</td>
<td>149</td>
<td>1.0</td>
<td>236</td>
<td>19.3</td>
<td>293</td>
<td>1.4</td>
</tr>
<tr>
<td>43</td>
<td>.4</td>
<td>196</td>
<td>.4</td>
<td>238</td>
<td>4.9</td>
<td>295</td>
<td>2.4</td>
</tr>
<tr>
<td>44</td>
<td>.3</td>
<td>198</td>
<td>22.7</td>
<td>239</td>
<td>16.4</td>
<td>296</td>
<td>4.6</td>
</tr>
<tr>
<td>45</td>
<td>.3</td>
<td>199</td>
<td>29.8</td>
<td>241</td>
<td>2.2</td>
<td>297</td>
<td>1.1</td>
</tr>
<tr>
<td>46</td>
<td>51.6</td>
<td>200</td>
<td>51.6</td>
<td>246</td>
<td>1.5</td>
<td>298</td>
<td>2.6</td>
</tr>
<tr>
<td>47</td>
<td>2.4</td>
<td>201</td>
<td>33.9</td>
<td>248</td>
<td>1.7</td>
<td>300</td>
<td>.7</td>
</tr>
<tr>
<td>48</td>
<td>65.5</td>
<td>202</td>
<td>65.5</td>
<td>249</td>
<td>30.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>.5</td>
<td>203</td>
<td>5.8</td>
<td>249</td>
<td>48.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>.8</td>
<td>204</td>
<td>14.2</td>
<td>250</td>
<td>79.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>.4</td>
<td>205</td>
<td>1.4</td>
<td>251</td>
<td>53.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>1.7</td>
<td>210</td>
<td>1.1</td>
<td>252</td>
<td>100.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>1.6</td>
<td>211</td>
<td>3.8</td>
<td>253</td>
<td>13.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>1.9</td>
<td>212</td>
<td>9.6</td>
<td>254</td>
<td>46.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>.4</td>
<td>213</td>
<td>31.0</td>
<td>255</td>
<td>.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>53.4</td>
<td>214</td>
<td>53.4</td>
<td>256</td>
<td>6.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**WORK AREA FRN 6005** + 42 + 42 + 42 - 5 - 5 - 5
could not be injected into the column due to overloading. Since all those compounds were dissolved in water, the water could not be removed from the interfacing jet separator and the vacuum system of the G.C.-M.S. had difficulty in maintaining enough vacuum due to the presence of water vapor in the system. The G.C.-M.S. was malfunctioning when running those compounds. Column bleeding was another problem. Figure 31 shows the mass spectrum from column bleeding without injecting any sample. Fortunately, the atomizer of the G.C.-A.A. system was able to decompose the bleeding material from column and gave selective detection.

The poor results obtained from G.C.-MS study served to emphasize the value of the G.C.-A.A. system which can accommodate column bleed, overlapping peaks and still provide quantitative data on the mercury compound.

f. RESULTS AND DISCUSSION

Urine and sweat samples were collected as described in the sample collection section. They were analyzed by using direct injection without sample pretreatment. As discussed previously, the quartz T A.A. was used to determine the total mercury contents in samples collected. G.C.-A.A. was used to determine the mercury form in those samples. Figure 32 shows gas chromatograms for typical urines and sweat samples studied. The mercury species detected in both types of samples had the same retention time as the mercuric chloride standard and no other mercury form was found in those samples. This indicates
**Figure 31.** DEGS Column Bleeding by G.C.-MS

<table>
<thead>
<tr>
<th>MASS</th>
<th>ABUND</th>
<th>MASS</th>
<th>ABUND</th>
<th>MASS</th>
<th>ABUND</th>
<th>MASS</th>
<th>ABUND</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>2.2</td>
<td>87</td>
<td>12.6</td>
<td>134</td>
<td>2.6</td>
<td>191</td>
<td>4.4</td>
</tr>
<tr>
<td>38</td>
<td>2.6</td>
<td>89</td>
<td>27.0</td>
<td>145</td>
<td>7.0</td>
<td>201</td>
<td>7.8</td>
</tr>
<tr>
<td>40</td>
<td>100.0</td>
<td>90</td>
<td>2.6</td>
<td>146</td>
<td>5.9</td>
<td>202</td>
<td>7.8</td>
</tr>
<tr>
<td>41</td>
<td>6.3</td>
<td>91</td>
<td>2.2</td>
<td>147</td>
<td>8.9</td>
<td>203</td>
<td>8.1</td>
</tr>
<tr>
<td>42</td>
<td>3.3</td>
<td>93</td>
<td>2.2</td>
<td>148</td>
<td>7.8</td>
<td>204</td>
<td>23.7</td>
</tr>
<tr>
<td>43</td>
<td>12.2</td>
<td>100</td>
<td>2.2</td>
<td>149</td>
<td>4.4</td>
<td>205</td>
<td>6.7</td>
</tr>
<tr>
<td>44</td>
<td>3.3</td>
<td>101</td>
<td>4.4</td>
<td>158</td>
<td>2.6</td>
<td>215</td>
<td>2.2</td>
</tr>
<tr>
<td>46</td>
<td>4.1</td>
<td>102</td>
<td>2.2</td>
<td>159</td>
<td>4.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>6.3</td>
<td>104</td>
<td>3.3</td>
<td>160</td>
<td>3.7</td>
<td>217</td>
<td>8.5</td>
</tr>
<tr>
<td>56</td>
<td>4.1</td>
<td>105</td>
<td>2.2</td>
<td>162</td>
<td>3.3</td>
<td>219</td>
<td>28.9</td>
</tr>
<tr>
<td>57</td>
<td>4.4</td>
<td>106</td>
<td>2.2</td>
<td>163</td>
<td>3.0</td>
<td>220</td>
<td>7.4</td>
</tr>
<tr>
<td>58</td>
<td>4.8</td>
<td>108</td>
<td>2.2</td>
<td>173</td>
<td>4.1</td>
<td>221</td>
<td>3.3</td>
</tr>
<tr>
<td>59</td>
<td>8.1</td>
<td>115</td>
<td>3.7</td>
<td>174</td>
<td>3.7</td>
<td>230</td>
<td>2.2</td>
</tr>
<tr>
<td>70</td>
<td>2.3</td>
<td>117</td>
<td>2.6</td>
<td>180</td>
<td>6.7</td>
<td>232</td>
<td>2.6</td>
</tr>
<tr>
<td>71</td>
<td>2.6</td>
<td>118</td>
<td>3.3</td>
<td>181</td>
<td>2.2</td>
<td>233</td>
<td>16.7</td>
</tr>
<tr>
<td>72</td>
<td>3.7</td>
<td>119</td>
<td>2.2</td>
<td>186</td>
<td>2.6</td>
<td>234</td>
<td>3.3</td>
</tr>
<tr>
<td>73</td>
<td>8.5</td>
<td>120</td>
<td>3.0</td>
<td>187</td>
<td>3.3</td>
<td>235</td>
<td>4.1</td>
</tr>
<tr>
<td>75</td>
<td>3.7</td>
<td>131</td>
<td>6.3</td>
<td>193</td>
<td>3.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>76</td>
<td>2.2</td>
<td>141</td>
<td>3.6</td>
<td>194</td>
<td>3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>77</td>
<td>4.4</td>
<td>142</td>
<td>4.4</td>
<td>199</td>
<td>4.8</td>
<td>247</td>
<td>5.3</td>
</tr>
<tr>
<td>79</td>
<td>2.2</td>
<td>132</td>
<td>4.4</td>
<td>190</td>
<td>4.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>86</td>
<td>2.2</td>
<td>133</td>
<td>7.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**CONT**
that mercury is excreted from urine and sweat predominately as
inorganic mercury.

Table 3 and 4 show results for mercury determination in
urine and sweat samples respectively. Both tables show
concentrations of inorganic mercury determined by G.C.-A.A. and
concentrations of total mercury determined by quartz T A.A. for
different samples.

Although there was no relationship among those samples,
some conclusion can still be made from these limited quantity
of data. Inorganic mercury with the same retention time as
mercuric chloride was the major chemical form for mercury among
those samples. The concentration differences between the
results from G.C.-A.A. and those from quartz T A.A. indicated
there were nonvolatile mercury species not detected by this
method. The mercury concentration in urine was higher than
that in the sweat. However, sweat should still be considered
as a major route of excreting mercury from human bodies since
large quantity of sweat was excreted daily (128).

The author did not try to correlate the data with other
factors such as diet, age and daily activity which might affect
the mercury levels in those sample. A more systematic study
using this method with larger number of sample will supply more
information. However, samples collected on the same day from
two studied subjects were analyzed.

Table 5 shows the mercury levels in both urine and sweat
collected at seven different day over a month period from the
Figure 32. Gas Chromatograms of Urine and Sweat By G.C.- A.A.

Column: 2-ft DEGS 5% on Chromosorb W. AW. 80-100 Mesh
Carrier Gas: Ar (70 ml/min)
Injection: 210 °C
Oven: 170 °C

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>%ABS</td>
<td>40.0</td>
<td>30.0</td>
<td>20.0</td>
<td>10.0</td>
<td>0.0</td>
<td>1 Min</td>
<td>1 Min</td>
<td>1 Min</td>
<td>1 Min</td>
<td>1 Min</td>
<td>1 Min</td>
<td>1 Min</td>
</tr>
<tr>
<td>Sweat</td>
<td>3 ul</td>
<td>3 ul</td>
<td>3 ul</td>
<td>1 ul</td>
<td>2 ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>1 ul</td>
<td>1 ul</td>
<td></td>
<td></td>
<td></td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td>Blank</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blank (Water)</td>
<td>A</td>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 32. Gas Chromatograms of Urine and Sweat By G.C.- A.A.
<table>
<thead>
<tr>
<th>URINE ALIQUOT #</th>
<th>PPM INORGANIC MERCURY</th>
<th>PPM TOTAL MERCURY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.80</td>
<td>2.50</td>
</tr>
<tr>
<td>2</td>
<td>1.00</td>
<td>2.00</td>
</tr>
<tr>
<td>3</td>
<td>2.20</td>
<td>3.00</td>
</tr>
<tr>
<td>4</td>
<td>0.00</td>
<td>1.80</td>
</tr>
<tr>
<td>5</td>
<td>3.30</td>
<td>3.30</td>
</tr>
<tr>
<td>6</td>
<td>0.50</td>
<td>1.20</td>
</tr>
<tr>
<td>7</td>
<td>2.60</td>
<td>3.20</td>
</tr>
<tr>
<td>8</td>
<td>0.90</td>
<td>1.50</td>
</tr>
<tr>
<td>9</td>
<td>3.10</td>
<td>3.70</td>
</tr>
<tr>
<td>10</td>
<td>0.30</td>
<td>0.60</td>
</tr>
<tr>
<td>11</td>
<td>1.60</td>
<td>2.60</td>
</tr>
<tr>
<td>12</td>
<td>1.20</td>
<td>2.00</td>
</tr>
<tr>
<td>13</td>
<td>0.80</td>
<td>1.50</td>
</tr>
<tr>
<td>14</td>
<td>2.50</td>
<td>3.40</td>
</tr>
<tr>
<td>15</td>
<td>0.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

**TABLE 3 MERCURY CONTENTS IN URINE SAMPLES**

Inorganic mercury determined by G.C.-A.A.

Total mercury determined by Quartz T.A.A.
<table>
<thead>
<tr>
<th>SWEAT ALIQUOT #</th>
<th>ppm INORGANIC MERCURY</th>
<th>ppm TOTAL MERCURY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.20</td>
<td>0.40</td>
</tr>
<tr>
<td>2</td>
<td>0.00</td>
<td>0.20</td>
</tr>
<tr>
<td>3</td>
<td>0.50</td>
<td>0.80</td>
</tr>
<tr>
<td>4</td>
<td>0.40</td>
<td>0.60</td>
</tr>
<tr>
<td>5</td>
<td>0.60</td>
<td>0.80</td>
</tr>
<tr>
<td>6</td>
<td>0.20</td>
<td>0.50</td>
</tr>
<tr>
<td>7</td>
<td>0.10</td>
<td>0.30</td>
</tr>
<tr>
<td>8</td>
<td>0.50</td>
<td>0.70</td>
</tr>
<tr>
<td>9</td>
<td>0.60</td>
<td>1.00</td>
</tr>
<tr>
<td>10</td>
<td>0.30</td>
<td>0.50</td>
</tr>
</tbody>
</table>

**TABLE 4 MERCURY CONTENTS IN SWEAT SAMPLES**

Inorganic mercury determined by G.C.-A.A.

Total mercury determined by Quartz T A.A.
<table>
<thead>
<tr>
<th>DATE</th>
<th>URINE (ppm Hg)</th>
<th>SWEAT (ppm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>INORG.</td>
<td>TOTAL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUBJECT 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11/7/83</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>11/9/83</td>
<td>1.4</td>
<td>2.0</td>
</tr>
<tr>
<td>11/11/83</td>
<td>0.8</td>
<td>1.5</td>
</tr>
<tr>
<td>11/14/83</td>
<td>1.8</td>
<td>2.8</td>
</tr>
<tr>
<td>11/16/83</td>
<td>0.9</td>
<td>1.5</td>
</tr>
<tr>
<td>11/18/83</td>
<td>0.5</td>
<td>1.2</td>
</tr>
<tr>
<td>11/21/83</td>
<td>1.8</td>
<td>2.5</td>
</tr>
<tr>
<td>SUBJECT 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11/7/83</td>
<td>1.2</td>
<td>2.5</td>
</tr>
<tr>
<td>11/9/83</td>
<td>0.5</td>
<td>0.9</td>
</tr>
<tr>
<td>11/11/83</td>
<td>0.7</td>
<td>1.2</td>
</tr>
<tr>
<td>11/14/83</td>
<td>0.4</td>
<td>1.2</td>
</tr>
<tr>
<td>11/16/83</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>11/18/83</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>11/21/83</td>
<td>2.5</td>
<td>3.4</td>
</tr>
</tbody>
</table>
two subjects. Wide fluctuations existed among those samples, for the same person on different days and there seemed to be no correlation of mercury level between the urine and the sweat samples.

9. RELIABILITY OF DATA

Based on repetitive analyses the standard deviation ($\sigma$) of the procedure for measuring the standard solutions was 0.02 ppm at 0.50 ppm level, or $2\sigma = 0.04$ ppm with 95% confidence.

For urine samples the standard deviation ($\sigma$) was 0.005 ppm at the 0.05 ppm level, or $2\sigma = 0.01$ ppm with 95% confidence.

For sweat samples the standard deviation ($\sigma$) was 0.01 ppm at the 0.25 ppm level, or $2\sigma = 0.02$ ppm with 95% confidence.
IV. SUMMARY AND CONCLUSION

A analytical method based on an interfaced G.C.-A.A. system was developed to speciate mercury in urine and sweat samples. This method enjoyed several advantages:

1. SENSITIVITY

The sensitivity for mercury by A.A. is relatively poor compared to other elements. The improved design of the atomizer in G.C.-A.A. offered marginally better sensitivity. The major reason is due to the improved interface with extend vertical piece. A longer vertical piece gave a better atomization since the vertical piece of the carbon T is actually responsible for most of the decomposition and atomization processes. One other reason was the relatively high temperature maintained in the whole G.C.-A.A. system which minimized any physical and chemical adsorption of the sample. The whole system was sealed tightly and the atomizer is the only exit for the sample. Sample loss was minimum. A better sensitivity required no sample preconcentration.

2. ANALYTICAL SPEED

Due to the efficiency of the G.C. column, the sample could be analyzed within about ten minutes after collection with little pretreatment. This greatly reduced error due to sample loss or contamination and gave more reliable results and decrease the time required for analysis. Direct analysis with minimal sample handling and pretreatment is one of the goals of trace element analysis.
3. SELECTIVITY

Using A.A. as a detector coupled with a chromatographic technique for analyzing metal species in biological samples avoided other interferences present in the samples. The designed atomizer offered the advantage of decomposing the sample before detection of the interested metal was made. This further avoided the interferences. The chromatogram obtained were much simpler than those from common nonselective G.C. detectors. This also minimized some of the sample pretreatment work, such as extraction, to move interferences.

4. SIMULTANEOUS DETERMINATION OF BOTH ORGANIC AND INORGANIC MERCURY

Many mercury compounds are volatile. Nevertheless, few G.C. detectors can be used to analyze both organic and inorganic mercury at same the time. Mass spectrometer and flame ionization detector were used unsuccessfully in this study. Most other detectors require isolation of specific chemical form or transformation into a certain chemical form before the final detection. This technique offered a rapid and reliable means to determine both volatile organic and inorganic mercury simultaneously.

