Speciation Studies of Biologically Active Compounds (Atomic Absorption, Ultrasonic Nebulization, Hplc, Organoleads, Aminoglycosides-Metal Complexes).

Edmund Douglas Boothe

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

Recommended Citation
https://digitalcommons.lsu.edu/gradschool_disstheses/3974

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.
Boothe, Edmund Douglas

SPECIATION STUDIES OF BIOLOGICALLY ACTIVE COMPOUNDS

The Louisiana State University and Agricultural and Mechanical Col. PH.D. 1984

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

Copyright 1985
by
Boothe, Edmund Douglas
All Rights Reserved
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, √.

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 ______
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
SPECIATION STUDIES OF BIOLOGICALLY ACTIVE COMPOUNDS

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
Edmund D. Boothe
B.S. in Chemistry, University of Alabama, 1970
M.S. in Chemistry, University of Alabama, 1974
August 1984
DEDICATION

To Mary
for her loving and patience

and

to Cathy and James Robert
who could always bring
a smile to my face.
ACKNOWLEDGEMENT

The author wishes to thank those members of the faculty and staff of the Department of Chemistry who helped and assisted him during these investigations.

The author wishes to thank the following for their generous support and donation of equipment, chemicals, and time:

Gerald Brooks and Perkin-Elmer Corporation for donation of a atomic absorption spectrophotometer, spray chambers, and burner heads used in the development work of the new flame atomizer-atomic absorption system.

Paul M. Rashba and Sono-Tek Corporation for their help and consultation on the ultrasonic nebulizer.

Vernitron Corporation for the help in with the piezoelectric material.

Joseph Grady and The Upjohn Company for the donation of the neomycin samples.

Allen Waitz and Schering Corporation for the donation of the gentamicin samples and for helpful discussions.

Albert Vulcano and Bristol Laboratories for the kanamycin and amikacin samples.

Burroughs Wellcome Co. for neomycin samples and their helpful information and discussions.
FOREWORD

Parts of this dissertation have been presented or are in submission for publication:


# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS.</td>
<td>iii</td>
</tr>
<tr>
<td>FOREWORD</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES.</td>
<td>xviii</td>
</tr>
<tr>
<td>LIST OF FIGURES.</td>
<td>xx</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>xxi</td>
</tr>
<tr>
<td>PART I. CHAPTER 1. DEVELOPMENT OF A NEW NEBULIZER-FLAME ATOMIZER-ATOMIC ABSORPTION SYSTEM DESIGNED FOR METAL SPECIATION.</td>
<td>1</td>
</tr>
<tr>
<td>1A. INTRODUCTION.</td>
<td>1</td>
</tr>
<tr>
<td>(a) General Introduction</td>
<td>1</td>
</tr>
<tr>
<td>(b) Nebulization and Atomization Processes</td>
<td>5</td>
</tr>
<tr>
<td>(1) Absorption of Radiation by Free Atoms</td>
<td>5</td>
</tr>
<tr>
<td>(2) Formation of Free Atoms</td>
<td>7</td>
</tr>
<tr>
<td>(c) Nebulization Processes and Efficiency</td>
<td>15</td>
</tr>
<tr>
<td>(d) Nebulizers Used in Atomic Spectroscopy</td>
<td>18</td>
</tr>
<tr>
<td>(1) Pneumatic Concentric Nebulizer</td>
<td>19</td>
</tr>
<tr>
<td>(2) Babington Type Nebulizer</td>
<td>25</td>
</tr>
<tr>
<td>(3) Cross-Flow Nebulizers</td>
<td>28</td>
</tr>
<tr>
<td>(4) Meinhard Concentric Nebulizer</td>
<td>30</td>
</tr>
<tr>
<td>(5) Glass Frit Nebulizer</td>
<td>33</td>
</tr>
<tr>
<td>(6) Ultrasonic Nebulizers</td>
<td>35</td>
</tr>
<tr>
<td>(e) Summary of Nebulizers and Nebulization Processes</td>
<td>40</td>
</tr>
<tr>
<td>1B. PROPOSED RESEARCH FOR THE DEVELOPMENT OF A NEW DRIVE...</td>
<td>5</td>
</tr>
</tbody>
</table>
### IC. EXPERIMENTAL: CHEMICALS, EQUIPMENT AND PROCEDURES

<table>
<thead>
<tr>
<th>(a) Chemicals</th>
<th>(b) Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. Atomic Absorption Spectrophotometer</td>
</tr>
<tr>
<td></td>
<td>2. Gas Regulation Systems</td>
</tr>
<tr>
<td></td>
<td>3. Burner Heads</td>
</tr>
<tr>
<td></td>
<td>4. Spray Chambers</td>
</tr>
<tr>
<td></td>
<td>5. Autotransformers</td>
</tr>
<tr>
<td></td>
<td>6. Thermocouple Vacuum Gauge Meter</td>
</tr>
<tr>
<td></td>
<td>7. Thermocouple Vacuum Gauge</td>
</tr>
<tr>
<td></td>
<td>8. Dual Seal Vacuum Pumps</td>
</tr>
<tr>
<td></td>
<td>9. High Performance Liquid Chromatographs</td>
</tr>
<tr>
<td></td>
<td>10. Miscellaneous Supplies and Equipment</td>
</tr>
</tbody>
</table>

### ID. DESIGN OF NEW ULTRASONIC NEBULIZER

<table>
<thead>
<tr>
<th>(a) Basic Design Concepts and Nebulizer Modifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Front and Rear Assembly Sections</td>
</tr>
<tr>
<td>(2) Piezoelectric Discs</td>
</tr>
<tr>
<td>(3) Center Electrode</td>
</tr>
<tr>
<td>(4) Sample Passage Tube</td>
</tr>
<tr>
<td>(5) Decoupling Sleeve</td>
</tr>
<tr>
<td>(6) Modifications to the Sample Passage</td>
</tr>
</tbody>
</table>
Tube and Decoupling Sleeve .................. 59

(i) Teflon or Polyethylene Inserts .. 59

(ii) Flared Glass Tubing ................. 60

(b) Operating Conditions for the Ultrasonic
Nebulizer .................................... 63

IE. EQUIPMENT AND PREPARATION OF STANDARD SOLUTIONS. 64

(a) Standard Solutions ...................... 64

(1) Copper .................................. 64

(2) Lead .................................... 64

(b) High Performance Liquid Chromatographs
Operating Conditions ..................... 64

(1) General Conditions ..................... 64

(2) Degassing ............................... 65

(c) Hollow Cathode Preparation and Operating
Conditions ................................... 65

(1) Copper ................................. 65

(2) Lead .................................... 66

(d) P-E 305B Atomic Absorption
Spectrophotometer ....................... 66

(1) Copper Analysis ....................... 66

(2) Lead Analysis ........................... 66

(e) Recorder ................................. 66

(f) Flame Conditions and Gasses for AA
Measurements Using Concentric Nebulizer .... 67

(1) General Conditions ..................... 67

(2) Gas Conditions for Three Burner Heads/
Perkin-Elmer Concentric Nebulizer ....... 67

(i) P-E Chamber/Burner Heads .......... 68

1. P-E Concentric Nebulizer /
Single Slot Burner Head........ 68

2. P-E Concentric Nebulizer/
   Three Slot Burner Slot........ 68

3. P-E Concentric Nebulizer/
   Single Slot Techtron Burner
   Head............................ 69

(ii) P-E Concentric Nebulizer/ Single
     Slot Techtron Burner Head.... 70

16. INTERFACING OF HPLC TO PERKIN-ELMER PNEUMATIC
    CONCENTRIC NEBULIZER............ 71

1G. NEBULIZATION STUDIES................................. 73

(a) Varian Techtron Spray Chamber/AB51 Burner
    Head.................................. 75
    (i) Incorporation of Ultrasonic Nebulizer.... 79
        (i) Tip Insertion into Chamber.......... 79
        (ii) Front Section Insertion into
             Chamber.......................... 82
        (iii) Entire Nebulizer Insertion........ 84
            1. Chamber Modifications.......... 84
            2. Nebulizer Insertion.......... 84
        (iv) Nebulizer with Cross-Flow Gas
             Introduction..................... 89
            1. Chamber Modification............ 89
            2. Gas Flow Studies.............. 89
    (v) Summary................................ 98

(b) Perkin-Elmer Spray Chamber, Model 303-0191.... 99
    (1) Incorporation of Ultrasonic Nebulizer
        via Rear Chamber Cap.............. 99
        (i) Tip Insertion into Chamber..... 99
        (ii) Front Section Insertion into
             Chamber........................... 102
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Spray Chamber Modifications</td>
<td>102</td>
</tr>
<tr>
<td>2. AA Measurements for Both Tip and Front Section Insertions</td>
<td>102</td>
</tr>
<tr>
<td>(iii) Rear Tip Insertion with Cross-Flow Gas Flow</td>
<td>106</td>
</tr>
<tr>
<td>1. Chamber Modifications</td>
<td>106</td>
</tr>
<tr>
<td>2. Atomic Absorption Measurements</td>
<td>106</td>
</tr>
<tr>
<td>3. Observations</td>
<td>106</td>
</tr>
<tr>
<td>(iv) A Separated Gas Flow Design</td>
<td>109</td>
</tr>
<tr>
<td>1. Chamber Modifications</td>
<td>109</td>
</tr>
<tr>
<td>2. Purpose</td>
<td>112</td>
</tr>
<tr>
<td>3. Observations</td>
<td>112</td>
</tr>
<tr>
<td>4. Additional Observations</td>
<td>112</td>
</tr>
<tr>
<td>Observations Utilizing Glass Chamber Body</td>
<td>112</td>
</tr>
<tr>
<td>(v) Summary for Nebulizer Insertion via Rear Chamber Cap</td>
<td>113</td>
</tr>
<tr>
<td>(2) Nebulizer Insertion via Burner Chamber Neck</td>
<td>114</td>
</tr>
<tr>
<td>(i) Chamber Modifications</td>
<td>114</td>
</tr>
<tr>
<td>(ii) Nebulization Studies</td>
<td>114</td>
</tr>
<tr>
<td>(iii) Attempted AA Measurement</td>
<td>115</td>
</tr>
<tr>
<td>(c) Perkin-Elmer Spray Chamber, Model 0040-0144</td>
<td>117</td>
</tr>
<tr>
<td>(1) Spray Chamber Modifications</td>
<td>117</td>
</tr>
<tr>
<td>(i) Rear Chamber Cap Insertion</td>
<td>117</td>
</tr>
<tr>
<td>(ii) Tip and Rear Insertion</td>
<td>118</td>
</tr>
<tr>
<td>(2) Side Insertion via Chamber Neck</td>
<td>122</td>
</tr>
<tr>
<td>(i) Chamber Redesign</td>
<td>122</td>
</tr>
</tbody>
</table>
(ii) Nebulizer Holder .......................... 122
(iii) AA Measurements .......................... 125
d) Final Spray Chamber Design .................. 126
   (1) Desolvation .................................. 126
      (i) Burner Neck Extensions .................. 127
      (ii) Spray Chamber Desolvation .............. 129
      (iii) Gas Flow Patterns and Inlet
             Redesign Studies ....................... 130
             1. Cross-Flow .......................... 130
             3. Flow Spoilers in Place ............ 133
             4. Gas Inlet Redesign ............... 134
e) Summary of Burner Chamber—Nebulizer
      Interfacing Design Studies ................... 137

PART I. CHAPTER 2. EVALUATION OF NEW NEBULIZER—
FLAME ATOMIZER FOR ATOMIC ABSORPTION ............ 141
2A. PROPOSED METHOD .................................. 141
2B. EXPERIMENTAL .................................... 142
   (a) Chemicals and Solutions .................. 142
   (b) Equipment and Procedures .................. 143
       (1) AA Spectrophotometer Settings ...... 143
       (2) Nebulizers ............................... 143
       (3) New Nebulization System: Spray Chamber/
            Nebulizer Configuration ............... 144
       (4) Spray Chamber Extensions ............. 144
       (5) Burner Head ............................. 145
       (6) Gas Settings and Controls ............ 145

X
(i) Pneumatic Concentric Nebulizer...... 145
(ii) Ultrasonic Nebulizer.................. 145

2C. RESULTS AND DISCUSSION.................. 147
   (a) Desolvation Studies..................... 147
   (b) Sample Flow/Burner Extension Studies.... 150
   (c) Flow Rate-Burner Extensions with
       Concentric Nebulizer...................... 159
   (d) Atomic Absorption Detection Limits........ 162
   (e) Atomic Absorption Sensitivity Limits....... 164

2D. GENERAL SUMMARY.......................... 166

PART II. CHAPTER 1. SPECIATION OF ORGANOLEAD COMPOUNDS
BY HPLC-AA AND TLC-COLORIMETRY............... 168

1A. GENERAL INTRODUCTION........................ 168
1B. ANALYTICAL DETERMINATION AND SPECIATION........ 171
   (a) General Analytical Determinations........... 171
   (b) Speciation Methods.......................... 173
      (1) Gas Chromatographic Methods............... 173
      (2) Liquid Chromatographic Methods............. 176
      (3) Other Chromatographic Methods.............. 177
1C. OBJECTIVES OF RESEARCH...................... 179
1D. EXPERIMENTAL............................... 181
   (a) Instrumentation and Supplies................ 181
      (1) High Performance Liquid Chromatographs... 181
      (2) HPLC Columns and Accessories.............. 181
         (i) Columns................................... 181
         (ii) Guard Columns........................... 181
(iii) Guard Column Packing ............... 182
(iv) Injector .................................. 182
(v) Syringes.................................. 182
(3) HPLC-AA................................. 182
   (i) Sample Injection ..................... 182
   (ii) Hollow Cathode Lamp................. 183
(4) Thin Layer Chromatography Supplies
   (i) Plates.................................. 183
   (ii) Chambers............................ 183
   (iii) Spotting Capillaries................. 184
   (iv) Air Gun............................. 184
   (v) Glass trays........................... 184
(5) Mass Spectrophotometer.................. 184
(6) Melting Point Apparatus................. 184
(b) Chemicals and Solutions............... 184
   (1) Chemicals............................ 184
   (2) Solutions............................ 185
(c) Experimental Procedures................. 186
   (1) Development of HPLC System for the Speciation of Lead Compounds...... 186
   (2) TLC Plate Development and Colorimetry....... 187
      (i) Application of Sample to Plate..... 187
      (ii) TLC Plate Development............. 187
      (iii) Sample Detection by TLC Colorimetry. 188
         1. Dithizone Colorimetry.............. 188
         2. PAR Colorimetry................... 188
3. PAR Solution Colorimetry........ 189