The major disadvantage using this method for mercury speciation is the poisoning of the G.C. columns. Direct injection of biological samples into the G.C. column does poison the column. This greatly reduced the column life time. The column was re-packed whenever the result was not
reproducible.

Since G.C. is a good separation method only for volatile compounds, nonvolatile mercury species can not be analyzed with this method. Interfacing a H.P.L.C. unit with a A.A. system should be a good complementary method for metal speciation studies.
PART B

INTERFACING H.P.L.C. AND A.A. FOR METAL SPECIATION STUDIES

I. CHAPTER 1 - INTRODUCTION

A. FLAME A.A. PROCESSES

The most commonly used atomizer is the flame, based upon the combination of a fuel gas with an oxidant. The sample solution is introduced into the flame using a nebulization system which produces an aerosol of sample. A flame is characterized by the gases involved, the temperature, the form in which the gases are mixed, the flow of the gases and also by its shape and size. All these aspects play essential roles in flame atomic absorption processes.

The height of the flame is very critical. The flame is not uniform along its full height. As it rises from the burner slot, the flame varies in shape, temperature and composition (108). From considering the processes which occur in the flame after a sample is introduced (evaporation of droplets, drying the solid residue, decomposition of the residue to free atoms, and subsequent recombination or ionization of atoms to form other products), it is clear that the life of the free atoms will be limited and their greatest concentration will be found at a certain height of the flame.

In normal flame A.A. operation, an optimum zone in the flame must be found, which produces the greatest absorption effect and thus the greatest sensitivity. If the atomic
absorption is measured at different heights in the flame, it is found to vary considerably. The relationship between height in the flame and atomic absorption signal is called the flame profile. As an example, flame profiles for lead and chromium are shown in figure 33 along with flame processes which will be discussed later. Nevertheless, in many instruments the beam crosses the flame only a few millimeter above the top of the burner. Different metals have different flame profiles. For optimum sensitivity, a study on flame profile is necessary to find the observation height in the flame.

Variation in the ratio of fuel to oxidant in the flame will affect the atomization processes in the flame. When excess oxidants are present in the flame, metal oxides form more rapidly, i.e., the higher rate of loss of free atoms. With excess fuel in the flame, the flame temperature drops and atoms don't form so fast. The available energy for atomization is less than in a stoichiometric flame. This also affects the number of free atoms produced. So, the oxidant-fuel ratio directly affects the flame temperature and the lifetime of neutral atoms in the flame. For reproducible results the ratio of oxidant to fuel in the flame must be kept constant.

Changes in oxidant gas flow will also produce differences in sample solution intake for most commercial nebulization systems. Changes in fuel gas flow should not notably affect the sample intake even that they do drastically change the flame conditions. When using flame as an atomization means,
FLAME A.A. PROCESSES AND FLAME PROFILES

Oxide Formation

Excitation and Ionization

Atomization

Decomposition

Fusion and Volitilization

Desolvation

Figure 33. Flame A.A. Processes and Flame Profiles
attention should be paid to gas regulation during operation. For most metals these ratios have been worked out and are published in the literature. Recommended flow rates are also suggested by burner manufacturers.

Flame temperature is another important factor controlling the efficiency of flame atomization. Of course, the hotter the flame, the more efficient it will be in reducing the sample to free atoms since the transformation from a sample aerosol to free neutral atoms is achieved by absorbing energy from flame.

The other advantage of high temperature flames is the removal of chemical interferences. Since the production of free atoms involves the liberation of those atoms from a salt residue, this involves breaking the chemical bond through which the sample element is attached to either an inorganic salt or an organic addend. A high temperature is more effective in breaking those chemical bonds and less subjected to chemical interferences.

The most commonly used flames are premixed air-acetylene flame and nitrous oxide-acetylene flame. Metals such as molybdenum, tin, and some alkaline earths are only partially atomized in air-acetylene flame. The rare earths and refractory elements including silicon, aluminum, and vanadium are not appreciably atomized at all in air-acetylene flame. They are better atomized in nitrous oxide-acetylene flame.

The flame temperature can be changed by changing the flame fuel-oxidant mixture. Different types of flame mixture will
give different maximum temperatures. A premixed nitrous oxide-acetylene flame is able to reach a peak temperature about 2900°C which is widely used for the analysis of refractory elements (137)(138). Acetylene oxygen flame was reported to achieve a temperature over 3000°C (137).

The disadvantage in using a high temperature flame is the marked increase in ionization of many metals (138). This can be overcome by adding an easily ionized metal such as sodium or potassium to suppress the ionization.

Flame path length is the most important dimension in the flame. The farther the beam travels across the flame, the more favorable to the absorption and sensitivity.

In A.A. spectroscopy, the amount of light absorbed by free atoms is expressed as:

\[
\text{Total absorption} = \int_{0}^{\infty} K_{\nu} \, d\nu = \left( \frac{\pi e^2}{m c} \right) N_{\nu} f
\]

where

- \( K_{\nu} \, d\nu \) is the total absorption by free atoms at frequency \( \nu \)
- \( e \) is the charge of the electron
- \( m \) is the mass of the electron
- \( c \) is the velocity of light
- \( f \) is the oscillator strength of the absorbed line
- \( N_{\nu} \) is the number of free atoms that can absorb at frequency \( \nu \)
The oscillator strength can be further expressed as:

\[ f = \frac{\lambda_0^2}{8\pi} \frac{g_e g_1^{-1}}{g_1} \]

where

- \( \lambda_0 \) is the wavelength of the line
- \( A_{e1} \) is the Einstein's coefficient of spontaneous emission
- \( g_1 \) and \( g_2 \) are the statistical weights of atoms in excited state \( (g_1) \) and ground state \( (g_2) \)

It can be seen that \( f \), the oscillator strength, is a constant for a particular resonance line for that element.

Therefore for a given set of flame conditions and for a particular element, the total amount of light absorbed is equal to a set of constants times the number of free atoms in the light path that are able to absorb. The sensitivity is thus proportional to the effective length of the flame. If the space occupied by the atoms has a cross-section greater than the beam, and if the density remains constant in the cross-section, the absorbance is proportional to the length of the atom-filled space, i.e., to the product of the concentration of atoms and the optical path length according to the Beer's law. The width is another important dimension. The concentration of atoms in the flame is inversely proportional to the horizontal cross section of the flame. A reduction in this section can be achieved by reducing the practical width of the flame. The
light beam from the source should sufficiently cover the width of the flame.

When a solution is introduced into a flame in the form of an aerosol, a series of physical and chemical reactions occurs, leading to atomization. Figure 31 shows these typical flame processes. In particular, these processes involve fusion, volatilization, dissociation or decomposition, resulting in the formation of free atoms and their excitation and recombination, especially with the combustion products.

The nebulization system in flame A.A. is responsible in producing the liquid-gas aerosol. The solvent of the sample solution is then removed in the desolvation step in the flame with the formation of a solid-gas aerosol. After the fusion step a liquid-gas aerosol is produced due to the fusion of the solid salt residue. The liquidified solid salt vaporizes due to the flame energy to produce a vapor from the initial solid residue. It is also possible to generate vapor form of the solid residue without going through the fusion step, i.e., through sublimation. The vapor form generates molecules which then decomposed and dissociated into the free atoms. The free atoms produced are responsible to give A.A. signals. Excess flame energy will cause the excitation and ionization of the produced free atoms which decrease the flame A.A. sensitivity.

In the flame, reactions between the studied metal and the flame constituents are also likely. Reaction of the metal with the oxidant will produce the metal oxide. Metal oxide may also
be produced from the interaction of metal with hydroxyl radicals and water produced in the flame combustion process. Reactions between the metal with hydroxyl radical will also produce metal hydroxide. Metal carbonate can be generated from the reaction between the metal oxide and the carbon dioxide in the flame.

One should realize that the flame is a very complicated medium in A.A. research. Increasing the efficiency in the production of free atoms will help to give a better flame A.A. sensitivity. Other reactions which consume the produced free atoms need to be controlled to maintain the sensitivity. In this study, efforts have been put in to increase the nebulization and desolvation efficiencies to improve flame A.A. sensitivities, which will be discussed in details in the following sections.

B. TYPES OF NEBULIZER IN ATOMIC SPECTROSCOPY

Nebulization is one of the essential steps in flame A.A. processes. It actually controls the first step of flame atomization, i.e., the generation of aerosol. Improvement in nebulization efficiency should theoretically improve the flame A.A. processes and the flame sensitivity. A small review and comparison among different nebulization systems used in atomic spectroscopy, mostly in flame A.A. and inductively coupled plasma emission (I.C.P.), will be given along with their advantages and disadvantages.

1. PNEUMATIC CONCENTRIC NEBULIZER
Following intensive studies by both academic and industrial researchers, the pneumatic concentric nebulizer is used almost exclusively in flame A.A. today. A schematic diagram of a commercial pneumatic concentric nebulizer is shown in figure 34. Sample solution is fed from the back of the nebulizer through a plastic capillary tubing. Nebulizing gas which is normally the oxidant (air, oxygen or nitrous oxide) enters the side arm and to the front of the nebulizer. This then controls the intake of the sample solution. A needle valve is used to control the nebulizing gas flow. The high flow of nebulizing gas causes collisions with the sample solution and small aerosol droplets are produced and leave the front of the nebulizer flowing with the nebulizing gas. An impact bead or sample spoiler may be placed in front of the outlet of the nebulizer to improve the mix and flow pattern for better combustion.

The advantages of pneumatic concentric nebulizers include their mechanical stability, corrosion resistance, ease in cleaning, freedom from blocking and ease in operating. Most of the commercial pneumatic concentric nebulizers are made of titanium which is a highly chemical resistant metal. Performance of the pneumatic concentric nebulizer is very reliable and is used by almost all major A.A. production companies, such as Perkin-Elmer, Jarrell-Ash and Varian Techtron.

The major disadvantages of the pneumatic concentric
1. Knurled Knob
2. Washer
3. Needle Assembly
4. Spring
5. O-Ring
6. Insert
7. Nebulizer
8. Venturi
9. Rear End Cap
10. O-Ring
11. O-Ring
12. Locking Ring

Figure 34. Perkin Elmer Pneumatic Concentric Nebulizer
nebulizer include the difficulty in precisely adjusting the sample flow rate and its low nebulization and transport efficiency. Precise adjustment in sample flow rate is difficult due to the difficulty in fine control of the nebulizing gas. For example, if a sample intake of 6 ml/min is required for certain A.A. application, an exact flow of sample feeding rate is very difficult to be obtained without a large number of trials. The achieved sample flow rate will vary due to any small fluctuation of flow rate of the nebulizing gas. The nebulizing gas control nut at the back of the nebulizer is just not efficient enough to precisely control the gas.

The nebulization and transport efficiency for pneumatic concentric nebulizer is about 6.6% to 14.4% (139) (140). This is mainly due to the non-uniform droplet size and high droplet momentum. The droplet size distribution using a pneumatic concentric nebulizer is wide (141). This affects the flame A.A. processes after the nebulization; it also affects the transportation of those produced droplets to the flame. Larger droplets may never reach the flame. The high momentum of the droplets is attributed to the higher nebulizing gas flow. The large momentum of the droplets increases the possibility of impacts between the droplets and the burner chamber and burner head. A poorly designed burner assembly may further increase the impact frequency and decrease the flame A.A. sensitivity.

The final goal of this study is to developed a good nebulization system to interface flame A.A. and H.P.L.C. and
sample flow rate control is important in H.P.L.C. system for best chromatographic resolution.

2. CROSS-FLOW NEBULIZERS

The cross-flow nebulizer was introduced by Valente and Schrenk (142). Since the introduction, it is almost used exclusively in I.C.P. because of its low gas flow requirement. Major I.C.P. production companies, such as Perkin-Elmer, Jarrell-Ash, Baird and Beckman use cross-flow nebulizer for their products. Figure 35 illustrates a commercial cross-flow nebulizer.

In a cross-flow nebulizer, two pieces of glass capillary tubes were mounted with a right angle to each other. The nebulizing gas passes through one of the two tubes and the other tube is for sample intake. Argon flowing from the top of the gas needle streams across the tip of the sample aspirator needle. Because of the cross sectional area of the gas needle is restricted, argon velocity increases, reaching a maximum just at the orifice. Then the high velocity argon streams across the sample aspirator needle, drawing the sample out. The argon collides with the sample and nebulize it. The orifices in the cross-flow nebulizer is only about 400 microns to maintain a high velocity and low volume flow of the gas. The nebulization efficiency is about 2.5% for a typical commercial product (143). The adjustable cross-flow nebulizer permits adjustment of the distance between the needles. An adjustable cross-flow nebulizer features a sample orifice of
CROSS-FLOW NEBULIZER

Figure 35. Cross-Flow Nebulizer
2000 microns.

Besides the low nebulization efficiency, orifice blockage is the major disadvantage of the cross-flow nebulizer when nebulizing solutions with high solid content. I.C.P. manufacturers usually recommend the users to use other types of nebulizer if particles larger than 45 microns are present in sample solutions.

3. BABINGTON NEBULIZER

The major advantage of Babington Nebulizer is its ability to nebulize solutions with a high solid content and high viscosity (144). I.C.P. production companies, such as BAIRD, uses Babington nebulizer for handling high solid content samples. It was also used to a small extent in atomic absorption spectroscopy (145).

Figure 36 shows the schematic of Babington nebulizer. Sample flows through the delivery tube and over a hollow glass or metal sphere with a small orifice in the side. A nebulizing gas such as argon passes through the orifice and collides with the sample solution. An aerosol is produced at the gas-solution interface. An impact bead can be placed in front of the nebulizer for further break-down of the droplets. A grooved ring can be constructed around the front of the nebulizer. Samples with a high solid content drips along the groove and across the argon stream where it is nebulized. A peristaltic pump is required to pump sample solution from the container to the groove through a tube (146). The average
Figure 36. Babington Nebulizer
nebulization efficiency of a Baington nebulizer is about 1.2% with the major advantage in handling high solid content samples.

4. THE MEINHARD CONCENTRIC NEBULIZER

Figure 37 illustrates the Meinhard Concentric Nebulizer which was initially designed for use in atomic spectrophotometers (147). Now, it is used by Beckman Instrument for the generation of aerosol in their I.C.P. Units.

It consists of two glass capillary tubes with one inside the other as shown in figure 35. The sample passes through the inside tube and is nebulized by the gas. It passes through the outside tube, at the nebulizer tip which has an orifice of the order of 400 microns. A nebulizing gas flow of 0.5 to 2.0 L/min is necessary for 2 to 3 ml/min sample uptake under normal operation. An impact bead and sample spoiler can be used for better results.

The meinhard nebulizer suffers from orifice clogging when nebulizing high solid content sample and low efficiency (139)(143).

5. ULTRASONIC NEBULIZERS

Aerosol generation by ultrasonic technique was first reported by Word and Loomis (148). Several workers have applied this method to atomic spectroscopy (149)(150). However, the ultrasonic nebulizer finds its major application in I.C.P. (151). Commercial I.C.P. from Baird and RF Plasma Products use ultrasonic nebulizer for aerosol generation.
Figure 37. Meihard Concentric Nebulizer
The mechanics of droplet production using an ultrasonic nebulizer is very complex and only approximate theoretical relationship are known. This will be further discussed in the following sections. The general principle in ultrasonic nebulization is that small droplets are produced by setting up high frequency standing waves on the nebulizing surface which can be either liquid or solid. Small drops break off from the tips of these waves, with sufficient power input, to form a cloud of aerosol above the surface.

Ultrasonic nebulization techniques have the greatest potential in aerosol generation for atomic spectroscopy. The droplet sizes produced are more uniform than from other methods, and the droplet size can be controlled by the ultrasonic frequency. Sample uptake is controlled by the input power. The nebulization process is precise and accurate. Nebulization efficiency is actually controlled by transportation of the produced droplets. The nebulization efficiency was reported to be 75% using a rather complicated set-up by Hoare et al. (152). This offers the highest nebulization efficiency among the nebulization systems. High solid content samples can be nebulized with proper nebulizer design. This study used a prototype ultrasonic nebulizer for improving flame A.A. sensitivities and interfacing H.P.L.C. with flame atomic absorption. More discussion on this device will follow.

C. REASONS OF THIS STUDY
As discussed earlier, the determination of the total content or concentration of an element is not often sufficient. It is frequently necessary to go a step further and identify the chemical form of the compound or element. This is true for both organic and inorganic compounds.

The first part of this dissertation described the potential of interfacing atomic absorption with chromatographic separation for metal speciation. Gas chromatograph was interfaced with a graphite A.A. for some metal speciation work. However, a serious limitation of G.C. is its inability to analyze thermally labile compounds and compounds with high boiling point because they will not pass the column. This limits the application of G.C. and the interfaced G.C.-A.A. system because there are many metal compounds which cannot be analyzed and determined by this method.

H.P.L.C. enables one to examine compounds with high boiling point under ambient conditions. Interfacing H.P.L.C. with atomic absorption allows one to extend this limit and study compounds which may not be studied by G.C.-A.A. unit. Such a system will be an important complementary method to G.C.-A.A. system.

The ideal interfacing should be between the H.P.L.C. and the graphite atomic absorption system because of the high sensitivities available from furnace atomizers. Unfortunately, the cyclic heating programs, drying, ashing and atomizing, used with commercial carbon furnaces create a compatibility problem
for an interfaced H.P.L.C.-furnace A.A. instrument. A continuously flowing eluent from H.P.L.C. with a furnace that is heated in cycles, was found to be impossible to interface directly because the atomizer was rapidly decomposed. Alternatively, fractions can be collected from the end of a H.P.L.C. column by an auto-sampler and the fractions are then introduced into the furnace individually. The total signal of a chromatogram is a set of peaks instead of a single one (153).

Attempts have been tried in our research group to interface H.P.L.C. with furnace A.A. system (153). In that study, the effluent from the H.P.L.C. column was fed into a cross-flow nebulizer, where it was nebulized by a stream of oxygen. A miniature oxy-hydrogen flame was used to burn the organic solvent before the sample passed into the carbon furnace. An inlet allowed argon to be introduced to the flame combustion chamber to prevent air from leaking into the interface.

This interfacing design was not successful. The major product of the oxy-hydrogen flame was water vapor which reacted with the carbon atomizer to form carbon monoxide and hydrogen. In a short time, the carbon furnace was eroded to the extent that its operation was not efficient and reproducible. The life time of the carbon furnace was only about 60 minutes. The project was then abandoned.

The decision was then made to interface H.P.L.C. with flame atomic absorption. One reason is that flame A.A. burners
normally utilize liquid samples in a continuous fashion instead of in cycles. However, the analytical sensitivities using flame A.A. are worse than furnace A.A. methods. The flow rate matching between H.P.L.C. and flame A.A. is another difficulty. Flame A.A. using a typical pneumatic concentric Nebulizer requires a flow rate of 5-6 ml/min for optimal sensitivity and this is not always true for best resolution in H.P.L.C. separation. Moreover, as described earlier, it is very difficult to control precisely the flow rate using a pneumatic concentric nebulizer.

The second part of this dissertation will describe the use of a prototype ultrasonic nebulizer to solve the problem involved in interfacing H.P.L.C. and flame atomic absorption. Sensitivity improvements will be illustrated and discussed. Several methods for metal speciation work dealing with biological and environmental aspects will be shown to illustrate the feasibility of combination of flame A.A. and H.P.L.C. using the ultrasonic nebulizer.

D. PRINCIPLE OF ULTRASONIC NEBULIZER

This study involved the use of an prototype ultrasonic nebulizer, shown as figure 38, for improving flame A.A. sensitivities and interfacing H.P.L.C. with a flame A.A. unit. The detailed principles of ultrasonic nebulizer will be discussed in this section for a better appreciation of this unique device. Although ultrasonic aerosol generation was used for I.C.P. and atomic absorption (149)(150)(151)(152), no other
device offered more advantages and convenience than the one used in this study.

In this ultrasonic nebulizer, two pieces of piezoelectric disks with their polarity facing each other were sandwiched between two pieces of ultrasonic horns, the front horn and the back horn. The opposite polarities of the two disks, when connected with electric power, created vibrations which were transmitted to the tip of the front horn. Samples of the solution to be nebulized was fed from the center of the back horn through the center of the nebulizer with a liquid passage tubing. The solution was transferred to the nebulizer tip surface where nebulization occurred.

When liquid is introduced onto a rapidly vibrating solid surface, a checkerboard like wave pattern appeared in the film, which formed as the liquid spread over the surface. When the input power was increased, the amplitude of the surface vibration was increased, the wave crest height in the film also increased. With sufficient amplitude, the wave crests in the film became unstable and collapsed. This caused a fine mist of micro-drops to be ejected from the surface. Because the nebulization process was unpresurized, the diameter of the feed tube's bore doesn't have to be restricted. This minimized the clogging problem. The droplet produced had a very low velocity of only 0.2 to 0.4 m/sec which was much lower than from a pneumatic concentric nebulizer (10 to 20 m/sec). The factor of 100 fold reduction in velocity was attributable to
ULTRASONIC NEBULIZER SCHEMATIC

Figure 38. Ultrasonic Nebulizer
the static nature of the ultrasonic nebulization process. In terms of kinetic energy, which was proportional to the square of the velocity, the difference was even more striking. An ultrasonically generated droplet carried with it only about $10^{-4}$ as much energy as a pressure generated droplet. This decrease is an important factor in flame A.A. sensitivity increases which will be discussed later.

Another important feature of the ultrasonic nebulizer was its ability to control the droplet size with ultrasonic frequency. The wavelength $\lambda$ of the liquid wave form on the nebulizing surface is given by the relationship (154)

$$\lambda = (8\pi Q/\rho g f^2)^{1/3} \quad \text{Equation A}$$

Where $Q$ is the surface tension and $\rho$ the density of the liquid if $f$ is the ultrasonic frequency. Each of the wavelets acted as a site for atomization. As the drive amplitude increased, the amplitude of the surface waves increased correspondingly until at some critical amplitude the crests of the waves became unstable and small droplets were ejected. It has been shown that the median droplet diameter, $D$ is linearly related to the wavelength $\lambda$ by

$$D = 0.34 \lambda \quad \text{Equation B}$$

Thus, in general $D=0.34 (8\pi Q/\rho g f^2)^{1/3} \quad \text{Equation C}$
The median droplet size was then proportional to \( f^{-2/3} \).
Theoretically, it is possible to decrease the droplet size by increasing the drive frequency. The threshold amplitude for atomization was a variable related to the drive frequency, fluid viscosity, surface tension and density. Mean droplet size could then be predicted with above known parameters. One thing needs to be mentioned here is the importance of the drive power control. Careful control of the drive power was essential. If the nebulizer power increased too much, an amplitude of motion was reached in which the liquid disturbance is no violent that large drops are ejected rather than small droplets. This phenomenon was termed cavitation and was the result of the excess energy ripping away large chunks of liquid from the main body of liquid.

The nebulizer design was very critical in obtaining good performance. Certain requirements must be met in designing the nebulizer. Figure 39 illustrates these requirements. The contact plane of the two pieces of piezoelectric disks was a nodal plane since the amplitude of motion is always zero. A point \( 1/4 \lambda \) away was the antinodal plane, the plane of maximum amplitude varied sinusoidally with distance. For best nebulization, a configuration in which the atomizing surface was at an antinode was necessary since the amplitude was maximum there. The two ultrasonic horns were configured to accomplish this. The back horn consisted of just enough metal,
PRINCIPLES OF ULTRASONIC NEBULIZER

\[ \lambda = \left( \frac{8\pi\sigma}{\ell f^2} \right)^{1/3} \]

\[ D = 0.34 \lambda \]

\[ D = 0.34 \left( \frac{8\pi\sigma}{\ell f^2} \right)^{1/3} \]

\( \lambda \) : WAVELENGTH OF DROPLET FORMATION

\( f \) : ULTRASONIC DRIVE FREQUENCY

\( \sigma \) : SURFACE TENSION

\( \ell \) : DENSITY OF LIQUID

\( D \) : AVERAGE DIAMETER OF DROPLETS

Figure 39. Principles of Ultrasonic Nebulizer
together with the disk coupled to it, to give $1/4 \lambda$ length. The front horn was $3/4 \lambda$ long. It provided the amplification of vibrational amplitude by virtue of a sharp transition in diameters at the $1/2 \lambda$ point from the contact plane. The amplification was equal to the ratio of the cross-sectional areas of the two sections. Amplification was increased either by increasing the diameter of the large section or reducing the diameter of small section.
CHAPTER II- H.P.L.C. THEORY

A. HISTORY

In 1903 the Russian chemist Tswett, who was born in Asti, Italy, in 1872, reported the separation of green plant pigments in a column filled with powdered chalk (155). That was the earliest report of an example of L.C. separation. After that report, little work has involved with the L.C. technique until in the 1930's, the technique was adopted by Reichstein for the isolation of natural products (156). The introduction of liquid partition chromatography by Martin and Synge to separate amino acids also gave a strong impact on L.C. advances (85). They were recognized by the 1952 Nobel Prize in Chemistry. In 1948, ion exchange chromatography was used by Moore and Stein for the separation of amino acids (157).

Liquid chromatography, until the mid-1960's, generally involved using a large column with a packed bed of adsorbent coated with a stationary liquid for partition applications. The force of gravity drove the solvents and sample through the bed for separation. The process of separation was monitored by collection of fractions and subsequently performing some method of identification and quantitation.

The re-emergence of liquid chromatography on a basis comparable to gas chromatography started from the publication by Huber and Hulsmann (158). In the paper, they suggested that the separation time in liquid chromatography can be reduced considerably if high pressure drop is acceptable. Further, the
column performance can be improved by using regular packing with very small particles. Theoretical aspects of separation time, solvent feed rate, pressure drop, and column dimension were also discussed in that paper.

Since then liquid chromatography has grown steadily and developments in this technique have now reached a mature and exciting stage. H.P.L.C. is now considered to be one of the most powerful separation technique in chemistry.

B. PRINCIPLES

Liquid chromatography involves the separation of the components of a mixture by the differences in interactions of the components between the liquid mobile phase and the stationary phase. Because of the differences in interaction forces, the separation of components of a mixture is achieved by establishing conditions under which the individual components flow at different rates through a column, under the influence of a moving liquid phase, the mobile phase. The interaction of components with the column is referred to as retention. For a given chromatographic system the degree of retention of a compound is a characteristic of that sample, since it depends on properties of that compound, such as the solubility, adsorption, size and ionization characteristic of that compound, in that specific environment of chromatographic system employed. The retention of a compound in a L.C. column is expressed quantitatively as the retention volume, which is defined as the volume, usually in milliliters, of mobile phase
which flows through the column system from the moment of sample introduction to the appearance of the maximum concentration of the eluting peak detected by the detector. The retention volume for a compound without any interaction with the column is said to be eluted in the dead volume of the column, usually represented by $V_0$. The retention volume, $V_R$, for any component having interaction with the column will be the sum of the dead volume and the volume of mobile phase necessary to overcome the interaction between the sample and the column material.

The capacity factor, $K'$, is used to express retention of a compound relative to the elution of a non-retained sample. It is defined as

$$K' = \frac{V_R - V_0}{V_0}$$

When a fixed flow rate for the mobile phase is used, the above expression can be considered as

$$K' = \frac{T_R - T_0}{T_0}$$

where $T_R$ and $T_0$ are the retention times of a retained and non-retained compound, respectively. The capacity factor is actually related to the mass of component in the mobile and the stationary phase in the column.

For any separation to be possible it is essential that each component have a different value for the capacity factor,
i.e., each component must be retained to a different extent.

The selectivity, \( \alpha \), of a chromatographic system is used to express the ratio of the capacity factors of the two components, A and B, of interest, i.e.,

\[
\alpha = \frac{K_b'}{K_a'} = \frac{t_{Rb} - t_{Ro}}{t_{Ra} - t_{Ro}}
\]

A separation between components A and B in a mixture will only be possible if the selectivity factor, \( \alpha \), has a value other than unity. However, the selectivity factor doesn't involve any sample diffusion along the separating process; a selectivity factor with a value other than unity merely indicates that the points of maximum concentration of the two components are not coincident.

The resolving power, often called as the resolution factor, \( R \), of a column is calculated as follows

\[
R = 2 \left( \frac{t_{Rb} - t_{Ra}}{W_b - W_a} \right)
\]

where \( T_{Ra}, T_{Rb}, W_a \) and \( W_b \) are the retention times and base widths, respectively, of compound A and B, and compound B is retained longer in the column. Because in practice the peak shapes approximate to a Gaussian distribution rather than an isosceles triangle, when \( R=1 \), there is still a slight overlap of the peaks (about 2%). A \( R = 1.5 \) (0.03% overlap) is normally considered to have a complete separation, the baseline
A general expression for the performance of a chromatographic system in terms of resolution can be integrated as follows:

\[ R = \frac{1}{4} \sqrt{N} \left( \frac{\alpha - 1}{\alpha} \right) \left( \frac{b'}{b' + 1} \right) \]

This single equation describes the resolution of a chromatographic system in terms of column efficiency (N, number of theoretical plates), selectivity (nature of chemical interactions related to the phases used), and capacity factor (giving the extent of phase interaction). It is easy to understand that improvement of resolution between two peaks by efficiency will require a considerable increase in the number of theoretical plates due to function of the square root of the column efficiency. On the other hand, resolution can be improved easier by using a different chromatographic system to change the selectivity and capacity factors. Based on this general equation for resolution, it is possible to estimate an optimum column length for separating two compounds of interest.

A new column should always be tested with a standard sample mixture under carefully standardised conditions. A record of such tests is invaluable in later date for troubleshooting work. It is also important to keep a record of the indicated inlet pressure necessary for the flow of a given solvent through the given column at the given rate. Any
significant change in resistance to flow of the column indicates materials being built up in the column. Any marked decrease in the resistance to flow may indicate loss of column packing material from the column. Sometimes, problems in column performance can be solved before they become serious and make the column no longer serviceable.

C. BASIC INSTRUMENTATION

The main components of a H.P.L.C. chromatograph are a high pressure solvent delivery pump, pulse dampeners to reduce pulsation from pumping action, an injector or injection valve for sample introduction, the separation column, the detection systems, and a data output device, such as integrator, recorder, or computer. Other components, such as auto sampler and fraction collector, may be necessary for different applications.

There are now two basic types of pump in common use: constant pressure pumps and constant volume pumps. A modern H.P.L.C. pump can normally apply a pressure up to 6000 psi and can deliver solvent flow rate with a range from 0.0 to 10.0 ml/min. The accuracy of solvent flow control should be better than 0.5%. Material such as stainless steel and Teflon must be used to assure chemical inertness. A pulse damper may be necessary to minimize the pulsation and maintain smooth delivery. A gradient elution device may also be necessary to carry out continuous solvent composition changes. A filter is always required for filtration of all solvents so no
particulates will enter into the system and plug the very narrow bore tubing used. A 2-micron stainless steel filter with a large surface area is normally used. A small area 2-micron frit is normally used on the top of the column for column protection.

There are basically three types of sample introduction devices in H.P.L.C., including on-column syringe injectors, valve-loop injectors and syringe-loop injectors. Normally on column syringe injection gives the highest separation efficiency although it is not convenient. Syringe injection can be made though a septum injector similar to G.C. or using a septum-less system. If a septum is used, it may be the pressure limiting factor, but normally the use of dual septa and stainless steel disks pressing down on the septa enables pressures up to 3000 psi to be used.

The septum-less injection is also called stop-flow injection. This involves either switching off the pump, allowing the pressure to fall to atmospheric, injecting the sample, and then re-starting the pump. A three-way valve may be used to divert the flow whilst the injection is made. However, the stop-flow technique may introduce retention time uncertainty and cause flow disturbances in some detector systems.

In a valve-loop injector, the solvent flow is by-passed into the column and an external or internal loop is filled with sample, which is then introduced into the column by a switching
valve. The sample loops are normally interchangable to give a wide range of injection volume (1-500 microliters). The main drawback of this type of injector is that excess injection volume is required to fill the loop completely. For example, it may take 100 microliter of sample to fill a 10 microliter loop.

A syringe-loop injector combines a loop injector with a microsyringe and zero volume fitting. It can be used either as a conventional fixed loop injector, or as a variable volume injector. The sample loop is initially filled with mobile phase. The zero volume fitting allows the sample to be injected into the sample loop with no loss of sample. The sample injected will displace an equal volume of mobile phase out of the drain tube. The valve is then rotated and the sample flushed onto the column by the flow of the mobile phase. The syringe-loop injector is the most sophisticated injection system in a H.P.L.C. unit. One of the injection units used in this study, the Water G K injector, belonged to this type.