(3) Conversion of TEL in Seawater over a Long Time Period................. 189

(i) Unboiled Seawater................. 190

(ii) Boiled Seawater.................. 190

(4) Decomposition of Diethyllead Dichloride in Solid Form.................. 190

1E. RESULTS AND DISCUSSION........................................ 192

(a) HPLC and TLC System Development for the Speciation of Lead Compounds............... 192

(1) 75 Ethanol/25 Water/0.10 Molar NaBr........ 203

(2) 55 ACN/45 Water/0.43 Molar NaBr........... 208

(3) 55 ACN/45 Water/0.10 Molar NaCl........... 210

(b) Conversion of TEL in Seawater over a Long Time Period.................. 219

(c) Decomposition of Diethyllead Dichloride in Solid Form.................. 225

1F. SUMMARY OF RESULTS............................................... 241

PART II. CHAPTER 2. SPECIATION OF ORGANOMERCURIALS BY HPLC-AA AND TLC-COLORIMETRY........ 243

2A. GENERAL INTRODUCTION.................. 243

2B. ANALYTICAL DETERMINATION AND SPECIATION........ 245

(a) General Analytical Determination........ 245

(b) Speciation Methods.................... 246

(1) Gas Chromatographic Methods.......... 246

(2) Liquid Chromatographic Methods........ 248

(3) Other Chromatographic Methods......... 250

2C. OBJECTIVES OF RESEARCH............... 252
2D. EXPERIMENTAL ........................................ 253
   (a) Instrumentation and Supplies ................... 253
      (1) HPLC-AA .................................... 253
      (2) Mercury Hollow Cathode Lamps ............. 253
      (3) HPLC and TLC Supplies ..................... 253
   (b) Chemicals and Solutions ........................ 254
      (1) Chemicals .................................. 254
      (2) Solutions ................................. 254
   (c) Experimental Procedures ....................... 254
      (1) Development of HPLC-AA Systems for the
           Speciation of Mercury and Organomercurial
           Compounds .................................. 254
      (2) TLC Plate Development and Colorimetry..... 255
          (i) Application of Sample to Plate ...... 255
          (ii) TLC Plate Development ............... 255
          (iii) Sample Detection by TLC-Dithizone
                 Colorimetry ............................ 256
2E. RESULTS AND DISCUSSION ............................ 257
   (a) HPLC and TLC System Development for the
       Speciation of Mercury Compounds ............. 257
      (1) TLC-Colorimetry Speciation of Mercury
          Compounds .................................. 257
          (i) Ethanol Based Solvent Systems ....... 257
          (ii) ACN Based Solvent Systems .......... 258
      (2) HPLC-AA Speciation of Mercury Compounds. 260
          (i) 55 ACN/45 Water/0.10 Molar NaCl .... 260
          (ii) 55ACN/45 Water/0.43 Molar NaBr .... 265
2F. SUMMARY OF RESULTS ............................... 272
(2) Aminoglycosides................................. 296
(3) Solutions........................................... 297
(c) Experimental Procedure........................ 297

(1) Development of HPLC-AA System for the Speciation of Aminoglycoside-Metal Complexes.......................... 297

(2) TLC Plate Development............................ 297
   (i) Application of Sample to Plate.............. 298
   (ii) TLC Plate Development..................... 298
   (iii) Sample Detection........................... 298

(3) Preparation of Aminoglycoside Complexes... 299

3E. RESULTS AND DISCUSSION.......................... 301
(a) HPLC and TLC System Development for the Speciation of Aminoglycoside-Metal Complexes... 301
   (1) Silica Gel Chromatography.................... 302
      (i) Water as Mobile Phase..................... 302
      (ii) Addition of Monovalent Salts to Mobile Phase.......................... 303
      (iii) Addition of Divalent Salts to Mobile Phase......................... 304
   (2) Reversed-Phase C18 Chromatography........ 306
(b) Speciation of Aminoglycosides by HPLC-AA and TLC-Colorimetry................................. 310
   (1) Introduction.................................... 310
   (2) Separation of Aminoglycoside Complexes by TLC-Colorimetry......................... 310
      (i) Silica Gel.................................... 310
         1. Complexation Studies with Copper(2+), Cobalt(2+), and Zinc(2+).................. 310
2. Additional Studies with Copper(2+) .......................... 314

(c) Reversed-Phase TLC of Selected Transitional Metals ............... 317

(d) HPLC-AA Studies of Aminoglycoside Complexation .................. 317

3F. SUMMARY .............................................. 323

REFERENCES ............................................... 324

VITA .................................................. 340
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Solvent Combustion Mechanism</td>
<td>12</td>
</tr>
<tr>
<td>2 Influence of Auxiliary Gas Flow on Transport Efficiency</td>
<td>21</td>
</tr>
<tr>
<td>3 Sensitivities and Detection Limits (mg/L) Measured in an Air/Acetylene Flame</td>
<td>23</td>
</tr>
<tr>
<td>4 Transport Efficiency for Selected Pneumatic Concentric Nebulizers-Spray Chamber Arrangements</td>
<td>24</td>
</tr>
<tr>
<td>5 Ultrasonic Nebulizers Used in Atomic Spectroscopy</td>
<td>39</td>
</tr>
<tr>
<td>6 Decrease in Absorbance Resulting from Cooling of Burner Extensions on Introduction of Aqueous Samples</td>
<td>148</td>
</tr>
<tr>
<td>7 Absorbance as a Function of Burner Extension Length and Sample Flow Rate</td>
<td>152</td>
</tr>
<tr>
<td>8 Relative Increase in Absorbance for New Nebulization System Versus Perkin-Elmer Pneumatic Concentric Nebulizer</td>
<td>155</td>
</tr>
<tr>
<td>9 Absorbance for Pneumatic Concentric Nebulizer Versus Burner Extension Length</td>
<td>161</td>
</tr>
<tr>
<td>10 Systems Evaluated for Separation of Organolead Compounds</td>
<td>198</td>
</tr>
<tr>
<td>I. Ethanol Based Systems</td>
<td>198</td>
</tr>
<tr>
<td>II. Acetonitrile (ACN) Based Systems</td>
<td>199</td>
</tr>
<tr>
<td>III. Mixed ACN/THF/Methanol/NaBr Systems</td>
<td>200</td>
</tr>
<tr>
<td>IV. Tetrahydrofuran (THF) Systems</td>
<td>200</td>
</tr>
<tr>
<td>11 Retention Factors (Rf) for Organolead Compounds in Ethanol Based Systems</td>
<td>201</td>
</tr>
<tr>
<td>12 Retention Factors (Rf) for Organolead Compounds in Acetonitrile Based Systems</td>
<td>202</td>
</tr>
</tbody>
</table>
13 Systems for Separation of Diethyllead Dichloride .................................................... 202
14 Mass Spectral Data for TEL ............................................................... 228
15 Mass Spectral Data for Decomposed Diethyllead Dichloride Standard ...................... 230
16 Mass Spectral Data for Decomposed Diethyllead Dichloride, Aqueous Solution .......... 232
17 Mass Spectral Data for Triethyllead Chloride Standard .............................................. 234
18 Mass Spectral Data for Triethyllead Chloride, Aqueous Solution .............................. 236
19 Retention Factors (Rf) for Mercury Compounds in Ethanol Based Solvent Systems ........ 259
20 Retention Factors (Rf) for Mercury Compounds in Acetonitrile Based Solvent Systems ... 259
21 Solvent Systems Useful for the Separation of Aminoglycoside-Metal Complexes ........... 309
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Flame Profile of Magnesium (Stable Oxide) and Silver (Weak Oxide)</td>
<td>9</td>
</tr>
<tr>
<td>2 Aerosol Generation. Model of Smith and Browner</td>
<td>16</td>
</tr>
<tr>
<td>3 Perkin-Elmer Pneumatic Concentric Nebulizer</td>
<td>20</td>
</tr>
<tr>
<td>4 Babington Type Nebulizer</td>
<td>26</td>
</tr>
<tr>
<td>5 Cross-Flow Nebulizer</td>
<td>29</td>
</tr>
<tr>
<td>6 Meinhard Concentric Nebulizer</td>
<td>30</td>
</tr>
<tr>
<td>7 Glass Frit Nebulizer</td>
<td>34</td>
</tr>
<tr>
<td>8 Optical System for Perkin-Elmer Atomic Absorption Spectrophotometer 305B</td>
<td>48</td>
</tr>
<tr>
<td>9 New Ultrasonic Nebulizer</td>
<td>56</td>
</tr>
<tr>
<td>10 Ultrasonic Nebulizer Schematic</td>
<td>57</td>
</tr>
<tr>
<td>11 Glass Tube Insert for Ultrasonic Nebulizer</td>
<td>62</td>
</tr>
<tr>
<td>12 Interface for HPLC to Perkin-Elmer Pneumatic Concentric Nebulizer</td>
<td>72</td>
</tr>
<tr>
<td>13 Signal to Noise Ratio as a Function of Backpressure of Pneumatic Concentric Nebulizer</td>
<td>74</td>
</tr>
<tr>
<td>14 Gas Flow Schematic for Techtron Spray Chamber with Pneumatic Concentric Nebulizer</td>
<td>76</td>
</tr>
<tr>
<td>15 Front and Top Views of Techtron Spray Chamber with Pneumatic Concentric Nebulizer</td>
<td>77</td>
</tr>
<tr>
<td>16 Side View of Techtron Spray Chamber with Pneumatic Concentric Nebulizer</td>
<td>78</td>
</tr>
<tr>
<td>17 Gas Flow Schematic for Techtron Spray Chamber with Ultrasonic Nebulizer</td>
<td>80</td>
</tr>
<tr>
<td>18 Techtron Spray Chamber with Ultrasonic Nebulizer in Tip Insertion Configuration</td>
<td>81</td>
</tr>
<tr>
<td>19 Techtron Spray Chamber with Ultrasonic Nebulizer in Front Section Insertion Configuration</td>
<td>83</td>
</tr>
</tbody>
</table>
36 Holder for Ultrasonic Nebulizer ................. 124
37 Cross-Section of Burner Neck Extensions ........... 128
38 Gas Inlet for Nebulization System ................. 136
39 Final Design for New Ultrasonic Nebulizer-Flame
Atomizer Atomic Absorption System ................. 138
40 Gas Flow Schematic for New Ultrasonic Nebulizer
-Flame Atomizer Atomic Absorption System ......... 139
41 Decrease in Absorbance with Time as Burner
Extensions Reach Steady State Heat Transfer ....... 149
42 Absorbance as a Function of Burner Extension
Length and Sample Flow Rate ....................... 154
43 Sensitivity for Copper with New Nebulizer-Flame
Atomizer Atomic Absorption System ............... 163
44 Speciation of Organoleads by HPLC-AA by
Reversed-Phase Chromatography. Mobile Phase:
75 Ethanol/25 Water/0.10 Molar NaBr ............. 204
45 Comparison of TLC-Colorimetry and HPLC-AA
Separations of Organolead Compounds. Mobile
Phase: 75 Ethanol/25 Water/0.10 Molar NaBr ....... 205
46 Speciation of Triethyllead Chloride for TEL by
HPLC-AA. Mobile Phase: 75 Ethanol/25 Water/0.10
Molar NaBr ....................................... 207
47 Speciation of Alkylleads by Reversed-Phase
HPLC-AA. Mobile Phase: 55 ACN/45 Water/0.43
Molar NaBr ....................................... 209
48 Separation of Organoleads by Reversed-Phase TLC
-Dithizone Colorimetry ............................ 211
49 Separation of Organoleads by HPLC-AA. Mobile
Phase: 55 ACN/45 Water/0.10 Molar NaCl .......... 213
50 Speciation of Organoleads by HPLC-AA. Mobile
Phase: 55 ACN/45 Water/0.10 Molar NaCl .......... 214
51 Speciation of Triethyllead Ion from TEL by
Gradient Elution HPLC-AA ......................... 215
52 Speciation of Alkylleads by HPLC-AA. Mobile
Phase: 55 ACN/45 Water/0.10 Molar NaCl .......... 217
53 Separation of Organoleads by TLC-Dithizone Colorimetry. Mobile Phase: 55 ACN/45 Water/0.10 Molar NaCl ............................................. 218

54 Conversion of TEL in Seawater Over a Long Time Period (50 Weeks). Detection of Triethyllead Chloride by HPLC-AA ............................................. 221

55 Conversion of TEL in Seawater Over a Long Time Period (50 Weeks). Detection of Triethyllead Chloride by TLC-Dithizone Colorimetry ............. 224

56 Mass Spectrum of TEL ............................................. 227

57 Mass Spectrum of Decomposed Diethyllead Dichloride Standard ............................................. 229

58 Mass Spectrum of Decomposed Diethyllead Dichloride Standard, Aqueous Solution ............. 231

59 Mass Spectrum of Triethyllead Chloride Standard ............................................. 233

60 Mass Spectrum of Triethyllead Chloride, Aqueous Solution ............................................. 235

61 Decomposition of Diethyllead Dichloride in Solid Form. Speciation of Triethyllead Chloride by HPLC-AA ............................................. 239