H.P.L.C. columns are normally made of stainless steel tubing filled with a tightly packed stationary phase. A normal analytical separation column may be from 3 cm to 30 cm long with a typical internal diameter of 2.1, 3.2, 4.1 or 4.6 mm. A semi-preparative and preparative scale separation may use a column up to 30.0 mm in internal diameter. Microbore columns with a internal diameter of 1.0mm up to 100cm in length have been introduced. Column packing materials used in H.P.L.C. may
have particle sizes from 5 microns to 30 microns. Slurry packing method is normally used for particle size lower than 20 microns. Particle size over 30 microns is seldom used in H.P.L.C. now.

Many different types of H.P.L.C. detector are available now in the market, depending on the applications. A separate section will be used to discuss those important detectors.

D. TYPES OF H.P.L.C.

There are four principal mechanisms in H.P.L.C. by which components of samples are selectively retained. These are the exploitation of differences in partition coefficients, adsorption effects on surfaces, dissociation of weak or strong electrolytes, or in molecular size or shape. Based on these mechanisms, branches in H.P.L.C. were developed independently. They are liquid-liquid chromatography, liquid-solid chromatography, ion-exchange chromatography and steric exclusion chromatography. These will be briefly discussed below.

Liquid-liquid partition chromatography involves the use of a liquid stationary phase, which is either coated on to a finely divided inert support or chemically bonded to the support material, and a liquid mobile phase. The sample to be analyzed is dispersed in the mobile phase and its components are partitioned between the stationary and mobile phases. This partitioning leads to a differential rate of migration and separation occurs. There are two major categories of
Liquid-Liquid partition chromatography (L.L.C.), the normal phase L.L.C. and the reverse phase L.L.C.

In normal phase L.L.C., a polar liquid stationary phase, such as polyglycols, ethers or nitriles, is used with the mobile phase which is relatively non-polar. It is normally used to separate polar species, e.g., phenols, amines, and heterocyclic compounds. If the stationary phase is non-polar, e.g., a hydrocarbon, and the mobile phase is polar, e.g., water, methanol and acetonitrile, the technique is referred to as reverse-phase L.L.C. Compounds separated by reverse-phase L.L.C. are those less polar compounds with molecular weight below about 2000.

In liquid-solid chromatography (L.S.C.), the separation is achieved with a liquid mobile phase and a solid stationary phase which reversibly adsorbs the solute molecules. The stationary phase may be either polar, e.g., silica gel, porous, glass beads or alumina, when the mobile phase would be relatively non-polar, or non-polar, e.g., polymer beads, when a polar mobile phase is used. This method is less sensitive to molecular weight differences between the solute species than L.L.C. is, but it is highly sensitive to compound type. A complex mixture can therefore be separated into classes of compounds having the same functional groups, whereas molecules with different lengths in alkyl chain would be poorly separated.

In ion exchange chromatography, the chromatographic
support contains ionic sites which are capable of being exchanged with ionic solutes in the mobile phase. Ion exchangers in H.P.L.C. normally utilize a bonded quaternary ammonium group for the separation of anions, and a bonded sulphonic acid group for the separation of cations. Two important variables controlling the success in ion exchange chromatography are the ionic strength of the eluent and the pH of the buffer solution. The pH of the buffer solution controls the ionization of acids and bases being chromatographed as well as the degree of ionization of the ion exchange groups. The classical ion-exchange resin is usually a styrene-divinyl benzene copolymer which has been cross-linked to provide mechanical rigidity, with ion exchange groups built into a matrix. These resins swell when placed in water, to a less extent, in organic solvents. The resin is also porous, so separation by size can also occur. Chemically bonded exchangers avoid the swelling problem. They consist of a non-porous silica matrix to which the functional group are bonded covalently to cross-linked silicone network. Ion exchange H.P.L.C. is used to separate acidic or basic ionizable species with molecular weight less than 2000.

Steric exclusion chromatography is the separation of sample components according to differences in their size or shape. This method of separation is unique among H.P.L.C. mechanisms as it relies entirely on the physical restriction of
molecules moving through a packed column rather than on any interactive action. The term gel filtration is used when separating samples in aqueous solution. Gel permeation chromatography describes similar separation processes performed in non-aqueous media. Both are sometimes called collectively as gel chromatography.

In practicing gel chromatography, the sample usually contains a mixture of components of differing sizes and the column packing will possess a range of pore diameters. If, for example, a two-component mixture is introduced it may have one of high molecular size and the other of low molecular size. The larger species will be unable to penetrate the pores of the column packing material. The small molecule is free to diffuse into the pools of mobile phase held in the internal pores of the column packing material; consequently, elution of the smaller species from the column will require a larger retention volume of mobile phase. Adsorption onto the gel may play some part in the separation processes, but in a properly designed system this effect should be minimal.

Retention time in gel chromatography is usually shorter than other H.P.L.C. mechanisms. Gradient elution is usually not required. The short retention time also gives narrow solute peaks which are easier to detect and do not give rise to the problems of detection limits in other forms of H.P.L.C. due to more sample diffusion. The retention time in this method is a function of molecular size. Because intermolecular
forces are absent in the separation process, the column does not accumulate strongly retained molecules. This means a long column lifetime, and precolumn is usually not required in this method. However, a molecular weight difference of 10% or more are usually required to obtain separation, so this technique does not give complete separation of complex mixtures. It is very useful to perform preliminary separation or as a method to determine the molecular weight distribution of polymers.

E. H.P.L.C. DETECTORS

Many types of detection system have been developed for H.P.L.C. practices, various reviews of H.P.L.C. detectors were published elsewhere (159)(160). More new detectors are under developing for H.P.L.C. applications. This section will briefly discuss several general detectors for H.P.L.C. practices.

1. REFRACTIVE INDEX DETECTORS

Refractive index detectors (R.I.D) function by measuring the change in refractive index in the eluent as the sample components flows through the sample cell. Since the refractive index of a compound depends on the temperature, for high sensitivity thermostating of the sample and reference cell is necessary to avoid drift. Refractive index detectors have the advantage that they are capable of detecting virtually all components in the sample provided that the refractive index of the sample component is different from that of the eluent. The detection limit of a modern R.I.D. is about $10^{-7}$ g/ml of column
effluent. It is an universal detector with moderate sensitivity and is sensitive to temperature and pressure fluctuation of the mobile phase.

2. PHOTOMETRIC DETECTORS

Detectors based on the absorbance of light in U.V. or visible regions of the spectrum are by far the most widely used detection systems in H.P.L.C. practices. These detectors measure the change in the light absorption as the solute passes through a flow cell in a U.V. or visible light transparent solvent. A low-pressure mercury lamp as the light source is the most widely used in this type of detector, due to its high intensity predominantly at a wavelength of 253.7 nm. This high-energy output has enabled simple yet high-performance detectors to be constructed with flow cells of quite low internal volume, normally less than 10 microliter with 10 mm optical path length.

Because different compounds have maximum absorption at different wavelengths. There is a demand for a photometric detectors with variable wavelength for best sensitivity. Commercially, a deuterium lamp is used to cover the spectrum from 190 to 370 nm and a tungsten source is used to cover up to the visible region. A temperature-controlled deuterium lamp can be used to cover the complete U.V. region with stable output.

The biggest breakthrough in H.P.L.C. photometric detector is the application of modern diode array technology. In
traditional detectors, continuous data can usually only be obtained at one pre-selected wavelength, which limits the amount of information available and a spectrum scan for a certain component in the sample can only be achieved under stopped flow status. This form of serial measurement is tedious and slow, taking time consuming seconds or minutes. And it also leads to band broadening, which adversely affects separation and virtually precludes quantification. In addition, stopped flow scanning is impossible with modern fast H.P.L.C. using small particle size column. The photo-diode array detection technique is based on a dynamic system using reversed optics. Quite simply, light is projected through the flow cell, dispersed via a holographic grating on to a number of photo-sensitive diodes, resulting in voltage. This is then converted to digital signals for further processing. Diode array technique allows for continuous spectral determination during elution, and because the process is rapid, the problem of stopped flow measurement are eliminated. The speed of photo-diode array detector can simultaneously access total spectrum information for each component in the sample. A output from this detector usually gives three or four dimensional information including retention times, absorption wavelengths, and extent of absorptions at different wavelengths. It is capable of multi-wavelength detection, wavelength variation, absorbance ratio calculation indicating peak purity and total wavelength chromatography. It is the
most advanced photometric detection system for H.P.L.C practices. The sensitivity of a photometric detector is about 1 ng/ml of effluent.

Of course, not all molecules possess a sufficiently strong UV or visible chromophore for satisfactory detection. Forming U.V. or visible absorbing derivatives of these compounds using either a pre-column or post-column reaction is often advantageous.

Although there has been some interest in a photometric detector operating in the infrared region of the spectrum, there appears to be little interest from commercial instrumental market due to the severe restriction on the solvents that may be employed as a mobile phase.

3. FLUORESCENCE DETECTOR

Fluorescence detectors are among the most sensitive detection systems in H.P.L.C. applications. They also enjoy good selectivity. In this detector, the component of interest is excited by a beam of strong U.V. radiation at a given wavelength, normally from a xenon lamp. The fluorescent energy which is emitted at a longer wavelength is detected. Sensitivity and selectivity can be increased by careful selection of both excitation and fluorescent wavelengths. Compounds which are not naturally fluorescent can be reacted with fluorogenic reagents to form fluorescent derivatives for detection. The intensity of fluorescent emission is dependent on the intensity of the excitation radiation. Since this
radiation is of necessity absorbed by the compounds present in the flow cell. The effective intensity of the source decreases when strong absorption occurs, leading to an apparent non-linearity of the detector. Therefore, quantitation by fluorimetric methods is best performed with dilute solutions and with U.V. transparent mobile phases.

4. ELECTROCHEMICAL DETECTORS

Many compounds in the presence of an electrical potential will be oxidized or reduced. The current resulting from these reactions is proportional to the quantity of the electroactive compounds eluting from the chromatographic column. This is the basis for electrochemical detection. Commercial products are now available on the market. Usually a dual-analysis porous graphite electrodes with large surface area are used for efficient electrochemical reactions (151). They are placed in series in the cell so that the column effluent flow through rather than pass the surface of the electrodes. The electrochemical reaction responsible for detection can be 100% complete even at high flow rate up to 4 ml/min. Each electrode produces an independent signal providing for increased flexibility in operation. The total flow cell volume can be as low as 5 microliters. Sensitivity is normally around 0.1 ng/ml of effluent. A major problem in this detector is the contamination of the electrodes by absorbed impurities; however, it is a sensitive and selective detector.

5. MASS SPECTROMETERS
The mass spectrometer is probably the most powerful H.P.L.C. detector on the market at present. H.P.L.C.-M.S. systems are now available from instrument companies such as Finnigan, V.G. Instrument, Hewlett-Packard Co. and Nermag, France at the time of this writing. It took several years to develop a G.C./M.S. interface that simply had to remove significant portions of helium gas from the G.C. eluent. The H.P.L.C./M.S. interface must either rapidly remove large portions of the eluent, which may include components as diverse as water and buffers, or allow direct introduction of the effluent into the high vacuum system of the mass spectrometer. Unlike the problem in G.C./M.S. interfacing, the H.P.L.C./M.S. interface must unite an instrument that normally operates in the liquid phase (1 ml/min, i.e. 1000 ml gas/min) with another instrument that normally operates at high vacuum (10^-7 torr).

Two major interfacing methods are currently used by instrument production companies, the moving belt transport method used by Finnigan and V.G. Instrument and the direct liquid introduction systems (D.L.I.) used by Hewlett-Packard Co. and Nermag in France (162).

In the D.L.I. method, effluent is vaporize through a 5 micron pinhole of a diaphragm directly into the ion source. When using normal bore column with a flow rate of 1 ml/min, a splitter allows 1% of the effluent to enter the mass spectrometer. On the other hand, the entire effluent can be introduces into the M.S. if the separation is conducted by
microbore H.P.L.C. at flow rates of 10 to 20 microliter per minute. However, operation of the mass spectrometer with D.L.I. is restricted to chemical ionization mode, which usually provides molecular mass information but is often of limited value for detailed structure characterization. Extension of the H.P.L.C.-D.L.I.-M.S. system to a tandem M.S. will solve this problem although it is more expensive. In the tandem M.S. condition, the effluent from H.P.L.C. can be introduced directly into the M.S. ion source. Protonated molecular ions are selectively transmitted through the first quadrupole and passed into a second chamber where they undergo collision-induced dissociation. The various ions are then subjected to conventional mass analysis by scanning the third quadrupole mass analyzer.

In the moving belt approach, the H.P.L.C. eluent is deposited on the surface of conveyor belt made of inert polymer (a polyimide material) and the solvent is vaporized at the belt surface. The belt traverses through a series of vacuum locks that provides the gradual drop from atmospheric pressure to high vacuum of the mass spectrometer. The solute remaining on the belt is vaporized into the M.S. ion source as it passes in front of the flash heater. This method actually treats the mass spectrometer as a vapor phase technique and allows its operation in the conventional modes (electron impact ionization or chemical ionization).

A new technique in interfacing H.P.L.C. with M.S. is the
thermospray method which the author encountered at the
Pittsburgh Conference, 1985 in New Orleans. It is the result
of the pioneering work of Marvin Vestal at the University of
Houston.

In this technique, the effluent from the H.P.L.C. passes
through a heated narrow tube, and enters into the ion source as
a fine jet spray. An expansion volume connected to a high-speed
mechanical pump is placed in the line of the jet stream to
remove the mobile phase. Ionization is initiated by
introducing organic solutes with ions present in the mobile
phase from ionic buffers or other modifiers. Protonated solute
ions or other adduct ions enter the vapor phase directly from
the condense phase. Ions in the jet stream are extracted into
the mass analyzer as they pass through the ion source. This
method allows H.P.L.C. flow rate up to 2 ml/min, even with
water as the solvent.

Using M.S. as H.P.L.C. detector enables one to take the
advantages of the good sensitivity of M.S. and its structure
identification capability.

Other detectors used in H.P.L.C. include radioactive
detectors, electrical conductivity detectors and electron
capture detectors. They are less popular in H.P.L.C.
practices.

Generally speaking, a good H.P.L.C. detector should have
the following characteristics: good sensitivity (10^{-9} g/ml or
better), continuous operation, insensitivity toward temperature
and mobile phase velocity change, having a low dead volume, and being reliable and convenient to use.
CHAPTER 3 IMPROVING FLAME A.A. SENSITIVITY BY ULTRASONIC NEBULIZATION

A. INTRODUCTION

The final goal of this study is to develop methods by using H.P.L.C.-A.A. for metal speciation studies. In metal speciation studies, unlike total concentration determination, one is looking at the chemicals which make up the total concentration. As generally known, available flame A.A. methods offer less sensitivity than furnace techniques. Also, as described earlier, difficulties were encountered when one tried to interface H.P.L.C. and furnace A.A. directly (153). The large quantity of mobile phase from the column shortens the life time of atomizers. It was therefore essential to improve flame A.A. sensitivities to enable direct analysis of metals in body fluids. With improved sensitivities, it would then be possible to speciate metal compounds in biological samples by interfacing flame A.A. with H.P.L.C. More discussion on metal speciation using a combination of H.P.L.C. and flame A.A will follow in the following chapters.

Nebulization and desolvation are essential steps in flame A.A. processes before sample atomization. It is possible to improve atomization efficiency and sensitivities by improving the nebulization and desolvation efficiencies. In this study, the ultrasonic nebulizer described earlier was used as the nebulization device with a commercial flame A.A. system to improve nebulization efficiency. Improving desolvation of
droplets was achieved by heating the A.A. burner chamber and burner neck before droplets reached the flame, i.e. desolvation outside the flame.

Although previous studies by a co-worker in our group indicated the possibility of better sensitivity in flame A.A. using a similar nebulizer made of aluminum operated at a frequency of 78.0 KHz (163), it was found that the aluminum ultrasonic nebulizer gave un reproducible performance due to chemical corrosion on the nebulizing surface. A titanium ultrasonic nebulizer operated at 57.7 KHz was then used in this study to overcome the corrosion problem. More elements were chosen in this study to give a systematic understanding of this unique nebulizer.

Flame processes actually involve a series of steps; however, improving nebulization and desolvation efficiencies seem to give better grounds for improving sensitivity because they are possibly the only two steps which can be performed outside the flame in flame A.A. methods. Once the sample reaches the flame, one can only change the flame composition i.e. the flame temperature, or add modifier in the sample to achieve better sensitivity. Also, from reviewing different nebulization systems, nebulization is the step that is very inefficient and has the highest potential for improvement in flame A.A.

B. CHEMICALS AND SOLUTIONS

1. CHEMICALS
Stock solutions of 1000 ppm were prepared from sources as the following commercial products:

a. Nitric Acid -- J. T. Baker Chemical Co.
b. Hydrochloric Acid -- J. T. Baker Chemical Co.
c. Ni Metal -- Alfa Product
d. Lead Nitrate -- J. T. Baker Chemical Co.
e. Cadmium Sulfate -- J. T. Baker Chemical Co.
f. Zinc Metal -- Alfa Product
g. Magnesium -- Alfa Product
h. Potassium Chromate -- MCB, Inc.
i. Calcium Carbonate -- MCB, Inc.
j. Cobalt -- Alfa Product
K. Iron Nitrate -- MCB, Inc.
l. Distilled dionized water -- prepared in the lab.

2. STANDARD SOLUTIONS

a. Cobalt standard solutions

Stock cobalt solution, 1000 ppm, was prepared according to the Perkin-Elmer procedure (114). Cobalt metal, 1.000 g, was dissolved in a minimum volume of (1+1) HCl, and diluted to 1 liter with 1% (V/V) HCl.

A standard test solution, 12 ppm, was made by appropriate dilutions of the 1000 ppm stock solution using volumetric flasks and de-ionized water.

b. Iron Standard Solutions

Stock iron solution, 1000 ppm, was prepared according to
the Perkin-Elmer procedure (114). Iron wire, 1.000 g, was dissolved in 50 ml of (1+1) HNO₃, and diluted to 1 liter with deionized water.

A standard test solution, 10 ppm, was made by appropriate dilutions of the 1000 ppm stock solutions using volumetric flasks and deionized water.

c. Nickel Standard Solutions

Stock nickel solution, 1000 ppm, was prepared according to the Perkin-Elmer Standard procedure (114). Nickel metal, 1.000 g, was dissolved in a minimum volume of (1+1) HNO₃, and diluted to 1 liter with 1% (V/V) HNO₃.

A standard test solution, 10 ppm, was made by appropriate dilutions of the 1000 ppm stock solutions using volumetric flasks and deionized water.

d. Chromium Standard Solutions

Stock chromium solution, 1000 ppm, was prepared according to the Perkin-Elmer procedure (114). Potassium Chromate, 3.735 g, was dissolved in 1 liter of deionized water.

A standard test solution, 5 ppm, was made by appropriate dilutions of the 1000 ppm stock solution using volumetric flasks and deionized water.

e. Copper Standard Solutions

Stock copper solution, 1000 ppm, was prepared according to the Perkin-Elmer standard procedure (114). Copper metal, 1.000 g, was dissolved in a minimum volume of 1:1 nitric acid, and
diluted to 1 liter with 1% (V/V) nitric acid.

A standard test solution, 5 ppm, was made by appropriate dilutions of the 1000 ppm stock solutions using volumetric flasks and deionized water.

f. Lead Standard Solutions

Stock lead solutions, 1000 ppm, was prepared according to the Perkin-Elmer standard procedure (114). Lead Nitrate, 1.598 g, was dissolved in 1 liter of 1% nitric acid.

A standard test solution, 20 ppm, was made by appropriate dilutions of the 1000 ppm stock solutions using volumetric flasks and deionized water.

g. Calcium Standard Solutions

Stock calcium solutions, 500 ppm, was prepared according to the Perkin-Elmer standard procedure (114). Calcium carbonate, 1.249 g, was dissolved in 50 ml of deionized water. HCl was then added dropwise to complete the dissolution of calcium carbonate. The solution was then diluted to 1 liter with deionized water.

A standard test solution, 2 ppm, was made by appropriate dilutions of the 500 ppm stock solutions using volumetric flasks and deionized water.

h. Magnesium Standard Solutions

Stock magnesium solution, 1000 ppm, was prepared according to the Perkin-Elmer procedure (114). Magnesium metal, 1.000 g, was dissolved in a minimum volume of (1+1) HCl, and diluted to 1 liter with deionized water.
A standard test solution, 1 ppm, was made by appropriate dilutions of the 1000 ppm stock solutions using volumetric flasks and deionized water.

i. Zinc Standard Solutions

Stock zinc solution, 500 ppm, was prepared according to the Perkin-Elmer procedure (114). Zinc metal, 0.500 g, was dissolved in a minimum volume of (1+1) HCl, and diluted to 1 liter with 1% (V/V) HCl.

A standard test solution, 0.75 ppm was made by appropriate dilutions of the 500 ppm stock solutions using volumetric flasks and deionized water.

j. Cadmium Standard Solutions

Stock cadmium solution, 1000 ppm, was prepared according to Perkin-Elmer procedure (114). Cadmium metal, 1.000 g, was dissolved in a minimum volume of (1+1) HCl, and diluted to 1 liter with 1% (V/V) HCl.

C. INSTRUMENTATION AND EQUIPMENT

1. Sample delivery pump: Laboratory Data Control Constamertic III and Water Associates 6000 M.

2. Titanium Ultrasonic nebulizer (Figure 36): Sono Tek Corporation, Model 8308-2-60 Tc, with power supply.

3. Atomic absorption spectrometer (Perkin-Elmer 303B), spray chamber and burner neck extention desolvation with power suppliers, also gas flow regulation, and gas inlet system as described by a co-worker in our group (164). A schematic diagram for the burner chamber, burner neck and the equipped
ultrasonic nebulizer is shown as figure 40.

4. Ultrasonic nebulizer holder: a teflon nebulizer holder, shown as figure 41, was machined from a solid piece of teflon. This design seals and protects the piezoelectric discs from sprayed liquids without affecting the performance of the nebulizer due to tightness.


6. Atomic absorption light sources: A demountable hollow cathode system designed and built in the lab was used for Ni, Pb, Cd, and Cu studies. Perkin-Elmer hollow cathode lamps were used for Zn, Mg, Ca, Fe and Co studies.

7. Sample Introduction and Connection: A capillary stainless steel tubing insert was connected to HPLC pump with a piece of teflon tubing, shown as figure 42. This design using regular H.P.L.C. solvent delivery tubing for sample introduction. The H.P.L.C. pump was used to drive the sample into the ultrasonic nebulizer.

8. Gas Flow Control and Connection: This ultrasonic nebulizer does not utilize gas for nebulization. Further the sample was introduced at a position slightly above the bend between the vertical and horizontal portions of the burner chamber. Consequently, a modification of the oxidant gas inlet was necessary when the ultrasonic nebulizer was used. This modification is shown as figure 43. The original sample inlet was modified with a piece of plastic tubing as the oxidant
Figure 40. Burner and Ultrasonic Nebulizer Assembly
TEFLON HOUSING FOR THE ULTRASONIC NEBULIZER

Figure 41. Teflon Ultrasonic Nebulizer Holder
inlet. This allowed a high volume of gas to be fed through the center of the burner chamber back, instead of from the side. It helped to improve the gas flow pattern and release flow restriction. The original oxidant inlet was used as fuel inlet. The fuel gas mixed well with the oxidant gas when using this change. The auxiliary oxidant inlet remained unchanged. Figure 44 shows the gas flow schematic and the position of ultrasonic nebulizer relative to all the gas inlets. All the gases were separately controlled using three different rotameters.

D. EXPERIMENTAL PROCEDURES

The sensitivities of ten different elements, including Ni, Mg, Ca, Zn, Cd, Pb, Cr, Fe, Co, and Cu, were studied under different sample feed rates, using the prepared test solutions. Results were obtained using:

a) a pneumatic concentric nebulizer,

b) a pneumatic concentric nebulizer and heating the burner chamber and burner neck extension,

c) a titanium ultrasonic nebulizer, and

d) the ultrasonic nebulizer and heating the burner chamber and burner neck extension.

All the studies using a Perkin-Elmer pneumatic concentric nebulizer used recommended P.E. flame A.A. procedures (114). The flame composition and position were adjusted and optimized to obtain optimum sensitivities and measure the effect of changing the sample flow rate.
Figure 42. Interfacing Sleeve of H.P.L.C.- A.A.
1. 1/8" Stainless Steel Tubing
2. Knurled Knob
3. Front and Rear Ferrules
4. Modified Insert
5. Locking Ring
6. Nebulizer Body
7. End Cap
8. O-Ring

Figure 43. Modified Gas Inlet for the Burner-Nebulizer Assembly
Figure 44. Gas Control Diagram for the Ultrasonic Nebulizer-Flame A.A. System
For studies using the ultrasonic nebulizer, sample solutions were introduced into the solvent reservoir of the H.P.L.C. pumping system. The ultrasonic nebulizer was connected to the solvent delivery system using a stainless steel sleeve coupled with teflon tubing (Figure 40). The ultrasonic drive power was adjusted and optimized by using a voltmeter as recommended by the manufacturer.

To increase the desolvation rate, the burner chamber and burner neck were heated to (a) 70° C and (b) 140° C. Sample solution was fed into the burner for fifteen minutes for the burner system to reach thermal equilibrium before data were collected.

When using the ultrasonic nebulizer, the gas flow rates were adjusted only when optimizing the flame composition. However, when using the pneumatic concentric nebulizer, oxidant flow adjustment was required to change the sample feeding rate and to adjust the flame composition.

Air-acetylene flames were used throughout this entire study. Oxidizing flames were used to study most of the elements except for Cr for which a strong reducing yellow flame was used. For the Ca study a mild reducing flame was used.

E. RESULTS AND DISCUSSION

1. EFFECTS OF ULTRASONIC FREQUENCY

As presented in the section describing the principles of the ultrasonic nebulizer, it is advantageous to have a small droplet size because this provides a high surface area
therefore facilitating easy vaporization and hence increases the atomization efficiency.

The drop size is proportional to $f^{-2/3}$, where $f$ is the ultrasonic drive frequency (Equation C).

The effects of ultrasonic frequency can be seen by comparison with the previous study by a co-worker in our group using a similar aluminum ultrasonic nebulizer operated at 78.0 KHz (164). Table 6 shows the calculated differences in droplet sizes, average surface areas of droplet, number of droplet per milliliter and sensitivity increases for Cu relative to those from pneumatic concentric nebulizer at two different frequencies.

One of the secondary effects of the ultrasonic frequency was the transfer of the droplets to the flame. Since lower ultrasonic frequency produced larger droplets, a higher gas flow rate was necessary to carry the droplets to the flame. Some droplets may be lost when a lower gas flow rate was used. A higher nebulization frequency is therefore desirable.

2. SENSITIVITY VS FLOW RATE

The results for sensitivities vs flow rates for the ten different elements studied are shown in Figure 45 to 54. For each element, four curves were obtained using a) a standard commercial pneumatic concentric nebulizer, b) a pneumatic concentric nebulizer and heating the burner chamber and burner neck extension, c) a burner using a titanium ultrasonic nebulizer, and d) a burner using an ultrasonic nebulizer and
<table>
<thead>
<tr>
<th>FREQUENCY</th>
<th>AVERAGE DROP VOLUME (cm²)</th>
<th>AVERAGE DROP SURFACE AREA (cm²)</th>
<th>NUMBER OF DROPLETS / MIN.</th>
<th>SENSITIVITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>78.0 KHz</td>
<td>6.2 x 10⁻⁹</td>
<td>1.6 x 10⁻⁵</td>
<td>1.6 x 10⁸</td>
<td>7.0</td>
</tr>
<tr>
<td>57.7 KHz</td>
<td>1.2 x 10⁻⁸</td>
<td>2.5 x 10⁻⁵</td>
<td>8.7 x 10⁷</td>
<td>3.0</td>
</tr>
</tbody>
</table>

**TABLE 6. EFFECT OF ULTRASONIC FREQUENCY ON THE NUMBER OF DROPS AND THEN VOLUME AND SURFACE AREA**
heating the burner chamber and burner neck extension.

A comparison was made between curve a) and d) for the elements at a typical H.P.L.C. flow rate of 1.0 ml/min, in table 7. The sensitivity was dependent on the sample flow rate. But using an ultrasonic nebulizer and improving desolvation by heating the burner chamber and burner neck gave sensitivity increases ranging from 1.67 X (for Cr) to 7.40 X (for Cd) at a flow rate of 1 ml/min.

3. DESOLVATION

The process of atomization involves evaporation of the solvent followed by decomposition of the residue and atomization of the sample. This is a time consuming process which may not be completed during the brief stay of droplets in the flame. Desolvation by heating the burner chamber and burner neck completes this step before the residue reaches the flame and this in turn enhances the efficiency of atomization process. This process was more efficient for the ultrasonic nebulizer than for the pneumatic concentric nebulizer because it produced a high density of droplets of similar size while the pneumatic nebulizer provides a wide range of sizes --some of which are simply too big to transport to the flame (141)(147).

A similar trend was seen on all the flow rate vs sensitivity curves. There was a decline of sensitivity at higher flow rates for the ultrasonic nebulizer. This was because a larger number of droplets were introduced into the
Figure 45. Sensitivity for Co
Figure 46. Sensitivity for Fe
Figure 47. Sensitivity for Ni
Figure 48. Sensitivity for Cr
Figure 49. Sensitivity for Cu
SENSITIVITY FOR Pb (20 ppm)
A. CONCENTRIC, EXTENSION NOT HEATED
B. CONCENTRIC, EXTENSION HEATED
C. ULTRASONIC, EXTENSION NOT HEATED
D. ULTRASONIC, EXTENSION HEATED

Figure 50. Sensitivity for Pb
SENSITIVITY FOR Ca (2 ppm)

A. CONCENTRIC, EXTENSION NOT HEATED
B. CONCENTRIC, EXTENSION HEATED
C. ULTRASONIC, EXTENSION NOT HEATED
D. ULTRASONIC, EXTENSION HEATED

Figure 51. Sensitivity for Ca
Figure 52. Sensitivity for Mg

SENSITIVITY FOR Mg (1 ppm)
A. CONCENTRIC, EXTENSION NOT HEATED
B. CONCENTRIC, EXTENSION HEATED
C. ULTRASONIC, EXTENSION NOT HEATED
D. ULTRASONIC, EXTENSION HEATED
Figure 53. Sensitivity for Zn

- A. CONCENTRIC, EXTENSION NOT HEATED
- B. CONCENTRIC, EXTENSION HEATED
- C. ULTRASONIC, EXTENSION NOT HEATED
- D. ULTRASONIC, EXTENSION HEATED
Figure 54. Sensitivity for Cd

SENSITIVITY FOR Cd (1 ppm)

A. CONCENTRIC, EXTENSION NOT HEATED
B. CONCENTRIC, EXTENSION HEATED
C. ULTRASONIC, EXTENSION NOT HEATED
D. ULTRASONIC, EXTENSION HEATED
<table>
<thead>
<tr>
<th>ELEMENTS</th>
<th>Co</th>
<th>Fe</th>
<th>Ni</th>
<th>Cr</th>
<th>Cu</th>
<th>Pb</th>
<th>Ca</th>
<th>Mg</th>
<th>Zn</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOILING POINTS</td>
<td>2870</td>
<td>2750</td>
<td>2732</td>
<td>2672</td>
<td>2567</td>
<td>1740</td>
<td>1484</td>
<td>1090</td>
<td>907</td>
<td>765</td>
</tr>
<tr>
<td>CONCENTRIC SENSITIVITY</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>ULTRASONIC SENSITIVITY AT 57.7 KHz</td>
<td>2.56</td>
<td>2.60</td>
<td>1.76</td>
<td>1.67</td>
<td>3.00</td>
<td>4.25</td>
<td>3.18</td>
<td>3.18</td>
<td>3.80</td>
<td>7.40</td>
</tr>
<tr>
<td>ULTRASONIC SENSITIVITY AT 78.0 KHz</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>7.00</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>
flame, which reduced the flame energy per droplet available for atomization. Further reduction of the droplet size by increased heating or an increase of the ultrasonic frequency may minimize this problem but clearly there will be a limiting flow rate, above which efficiency decreases.

4. EFFECT OF BOILING POINTS AND ANALYTICAL SENSITIVITIES

A qualitative relation seemed to exist between the boiling point of element and the sensitivity increase (Table 7). The sensitivities of volatile elements such as Cd, Zn, Mg, and Ca increased more than less volatile elements such as Ni, Fe, Co, and Cr. Perhaps the decomposition of the solid residue, which controls chemical interferences, is a more important rate determining step with less volatile (less easily atomized) elements than with more volatile metals.