62 TLC-Colorimetry of Decomposed Diethyllead Dichloride ............................................. 240

63 HPLC-AA of Mercury Compounds. Mobile Phase: 55 ACN/45 Water/0.10 Molar NaCl ................... 261

64 Speciation of Mercury Compounds by HPLC-AA. Mobile Phase: 55 ACN/45 Water/0.10 Molar NaCl ....... 262

65 Speciation of Mercury Compounds by TLC-Dithizone Colorimetry. Mobile Phase: 55 ACN/45 Water/0.10 Molar NaCl ............................................. 263

66 Speciation of Mercury Compounds by TLC-Dithizone Colorimetry. Mobile Phase: 55 ACN/45 Water/0.43 Molar NaBr ............................................. 266

67 HPLC-AA of Mercury Compounds. Mobile Phase: 55 ACN/45 Water/0.43 Molar NaBr ............................................. 267
68 Speciation of Mercury Compounds by HPLC-AA.
Mobile Phase: 55 ACN/45 Water/0.43 Molar NaBr... 268

69 Separation of Arylmercury Compounds by HPLC-AA.
Mobile Phase: 55 ACN/45 Water/0.43 Molar NaBr.... 270

70 Speciation of Arylmercury Compounds by HPLC-AA.
Mobile Phase: 55 ACN/45 Water/0.53 Molar NaBr.... 271

71 Structures of Kanamycin A, B, and Amikacin....... 275

72 Structures of the Gentamicins...................... 276

73 Structures of Neomycin B and C.................... 277

74 Retention Factors (Rf's) for Aminoglycosides on Silica Gel Plate for Three Monvalent Cationic Salt Solutions (2.50 Molar).................. 305

75 Retention Factors for Aminoglycoside on Silica Gel Plates as a Function of Magnesium Chloride Concentration (0.10 - 4.50 Molar)........... 307

76 Separation of Uncomplexed and Complexed Aminoglycoside by TLC. Detection of Aminoglycoside Moiety.......................... 311

77 Detection of Uncomplexed and Complexed Metal
-Aminoglycosides by TLC. Detection of Metal Portion with Dithizone.......................... 313

78 Separation of Uncomplexed and Complexed Amikacin as a Function of Ratio of Copper to Amikacin.... 315

79 Separation of Uncomplexed and Complexed Kanamycin as a Function of Ratio of Copper to Kanamycin.... 316

80 Complexation of Metals with Amikacin. Separation by TLC. Detection of Metal Cation with Dithizone. 318

81 Separation of Some Transition Metals by Reversed Phase TLC. Detection with Dithizone............... 319

82 HPLC-AA of Cobalt(2+) Not Complexed by Kanamycin A........................................... 321

83 Plot of Cobalt(2+) Not Complexed by Kanamycin A as a Function of Ratio of Kanamycin A to Cobalt(2+)...............................322
ABSTRACT

Part I. Development of a new nebulizer-flame atomizer atomic absorption system designed for metal speciation.

A new flame atomic absorption system has been designed and developed. A new, highly efficient ultrasonic nebulizer was used for sample introduction and aerosol generation. Extensive evaluations and modifications of commercially available atomic absorption spray chambers and burner heads were conducted. Observations made during these studies centered on some of the fundamental properties of aerosol transport within the atomic absorption nebulization system.

The new flame atomic absorption system consisted of the ultrasonic nebulizer incorporated into a modified spray chamber. The aerodynamic properties of the chamber were designed to give maximal aerosol transport. Burner extensions were incorporated into the chamber and could be resistively heated to aid in desolvation of the aerosol.

This system exhibited an increase in sensitivity, depending on the conditions and flow rates, of approximately 5 to 7 fold, and an increase in detection limits of approximately 2 to 4 fold over that reported for commercial flame atomizers.
Part II. The speciation of organoleads, mercury compounds, and metal complexes of aminoglycosides.

A number of physiologically and environmentally important organoleads and mercurials were speciated by high performance liquid chromatography-atomic absorption (HPLC-AA). HPLC systems were developed to separate these compounds by using complementary thin layer chromatographic (TLC) methods with colorimetry detection. These systems were used to study the conversion of tetraethyllead in seawater over a long period of time and the decomposition of diethyllead dichloride. Results showed that the triethyllead chloride was stable in seawater for at least one year, and that diethyllead chloride decomposes into triethyllead chloride and lead(2+) compounds.

Metal complexation with a class antibiotics, aminoglycosides, was investigated both by HPLC-AA and TLC-colorimetry. New HPLC and TLC systems were developed to separate the metal/complexes or the uncomplexed aminoglycoside. The formation of the copper and cobalt complexes was shown to be a function of the ratio of metal to aminoglycoside.
PART I. CHAPTER 1. DEVELOPMENT OF A NEW NEBULIZER-FLAME ATOMIZER ATOMIC ABSORPTION SYSTEM DESIGNED FOR METAL SPECIATION

1A.(a) General Introduction

In the past several decades, chemistry has greatly benefited mankind with a wondrous array of new products including pharmaceuticals, building and housing materials, electronic, computer, and telecommunication devices, and healthcare products. The chemical profession has been faced with a number of tremendous opportunities, but also with a number of great challenges. Along with the tremendous gifts of chemistry has come a number of problems.

With the technological advances also have come concerns over the impact on the environment and the potential toxicity and a number of health hazards. Some of the problems are real, but others are simply fears of the unknown. The concerns of the public are real, whether the perceived danger is or not. Only through the application of unbiased, scientific investigation can an understanding and a solution be found.

The determination of the total content or concentration of a compound or 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 satisfactory control and application of chemicals requires a knowledge and understanding of the chemical itself, as well as of its toxic or harmful effects. In order to gain this knowledge, it is necessary first to have some means of isolating, identifying, and quantifying the compound of interest. This is the tremendous challenge facing the modern-day analytical chemist.

There are a number of examples illustrating the need for metal speciation. Numerous studies attempting to correlate the toxicity of one form of chromium over another have been reported. Arsenic trioxide has been linked to lung cancer, whereas organoarsenicals have not. A number of studies have correlated the presence of chelating agent in the waterways with the toxicity and concentration of free metal ions present. Some examples of these studies include the toxicity of free zinc ions to algae, rather than the total amount of water soluble zinc, including the complexed form (1). Similarly free cadmium ions have been correlated to toxicity to grass shrimp (2). It has been found that some complexing agents actually increase the uptake of a metal (3, 4). Another area of great concern is that of specific aluminum species present in enviromental water, caused by the leaching of aluminum from ground clays by "acid rain" (5).

Not only are the challenges there to understand and
solve old problems, but the opportunities to help mankind through new and innovative chemistry, techniques, and understanding. This could be in the form of new catalysts for processes and helping solve our energy problems, design and manufacture of new pharmaceuticals and healthcare products for the treatment and rapid testing for diseases. The list is as infinite as man's imagination.

The challenge to the analytical chemist is to develop methods that can not only separate the specie of interest, but can quantitate it at the level that it is present in the environment or in physiological fluids.

This dissertation presents the results of the interfacing of two highly efficient technique for the speciation of a number of metal species. The two techniques were high performance liquid chromatography (HPLC) which was selected for its potential to separate a number of compounds; and atomic absorption spectroscopy (AAS) for its sensitivity and specificity for the metal of interest.

The broader scope of this work was to investigate some of the fundamental processes and problems involved in the instrumentation necessary for quantitation by atomic absorption. The results of this would have far reaching effects, not only in the ability to speciate, but in the potential of atomic absorption as an analytical method.

To illustrate the potential of the system designed,
three speciation projects were conducted. The first two
served to illustrate the potential of speciation in the
area of environmental and toxicology. These are the
speciation of a number of organol leads and
organomercurials. The third project illustrates the
potential that speciation presents in pharmaceutical
processing and drug design. There are broad implications
of the study to pharmaceutical and chemical processing,
as well as to the correct therapeutic treatment for
patients. After all, the purpose of chemistry is to help
mankind.

Part I, Chapter 1 deals with the development of a new
nebulizer-flame atomic absorption system. Chapter 2 is
the evaluation of this system as an analytical technique.

Part II, Chapter 1 is devoted to the development of
HPLC and TLC solvent systems for the speciation of
organolead compounds. These methods are used to study the
conversion of tetraethyllead (TEL) in seawater, and the-
decomposition of diethyllead dichloride. Based on the
results obtained some conclusions are stated that could
aid in elucidation of the impact that alkyl lead
compounds could have when released into the environment.
Part II, Chapter 2 deals with the speciation of a number
of mercury compounds by HPLC-AA. Chapter 3 deals with the
development of HPLC and TLC methods for the separation of
some transition metals and their complexes with a group of
antibiotics called aminoglycosides. The results of the
studies are significant in their implications to therapeutic use and to the chemical processing of these antibiotics.

1A. (b) Nebulization and Atomization Processes

This section examines some of the fundamental processes in atomic absorption spectroscopy that allows it to be a quantitative, analytical technique. After critical assessment of these processes, an approach was formulated to maximize the potential of interfacing high performance liquid chromatography to atomic absorption spectroscopy as a speciation technique.

There are two major processes in flame atomic absorption spectroscopy. The first is the formation from the sample of free atoms in the flame and the second is the absorption of radiation by these free atoms. Each of these steps is highly dependent on a number of variables, and interrelated processes. An understanding of these processes was essential for the improvement of atomic absorption spectroscopy as an analytical technique and its development as a method for speciation.

(1) Absorption of Radiation by Free Atoms

In atomic absorption spectroscopy, the amount of light that is absorbed by a free atom is given by Equation 1 (6):
Total Absorption = $\int_0^\infty K_v \, dv = \frac{\pi e^2 f N}{mc}$

$K_v \, dv$ is the total amount of light absorbed at frequency $v$

$e$ is the charge on the electron

$m$ is the mass of the electron

$c$ is the velocity of light

$f$ is the oscillator strength of the absorption line

$N$ is the number of free atoms which can absorb at frequency $v$

In this equation only $f$, the oscillator strength, and $N$, the number of free atoms in the light path, are variables; all other terms are constants.

The oscillator strength for an element is also a constant for a particular resonance line for that element.

Thus 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:
Total Absorption = (Constants) \times (Number of Free Atoms)

(2) Formation of Free Atoms

The number of free atoms produced in the flame is dependent on a number of factors and processes. The sample to be determined by flame atomic absorption spectroscopy is usually in a liquid. Therefore this discussion is oriented to the variables affecting the generation of free atoms in analysis region of the flame from the original liquid sample.

The sample element generally exists either in the ionic state in aqueous solutions, or in a molecular form in organic and some inorganic solutions. It is therefore necessary to convert the element of interest from its original ionic or molecular state to a free atomic state. This is the purpose of the flame.

The number or population of free atoms at any instant in the flame is a function of several dynamic processes. These processes can be summarized as
The rate of formation of free atoms depends on the concentration of the element in the original sample time the overall efficiency of atomization. Some free atoms may also be in an equilibrium with their ionic form in the flame, but as a rule this is only significant for easily ionized atoms such as sodium (1).

The rate of loss \((k_2, k_4, k_5)\) of free atoms depends on the chemical reactivities of the free atoms within in the environment of the flame. The absorption profiles vertically through a flame for two different elements, for example, magnesium and silver, serve to illustrate this. (Figure 1). This shows the relative abundance of free atoms versus flame height for magnesium and silver. At the base of the profile the free atoms start to form. The population reaches a maximum for the magnesium and then diminishes in the upper portion of the flame, where the population is controlled by \(k_5\). The population increases steadily up the flame for silver whose oxides are not easily formed in the flame. The oxides do form after the silver atoms have left the confines of the flame.
This further serves to illustrate that the development and control of conditions that minimize the loss of the free atoms are essential to producing accurate, and precise data.

(3) Factors Affecting Atomization Efficiency

The production of free atoms, i.e., the atomization efficiency, is crucial in atomic absorption spectroscopy since the number of free atoms sets the limits for the degree of absorption.

The overall efficiency of atomization is
dependent upon several different processes. It is generally defined as the ratio of the rate at which analyte (sample) passes through the cross section of the flame at the mean observation height as free atoms or ions to the rate at which it is aspirated (8).

The efficiency of nebulization will be discussed in great detail for a number of nebulizers in Chapter 2. However, the efficiency of nebulization is the ratio of the amount of analyte entering to the amount aspirated. Nebulizers produce aerosols which generally vary tremendously in droplet size and number (9, 10, 11). In atomic absorption spectroscopy only those droplets that are less than 10 micron in size can be effectively utilized by the other processes to yield atoms in their free state. In practice most commercial pneumatic concentric nebulizers are designed to give droplets with a medium value of 5 microns (9).

In the flame not all of the droplets may be desolvated. In fact, the large droplets (>10 microns) may traverse the flame without being fully desolvated because of the short residence time in the flame. The degree of desolvation also depends upon the solvent and the temperature of the flame. Therefore only a fraction of the sample may be desolvated.

Also not all of the sample that is desolvated may be broken into its constituent atoms, i.e., atomized. This depends on the chemical form that its in, such as
chloride, oxide, etc. The energy necessary for sample decomposition depends on the particular molecules present and the bond energies. This also depends upon the matrix that the sample is in. If the element of interest is entrained in a residue which does not permit efficient energy transfer, the amount of analyte volatilized will be diminished.

The understanding of the parameters which can affect the overall atomization efficiency is necessary before any improvements can be achieved. These factors are discussed as follows:

(i). Solvent

The solvent or analyte matrix affects a number of parameters. The sample is generally introduced into a flame as a fine aerosol. The solvent, whether aqueous or organic, affects the aerosol droplet size due to its surface tension.

These aerosol droplets evaporate at the base of the flame yielding a residue. This residue is then thermally decomposed by the flame, liberating the free atoms. The solvent directly affects the composition and combustion products of the flame. This can also affect the rate of loss of the free atoms.

If the sample is aqueous both the evaporation and the residue destruction step are endothermic, requiring significant amounts of energy from the flame. This can
directly affect the efficiency of sample decomposition and free atom generation.

If an organic solvent is used, the evaporation step can involve combustion of the solvent and the process is exothermic and rapid. The residue that remains is usually more combustionable, resulting in better flame atomization efficiency and thus yielding a greater number of free atoms.

These overall processes for nebulization/combustion/ and atomization is illustrated in Table 1.

Table 1: Solvent Combustion Mechanisms

<table>
<thead>
<tr>
<th>Nebulization/Combustion/Atomization</th>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aqueous solvent</strong> (endothermic, slow reaction)</td>
<td>Ions in water</td>
<td>Evaporation</td>
<td>Residue</td>
<td>Free Atoms</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hydrated</td>
<td>Dehydrated</td>
<td>Absorption</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Residue</td>
<td>Atoms Form</td>
<td></td>
</tr>
<tr>
<td><strong>Organic solvent</strong> (exothermic, fast reaction)</td>
<td>Metals, complexes in organic solvent</td>
<td>Burns</td>
<td>Organic</td>
<td>Free Atoms</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Addend</td>
<td>Absorption</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Burns</td>
<td></td>
</tr>
</tbody>
</table>


In ordinary pneumatic nebulizers the aspiration rate is affected by the solvent viscosity. Serious errors can result from mismatched sample and standards that are not delivered to the flame at the same mass transfer rate.

Improvement of the evaporation/desolvation step would significantly improve the atomization and thus the use of atomic absorption spectroscopy as an analytical technique for the determination of metals.

(ii) Temperature

The flame provides the energy necessary for the evaporation of the solvent and for the formation of free atoms. Generally, the hotter the flame, the better the atomization efficiency because of the increased ability of the flame to break the chemical bonds of the analyte into its free atomic form.

(iii) Flame Composition

As discussed previously the flame temperature has a direct affect on the rate of atomization of a sample. In addition to the temperature, the composition of the flame with respect to ratio of oxidant and fuel affects the atomization process. There are three types of flame: 1) stochiometric, in which the oxidant and fuel are in equivalent amounts; 2) oxidizing, in which there is an excess of oxidant; and 3) reducing, in which there is an excess of fuel.
The analyte and its chemical properties determine the best flame for its analysis. For example, some elements, such as aluminum, form oxides rapidly in the flame and thus a reducing flame is normally used for their analysis.

(iv) Chemical Form

The ease at which free atoms are formed depends to a large degree on the chemical form of the analyte in the flame, i.e., oxide, chloride, organic complex, etc. Some chemical forms are more stable than others and greater energy is necessary to break them up.
The efficiency of nebulization directly affects the number of free atoms since it is an integral part of the overall nebulization process. It represents though one factor that can be improved through the design of more efficient nebulizers and nebulization systems. Consequently a tremendous amount of research effort has been directed toward nebulizer design and improvement, and toward trying to improve nebulization efficiency. Any technique that would improve the nebulization efficiency would increase the sensitivity of atomic absorption spectroscopy.

The efficiency of nebulization is defined as the ratio of the amount of analyte entering the flame to the amount aspirated (9). Alternatively, a better description the overall total "transport efficiency" of the analyte from initial nebulization through the spray chamber to the flame (12).

A number of studies have been published in which the efficiency of nebulization were determined for flame systems (13, 14, 15, 16). Several models depicting aerosol generation for flame atomic absorption spectrophotometers generally divide the processes into at least three categories. One such model by Smith and Browner (17) is given. (Figure 2)
Figure 2. Aerosol Generation Model of Smith, Boorn and Browner.
Aerosol generation is generally broken down into three categories: primary, secondary, and tertiary. The first of the three categories is the primary aerosol generation step where the bulk liquid is initially scattered by pneumatic, ultrasonic, or other means into an aerosol. This usually results in a polydisperse droplet distribution. At this stage the droplet distribution can be considered as the "primary" distribution.

In many systems a "secondary" process of impaction, usually on impact beads or flow spoilers, occurs. For the impaction process the large droplets, on striking the impact surface, will generally adhere to the impaction surface and run to waste; small droplets will tend to follow the air stream around the surface without change; and intermediate droplets with sufficiently high velocity may shatter and generate additional small droplets. The impaction surface, therefore, acts to modify the size distribution by shifting it to smaller median diameter, and the aerosol in now in its secondary drop size distribution.

The "tertiary" stage involves those processes which modify the drop size distribution in the region beyond the impaction surface but prior to the flame. These processes are generally impaction, turbulent and centrifugal loss, gravitational settling, and evaporation. These processes generally shift the aerosol size distribution to smaller droplets. Impaction and settling processes result in the
preferential loss of the larger droplets. Evaporation from the walls, etc., produces small droplets.

The tertiary drop size distribution is altered by changing the physical configuration of the spray chamber, impact bead position, and incorporation of any paddles or baffles. In addition to the impact bead, the right angle at the end of the spray chamber can be another major site for impaction loss.

The auxiliary air flow incorporated into conventional atomic absorption spectrophotometers induces turbulent loss, which may be a major loss mechanism for the system. In addition, the paddle or baffle incorporated into several chamber designs, such as the Perkin-Elmer models, impacts a centrifugal motion to the aerosol which has the effect of removing larger droplets (17). Therefore, it is quite evident that the sensitivity of atomic absorption techniques is highly dependent on a number of parameters, including the design and type of nebulizer, gas flow patterns and aerodynamics, as well as the physical dimensions of the spray chamber.

1A.(d) Nebulizers Used for Atomic Spectroscopy

Characterization of the nebulization efficiency of various nebulizers and nebulization systems currently available for atomic absorption spectroscopy served as the base for determining the area with most potential for
improvement.

(1) Pneumatic Concentric Nebulizer

Most flame atomic absorption spectrophotometers use the pneumatic concentric nebulizer for sample nebulization and introduction. The reason for the dominance in the field appears to be due to several reasons. They are mechanically stable, corrosion resistant, difficult to block and easy to clean.

Also the flame typically utilizes air, or nitrous oxide as the oxidant. The oxidant flow rate needed to maintain the flame is normally much greater than the flow rate needed by the pneumatic concentric nebulizer to efficiently nebulize a significant proportion of the sample. Thus the pneumatic concentric nebulizer can make maximal use of the high gas flow rates necessary for the flame.

Pneumatic concentric nebulizer/spray chamber arrangements have been intensely investigated by both academic and industrial laboratories. Consequently better pneumatic nebulizers and their support systems developed as atomic absorption spectroscopy evolved.

Aerosol transport models for the Perkin-Elmer concentric pneumatic nebulizer/spray chamber have been proposed. Droplets produced by the Perkin-Elmer and Jarrell-Ash concentric adjustable flow nebulizers were characterized in regards to capillary position, analyte
1 Knurled Knob  6 Insert  11 O-ring
2 Washer  7 Nebulizer  12 Locking Ring
3 Needle Assembly  8 Venturi
4 Spring  9 Rear End Cap
5 O-ring  10 O-ring

Figure 3. Perkin-Elmer Pneumatic Concentric Nebulizer.
aspiration rate, nebulization gas flow rates, and chamber configurations (11). The design, performance, and aerosol produced by the Varian Techtron pneumatic concentric nebulizer was characterized (18). It was found that droplet number density and size distribution were correlated with the position of the impact bead, spray chamber conditioning, burner head temperature, nebulizer air pressure, and solution aspiration rate.

The transport efficiency for a typical Perkin-Elmer atomic absorption concentric pneumatic/spray chamber /burner head combination was 6.6+0.3% (12). The transport efficiency at different auxiliary gas flow rates and with either the paddle or impact bead yielded values from 3.3+0.2% to 14.4+7% (17) (Table 2).

Table 2. Influence of Auxiliary Gas Flow on Transport Efficiency

<table>
<thead>
<tr>
<th>Auxiliary Flow L/min</th>
<th>Nebulization Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>6.4+0.3</td>
</tr>
<tr>
<td>4.2</td>
<td>8.5+0.4</td>
</tr>
<tr>
<td>8.8</td>
<td>9.8+0.5</td>
</tr>
<tr>
<td>without</td>
<td>3.3+0.2</td>
</tr>
<tr>
<td>paddle</td>
<td>4.6+0.3</td>
</tr>
<tr>
<td>bead</td>
<td>4.4+0.2</td>
</tr>
</tbody>
</table>

Values indicated are +2 standard deviations
b spray chamber operated without impact bead or paddle in place
The improved transport efficiency with the use of the impact bead versus the flow spoiler resulted in approximately a two-fold improvement in sensitivity and detection limits for a number of elements (19) (Table 3). Commercial Perkin-Elmer atomic absorption spectrophotometers currently provide the flexibility of operation using either the flow spoiler (paddle) or impact bead. Use of the impact bead provided improved sensitivity and detection limits for most elements with an air/acetylene flame. However, for the analysis of very concentrated solutions, the flow spoiler was recommended to minimize memory effects and clogging. For operation with the nitrous oxide/acetylene flame, the flow spoiler was used to minimize flame "flutter" but gave poorer data precision. The flow spoiler was used with solutions with high levels of dissolved solids to minimize clogging of the burner head.

One study measured the transport of ground mesh-sized iron particles suspended in oil (20). For a Perkin-Elmer nebulizer and spray chamber with the flow spoiler removed, transport efficiencies of 7.8% for 3.0 μm, and 11.4% for 1.2 μm sized particles were found on collection.

Another extensive study of 20 different sample introduction systems and the factors influencing the transport of ground mesh-sized metallic particles suspended in lubricating oils through an atomic absorption spray chamber was highly dependent on the mesh size of
Table 3: Sensitivity and Detection Limits in Atomic Absorption for Flow Spoiler Versus Impact Bead

<table>
<thead>
<tr>
<th>Element</th>
<th>(nm)</th>
<th>Flow Spoiler</th>
<th>Impact Bead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>328.1</td>
<td>0.07</td>
<td>0.002</td>
</tr>
<tr>
<td>Ca</td>
<td>422.7</td>
<td>0.05</td>
<td>0.002</td>
</tr>
<tr>
<td>Cd</td>
<td>228.8</td>
<td>0.02</td>
<td>0.001</td>
</tr>
<tr>
<td>Co</td>
<td>240.7</td>
<td>0.17</td>
<td>0.01</td>
</tr>
<tr>
<td>Fe</td>
<td>248.3</td>
<td>0.07</td>
<td>0.004</td>
</tr>
<tr>
<td>Li</td>
<td>670.7</td>
<td>0.04</td>
<td>0.0008</td>
</tr>
<tr>
<td>Mg</td>
<td>252.8</td>
<td>0.004</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mn</td>
<td>279.5</td>
<td>0.06</td>
<td>0.002</td>
</tr>
<tr>
<td>Pb</td>
<td>283.3</td>
<td>0.50</td>
<td>0.03</td>
</tr>
<tr>
<td>Se</td>
<td>196.0</td>
<td>0.74</td>
<td>0.13</td>
</tr>
<tr>
<td>Zn</td>
<td>213.8</td>
<td>0.014</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Sensitivities and detection limits (mg/L) measured in an air/acetylene flame

Model 703 Perkin-Elmer atomic absorption spectrophotometer

Sensitivity values in mg/L producing a signal of 0.0044 absorbance

Detection Limits is that signal that can be detected at twice the noise level.
the particle (21). Transport efficiencies for the particles with 60 um diameters ranged from 1.2+0.2 to 22.1+2.4%, depending upon the system.

The transport efficiency for a number of other pneumatic concentric nebulizers-spray chamber arrangements for atomic fluorescence and atomic emission flame spectrometry gave values in the same range of 5 - 15% (Table 4). The studies utilized an air/acetylene flame with a capillary burner head, not a conventional slot burner head (22).

Table 4: Transport Efficiencies for Selected Pneumatic Concentric Nebulizers-Spray Chamber Arrangements

<table>
<thead>
<tr>
<th>Nebulization System</th>
<th>Impact Bead</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-E capillary burner</td>
<td>NA</td>
<td>8.5</td>
</tr>
<tr>
<td>Varian fixed flow nebulizer/ capillary burner</td>
<td>No</td>
<td>5.0</td>
</tr>
<tr>
<td>Varian fixed flow nebulizer/ capillary burner</td>
<td>Yes</td>
<td>10.1</td>
</tr>
<tr>
<td>Varian adjustable nebulizer/ capillary burner</td>
<td>No</td>
<td>8.4</td>
</tr>
<tr>
<td>Varian adjustable nebulizer/ capillary burner</td>
<td>Yes</td>
<td>13.0</td>
</tr>
<tr>
<td>Instrumentation Laboratories/ capillary burner</td>
<td>Yes</td>
<td>15.4</td>
</tr>
</tbody>
</table>

a NA, not applicable; Yes, impact bead optimized; No, impact bead not in position.
b Perkin-Elmer Model No. 0303-0299
d Varian Instrument Model No. 01-1000066-00
e Varian Instrument Model No. 01-1000067-00
f Instrumentation Laboratories Model No. 01-100066-00
Although the pneumatic concentric nebulizer is utilized almost exclusively in conventional atomic absorption spectroscopy for the aerosol generation, the overall objectives of this research project was to achieve greater sensitivity primarily through improvement of the nebulization system. Thus a number of other nebulization systems used in atomic spectroscopy, primarily inductively coupled plasma spectroscopy, were surveyed as potential aerosol generation systems. A comparison of each nebulizer type with their advantages, disadvantages, and nebulization efficiencies is given below.

(2) Babington Type Nebulizer

The Babington nebulizer (23) operates by flowing the sample over a hollow glass or metal sphere which has a small orifice in the side (Figure 4). Gas is passed through the orifice forming an aerosol at the gas-solution interface.

The unique advantage of this approach is the ability of the nebulizer to nebulize very viscous or high-solids content samples without clogging the orifice.

The Babington nebulizer has been used to a small extent in atomic absorption spectroscopy (24, 25). Measurements of nebulization efficiencies in this system was 1.2±0.2% for 0.02 inch orifice, and 4.8% for a 0.004 inch orifice (26).