5. MEMORY EFFECTS

Flame A.A. often suffers from memory effects at high flow rate due to the solution left in the spray chamber from previous sample. This is because the spray chamber is located down stream from the air and sample inlet (the nebulizer) and the restrictions on droplets from leaving the burner slot freely.

In this study, the ultrasonic nebulizer was located at the neck of the burner chamber and the burner chamber was up stream from the sample inlet. The gas flow was able to carry most of the droplets into the flame and memory effect were minimal.
E. GAS FLOW CONTROL

In pneumatic nebulizers, the flow rate of the oxidant was adjusted to change the sample feeding rate. In order to maintain the same flame composition, fuel and auxiliary oxidant flows may have to be readjusted, which in turn affect the optimal flame position for the best sensitivity. Using the ultrasonic nebulizer, gas flow control was much easier due to more uniform droplet size (154)(165), usually no gas flow change was necessary when the sample flow rate was changed.

Also at increased flow rate, greater quantities of thermal energy were required to dissolve the sample. Thermal saturation may occur with the design used. This can be improved in future designs.

F. SUMMARY

Several advantages from this titanium ultrasonic nebulizer for flame A.A. are summarized as follows.

a. Better A.A. sensitivity was obtained than the widely used pneumatic nebulizer but it was worse than the aluminum nebulizer used previously (164). This was probably because of the increased droplet size. However, this can be improved by changing the size of the titanium ultrasonic nebulizer and increasing frequency.

b. This study showed the feasibility of desolvating the sample outside the flame to improve A.A. sensitivities.

c. It was easier to control the sample feed rate using the ultrasonic nebulizer and the control was precise because a
H.P.L.C. pump controlled the flow accurately. The sample feed rate was therefore independent of the flame gas flow rate.

d. The droplet size was frequency dependent. As long as there is no mechanical limitation in producing a small size ultrasonic nebulizer, the ultrasonic frequency can always be further increased. This will in turn give better sensitivities.

e. The memory effect was minimal. This makes the ultrasonic nebulizer an ideal device for interfacing flame A.A. with H.P.L.C. since the memory effect from pneumatic concentric nebulizer may give poor resolution and ghost peaks on liquid chromatograms. More results and discussion will be described in the following chapters to illustrate the usefulness of the newly developed H.P.L.C.-A.A. system.

f. The corrosion resistance of titanium made the performance of ultrasonic nebulizer more reproducible than the aluminum one. This was because the nebulizing surface of the nebulizer was not affected by most chemicals in the sample and enabled it to give good stability which was important for flame A.A. applications and H.P.L.C.-A.A. interfacing since a liquid chromatogram may take a long time to be completed.
IV. CHAPTER 4-SPECIATION OF CHROMIUM BY H.P.L.C.-A.A.

A. TOXICITY AND BIOLOGICAL IMPORTANCE OF CHROMIUM

In one form, chromium is now considered to be an essential element. However it can be toxic, depending on the concentration and and chemical form especially the valence state. The essential form of chromium is the trivalent state; however, hexavalent chromium is toxic, or even carcinogenic. Speciation of chromium is therefore very important because simple analysis of total chromium provides little on the behavior of the metal.

Chromium (III) was found to be involved in the synthesis of cholesterol and fatty acid from acetate in rats (166)(167)(168). Chromium ion enhanced the rate of synthesis. It also inhibited the increase of the cholesterol level in serum with age.

Chromium also plays an important role in insulin metabolism as part of the glucose tolerance factor. Experiments showed that deficiency in chromium (III) gave impaired glucose tolerance in rats (169)(170). It was hypothesized that chromium (III) acts as a cofactor with insulin at the cellular level through the formation of a ternary complex between membrane sites, insulin, and chromium. Supporting evidence that chromium is involved in glucose tolerance in man has been documented (171)(172).

Chromium (III) is also related to protein synthesis. Experiments showed that chromium deficiency in rats inhibited
the capability to incorporate the amino acids, glycine, serine, methionine and aminobutyrate into the protein of their hearts (173)(174). This incapability could be repaired by supplementation with both chromium (III) and insulin, however, supplementation with only insulin gave only slight improvement.

The chromium level in liver, blood and hair were lower for diabetics than normal people (175)(176)(177). The concentration of chromium in normal human blood is estimated to be 0.5-5 microgram per liter and 5-10 microgram per liter in urine. The daily chromium intake by man ranges from 5-100 microgram (178). Chromium in food is usually in complex forms with nicotinic acid, glycine, glutamic acid and cysteine. They can be better absorbed than inorganic chromium. Animal protein is the best source of chromium (179).

That the toxicity of chromium depends on its valence state has been known for years. Trivalent chromium at low concentration is essential to man unlike hexavalent chromium which can be carcinogenic (180). Calcium chromate, lead chromate and zinc potassium chromate are carcinogens. NIOSH recommends that chromium (VI) in the workplace be considered to be carcinogenic unless evidence can demonstrate that only noncarcinogenic chromium (VI) compounds are present (181).

Chromium (VI) compounds are irritants and corrosive. They can enter human body by ingestion, inhalation, and through the skin. Acute exposures to the dust or mist may cause coughing
and wheezing, headache, dyspnea, pain on deep inspiration, fever, and loss of weight. Chronic exposures may cause a variety of symptoms including lacrimation, inflammation of the conjunctiva, nasal itch and soreness, ulceration and perforation of the nasal septum, congested nasal mucosa and turbinates, chronic asthmatic bronchitis, dermatitis and ulceration of the skin, and possibly lung cancer and hepatic injury (182).

B. ANALYTICAL DETERMINATION AND SPECIATION OF CHROMIUM

A wide range of techniques has been used to analyzed chromium in different samples; however, only a few indirect method were developed to speciate chromium (III) and chromium (VI).

The most popular method to determine chromium colorimetrically is by tranformation of chromium into chromate and complexing it with diphenylcarbazide. This method has detection limits ranging from 12 ng ml⁻¹ to 1.2 ng ml⁻¹ depending on the procedure used. In general a solution of the reagent is added to a digest of the sample in sulfuric acid (about 0.2N). Diphenylcarbazide reacts with chromium (VI). The absorbance of the red-violet complex is measured at 540 nm. The major interferences for this method are from iron and vanadium (183)(184)(185).

Conversion of chromium into volatile species following by the determination with gas chromatography is another popular technique. Chromium in biological samples can be wet digested
and converted to a trifluoroacetate which is then extracted into benzene and determined by G.C.-E.C.D. (186). Different reagents can be used to react with chromium for better recovery. However, 1, 1, 1-trifluoro-2,3-pentanedione was the best reagent reported to give a chromium recovery of 91% (187).

Neutron activation analysis (188)(189), X-Ray fluorescence (190) and atomic emission (191) all have been utilized to determine chromium in different types of samples, such as biological materials, foods and water.

However, the most popular instrumental technique for chromium determination is atomic absorption. Much effort has been devoted to the determination of chromium in biological samples using atomic absorption. Widely divergent values exist from different sources. A good review on determination of chromium in biological samples using atomic absorption was given by Jons and Jensen (191). They attributed the existing discrepancies to a) loss by volatilization during sample drying and ashing giving low results b) contamination during sample collecting and handling processes giving high results.

Atomic absorption was used in a standard method by NIOSH for speciation of chromium (III) and (VI) (192). In this method, hexavalent chromium is separated from the trivalent chromium by extracting the chelated complex of hexavalent chromium with ammonium pyrrolidine dithiocarbamate into methyl isobutyl ketone and determined by atomic absorption. Total
chromium was also determined by atomic absorption. The
difference between the two determination is attributed to
chromium (III). This method was not subjected to the
interferences which affect the diphenylcarbazide colorimetric
method.

The Environmental Protection Agency also published a
standard method for chromium speciation in which atomic
absorption was also used as a final determination tool (193).
Chromium (VI) from solution was coprecipitated as lead chromate
with lead sulfate in a solution of acetic acid. After
separation, the supernate, containing chromium (III), was drawn
off and the precipitate was washed to remove occluded chromium
(III). The chromium(VI) was then reduced and resolubilized in
nitric acid, and quantified as chromium (III) by atomic
absorption spectroscopy.

The use of H.P.L.C. in separating metal compounds is
rapidly growing in popularity. The possibility of speciating
Cr(III) and Cr(VI) using H.P.L.C. has been reported. Ion
exchange chromatography was used to speciate Cr(III) and Cr(VI)
in water samples with a conductivity detector (194). Chromium
(III) was separated in form of Cr(EDTA)⁻ and chromium (VI) was
eluted as chromate ion. This method required interfering ions
to be removed in sample preparation steps because of the use of
a non-selective detector. The interesting possibility of
simultaneously determining Cr(III) and Cr(VI) by complexation
following reverse phase H.P.L.C. detection was examined by
different researcher using different reagents (195)(196).

Results always suffered from lack of specificity of the reagent, low sensitivity, interferences from other metals and sample decomposition.

In this study, a method was developed to determine both Cr(III) and Cr(VI) simultaneously by the interfaced H.P.L.C.-A.A. system, using the described ultrasonic nebulizer. Advantages were taken from the high metal specificity of A.A. and high resolution power of H.P.L.C. unit. Results are good illustration of the capability of this developed H.P.L.C.-A.A. system which will be discussed more in latter sections.

C. EXPERIMENTAL

1. INTERFACING H.P.L.C. AND A.A.

The instrumentation and equipment used for interfacing H.P.L.C. and A.A. were the same as those used in sensitivity studies (chapter 3) of this dissertation.

Interfacing H.P.L.C. with the A.A. was achieved similarly by the technique used previously with the ultrasonic nebulizer except that a H.P.L.C. guard column and a H.P.L.C. separation column were incorporated into the system for separation purposes.

An ultrasonic nebulizer was used throughout this study. The 7'' burner head extension was used and heated to the desired temperatures with the burner chamber, i.e., 140° C and 70° C respectively.

2. CHEMICALS AND SOLUTIONS
a. Chemicals and their commercial sources

(i). Chromium (III) nitrate — J.T. Baker
(ii). Potassium chromate — MCB, Inc.
(iii). Potassium dichromate — MCB, Inc.
(iv). Ammonium pyrrolidinedithiocarbamate (APDC) — Aldrich chemical
(v). Acetonitrile — J. T. Baker
(vi). CIS reversed -phase analytical T.L.C. Precoated Plates — Whatman
(vii). Acetic acid — J. T. Baker
(viii). Sodium Acetate — MCB, Inc.
(ix). Dithiozone — Fisher Scientific
(x). Chloroform — MCB, Inc.

b. Solutions

Solutions used in this study were prepared from above sources.

(i) Chromium (III) standard solutions: Stock 1000 ppm
Cr(III) solution was prepared by dissolving 7.6930 g 
Cr(NO₃)₃·9H₂O in a 1000 ml volumetric flask using deionized water prepared in our lab. Dilute solution of Cr(III), 50 ppm, was prepared from the stock solution using volumetric flasks and deionized water.

(ii). Chromium (VI) standard solutions: Stock solution of Cr(VI), 1000 ppm, was prepared by dissolving 5.6581 g of K₂Cr₂O₇ in a 1000 ml volumetric flask using deionized water. Dilute solution of Cr(VI), 50 ppm, was prepared by
dilution of the stock solution using volumetric flask and deionized water.

(iii). Standard 0.2% APDC solution was prepared by dissolving 0.2000 gram of APDC in a 100 ml volumetric flask using deionized water.

(iv). A buffer solution of pH 4.5 was prepared by adding acetic acid dropwis into a 0.2 M sodium acetate solution. The pH of the solution was monitored during the addition of acetic acid using a standardized pH meter.

(v). Spray dithizone solution 0.05% was prepared by dissolving 0.0500 gram of dithizone in a 100 ml volumetric flask using chloroform.

(vi). Acetonitrile / Water solutions for T.L.C. development and H.P.L.C. were prepared when needed by mixing acetonitrile and water to the desired compositions.

3. DEVELOPING H.P.L.C. SOLVENT SYSTEM BY REVERSED-PHASE T.L.C.

a. EXPERIMENTAL PROCEDURE

(i). Preparation of Cr(III)-APDC complex solution:
standard Cr(III) 50 ppm solution, 10 ml, was mixed with 20 ml pH 4.5 buffer solution and 10 ml 0.2% APDC solution. The mixture was then heated in a 55° C water bath for twenty minutes; a pale blue precipitate of Cr(III)-APDC complex formed during the heating process. The complex was extracted from the solution using 10 ml ethylacetate. The two layers were separated by using a separatory funnel. The extraction
step was repeated three times. The organic ethylacetate phases containing the complex were combined together and ethylacetate was removed by a vacuum evaporator under room temperature. The residue which contained the complex was then dissolved in 5 ml acetonitrile to form a blue solution.

(ii) Preparation of Cr(VI)-APDC complex solution:
Standard Cr(VI) 50 ppm solution, 10 ml, was mixed with 20 ml pH 4.5 buffer solution and 10 ml 0.2% APDC solution. The mixture was shaken for 10 minute at room temperature. A white precipitate of the Cr(VI)-APDC complex formed during shaking. The complex was then extracted from the solution using 10 ml ethylacetate. The two layers were separated using a separatory funnel. The extraction step was repeated three times. The organic phases were combined together and ethylacetate was removed by a rotary vacuum evaporator under room temperature. The residue which contained the Cr(VI)-APDC complex was then dissolved in 5 ml acetonitrile to form a green solution.

(iii). Preparation of Cr(III)-APDC and Cr(VI)-APDC mixture solution: Solution mixture of Cr(III) and Cr(VI) was prepared by mixing 50 ppm Cr(III) and 50 ppm Cr(VI) solutions to give a total volume of 10 ml. The solution mixture containing both Cr(III) and Cr(VI) was added with 20 ml pH 4.5 buffer and 10 ml 0.2% APDC aqueous solution. Ten minute reaction time was allowed at room temperature to for the Cr(VI) complex. The solution was shaken occasionally during the reaction. The solution was placed into a 55°C water bath for twenty minutes
to complex the Cr(III). The pale blue precipitate was extracted with 10 ml ethylacetate and the two layers were separated. The aqueous layer was extracted twice more time with ethylacetate. The three organic phases were combined together. Ethylacetate was then removed by using a rotary vacuum evaporator under room temperature. The residue containing Cr(III) and Cr(VI) was dissolved in 5 ml acetonitrile.

(iv). T.L.C. Separation and Development

The purpose of this T.L.C. study was to develop solvent systems which can be used in H.P.L.C.-A.A. separation. It is easier and convenient using T.L.C. to evaluate solvent systems. Any solvent system can be tested by T.L.C. in about one hour; however, using H.P.L.C. directly to find a good solvent system may be very time consuming. It is not unusual that one may spend days without finding a good solvent system using H.P.L.C. directly since changing solvent systems in H.P.L.C. involves preparation of solvent systems, testing and flushing the H.P.L.C. system with stepwise polarity change in flushing solvents. This process may have to be repeated several times for finding best solvent system. It is also possible to exert harmful effect on the column.

T.L.C. plates of 5x10 cm were cut from commercial 5x20 cm precoated plates using a glass cutter and a ruler. APDC complexes of Cr(III), Cr(VI) and mixture of Cr(III) and Cr(VI) were spotted separately on the plates and developed by
different mobile phases prepared from deionized water and acetonitrile. Detection of T.L.C. spots were made by either spraying the plates with 0.05% dithizone solution or placing the plates under the room light since both complexes were slightly colored. To ensure the best detection, both methods were applied in this study.

b. RESULTS

Figure 55 and 56 shows the separation of Cr(III)-APDC and Cr(VI)-APDC using two different solvent systems, with indicated Rf values. When dithizone solution (0.05% in chloroform) was used as detecting agent, Cr(III) gave a dark purple color and Cr(VI) gave a orange yellow color on light purple background after the plate was heated in oven at 100° C for fifteen minutes.

Similar solvent systems were then used in later H.P.L.C.-A.A. studies.

4. SPECIATION OF Cr(III) AND Cr(VI) BY H.P.L.C.-A.A.

a. INSTRUMENTATION AND EQUIPMENTS

All the instrumentation and equipments used were the same as those described previously in chapter 3 of part B in this dissertation. H.P.L.C. was interfaced with the flame A.A. unit by using the previously described techniques.