The Babington nebulizer has been adapted readily to
Figure 4. Babington Type Nebulizer.
inductively coupled plasma spectroscopy for the analysis of natural waters (27). Modifications of the basic Babington design has included the incorporation of an impact bead for high performance liquid chromatographic-inductively coupled plasma of nucleotides (28) and of set-set liquid slot-gas orifice design for ICP (29).

Grooved Babington type nebulizers constructed of nickel or teflon coated have been used for slurry nebulization into inductively coupled plasmas or DC plasmas (30, 31).

Overall the Babington nebulizer has a low nebulization efficiency (1.2%) as compared to the pneumatic concentric nebulizer (6.6%). It utility comes from its low gas flow rates, which are essential if a plasma is to be maintained. The low nebulization efficiency of the Babington nebulizer is more than compensated for by the relatively high atomization efficiency of a plasma versus a flame.
(3) Cross-Flow Nebulizers

The cross-flow nebulizer, first described by Valente, consists of two glass capillary tubes mounted at a right angle with gas passing through one, and the sample solution passing through the other (Figure 5).

The cross-flow nebulizer has been utilized almost exclusively in ICP because of the stringent requirements on gas flow rates for ICP. Nebulizing gas flow velocities in excess of 500 m per second are required by pneumatic nebulizers for production of the small droplets required. To meet the requirements for nebulization, but low gas flows through the ICP necessitates that the orifices in the cross-flow capillaries be 0.1 mm or smaller (33).

Consequently, cross-flow nebulizers suffer from plugging of the sample capillary and even blockage of the gas capillary when high solids or concentrated solutions are aspirated (34).

Another disadvantage of the cross-flow nebulizers in general is that they are difficult to align (35). Recently, however, fixed-position cross-flow nebulizers have become available (34, 36, 37). The alignment in the fixed-position is one that will generally give good nebulization for routine aqueous samples, but results may suffer somewhat for the non-routine matrix sample.

A nebulization efficiency of 2.5 ±0.1% was obtained for a commercial model (26). Once again the low
nebulization efficiency of the cross-flow nebulizer versus the pneumatic concentric nebulizer is compassed for by the higher atomization efficiency of the plasma as an atomization and excitation source.

Figure 5. Cross-Flow Nebulizer.
(4) The Meinhard Concentric Nebulizer

The concentric nebulizer was designed for use in atomic spectrophotometers by Meinhard (38). The Meinhard concentric nebulizer consists of two glass capillaries tubes configured so that gas passing through the external tube produces a droplet mist from the sample passing through the inner glass tube (Figure 6). The concentric nebulizer requires that the sample solution pass through a small orifice on the order of 0.1 mm or less, in order to nebulize the sample.

![Figure 6. Meinhard Type Concentric Nebulizer](image)

A mathematical model for aerosol generation has been developed for nebulization systems employing concentric nebulizers (39). The model was used to calculate the cutoff diameter of the droplets, the droplet distribution of the aerosol, and the efficiency of the nebulization.
system. A maximal efficiency for the concentric nebulizer in this case was 4.25%.

A study of transport efficiencies utilizing ground mesh-sized metallic particles gave a value of a Meinhard concentric nebulizer of 8.9 +0.4% (26). However, a value obtained by both cascade impactor and filter collection procedures was 1.1 +0.1% for a Perkin-Elmer ICP Meinhard nebulizer/spray chamber/torch combination (12). These values were supported by additional studies by Ripson and de Galan with values of 1.0 - 1.4% (40, 41).

Concentric nebulizers have been widely used for droplet generation for inductively coupled plasma spectroscopy (42, 43).

However, there are some disadvantages for the concentric nebulizer as an aerosol generation device. The concentric nebulizer requires that the sample solution pass through a small orifice, on the order of 0.1 mm or less, in order to nebulize the sample. This has led to severe clogging and alteration of the droplet distribution pattern.

Concentric nebulizers have also suffered from memory effects due to their design. However, the Meinhard concentric nebulizer has been modified recently to minimize memory effects (44).

Recent articles (45, 46) have illustrated a potential problem in ICP that has not been adequately studied. The emission signal from an ICP was highly sensitive to very
slight changes in spray chamber pressures. Fluctuations in spray chamber pressures distort the plasma and consequently the signal. The pressure in a spray chamber is dependent on a number of parameters, but primarily gas flow rates. Therefore any constriction or alteration of the gas flow will affect not only the nebulization efficiency for that particular nebulizer, but will lead to changes in spray chamber pressures and signals.

A significant development in concentric nebulization designs was introduced recently in the form of a microconcentric nebulizer for direct liquid sample introduction into the plasma (47). The microconcentric nebulizer was shown to be applicable to flow injection analysis and for direct injection of high performance liquid chromatographic eluent into the plasma. However, because of the high linear velocity of the nebulizing gas through this design, a high fraction of the sample traverses the plasma untouched. The net result was that the sensitivity was not improved over conventional ICP. Nevertheless this represents a significant improvement over previous attempts at direct introduction of liquid samples in which severe plasma distortions and plasma blowouts were observed.
(5) Glass Frit Nebulizer

The glass frit nebulizer (48) is based upon the same principle as the Babington type nebulizer. The sample is allowed to flow across a fritted glass surface (Figure 7). The nebulizing gas is passed through the multitude of small holes in the glass disc.

The advantages of the glass frit nebulizer is that it was not prone to clogging. It was highly efficient, but only at low sample flow rates (< 70 ul/min.). The disadvantages were that the frit becomes quickly saturated with sample at higher sample flow rates. At this point it ceases to nebulize. It was also subject to severe memory effect and consequently signal drift.

Nebulization was highly dependent on gas flow rates through the frit. Nebulization increases with gas flow up to a point, but high gas flows the sample is blown away from the surface before nebulization could occur.

In comparison to the Babington type nebulizer, the glass frit nebulizer was found to be about 2.4 times as efficient, but only at very low sample flow rates.
Figure 7. Glass Frit Nebulizer.
(5) Ultrasonic Nebulizers

Although ultrasonic nebulizers have been used in atomic absorption spectroscopy, they have been used more extensively for the generation of aerosols for inductively coupled plasmas where they display a number of advantages.

First, the rate of aerosol generation does not depend on the carrier-gas flow, in contract to pneumatic, concentric, or cross-flow nebulizers. Thus the aerosol production rate and the carrier-gas flow rates may be varied independently to optimize the analytical performance of the system.

Second, ultrasonic nebulizers can produce aerosols of greater droplet density (49) and of more uniform particle size than pneumatic nebulizers (50).

Third, the mean size of the droplets produced by the ultrasonic nebulizer is frequency-dependent (51). Thus smaller droplets can be produced by increasing the ultrasonic frequency employed. The advantage is that smaller aerosol particles are more efficiently transported, and are more rapidly desolvated and atomized. This produces an increase in the atom population in the flame or plasma, and consequently, an increase in the analytical signal.

Sample introduction and delivery using ultrasonic nebulizers have been done in two general modes: continuous feed, or by discrete sampling (batch).

In the continuous mode, the analyte solution is
pumped continuously onto the transducer surface or transfer plate of the ultrasonic nebulizer. Continuous ultrasonic nebulizers are generally preferred for routine analysis because rapid sample interchange can be achieved and the cleanout time required to minimize sample memory effects is relatively short.

In the discrete or batch mode a known amount of sample is nebulized. The principle advantage of batch nebulization is the higher efficiency achieved due to the greater surface contact between the ultrasonic disks and the sample, and consequently a greater transfer of energy for nebulization.

The batch mode results in significant enhancement of analytical signal in case of limited sample size. The batch nebulizer however suffers from inconvenient sample changeover procedures, and memory effects unless the vessel is throughly cleaned.

Ultrasonic nebulizers have been used sparsely for aerosol generation for atomic absorption spectroscopy dispite findings of sensitivity enhancements on the order of 2 to 4 (52). This study employed a system in which the analyte stream was feed continuously by a pump onto the face of an ultrasonic disc.

No examples of batch sample introduction via ultrasonic nebulization were found. This is due to the available of the more efficient graphite furnace instrumentation designed for minute sample sizes.
In contrast to atomic absorption spectrophotometers, ultrasonic nebulizers have been used extensively in inductively coupled plasma spectrophotometers. A model is offered commercially on the Plasms-Therm ICP (53).

Improvements in detection limits of approximately tenfold over conventional pneumatic nebulization were achieved for seventeen elements in a dilute acid matrix using ultrasonic nebulization coupled with aerosol desolvation (49).

The Plasma-Therm model with aerosol desolvation was used for the simultaneous determination for iron, manganese, copper, zinc, and nickel in seawater (54). The procedure utilized Chelex 100 resin to preconcentrate the metals of interest and to remove the large amounts of calcium, magnesium, and sodium present in the seawater. The high content of calcium, magnesium and sodium had to be reduced to prevent salt buildup on the plasma torch and to minimize spectroscopic interferences.

Priority pollutant metals as designated by the Environmental Protection Agency were analyzed using a modified Plasma-Therm ultrasonic nebulizer with aerosol desolvation (55). Samples included drinking water and municipal sewage influent and effluent waters. Advantages included high sample throughput, wide dynamic range of operation, acceptable precision and accuracy, and simultaneous quantitative capabilities. Moreover, the ultrasonic nebulization technique did not require sample
preconcentration which can introduce a source of contamination.

Batch nebulization generally take two forms. The first is a "steady state" nebulization from the surface of a large sample volume. Batch nebulization of this type was used to study the feasibility of plasmas as spectroscopy methods for the analysis of metals (56).

Signals exhibiting good stability (deviation of less than 1%) have been achieved for a batch nebulization process (50).

The second type of batch nebulization is discrete. A system for the batch nebulization of a 300 ul size sample has been described (57). Due to the variable signal profile over the 60 second nebulization cycle, a 20 second integration period was employed.

A summary of ultrasonic nebulizers used in aerosol generation for atomic spectroscopic analysis is give in Table 5.
Table 5. Ultrasonic Nebulizers Used in Atomic Spectroscopy

<table>
<thead>
<tr>
<th>Type</th>
<th>Ultrasonic Frequency</th>
<th>Dect.</th>
<th>Comments</th>
<th>Sensitivity Enhancement</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous</td>
<td>1.4 MHz</td>
<td>AAS</td>
<td>Disc</td>
<td>2-4</td>
<td>52</td>
</tr>
<tr>
<td>Continuous</td>
<td>1.45 MHz</td>
<td>ICP</td>
<td>Disc</td>
<td>10</td>
<td>49</td>
</tr>
<tr>
<td>Continuous</td>
<td>1.4 MHz</td>
<td>ICP</td>
<td>Disc</td>
<td>9</td>
<td>53</td>
</tr>
<tr>
<td>Plasma-Therm</td>
<td>1.4 MHz</td>
<td>ICP</td>
<td>Desolv.</td>
<td>13</td>
<td>54</td>
</tr>
<tr>
<td>Plasma-Therm</td>
<td>1.4 MHz</td>
<td>ICP</td>
<td>Desolv.</td>
<td>13</td>
<td>55</td>
</tr>
<tr>
<td>Continuous</td>
<td>.83 MHz</td>
<td>ICP</td>
<td>Water-Membrane</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Batch</td>
<td>1.72 MHz</td>
<td>ICP</td>
<td>300 ul Sample</td>
<td>5</td>
<td>56</td>
</tr>
<tr>
<td>Batch</td>
<td>.87 MHz</td>
<td>ICP</td>
<td>Focusing</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Batch</td>
<td>.30 MHz</td>
<td>ICP</td>
<td>Water-Membrane</td>
<td>57</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity Enhancement: Degree of signal enhancement (X Times)

AAS: Atomic Absorption Spectroscopy
ICP: Inductively Coupled Plasma Spectroscopy
Disc: piezoelectric ultrasonic disc
Plasma-Therm: Commercial ultrasonic nebulizer by Plasma-Therm
Desolv.: Thermal desolvation employed
Focusing Lens: Ultrasonic discs aligned to focus waves
Water-membrane: Water used for transfer of ultrasonic waves to the metal membrane used as nebulization surface.
1A.(e) Summary of Nebulizers and Nebulization Processes

The survey of available nebulizers indicated that ultrasonic nebulization offered the greatest nebulization efficiency and the most potential for improvement, and incorporation into a technique for the speciation of metals. This was based on the facts that 1) the number and size of the droplets is dependent upon the frequency of the nebulizer, 2) nebulization is independent of the gas flow, and therefore the aerodynamics and flow rates can be varied and maximized, and 3) a number of parameters may be maximized in the design of the nebulizer.
1B. PROPOSED RESEARCH FOR THE DEVELOPMENT OF A NEW NEBULIZATION SYSTEM FOR FLAME ATOMIC ABSORPTION SPECTROSCOPY

1B. (a) Introduction

Several factors have been shown to affect the number of free atoms in the flame. The number of free atoms in the flame in turns sets the limits for the degree of absorption. Theoretically as few as $10^7$ atoms, or approximately $10^{-16}$ grams of an element should result in 1% absorption. In practice, about $10^{16}$ atoms, or $10^{-7}$ grams, typically gives 1% absorption. This difference represents potential for tremendous gains in sensitivity and detection in atomic absorption spectroscopy.

The need for greater sensitivity and better detection limits in atomic absorption spectroscopy actually spawned two other major techniques: graphite furnace atomic absorption, and plasma emission spectroscopy. Graphite furnace atomic absorption spectroscopy increases sensitivity through greater atomization efficiency. However, this is limited to extremely small sample size, generally less than 20 ul. Thus graphite furnace AA is not compatible to most liquid chromatographic techniques, including conventional high performance liquid chromatography.

Plasma emission spectroscopy, both inductively coupled (ICP) and directly coupled (DCP) plasma, increase
sensitivity through greater atomization due to the extremely high temperatures present in the plasma. Temperatures of 12,000 - 15,000 degrees K are routinely produced on most commercial plasma emission spectrophotometers.

A tremendous need exists though to increase the sensitivity of the conventional flame laboratory atomic absorption spectrophotometer. Reasons include 1) the easy of operation for most commercial atomic absorption spectrophotometers; 2) potential for interfacing with speciation techniques that typically operate at sample flow rates comparable to conventional AA's; and 3) cost.

1. Atomic absorption spectroscopy has gained wide acceptance because of its applicability to the detection and quantitation of a large number of elements. Consequently a great number of experimental procedures for different samples and sample matrixes have been developed, and the instrument developed for ease of operation.

2. Atomic absorption spectroscopy also has a tremendous potential for speciation analysis because of the sample flow rates of many liquid separation methods are very compatible to sample uptake by the AA.

3. The fourth advantage of atomic absorption spectroscopy is its relatively low cost. Conversely, the graphite furnace is added as accessory to an AA at a cost of approximately $20,000 (1984, Perkin-Elmer Corporation). Commercial plasma emission spectrophotometers are purchased
as separate instruments and cost in the range of $55,000 (1984, Instrumentation Laboratories Inc.) to $70,000 (1984, Perkin-Elmer Corporation).

1B.(b) Proposed Research Design

This research project sought to increase the sensitivity of atomic absorption by addressing the one fundamental process that offered the greatest potential: increasing the overall nebulization process. In atomic absorption spectroscopy the nebulization efficiency is only approximately 6% (94% of the sample lost!). Thus any technique that could significantly increase the nebulization efficiency would have the potential for a tremendous increase in sensitivity and analytical productivity of atomic absorption spectroscopy.

The survey of nebulizers showed that nebulizers of the ultrasonic type have a number of distinct advantages over the other nebulizers. These were 1) overall greater nebulization efficiency versus other types of nebulizers, 2) the number and size of the droplets can be controlled by the ultrasonic oscillator, 3) nebulization is not dependent on a nebulizing gas flow which allows for flexibility for another parameter in the design, and 4) a number of parameters, such as frequency, power, etc., can be designed into the nebulizer.

This section therefore presents:

1. the design and incorporation of a new, prototype
ultrasonic nebulizer into a conventional atomic absorption spectrophotometer.

2. the evaluation of a number of commercially available spray chambers for incorporation of the new ultrasonic nebulizer

3. an extensive study of the gas flow patterns and aerodynamics of the systems.

4. design of accessories and procedures for desolvation prior to the flame in order to increase the overall nebulization efficiency
1C. EXPERIMENTAL: CHEMICALS, EQUIPMENT AND PROCEDURES

1C. (a) Chemicals.
   Acetylene: Lincoln Big Three Inc.
   Copper metal: Fischer
   Lead metal: Fischer
   Nitrogen: Lincoln Big Three Inc.

1C. (b) Equipment
   (1) Atomic Absorption Spectrophotometer

   The atomic absorption spectrophotometer was a Perkin-Elmer Model 305B. This was a double-beam system with a single detector shared by both beams. The hollow cathode light source was modulated and the flame light was unmodulated.

   (i) Monochromator: high-dispersion Czerny-Turner system with ultraviolet dispersion about 0.65 nm per millimeter; visible dispersion about 1.3 nm per millimeter. Focal length was 400 millimeters. Ultraviolet grating ruled at 2800 lines/millimeter, blazed at 210.0 nm. Visible grating was ruled at 1440 lines/millimeter, blazed at 600.0 nm. Wavelength range was 188 to 900 nm. Wavelength as read on the Wavelength Counter was accurate ±1 nm. The counter read directly in nanometers when the Range Control knob read UV. When the Range Control knob was at VIS, the wavelength reading was multiplied by a
factor of two.

(ii) Slit Openings: 0.03, 0.1, 0.3, 1.0, 3.0, and 10.0 millimeters

(iii) Maximum Spectral Band Width: The minimum spectral band width in the ultraviolet was about 0.02 nm; in the visible it was about 0.04 nm.

(iv) Readout: Direct readout was available in absorbance (1X), with recorder scale expansion of 3X, 10X and 30X.

(v) Hollow Cathode Source Current: Adjustable from 0 to 50 ma. Both commercially sealed hollow cathode lamps and laboratory constructed lamps were operational on the Model 305B.

(vi) Hollow Cathode Lamps

1. Commercial Hollow Cathode Lamps: Perkin-Elmer Intensitron No. 303-6103 for copper analysis. Current was 30-35 ma. The 324.8 nm copper line was used.

The hollow cathode lamp used for each element will be given in the Experimental Section covering that particular study.

2. Demountable Hollow Cathode Lamp: Two demountable hollow cathode lamps (HCL) were constructed
according to specification as detailed previously (94).

Because the demountable hollow cathode lamp was smaller in diameter than the commercial sealed HCL, the instrument bracket that holds the HCL in the instrument had to be modified. Two half inch holes were drilled through the top of the bracket for accessibility to the lamp's electrode connections. Then spacers were placed and adjusted on the lamp bracket so that maximal optical alignment was achieved.

(vii) Electrical Connections for the Demountable Hollow Cathode Lamps

New electrical connections between the terminals of the demountable hollow cathode lamps and the power source in the Model 305B were made. The electrical leads were connected depending on the operating current needed for the lamp. Leads from the A terminal to each successive lead were as follows depending on the current required: terminal B, 5-10 ma; terminal D, 5-25 ma; terminal E, 5-40 ma; and terminal F, 10-45 ma.

(viii) Power Requirements: 105-125 volts. A new high-voltage transformer, L 201, Perkin-Elmer Part No. 997-3018 was installed prior to use. The major electronic circuits were checked prior to use by the Department of Chemistry Instrument Shop.
Optical System: The optical system is shown in Figure 8. A new grating change motor was installed prior to use. The grating change motor facilitates change to and from ultraviolet to visible modes. The optical system was inspected and cleaned prior to use by the Department of Chemistry Instrument Shop.

Figure 8. Optical System for Perkin-Elmer Atomic Absorption Spectrophotometer 305B.
Deuterium Background Corrector: designed for use in the Model 305B to remove the effect of unwanted absorption or light scattering. The continuum radiated by the arc replaced the ordinary reference beam of the double beam system, which was automatically shut off by a shutter when the arc was turned on. A motor driven variable attenuator, a graded neutral density filter, served to balance the reference beam energy radiated by the deuterium arc and the sample beam energy of the hollow cathode lamp. Since the unwanted background absorption was of equal intensity in the two beams, the effect on the signal was cancelled when the two beams were balanced. This left the sample beam absorption due to the element of interest as a corrected signal.

During the operation of the Background Correction Accessory, the normal double beam functioning of the spectrophotometer was automatically suspended. Therefore, prior to analysis the operator had to decide whether to use the Background Corrector or the ordinary double beam.

Energy Meter: indicates the energy level of the reference beam with the Phase Switch at NORMAL. For absorbance measurements the needle is kept in the green region of the meter with the use of the GAIN Control.

Auto Zero: when pressed, sets the Readout Meter to zero.
(xiii) Optional Features of the Perkin-Elmer Model 305B: These were not used during the course of these investigations but were available.

1. Filter: an order-separating filter used for analysis with wavelengths greater than 650 nm.

2. EM Chopper: an electronic chopper for flame emission work.

3. Emission: for flame emission work.

(xiii) Instrument Modifications: Brackets to allow vertical movement of the entire burner and mount assembly (Perkin-Elmer, part No. 303-0826) were installed. This installation necessitated removed of the flame shield, and the drilling of four holes in the body of the instrument. The positioning of the holes was determined by comparison to a Perkin-Elmer Model 403 available in the department.

Two sets of 12 inch brackets (Ace Hardware Store) with 1/2 inch slots spaced at 1 inch intervals were secured by sheet-metal screws to the instrument body. This allowed for the entire burner and mount assembly to be positioned over the entire 12 inch range.

A variable height adjustment assembly is also incorporated into each of the burner used for these investigations. These allowed for additional vertical adjustment from 2 to 3 inches, depending on the burner assembly used.
(2) Gas Regulation Systems

Several different gas flow systems were used during the course of these investigations, depending on the nebulizer and the spray chamber utilized. Each of these systems will be described in detail in the appropriate section.

Because the Perkin-Elmer gas control unit was not available with the unit, the gas flow was controlled by a series of regulators and values assembled by the author. The air supply source was the Department of Chemistry laboratory air supply (70-80 psig., variable). This was regulated to 35 psig. by a Beckman Regulator DB, Model 9220. The air was then split via a tee in the line into a primary air supply and an auxiliary air supply.

Rotameters were used to control both primary and auxiliary air flows. The primary air flow to the Perkin-Elmer nebulizer had an additional quick shutoff value. This value was used as a convenient "on - off" control which was useful during the nebulization design experiments. The value allowed the flow rate to be set at the rotameter and then for the system to shut off, and then to be conveniently returned to the same flow rate by opening the value.

The acetylene flow was regulated at the tank by a standard acetylene regulator to 12-13 psig. This was regulated further by the Beckman Regulator DB, Model 9220
to 10 psig. A rotameter was then placed in line to regulate the flow rate. A quick shutoff Nupro valve was installed in the line approximately 6 inches from the spray chamber inlet. This valve was incorporated as an additional safety feature to assure quick cutoff of the acetylene as close to the spray chamber as possible in case of a fire or flashback.

(3) Burner Heads: Varian Techtron AB-51, 4" x 0.020" slot for air/acetylene or air/hydrogen; Perkin-Elmer single-slot burner head No. 303-0418; and Perkin-Elmer three-slot burner head No. 303-0401.

(4) Spray Chambers: Varian Techtron Model AA-5, Part No. 5-133; Perkin-Elmer No. 303-0191 (plastic); and Perkin-Elmer No. 040-0146 (stainless steel).
(5) Autotransformers: Powerstat, Type 116, 10 ampere, 0-140 volt, The Superior Electric Co.;
Adjust-A-Volt, Type 500B, 10 ampere, 0-140 volt, Staco, Inc.

(6) Thermocouple Vacuum Gauge Meter: 0-1000 microns, Consolidated Vacuum Corporation.

(7) Thermocouple Vacuum Gauge: Varian, Model NCR531

(8) Dual Seal Vacuum Pump: Welch Scientific Company, Model 1402

(9) High Performance Liquid Chromatographs:
Laboratory Data Control, ConstaMetric III, variable flow 0.00-10.00 ml/min, 0-6000 psi; Waters Associates, Model 6000, 0.0-9.9 ml/min in 0.1 ml/min increments, 0-6000 psi.

(10) Miscellaneous Supplies and Equipment: Nichrome wire, 32/1000 inch, 20 gauge.
    Abestos insulation: 1 inch wide abestos strip, and 1/16 inch abestos twine to wrap and insulate spray chamber and extensions.
1D. DESIGN OF NEW ULTRASONIC NEBULIZER

A prototype aluminum ultrasonic device from Sono-Tek Corporation, Model STC-027-S with power supply, was investigated for use a new nebulizer for atomic absorption spectrophotometry. Several design modifications were made to the original Sono-Tek model during the course of the study. These modifications will be described in detail as well as the construction and design principles.

It was found during the course of the study that material composing the ultrasonic nebulization surface (aluminum) was subject to severe corrosion. The author worked closely with Sono-Tek Corporation in repair of the nebulizer and in coating it to impede additional corrosion.

As a result of these investigations the original prototype ultrasonic nebulizer was substantially redesigned and modified to meet the criteria necessary for use in atomic absorption spectroscopy.

1D.(a) Basic Design Concepts and Nebulizer Modifications

A number of new design concepts were used in the development of the nebulizer. These concepts incorporated several features to maximize the transfer of the ultrasonic energy needed for nebulization. The nebulizer was broken into two major sections: the front
and the rear assemblies (Figures 9, 10).

(1) Front and Rear Assembly Sections

The rear assembly section is referred to as double-dummy ultrasonic horn. In the initial construction, an actual double horn assembly was assembled and the resonant frequency measured. The front half-wavelength section which includes an amplification step was then designed to match the rear section. The final assembly combines the two into a single unit.

(2) Piezoelectric Discs

A pair of annular piezoelectric discs are sandwiched between the double-dummy ultrasonic horns of the rear section. The piezoelectric discs are available from Vernitron Corporation, Cleveland, Ohio. An electrode is positioned between the discs and serves as the terminal for ultrasonic oscillator.

The piezoelectric discs are securely clamped between flanged portions of the horn sections by a series of eight bolts. These bolts are electrically isolated from the electrode by insulating O-rings.

The piezoelectric discs are centered easily between the front and rear horns due to the axial openings in the discs and the electrode. O-rings are disposed around the outer periphery of the discs.

(3) Center Electrode
Figure 9. New Ultrasonic Nebulizer.
Figure 10. Ultrasonic Nebulizer Schematic.
The center electrode with serves as the terminal for the ultrasonic oscillator power supply is electrically isolated from the sample tube which runs through the center. Nylon or other insulating medium can be used to electrically isolate the electrode. This insulation extends into the openings of the piezoelectric discs and shields the sample tube from the inner surface of the discs.

(4) Sample Passage Tube

The rear section has an axially-extending bore passing through the back dummy horns, the piezoelectric discs, and extending to the nodal plane. The front section has an axially-extending, threaded bore which is connected to the analogous bore in the rear section. A threaded tube is passed through the front section and a sealing compound or joint compound is applied to the joint to insure that there is not leakage.

The attachment of the analyte sample passage tubes at the nodal plane has several advantages. First, this arrangement provides a means of securing both sections of the nebulizer prior to applying torque to the bolts. The second advantage is a reduction in vibration in the sample tube. Parameters such as uneven tightening of the bolts can result in inefficient coupling of the piezoelectric discs to the nebulizing horn, and consequently a decrease in the nebulizing efficiency.
(5) Decoupling Sleeve

A decoupling sleeve was incorporated into the front section. A decoupling sleeve composed of non-acoustically coupled aluminum was inserted in the front section such that its tip extends just inside of the face of the nebulizer. Teflon and other non-acoustically coupled material such as steel, copper, etc., may be used.