The ultrasonic nebulizer with the interfacing sleeve shown as figure 42. Figure 40 shows the complete set up of flame burner and nebulizer combination.

Based on the the studies using T.L.C., a Partisil C8
Stationary Phase: Whatman C18 Plate
(5 x 10 cm)
Mobile Phase: 80% Acetonitrile
20% Water
Detection: Visual and Dithizone

$R_f$ Values:
- Cr(III)-APDC 0.43
- Cr(VI)-APDC 0.52

Figure 55. Separation of Cr(III)-APDC and Cr(VI)-APDC Using T.L.C.
Stationary Phase: Whatman C18 Plate
(5 x 10 cm)
Mobile Phase: 70% Acetonitrile
30% Water
Detection: Visual and Dithizone

R_f Values:

Cr(III)-APDC  0.26
Cr(VI)-APDC  0.36

Figure 56. T.L.C. Separation of Cr(III)-APDC and Cr(VI)-APDC
reversed-phase column purchased from Whatman was used as the separation column. A 5 cm guard column packed by the same column material was prepared by the author to protect the separation column.

A Waters Associates Model U6K injector was used for sample injections. This injector allowed sample volumes up to two milliliters to be injected into the system using Waters syringe needles of designated size. Although its construction is sophisticated, it is an excellent device and easy to use.

b. EXPERIMENTAL PROCEDURES

Cr(III)-APDC, Cr(VI)-APDC and mixtures of the two complexes were prepared by procedures described in the previous section.

The A.A. spectrometer with Perkin Elmer Cr hollow cathod lamp was turned on and warmed up for five minutes. The intensities of Cr lamp and D_2 lamp used for background correction were adjusted and matched according to Perkin Elmer procedures (114).

The flame and burner was adjusted to a proper position by visual inspection and then by checking with A.A. using a pneumatic concentric nebulizer. A chromium solution was constantly fed into the flame. The proper flame position was obtained when maximum atomic absorbance was seen on the absorbance indicator of the spectrometer. A strong yellow reducing flame was used for this study.

The gas control system was the switched to the system used
for ultrasonic nebulizer, shown in figure 42. The ultrasonic nebulizer interfaced with H.P.L.C. unit was applied. The H.P.L.C. pump was turned on and the flow rate was adjusted to desired value. Method for determining flow rate will be discussed in later section.

Power was applied to the ultrasonic nebulizer. Voltage adjustment was carried out by following the procedures recommended by manufacturer. Two volts above the minimum voltage required to start nebulization was used to operate the ultrasonic nebulizer.

The chart recorder was turned on with chart paper moving and the spectrometer was rezeroed when only the mobile phase was pumped into the flame. The Sample to be analyzed was then injected into the H.P.L.C.-A.A. system. Slight adjustment and checking on flame position were made before chromatograms to be collected.

c. INSTRUMENTAL PARAMETERS

The sensitivity of the H.P.L.C.-A.A. system depended on the flow rate of the mobile phase according to the sensitivity studies in previous chapter. Also the chromatographic resolution is also flow rate dependent. A study to find optimal flow rate in H.P.L.C.-A.A. study was necessary to elucidate optimum conditions.

Figure 57 shows the results obtained by injection of Cr(III) and Cr(VI) complexes into the system at different flow rates. The optimal flow rate was found to be around 1.0 to 1.2
FLOW RATE STUDY OF Cr-APDC COMPLEXES

Figure 57. Flow Rate Study of Cr-APDC Complexes Using H.P.L.C.- A.A.
ml/min. At lower flow rates, the signals were broadened and peak heights were lower. This was due to diffusion of the sample in the column. At higher flow rates, signals remained sharp; however, signal heights decreased due to poor sensitivity at high flow rates. This matched with the results obtained from sensitivity studies from previous chapter. At higher flow rates, a larger number of droplets were produced but the energy from heating the burner extension and flame was not sufficient for efficient atomization. A decrease in sensitivity resulted.

All instrumental parameters used in this study are as follows

\( \text{(i)} \). Cr hollow cathode current : 15 mA

\( \text{(ii)} \). Absorption Wavelength : 357.9 nm

\( \text{(iii)} \). Slit setting : 4 (0.7nm)

\( \text{(iv)} \). Flame type : air-acetylene flame, rich yellow reducing flame.

\( \text{(v)} \). Guard column : 5.0 X 0.46 cm partisol C8, 10 micron packing.

\( \text{(vi)} \). Column : 25.0 X 0.46 cm partisol C8, 10 micron packing.

\( \text{(vii)} \). H.P.L.C. flow rate : 1.2 ml/min.

\( \text{(viii)} \). Mobile phase : Acetonitrile / Water.

\( \text{(ix)} \). Ultrasonic frequency : 57.7 KHz.

\( \text{(x)} \). Ultrasonic voltage : 14 volts.

d. RESULTS
Chromatograms for Cr(III), Cr(VI) and mixtures of Cr(III) and Cr(VI), along with their retention times, are shown in figure 58 to 60 respectively. The retention time for Cr(III) was longer than that of Cr(VI). No other interference peak was observed.

5. SPECIATION BY H.P.L.C.-U.V.

A U.V. detector was used for the same chromatographic system in chromium speciation studies. However, this method proved to be less selective and subjected to interferences. Conditions and results will be described as follows.

a. INSTRUMENTATION AND EQUIPMENT

One of major changes in instrumentation was the use of a Perkin-Elmer UV-90 H.P.L.C. detector instead of the A.A. detection system interfaced with ultrasonic nebulizer.

The H.P.L.C. pump was from Laboratory Data Control, Model Constametric III. Injector was from Rheodyne, Model 7125, with a 100 microliter loop. Guard column and separation column were the same as those used in H.P.L.C.-A.A. studies.

b. EXPERIMENTAL PROCEDURES

Cr(III) and Cr(VI) APDC complexes and mixtures were prepared by the methods described in earlier sections. They were then injected into the H.P.L.C.-U.V. system after sufficient instrument warm-up time. Chromatographic conditions were the same as those used in H.P.L.C.-A.A. studies.

c. INSTRUMENTAL PARAMETERS

(i). Guard column : 5 X 0.46 cm Partisol C8, 10 micron
Cr(III)-APDC

Retention Time:
4.2 min
2.0 ug

Column: Whatman Partisil PXS
10/25 C8
Mobile Phase: 70% Acetonitrile
30% Water
Flow Rate: 1.2 ml/min

Figure 58. Chromatogram of Cr(III)-APDC
Using H.P.L.C.- A.A.
Figure 59. Chromatogram of Cr(VI)-APDC
Using H.P.L.C. - A.A.
Figure 60. Chromatogram of Cr(III)-APDC and Cr(VI)-APDC Mixture Using H.P.L.C.-A.A.
packi
g.

\( \text{(ii). Column : 25} \times 0.46 \text{ cm Partisil CS 10 micron packing.} \)

\( \text{(iii). H.P.L.C. flow rate : 1.2 ml/min.} \)

\( \text{(iv). Mobile phase : Acetonitrile / Water.} \)

\( \text{(v). U.V. wavelength : 254 nm.} \)

\( \text{(vi). U.V. detector scale : 250} \times 0.01 \text{ absorbance unit full scale on recorder.} \)

d. RESULTS

Figure 61 shows the liquid chromatogram for Cr(III). Two major signals were recorded for Cr(III)-APDC complex with retention times 5.75 min. and 4.50 min. respectively. Figure 62 shows the chromatogram for Cr(VI). Three major signals were detected. Retention times were 5.70 min., 4.55 min., and 4.05 min. respectively. Figure 63 is the chromatogram or Cr(III) and Cr(VI) mixture. Three major signals were recorded for the mixture with same retention times as those in figure 60.

These chromatograms indicated other U.V. active compounds in the samples besides chromium complexes. No further attempt was tried to identify those extra signals; however, they must be due to the excess reagent and impurities in the samples.

This study illustrates the difficulty in using H.P.L.C.-U.V. for chromium speciation using APDC complexation method. The difficulty is mainly due to selectivities of detection systems.

6. DISCUSSION

Using the developed H.P.L.C.-A.A. and APDC complexation
Column: Whatman Partisil PXS 10/25 C8
Mobile Phase: 70% Acetonitrile
            30% Water
Flow Rate: 1.2 ml/min
Wavelength: 254 nm

Figure 61. Chromatogram of Cr(III)-APDC Using H.P.L.C.-UV
**ABSORBANCE**

Column: Whatman Partisil PXS 10/25 C8  
Mobile Phase: 70% Acetonitrile  
30% Water  
Flow Rate: 1.2 ml/min  
Wavelength: 254 nm

---

**Cr(VI)-APDC**

Retention Time:  
4.55 min  
4.05 min  
5.70 min

---

*Figure 62. Chromatogram of Cr(VI)-APDC Using H.P.L.C.- UV*
Cr(III)-APDC Cr(VI)-APDC Mixture

**ABSORBANCE**

Column: Whatman Partisil PXS 10/25 C8  
Mobile Phase: 70% Acetonitrile  
30% Water  
Flow Rate: 1.2 ml/min  
Wavelength: 254 nm

Figure 63. Chromatogram of Cr(III)-APDC and Cr(VI)-APDC Mixture Using H.P.L.C.- UV
method for chromium speciation work was proved to be feasible. Using T.L.C. to develop a H.P.L.C. solvent system enables one to achieve H.P.L.C. separation with minimal effort.

This method enables one to detect both Cr(III) and Cr(VI) simultaneously. Because of the selectivity of A.A., interferences from other metals and reagents can be avoided.

Comparison between liquid chromatograms from a U.V. detector and the interfaced A.A. detector indicates there was no loss in chromatographic resolution in H.P.L.C.-A.A. interfacing section. Signals from H.P.L.C.-A.A. remained reasonably sharp. This was due to minimal dead volume in the interfacing section.
A. INTRODUCTION

1. BIOLOGICAL IMPORTANCE OF ZN

Zinc has been known to be essential for animals for over fifty years (197). The importance of Zinc for humans has been suspected since 1961 (198). First Zinc metalloenzyme, carbonic anhydrase, was reported in 1940 by Keilin and Mann (199). Now, Zinc metalloenzymes were found to be more than 70 from different species (200). During the past fifteen years, rapid advances have been made with respect to the role of Zinc in human metabolism.

Zinc has its primary effect on zinc-dependent enzymes that regulate the biosynthesis and catabolic rate of RNA and DNA (201)(202).

Zinc deficiency was known to produce growth retardation and maturation delay (203). Zinc-deficient dwarfs have been reported in different countries (204). There is no doubt that nutritional zinc is essential for the proper growth of man.

Major functions of zinc in human and animal metabolism appear to be enzymatic. Zinc enzymes are known to participate in a variety of metabolic processes including carbohydrate, lipid, protein, and nucleic acid synthesis or degradation. The metal is present in several dehydrogenases, aldolases, peptidases, and phosphatases (200)(205).

Zinc may also play a part in nonenzymic, free-radical reactions. In particular, zinc is known to protect against
iron-catalyzed, free-radical damage (206).

Zinc in the plasma is mostly present as bound to albumin, but other proteins, such as macroglobulin, transferrin, ceruloplasmin, haptoglobin, and gamma globulins, also bind significant amounts of zinc (207). A small fraction of zinc in the plasma is bound to amino acids, and some exists as the ionic form. Histidine, glutamine, threonine, cystine, and lysine appear to have significant zinc-binding ability (207).

Normal zinc intake in a well-balanced American diet with animal protein is approximately 12-15 mg/day. Approximately 20-30% of ingested dietary zinc is absorbed. Excretion of zinc can be from gastrointestinal tract, sweat and urine (207).

Excess excretion of zinc, hyperzincuria, has been related to different diseases. It is well known that alcohol induces hyperzincuria. The mechanism is unknown (208). Cirrhosis of the liver causes abnormal zinc metabolism and diminishes hepatic zinc (209). Some patients with diabetes mellitus have been found to have increased urinary losses of zinc (210). A deficiency of zinc in patients with sickle cell disease, a genetic disorder, has been recognized (211). It was also reported that zinc in plasma, erythrocytes, and hair was decreased and urinary zinc excretion was increased in sickle-cell-anemia patients as compared to controls.

In summary, zinc is involved in many biochemical functions. Many zinc metalloenzymes have been recognized in the past fifteen years. Many different diseases are known to
be related to zinc deficiency. Determination of zinc in urine is of additional help in diagnosing those diseases. Besides being used to diagnose disease, the determination of zinc in urine may be of additional help in finding zinc deficiency and preventing other problems.

It is the goal of this study to take advantage of the developed H.P.L.C.-A.A. to initiate zinc speciation studies in urine samples and other biological samples. Although many methods have been developed to determine total zinc concentrations in different samples, there is a definite need to develop an efficient method for zinc speciation in biological samples. Metal speciation in biological samples are often complicated by matrix effects in those samples. Since the developed H.P.L.C.-A.A. system is a metal specific tool, it can also be excellent instrument for metal speciation in biological samples. Separation of zinc species in urine samples will be illustrated as follows.

2. DETERMINATION AND SPECIATION METHODS OF ZINC

Many methods for zinc determination can be found in the literature. Few are related to zinc speciation work. This section will cover some representative methods for zinc determination and speciation.

Colorimetric methods have been used extensively for zinc determination. Dithizone and naphthothiocarbazono are the primary reagents that have been employed in the determination of zinc in biological samples (212)(213). These methods are
highly pH dependent. Interferences occur from cadmium, lead, thallium and other heavy metals.

Fluorimetry have been employed for measuring zinc concentrations in biological materials. Formation of fluorescent complexes can be accomplished by using reagents such as 8-hydroxyquinoline (214), and 8-hydroxyquinoline-5-sulfonic acid (215).

Chromatographic techniques have been employed alone or in conjunction with other techniques in determining zinc in various materials. These included a combination of anion exchange chromatography with colorimetry (216), combination of anion exchange with EDTA titration (217), and combination of cation exchange and polarography (218). Zinc acetylacetone complex along with other 42 metal complexes were eluted by employing high pressure gas chromatography at 115° C and 800-1000 psi (219).

Differential pulse anodic stripping voltammetry has been used as a screening procedure to identify zinc and other heavy metals in urine (220).

X-ray fluorescence spectrometry was used for the analyses of zinc for clinical samples (221). Matrix effects challenge the accuracy and precision of analysis. For example, iron can interfere since it has an absorption upon the zinc line.

Atomic emission and atomic absorption techniques are by far the most popular methods for zinc determination. A review article by Jensen and coworkers covers many methods involving
both techniques for zinc determination in biological samples (222). These methods include flame A.A., non-flame A.A. and ICP methods. Speciation of zinc by atomic absorption in biological samples involving the use of gel filtration with sephadex G-100 to partition zinc-containing proteins followed by fraction collections and A.A. determination without sample pretreatment has been reported by Gardiner and co-workers (223). The results have confirmed the known association of zinc with 2-macroglobuline, albumin and other proteins.

B. EXPERIMENTAL

1. INSTRUMENTATION AND CHEMICALS

(a). INSTRUMENTATION

This study used the developed H.P.L.C.-A.A. for speciation of zinc in urine samples. All components for the H.P.L.C.-A.A. system were the same as those described in earlier sections (Chapter 3 and 4) except for the guard column and separation column. A high speed cation exchange column (Catalog No.269-024) and a guard column (Catalog No. 269-005) from Wescan Instruments, Inc. were incorporated into the system for separating the zinc species in urine samples.

(b). CHEMICALS

(i). Zinc metal - Alfa Product
(iii). Tartaric acid - MCB. Inc.
(iv). Dionized Water - prepared in the lab.
(v). Hydrochloric Acid - J.T. Backer Chemical Co.
2. EXPERIMENTAL PARAMETERS

Instrumental parameters used in this study are as follows

<i>. Zn hollow cathode current: 9 mA

<i>i>. Wavelength: 213.9 nm

<i>iii>. Slit setting: 4 (0.7nm)

<i>iv>. Flame type: Air-acetylene flame, lean blue oxidizing flame.

<i>v>. Guard column: Wescan Ion Guard Cation-269-005

<i>vi>. Separation Column: Wescan Cation 1 HS-269-024

<i>vii>. H.P.L.C. flow rate: 1.0 ml/min.

<i>viii>. Mobile phase: 2.0 mM ethylene diamine, 0.5 mM tartaric acid aqueous solution with pH 3.9

<i>ix>. Ultrasonic frequency: 57.7 KHz.

<i>x>. Ultrasonic voltage: 12 volts.