The decoupling sleeve was inserted so that it made just slight contact with the analyte sample passage tube. This avoided a force fit which could cause some distortion in the tube or pressure on the discs.

(6) Modifications to the Sample Passage Tube and the Decoupling Sleeve

Several additional modifications were made to the sample passage tube and the decoupling sleeve during the course of these investigations.

In the original design, the sample passage had an internal diameter of 3/32 inch. For routine atomic absorption analyses where homogenous solutions are nebulized, this design would be sufficient. However, for applications in which dead volume and memory effects are critical, or where discrete nebulization is desired, this size passage was not desirable.

(i) Teflon or Polyethylene Inserts

A simple method for improving sample delivery was to
insert a Teflon or polyethylene tubing having an outside diameter of 3/32 inch through the nebulizer sample passage. The Teflon or polyethylene tubing also protects the sample passage from corrosive liquids. The sample can be easily fed to the nebulizer by insertion of a hypodermic needle into the portion of the Teflon tubing extending from the rear of the nebulizer.

The Teflon tubing inserts also decreased the internal dead volume of the nebulizer which was important during the chromatographic portions of this research.

(ii) Flared Glass Tubing

A different modification to the sample delivery tube proved to be particularly suited to the delivery of low sample flow rates (0.10 to 1.0 ml/min) with the nebulizer in a horizontal position.

A 3/32 inch outer diameter soft glass tubing was heat-flared such that the diameter of the flare approached the inner diameter edge of the nebulizer tip (Figure 11). No contact between the flared portion of the glass tubing and the surface of the nebulizer tip should occur. Such contact was very deleterious to the performance of the nebulizer.

This modification offered its most significant advantages at low sample flow rates. The "wetting" property of the glass allowed for more even distribution of the sample to the face of the nebulizer.
The nebulizer was operated in a horizontal position for most of the studies due to physical limitations imposed by spray chamber designs.

For high sample flow rates (> 1.0 ml/min) with the nebulizer in a horizontal position, little difference was evident in the form or appearance of the nebulization cloud. At high sample flow rates, an even stream of sample was delivered across the face and nebulization from the face was uniform.

However, at low sample flow rates (< 1.0 ml/min), delivery was more in the form of individual drops. Low flow rates allow for the formation of large drops at the exit of the sample passage tube unless the surface tension, etc., was modified by some means. The "wetting" effect of the glass provided changed the drops to a thin film and dispersed the sample to the face of the nebulizer from which it was nebulized away.

Also in the horizontal position at low flow rates, single drops would initially form at the end of the sample passage and due to gravitational forces would run onto the lower section of the nebulizer face. This resulted in non-uniform distribution of the sample to the surface and preferential nebulization from the lower portion of the nebulizer.

The glass tubing also decreased the internal dead volume of the nebulizer which was important during the chromatographic portions of this research.
Figure 11. Glass Tube Insert for Ultrasonic Nebulizer.
1D. (b) Operating Conditions for Ultrasonic Nebulizer

The nebulizer was first connected to the oscillator power supply, with the power off. The HPLC was turned on, and the flow rate set. Sufficient time was allowed for the sample to reach the face of the nebulizer if there was delay volume in the nebulizer and/or the HPLC system.

The voltage was turned on to the nebulizer. The voltage was turned up (25 volt maximum). This was necessary for the initial nebulization of the liquid away from the nebulizer tip. The voltage was then adjusted downward until maximum analyte signal was obtained.

If the voltage was adjusted too low, nebulization ceased due to insufficient energy for the process to occur. In this case, the voltage was sufficiently high again for the initial nebulization burst, and then readjusted to the maximum.

To stop nebulization the voltage to the nebulizer was turned off. The HPLC pump was then stopped.
1E. PREPARATION OF STANDARD SOLUTIONS AND EQUIPMENT

1E. (a) Standard Solutions

(1) Copper Standard Solutions

Stock copper solution, 1000 ppm, was prepared according to the Perkin-Elmer standard procedure (66). Copper metal, 1.000 g, was dissolved in a minimum volume of 1:1 nitric acid, and diluted to 1 liter (volumetric flask) with 1% (v/v) nitric acid.

Appropriate dilutions of the 1000 ppm stock solution were made using volumetric flasks and de-ionized water.

(2) Lead Standard Solutions

Stock lead solution, 1000 ppm, was prepared according to the Perkin-Elmer standard procedure (66). Lead nitrate, 1.598 g, was dissolved in 1 liter (volumetric flask) with 1% nitric acid.

Appropriate dilutions of the 1000 ppm stock solution were made using volumetric flasks and de-ionized water.

1E. (b) High Performance Liquid Chromatographs Operating Conditions

(1) General Conditions

Two high performance liquid chromatographs were utilized during the nebulizer development segment simply as a means to deliver solution to the Perkin-Elmer concentric nebulizer and to the new ultrasonic nebulizer at a constant rate.
A detailed description of the chromatographic conditions and equipment used for the metal speciation studies will be given in the chapter covering the investigations.

Since no chromatographic columns were used during the nebulizer development studies, the pumps operated at essentially zero backpressure.

(2) Degassing

Solutions were not degassed prior to pumping. Degassing was not necessary because bubbles do not form in the detector chamber, i.e., the flame, as what sometimes happens with other conventional HPLC detectors (ultraviolet and refractive index). Also since the pumps were operating at room pressure, the formation of bubbles during the pumping process was unlikely.

Solutions were filtered through a 2 micron size stainless steel frit at the solvent flask.

The Laboratory Data Control ConstaMetric II was operable over the flow range of 0.0 to 10.00 ml/min. The Waters 6000 pump operated at flows from 0.0 to 9.9 ml/min, in 0.1 ml/min increments.

IE.(c) Hollow Cathode Preparation and Operating Conditions

(1) Copper: the hollow cathode was prepared by cleaning copper foil with dilute nitric acid, washing with de-ionized water, and fitting the metal foil into a
brass cup. A d.c. current of 20-25 mA was used. The power supply was the Model 305B. The analytical line was the 324.8 nm copper line.

A Perkin-Elmer Intensitron sealed hollow cathode lamp when used was operated with a d.c. current of 15-20 mA, in accordance with the operating current instructions imprinted on the lamp.

(2) Lead: the hollow cathode was made by melting lead metal into a brass cup and the cathode drilled (7/64 inch diameter). A d.c. current of 18-25 mA was used. The power supply was the Model 305B. The analytical line was the 283.3 nm lead line.

A Perkin-Elmer Intensitron No. 303-6039 sealed hollow cathode lamp when used was operated with a d.c. current of 10-12 mA, in accordance with the operating current instructions imprinted on the lamp.

1E.(d) Perkin-Elmer 305B Atomic Absorption Spectrophotometer

(1) Copper Analysis

The Model 305B was operated according to standard Perkin-Elmer procedures for copper (66).

Slit Setting was number 4 (0.7 nm).

The instrument was used in the absorbance mode, double beam, with continuous readout to the recorder.

1E.(e) Recorder
The recorder was calibrated to 10 mv full scale for 100% absorbance. The recorder was operated in 2.5, 5.0 and 10 mv. full scale.

Scale expansion of 3X, 10X, and 30X were available on the Perkin-Elmer Model 305B. Only the normal mode 1X, or the 3X was used.

IE.(f) Flame Conditions and Gasses for Atomic Absorption Measurements Using Concentric Nebulizer

(1) General Conditions

Flame conditions were those recommended in the Perkin-Elmer procedure manual for the analysis of the particular element.

The flame was lit using a flint striker because the unit did not have the original gas control unit with it. These Perkin-Elmer gas control unit has an interlock system with the atomic absorption unit. Without the P-E gas control unit, the autolight system of the AA unit could not be activated.

The gas control system was laboratory assembled by the author. Rotameter flow values had been calibrated previously versus a Perkin-Elmer gas control unit, Model No. 040-0139, that was available on a Perkin-Elmer atomic absorption spectrophotometer Model 403 that was available in another laboratory.

(2) Gas Conditions for Three Burner Heads/Perkin-Elmer
Concentric Nebulizer

Three different burner heads were used during the course of these studies. The gas flow rates are set for a particular flame desired for analysis. For example, a lean, blue oxidizing flame for copper and lead; and for a particular type of burner head employed.

(i) P-E Spray Chamber/Burner Heads

Two different burner heads were used with the Perkin-Elmer spray chambers. They were a 4-inch single slot (model 303-0418), and the three slot (model 303-0401) burner heads.

1. P-E Concentric Nebulizer/Single Slot Burner Head

Laboratory air supplied at 70-80 psig, regulated to 35 psig by Beckman DB regulator.

Nebulizing Air, Rotameter Setting: 8.0 (24 L/min)

Auxiliary Air, Rotameter Setting: 8.0 (24 L/min)

Acetylene regulated 12-13 at tank and to 10 psi by Beckman DB regulator.

Acetylene, Rotameter Setting: 3.5 (4.8 L/min)

2. P-E Concentric Nebulizer/Three Slot Burner Head

Laboratory air supplied at 70-80 psig, regulated to 35 psig by Beckman DB regulator.

Nebulizing Air, Rotameter Setting: 9.0 (28 L/min)

Auxiliary Air, Rotameter Setting: 9.0 (28 L/min)

Acetylene regulated at 12-13 psig at tank and to 10
psig by Beckman DB regulator.

Acetylene, Rotameter: 4.0 (5.5 L/min)

3. P-E Concentric Nebulizer/Single Slot Techtron Burner Head

Only the Varian Techtron Model AB51 was used with the Varian Techtron chamber. Burner heads could not be interchanged between different Perkin-Elmer models and the Varian Techtron due to different size burner necks.

(ii) P-E Concentric Nebulizer-Techtron AB-51 Burner Head

Laboratory air supplied at 70-80 psig, regulated to 35 psig by a Beckman DB regulator.

Nebulizing Air, Rotameter Setting: 8.0 (24 L/min)
Auxiliary Air, Rotameter Setting: 8.0 (24 L/min)
Acetylene regulated to 12-13 psig at tank and to 10 psig by Beckman DB regulator.

Acetylene, Rotameter Setting: 3.5 psig (4.8 L/min)

Additional fine adjustments, as recommended by the Perkin-Elmer procedure manual, were necessary during all atomic absorption measurements to obtain optimum sensitivity. A blank solution was aspirated and the instrument zeroed. A standard solution of copper was then aspirated and the fuel or oxidant flow was adjusted to obtain a maximum signal reading. The blank solution was used then re-adjusted to the instrument to zero.
(The flame and gas conditions for atomic absorption measurements with the new ultrasonic nebulizer will be described in detail in a later section. This format was chosen because of the extent of the design changes made to the burner chambers and the gas inlet systems for the final system.)

(After these design changes are presented in the following text, the flame and gas conditions for atomic absorption measurements with the new system will be described.)
INTERFACING OF THE HPLC TO A PERKIN-ELMER PNEUMATIC CONCENTRIC NEBULIZER

An interface from the HPLC to the Perkin-Elmer pneumatic concentric nebulizer was constructed by the author. A piece of polyethylene tubing, whose inside diameter matched the nebulizer inlet, was inserted through a piece of 1/16 O.D. stainless steel tubing. The inner diameter of the tubing matched the outside diameter of the polyethylene tubing.

The ends of the polyethylene tubing were then momentarily exposed to heat (a Burnsen burner flame). The heat caused the polyethylene to end to flare. The hot polyethylene was then pressed against a flat surface resulting in the polyethylene molding around the stainless steel tubing. On cooling, a water-proof seal between the polyethylene tubing and the stainless steel tubing formed.

Swagelok ferrules and caps were used to connect the stainless steel tubing to HPLC pump outlet or to the HPLC columns. (Figure 12)

The Perkin-Elmer pneumatic concentric nebulizer was a variable flow model. The nebulizer can be used in three modes during conventional AA work: aspiration (normal), non-aspiration (sample is neither aspirated nor is air blown out the inlet), or in backpressure mode (air is blown out the sample aspiration inlet).

For these studies the nebulizer was used in the
Figure 12. Interface of HPLC to Perkin-Elmer Pneumatic Concentric Nebulizer.
backpressure mode. The nebulizer flow adjustment knob was turned until a very slight stream of air came from the nebulizer inlet. This was determined by attaching a short piece of polyethylene tubing to the nebulizer inlet, and then placing the other end of the tubing into a beaker of water.

The backpressure mode was chosen following an evaluation of the signal versus operation mode. A 20 ppm Pb sample was introduced into the concentric nebulizer using the HPLC pump for constant delivery. The best signal was obtained when the nebulizer exerted a light backpressure (Figure 13). The HPLC pump had no difficulty overcoming the very slight backpressure used. The results were consistent with the observations of Koropchak and Coleman (60). However, these results are highly dependent on the specific design of the nebulizer, and even to the individual nebulizer used. A difference of 30% in nebulization efficiency between any two Perkin-Elmer nebulizers is within operational norms (61).

16. NEBULIZATION STUDIES

The primary objective of this research was the development of a nebulization system that would provide sufficient sensitivity, etc., for speciation of trace metals in various environmental and physiological samples.
Figure 13. Signal to Noise Ratio as a Function of Backpressure of Pneumatic Concentric Nebulizer.
The secondary objective was to try to incorporate the basic nebulization process or nebulizer into an existing atomic absorption spectrophotometer. The reasons were because 1) a commercial atomic absorption spectrophotometer was available to us, and 2) the finding would be more easily transferable to other laboratories.

Three commercially available spray chambers were also chosen for evaluation during these studies. They were chosen because of 1) their extensive use in existing atomic absorption spectrophotometers. Extensive nebulization efficiency/transport studies have been done for these chambers; thus they provided a baseline for some of the nebulization studies.