3. EXPERIMENTAL PROCEDURES

Because of the difficulty in finding suitable organic zinc compounds, ionic zinc was the only standard used in this study. Standard 500 ppm ionic zinc solution was prepared by dissolving 0.500 g of zinc metal in a minimum volume of (1 + 1) HCl, and diluting to 1 liter with 1% (V/V) HCl. Testing solutions of desired concentrations were then diluted from the standard stock solution.

Ionic zinc solution was injected into the H.P.L.C.-A.A. system to obtain chromatograms. Chromatographic conditions and mobile phase were chosen to properly elute ionic zinc from the column.
Chromatograms of urine were obtained by direct injection of urine samples into the system after centrifugation without any other pretreatment.

After each urine sample injection, the column was cleaned by continuously running mobile phase through it. Normally 500 ml of mobile phase was required to clean the column.

C. RESULTS AND DISCUSSION

Figure 64 shows the chromatogram of ionic zinc and its retention time. Dilute nitric acid with pH as low as 2.0 has been tried to elute ionic zinc from the column without any success. Ethylene diammonium cation and tartrate anion were then added as auxiliary complexing reagent to elute ionic zinc from the column (224). The ethylene diammonium cation exchanged with zinc from resin and tartrate anion further complexed with the exchanged zinc to increase the elution speed. The method was successful and elution of zinc was achieved.

Advantages of using ion exchange column to speciate metal in urine sample is obvious. The mobile phase is a aqueous solution with minor organic modifier. This allows body fluids to analyzed without pretreatment. It also prevents the mobile phase from changing the nature of the sample.

Figure 65 shows a liquid chromatogram of urine using the H.P.L.C.-A.A. system. Most of the zinc species were eluted in twenty minutes. Two major signals were found to have retention times shorter than the ionic zinc. They may be non-ionizable
Figure 64. Chromatogram of Zinc Ion Using H.P.L.C.-A.A.
LIQUID CHROMATOGRAM OF URINE USING AA AS Zn SPECIFIC DETECTOR

COLUMN - WESCAN HS/CATION
FLOW - 1 ml/min
MOBILE PHASE - 2.0 mM EDA
0.5 mM Tartaric acid pH 3.9

FIGURE 65

RETENTION TIME (min)
zinc species and monovalent zinc species and may be explained by their weaker interactions with the resin; however, further identification using standard compounds may be necessary.

This study was the first direct speciation of zinc in urine. No attempts were tried for identifying and quantifying those signals due to difficulty in obtaining standard compounds. Nevertheless, the developed H.P.L.C.-A.A. system was proved to be a good method for biological zinc speciation.
VI. CHAPTER 6 - DIRECT SPECIATION OF MAGNESIUM IN URINE BY H.P.L.C.-A.A.

A. INTRODUCTION

1. BIOLOGICAL IMPORTANCE OF MAGNESIUM

It was reported that magnesium forms about 2.5% of crust of the Earth. Magnesium is widely distributed in nature in a variety of compounds. Important magnesium ores are magnesite (MgCO₃), dolomite (MgCO₃·CaCO₃), brucite Mg(OH)₂, Olivine (MgFe)₂SiO₄, and many others. Magnesium exists in sea water at a concentration of 1.27 g/Kg (225).

Chlorophyll, the magnesium chelate of porphyrin, was reported by Willstatter and Stoll in 1903. They also found that the magnesium ion occupies the central location in the chlorophyll molecule (225). Since then, a lot of magnesium compounds have been found in biological systems. The misconception that magnesium is a trace element in the body has been dispelled. This metal is responsible for many biochemical roles in cells: it activates enzymes; it maintains the conformation of nucleic acids; and it regulates a number of important biological processes.

The stored chemical energy to maintain life for both plant and animal cells is released by oxidative phosphorylation which is the primary function of all mitochondria. Adenosine triphosphate, ATP, the main fuel of life, is produced in oxidative phosphorylation. All enzyme reactions that are known to be catalyzed by ATP show an absolute requirement for
magnesium. These reactions encompass a very wide range of synthetic processes (226). All components in processes known to be dependent on ATP are capable of chelating with magnesium. Enzyme /ATP/Mg$^{2+}$ ternary complexes with intramolecular stacking have been demonstrated by nuclear magnetic resonance (227). Magnesium is thus required for the action of numerous enzyme systems and for the contraction of muscular tissue. Also, it has been known for many years that the concentration of magnesium is higher in the interior of cells, especially in mitochondria, than the intercellular medium (228). The reverse situation holds for calcium. This explains why magnesium is important enzyme activator inside the cell, while calcium activates extracellular enzymes.

Studies also suggested that magnesium in the inner mitochondrial membrane plays a role in permeability of monovalent cations. For example, the permeability of potassium was determined primarily by the amount of magnesium associated with the red cell membrane (229).

DNA (deoxyribonucleic), which is almost exclusively confined to the cell nucleus, is the carrier of genetic information. Variations in the concentration of magnesium in vivo must exert a control on DNA synthesis (230). It is reasonable to visualize Mg$^{2+}$ as an intermediate complexing agent responsible for the unwinding of the parent DNA chain, a step that must take place before the DNA content is doubled and cell duplication occurs, or to theorize that magnesium plays a role
in stabilizing polynucleotide structure formation of RNA on a double-stranded DNA template (231).

Many different diseases have been related to magnesium. There is a large and rapidly growing body of literature on the importance of magnesium in biochemical and physiological processes. Seeling made a thorough review on these subjects (232). Unquestionably magnesium related diseases are the result of a disturbance in one of the other electrolytes. However, they may also occur when the concentrations of other electrolytes are normal (233).

Symptomatic hypomagnesemia is related to gastronintestinal disorders, alcoholism, cirrhosis, acute pancreatitis, kidney diseases, endocrine disorders, resistant rickets, pellagra, malignant osteolytic disease, excessive lactation and absorption-excretion defects.

Data indicate the body content of magnesium for a man weighing 70 Kg would be on the order of 2000 meq (24g). About 89% of all the magnesium in the body resides in bone and muscle. Bone contains about 60% of the total body content of magnesium. Most of the remaining magnesium is distributed equally between muscle and nonmuscular soft tissues. Approximately only 1% of the total body content of magnesium is extracellular (225).

The U.S. National Research Council recommends a magnesium allowance of 25 to 42 meq/day (300 to 500 mg/day) for adults. A daily intake of 17 meq (0.257Kg) may meet nutritive
requirements provided that the individual remains in positive magnesium balance (225). Most of that portion of the magnesium which is absorbed into the body is excreted by the kidney (234). Excessive intake of vitamin D increases renal magnesium excretion (232). There is no evidence that significant amounts of magnesium is excreted by the bowel in most animals or in people (225)(235).

Since urine is the major source of magnesium excretion and the element has been related to many diseases, it may be interesting and important to investigate magnesium species in urine samples. It is then the goal of this study to speciate magnesium in urine samples by the developed H.P.L.C.-A.A. system using direct injection method.

2. DETERMINATION METHODS OF Mg

Although the importance of magnesium in the body has been known since 1920's for many years research was severely hampered because magnesium could not be measured accurately in biological systems. Many of the early studies were nutritional or kinetic. The effects of varying the amount of the metal in the diet of animals were observed, or its role in isolated biochemical systems was investigated.

The great variety of methods used in the past for determining magnesium in biological materials testifies to the fact that none of them was completely satisfactory. Most were cumbersome, indirect, or even inaccurate. The accuracy of magnesium determination was greatly improved after the
introduction of atomic absorption spectroscopy in the late 1950's by Walsh (103). It is still the current method of choice. It is thus the intention of this study to take advantages of A.A. and to develop a direct method for magnesium speciation. Nevertheless, some other methods for magnesium determination will be summarized in this section.

One of the earliest methods to estimate the concentration of magnesium in biological samples was to weigh the precipitate of magnesium ammonium phosphate (236). Later, a colorimetric method was incorporated to estimate magnesium as the formed phosphate by the reduction of phosphomolybdic acid to molybdenum blue which can be determined colorimetrically (237). Due to the instability of this blue substance, a better method was developed to determine the formed phosphate as the yellow molybdivanadophosphoric acid which is stable over hours (238). Other notable reagents used for magnesium determinations include Titan yellow, 8-hydroxyquinoline and Eriochrome Black T (239)(240)(241).

Instrumental spectroscopic methods for magnesium determination which were reviewed by Jensen and co-workers include atomic absorption (flame and nonflame), flame emission and ICP-emission (222). However, atomic absorption spectrometry is currently the method of choice for routine quantitative analysis due to its specificity, easy sample preparation, good sensitivity, reasonable analysis speed and good precision and accuracy.
B. EXPERIMENTAL

1. INSTRUMENTATION AND CHEMICALS

a. INSTRUMENTATION

Instrumentation used in this study was the same as those described in the previous chapter including H.P.L.C. columns.

b. CHEMICALS

<i>. Magnesium ribbon -- Alfa Product.

<i>i>. Ethylene diamine -- J. T. Baker Chemical Co.

<i>i>i>. Hydrochloric Acid -- J. T. Baker Chemical Co.

<i>i>iv>. Deionized water -- Prepared in the lab.

2. EXPERIMENTAL PARAMETERS

Instrumental parameters used in magnesium study are as following

<i>. Mg hollow cathode current: 10 mA

<i>i>. Wavelength: 285.2 nm

<i>i>i>. Slit setting: 4 (0.7 nm).

<i>i>iv>. Flame type: lean, blue oxidizing air-acetylene flame.

<i>v>. Guard column: Wescan Ion Guard Cation -- 269-005

<i>vi>. Separation Column: Wescan Cation / HS-269-024.

<i>vii>. H.P.L.C. flow rate: 1.0 ml/min.

<i>viii>. Mobile phase: 2.0 mM ethylene diamine aqueous solution with pH 6.1.

<i>i>ix>. Ultrasonic frequency: 57.7 KHz

<i>x>. Ultrasonic power supply voltage: 12 volts.

3. EXPERIMENTAL PROCEDURES
Magnesium stock standard solution, 1000 ppm, was prepared by dissolving 1.000 g of magnesium ribbon in a minimum volume of (1 + 1) HCl. Testing solutions of desired concentrations were prepared by dilution of the stock solution using deionized water and volumetric flasks.

The mobile phase was prepared by dissolving 0.1202 gm of ethylene diamine in a 1000 ml volumetric flask with deionized water.

Inorganic magnesium solution was the only standard used in this study. Chromatographic conditions and mobile phase were determined by elution of magnesium ion from the column.

Urine analyses were performed by direct injections into the H.P.L.C.-A.A. system after centrifugation without further chemical treatment.

After each urine sample injection, the column was cleaned by continuously running mobile phase through it. Normally 500 ml of mobile phase was required for this process.

C. RESULTS AND DISCUSSION

A chromatogram of inorganic magnesium ion is shown as figure 66. Only a simple solution of ethylene diamine was necessary to elute magnesium ion from the column. Unlike elution of zinc compounds, tartaric acid was not necessary. This has been due to less interaction between magnesium and the resin than zinc and the resin.

Figure 67 shows a chromatogram of urine samples. Three peaks were detected. The major signal with the same retention
Figure 66. Chromatogram of Magnesium Ion Using H.P.L.C.-A.A.
LIQUID CHROMATOGRAM OF URINE
USING AA AS Mg SPECIFIC DETECTOR

COLUMN-WESCAN HS/CATION
MOBILE PHASE-2.0 mM EDA
pH 6.1
FLOW-1.0 ml/min

Figure 67. Liquid Chromatogram of Urine Using AA as Magnesium Specific Detector
time as magnesium ion indicated that majority of magnesium in urine samples existed as magnesium ion. Two minor signals were also detected. One had shorter retention than magnesium ion; the other was retained longer in the column than magnesium ion.

No effort was put into to further identify the two minor signals. Wide range of clinical samples will be necessary to bring more significance to this study. Nevertheless, developing H.P.L.C.-A.A. allows one to step further in biological metal speciation work.
VII. CHAPTER 7 - SUMMARY

Further improvement of flame A.A. sensitivities was proved to be possible using the ultrasonic nebulizer to increase nebulization efficiency. Desalvation of droplets by heat outside the flame area can further improve flame A.A. sensitivity. The ultrasonic frequency which controls the droplet size can be increased to further improve flame A.A. sensitivities. Although other factors, such as droplet transportation, desoluation of droplet, gas flow control and chemical interference may also affect the improvement.

Interfacing H.P.L.C. with flame A.A. using ultrasonic nebulizer offers many advantages, such as easy flow rate matching, minor loss of chromatographic resolution and ease in operation, besides better sensitivities. H.P.L.C.-A.A. proved to be a useful system in metal speciation work of environmental and biological samples.

It was used in the second part of this dissertation study to speciate chromium (III) and chromium (VI) as well as zinc and magnesium in urine samples. Both reversed-phase H.P.L.C. and ion chromatography technique were used.

This dissertation reports the first direct zinc and magnesium speciation studies in urine. With further improvement of sensitivities for other metals, more metal speciation works will be possible. Information from these studies will have great values in helping diagnoses of metal related diseases.
It is no doubt that further improvement of this system is possible by using a higher ultrasonic frequency with a better design and more applications of this system in metal speciation studies will be successful with this H.P.L.C.-A.A. system.
BIBLIOGRAPHY


22. Imperial Chemical Industries, Mercury as an
Environmental Pollutant, A Bibliography, Brixham Laboratory, England (August 1973).


29. Fujiki, M., Kumamoto Igk. Z., 37, 10 (1963).


768 (1940).


60. Braman, R. S., Anal. Chem. 43 (11), 1462 (1971).


83. Cremer, E., and Muller, R., Mikrochemie Ver.


114. Perkin Elmer Corporation, Analytical Method for
Atomic Absorption Spectrophotometry.

115. Friberg, L., and Vostal, J., 'Mercury in the


118. Miettinen, J. K., Rahola, T., Hattula, T., Rissanen,

7, 13 (1937).

120. Kadowaki, H., Okamoto, K., and Nakajima, M., J.

121. Brooks, E. J., Gates, O. R., and Nottingham, M.,

122. Matusiak, W., Cefola, N., Dal Cortivo, L., and


124. Analytical Methods Committee, Soc. for Analytical
Chemistry, Analyst, 90, 515 (1965).


17, 171 (1965).


172. Levine, R. A., Stretten, D. H., and Doisy, R. J., 

173. Mertz, W. M., and Roginski, E. E., J. Nutr., 97, 

174. Roginski, E. E., and Mertz, W. M., J. Nutr., 97, 
525 (1967).


176. Hambridge, K. M., Rodgerson, D. O., and O'Brien, D., 
Diabetes, 17, 517 (1968).

177. Jiejeebhoj, K. N., Chu, R. C., Marliss, E. E., 
Greenberg, G. R., and Bruce-Robertaon, A., Amer. J. 


179. Mertz, W. M., Toepfer, E. W., Roginski, E. E., and 


181. National Institute for Occupational Safety and 
Health, Criteria for a Recommended Standard: 
Occupational Exposure to Chromium (VI), NIOSH Doc. 
No. 76-129 (1976).

182. Langard, S., and Norseth, T., Handbook on the 

1317 (1950).

1341 (1952).
Physiol., 107, 146 (1934).


209. Vallee, B. L., Wacker, W. E. C., Bartholomay, A. F.,


224. Fritz, J. S., Gjerde, D. T., and Pohlant, C., "Ion


VITA

Jia-Cherng Wu was born January 7, 1954 in Feng Shan, Taiwan, R. O. C. He graduated with honors from Tso Ying Senior High School in 1972 with a College Preparatory Curriculum.

He entered National Taiwan Normal University in the fall of 1973, majoring in chemistry. During the four years of study in N.T.N.U., he was awarded scholarship every semester based on his academic performance. He was an honored graduate in June, 1977 with a Bachelor of Science degree. Because of his academic achievements, he was assigned a teaching position in the Department of Chemistry of N. T. N. U. where he served as a lab instructor for two years.

He entered graduate school at Louisiana State University in the fall of 1979 to pursue the Ph.D. degree. During this time he got married in 1980 and his first child was born in 1983.

Having completed a major in analytical chemistry and a minor in organic chemistry, he is currently a graduate assistant in the Chemistry Department and a candidate for the degree of Doctor of Philosophy.
Candidate: Jia-Cherng Wu

Major Field: Analytical Chemistry


Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Wayne J. Mathies

F.E. Castle

Norman Bhaere

Mary W. Birt

Date of Examination:

July 16, 85