The use of these spray chambers allowed for 2) research to be done within the existing spacial constraints of the Perkin-Elmer 305B system.

1G. (a) Varian Techtron Spray Chamber/ABS1 Burner Head

The Techtron spray chamber had two distinct features: 1) a tapered chamber and 2) the auxiliary air and acetylene enter from towards the rear along the sides, and must consequently turn 180 degrees to get to the flame. The gas flow schematic is shown in Figure 14. The chamber with the concentric nebulizer in place is illustrated in Figures 15 and 16.
Figure 14. Gas Flow Schematic for Techtron Spray Chamber with Pneumatic Concentric Nebulizer.
Figure 15. Front and Top Views of Techtron Spray Chamber with Pneumatic Concentric Nebulizer.
Figure 16. Side View of Techtron Spray Chamber with Pneumatic Concentric Nebulizer.
(1) Incorporation of Ultrasonic Nebulizer

The gas flow schematic for the ultrasonic nebulizer with the Techtron chamber is shown (Figure 17). The air and acetylene entered through the two rear gas entrances.

(i) Tip Insertion into Chamber

The inlet hole for the concentric nebulizer was enlarged to 0.340 inch such that the tip of the ultrasonic nebulizer protruded just inside of the chamber. (Figure 18)

Deionized water was pumped at a rate of 1.0 ml/min to the nebulizer.

Gas Flow Studies: Despite generating a finely dispersed while free-standing in the laboratory, essential no aerosol was evident as emerging from either the burner head slot, or from the burner chamber neck.

With the burner head removed and no gasses flowing, a fine aerosol could be seen at the base of the burner chamber neck. However, as soon as any gas (either acetylene or air) was introduced into the chamber, no aerosol was visible at the top of the burner chamber neck.

It was apparent that the gasses, even a very low flow rates, were interfering with the nebulization processes occurring on the face of the nebulizer.
Figure 17. Gas Flow Schematic for Techtron Spray Chamber with Ultrasonic Nebulizer.
Figure 18. Techtron Spray Chamber with Ultrasonic Nebulizer in Tip Insertion Configuration.
(ii) Front Section Insertion into Chamber

The teflon nebulizer holder was then drilled out to 0.625 inch. This allowed the tip of the nebulizer to protrude into the center of the chamber and the flange portion of the of the nebulizer to be flush against the back of the spray chamber. (Figure 19)

The nebulizer was turned on and water was delivered at a rate of 1.0 ml/min.

At zero gas flow a fine aerosol could be seen at the base of the burner neck.

As the air flow was slowly increased, a portion the aerosol at the base of the burner neck could be seen moving vertically up the passage.

As the air flow rate was increased to that typically encountered during routine operation of an AA (up to 48 L/min), the aerosol ceased to be fine and any liquid emerging from the neck was as large droplets. It was quite evident that the total amount of sample existing the neck was also decreasing as the air flow rate increased.

The was evident that the nebulization was being affected severely by the increased air flow.
Figure 19. Techtron Spary Chamber with Ultrasonic Nebulizer in Front Section Insertion Configuration.
(iii) Entire Nebulizer Insertion into Chamber

1. Chamber Modifications

The Varian Techtron chamber was modified to allow the entire nebulizer assembly to be inserted into the chamber up to a point where the nebulizer tip was directly under the burner chamber neck.

The spray chamber was machined by the author to an inner diameter of 1.125 inches by 1.60 inches deep (Figure 20). The existing solvent drain was plugged using a teflon septum. The was done to minimize air or acetylene leaks in the system.

A new solvent drain was added by drilling a new 3/8 inch hole into the bottom of the chamber and then a 3/8 Swagelok fitting was inserted. Because the hole was smaller than the outside diameter of the fitting, an air-tight seal was achieved.

2. Nebulizer Insertion

To assure an air-tight rear seal around the nebulizer, a teflon seal was machined by the author. The seal was 1.125 inch o.d., 0.875 inch i.d., and 0.125 inch thick. The teflon seal provided seated snug against the flange on the nebulizer. The seal prevented gasses from escaping around the nebulizer, and sample from coming into contact with the flanges holding the piezoelectric discs (Figure 21).

The nebulizer was aligned in the chamber such that the tip in line with the inner parameter of the spray
Figure 20. Techtron Spray Chamber Modified for Entire Insertion of Ultrasonic Nebulizer.
Figure 21. Techtron Spray Chamber with Ultrasonic Nebulizer Tip Positioned Directly Beneath Burner Head.
chamber neck.

This position was chosen to allow for maximum sample pickup by the gasses just prior to entering the burner chamber neck. Thus, as any sample was nebulized, the aerosol would be picked up and transported up the burner neck (to the flame).

A sample flow of 1.0 ml/min was delivered to the nebulizer.

1. Zero gas flow rate

At zero gas flow rate the sample was observed to nebulize and then to coalesce onto the inner walls of the spray chamber. This was due to the short distance between the tip of the nebulizer and the walls.

2. Increasing gas flow rates

At low gas flow rates (< 5 L/min) only a fraction of the aerosol was observed to be transported up the burner chamber neck. As the sample was nebulized, the aerosol was being picked up and swept along by the gasses.

As the gas flow rate was increased to that typically encountered in routine operation of an AA (up to 48 L/min), less and less aerosol was being transported through the burner chamber. Some aerosol was still visible even at the higher gas flows but it was not a fine, uniform mist, but was non-uniform and contained a significant number of large droplets.

The gasses had to make a 90 degree turn at the end of the chamber, just past the tip of the nebulizer. Therefore
the droplets entrained in the gas also had to make the 90 degree turn.

The 90 degree turn at the end of the chamber proved to be a major source for the loss of aerosol droplets. The turn provided for an area for impaction of the droplets; and also for turbulent mixing of the gasses and consequently for droplet collisions.

Therefore, a new design for the introduction of the gasses into the chamber was sought.
(iv) Nebulizer with Cross-flow Gas Introduction

1. Chamber Modifications

A 5/8 inch hole was drilled by the author directly beneath the burn chamber neck. The center connector on a 5/8 inch Swagelok tee was machined to 0.250 inch. The Swagelok tee was then inserted into the hole in the bottom of the chamber. This configuration provided for a direct path between the gas outlet of the tee and the burner head directly above it. The gas flow schematic for this configuration is shown (Figure 22).

The burner chamber neck was enlarged to 0.625 inch i.d. This was to provide for greater burner chamber volume for greater desolvation, and a greater mean free path available for the sample.

2. Gas Flow Studies

The nebulizer was positioned so that the tip was just at the inner parameter of the burner chamber neck (Figures 23, 24).

The sample was delivered to the nebulizer at 1.0 ml/min.

a. Low flow rates: At low flow rates (< 5 L/min), it was observed that the aerosol was being picked up by the gas flowing past the tip. There was some loss of sample to the walls primarily in the areas immediately adjacent to the nebulizer.

b. High flow rates: At gas flow rates (up to 48 L/min) typical of those encountered during flame
Figure 22. Gas Flow Schematic for Techtron Spray Chamber with Gasses in Cross-Flow Configuration.
Figure 23. Techtron Spray Chamber with Ultrasonic Nebulizer in Cross-Flow Gas Configuration.
Figure 24. Top View of Chamber-Nebulizer in Cross-Flow Gas Configuration.
conditions, the sample was rapidly entrained by the gasses flowing past.

However, it was observed that the aerosol emerging from the tip of the burner chamber neck was not evenly dispersed or uniform. There were a numerous large sample or aerosol droplets. These were thought to be due to either the gas interfering with the generation of the aerosol at the nebulizer tip and/or through coalescing of smaller droplets in the turbulent airstream.

Attempts were made to smooth the turbulent flow by insertion of a "buffering" material into the gas outlet. Two methods were attempted.

Glass frit: a glass frit fashioned from a piece of medium porosity sintered glass was inserted and secured with epoxy glue into the gas outlet opening.

This failed to produce a more uniform flow. The glass frit became "water-logged" rapidly due to the sample runoff from the walls of the chamber. Consequently there was unevenly dispersal of gas going through the frit, and also sputtering of sample droplets from the frit. The behavior was thus analogous to that reported earlier for the glass frit nebulizer.

(3) Glass wool: insertion of a plug of glass wool into the gas outlet also was unsuccessful. The glass wool rapidly became saturated with sample runoff from the walls.

There also was not a means to easily secure the glass
wool in the outlet, and consequently was dislodged at high gas flows.

(5) Rear and Tangential Flow Studies

Further attempts to improve sample transport included the introduction of an auxiliary gas, nitrogen, at the rear of the chamber via the original gas outlet. Gas tubing, 0.12 inch o.d., was cut and/or bent by the author into various shapes (Figure 25).

![Figure 25. Glass Bends Used in Tangential Flow Studies](image)

The nitrogen flow was regulated via a pressure control valve at the tank, and a Nupro fine metering valve in the line going to the chamber. A tee was placed in the line and the nitrogen gas flow split to each of the two original gas inlets.

The glass tubings were inserted into the gas inlets inside of the chamber and positioned so that the gasses entered the chamber next to the teflon seal at the rear of the chamber.
Straight and curved glass configurations were evaluated. The straight glass pieces allowed for the auxiliary gas to enter at about 1/4 inch in front of the nebulizer and teflon seal.

a. Straight glass inserts

No improvements in sample transport was evidenced with the straight segments. This was not surprising since this gas inlet configuration was analogous to the original gas flow pattern.

b. Curved glass inserts

The curved portion of the glass tubing was one-quarter circumference. This was selected because there were two outlets; and the two glass inserts were aligned with the curvature of the chamber. Thus the glass tubing did not restrict each other.

In addition to the quarter circumference of the tubing, slight spirals (angles for 10 to 20 degrees) were added to several sets. This allowed for the additional of a slight spiral to be added to the tangential flow.

Gas flow results: With the addition of the auxiliary gas in a tangential or spiral, a significant improvement was observed in the aerosol pattern emerging from the burner chamber neck.

The tangential flow from the rear is believed to have imparted a slight tangential flow upon the overall general gas flow pattern. The net result was that the sample aerosol appeared to be swept away more from the walls of
spray chamber. Consequently there was less loss of aerosol to the walls of the chamber.

(6) Atomic Absorption Analyses

Satisfactory results by atomic absorption spectroscopy using this arrangement were not achieved. Severe clogging of the burner head slot by aerosol droplets occurred under actual atomic absorption experimental conditions.

Only one Varian Techtron burner head was available for evaluation. This was a single-slot Model A851. This spray chamber and burner head had been used previously for conventional atomic absorption analysis utilizing the Perkin-Elmer pneumatic concentric nebulizer with very satisfactory results.

However, with the ultrasonic nebulizer, the Varian Techtron single-slot burner head was not able to handle the high mass transfer of sample through the thin, narrow (0.040 inch) slot. If the flame was already lighted and then the nebulizer was turned on, the flame would often be lifted entirely off the head of the burner. On the other hand, when the flame was lighted and the nebulizer was nebulizing efficiency, if the nebulizer was turned off, the flame would flashback. This was due to the requirements that a burner head must have a high linear gas velocity across the slot to prevent flashback, and the slot must also be able to handle the mass transfer.
Single-slot burners, such as the Model AB51, are not designed to handle the high mass transfer produced by a highly efficient nebulizer. (Normally only about 5\% of the sample reaches the burner slot, instead of close to 100\%. This is a 20 fold mass difference.)

Similiar results were observed during desolvation studies in atomic absorption utilizing an infrared radiation-heated spray chamber and for a 3-slot burner utilized to handle sample desolvated by resistively heated coils inside the burner chamber (65).

It was also observed that a portion of the aerosol rapidly condensed in the slot even with the flame on. This was accentuated due to the presence of large droplets which rapidly clogged the thin slot and provided a surface for the condensation of smaller droplets. A flame suitable for consistant atomic absorption sensitivity results, etc., was never achieved.
(v) Summary of Results for the Incorporation of the Ultra sonic Ne bulizer into the Varian Techtron Spray Chamber Designs

Although the Varian Techtron spray chamber arrangements did not allow for complete atomic absorption results with the new nebulizer, there were a number of important findings:

1. The gas flow patterns and aerodynamics of the spray chamber were the most crucial factors determining the throughput of the sample.

2. Tangential or spiral gas flows improved sample transport by minimizing loss to walls.

3. Turbulent gas flow patterns were detrimental to aerosol transport.

4. Positioning of the nebulizer with respect to possible aerosol impaction surfaces was important to the degree of aerosol loss.

5. A burner head with high linear velocity to prevent flashbacks and capable of high mass transfer transport across the slot(s) will be required for additional studies.
1G. (b) Perkin-Elmer Spray Chamber Studies (No. 303-0191)

The Perkin-Elmer spray chamber model No. 303-0191 was a plastic model used in the early Perkin-Elmer atomic absorption models 290, 303, and early 305A and 305B models. The main spray chamber body was cylindrical with a burner chamber neck at 90 degrees to the main body. The concentric nebulizer was centered in the rear cap assembly. Auxiliary air, and the acetylene entered in the rear cap assembly (Figure 26).

(1) Incorporation of Ultrasonic Nebulizer into Assembly

Several different configurations incorporating the new ultrasonic nebulizer were evaluated for maximization of the aerosol transport.

(i) Tip Insertion into Chamber

1. Spray Chamber Modifications

The inlet hole for the concentric nebulizer in the rear cap assembly (part no. P-E 303-0192) was modified by the author to permit intrusion of the tip of the ultrasonic nebulizer to just inside of the chamber (Figure 27). The inlet hole was enlarged to 0.340 inch for tip insertion, and the rear cap assembly drilled out to 0.625 inch i.d. and 0.12 inch deep to accommodate the step section of the horn.

Sections of teflon septums were cut to fit the slot
Figure 26. Gas Flow Schematic for Perkin-Elmer 303-0191 Spray Chamber with Pneumatic Concentric Nebulizer.
Figure 27. P-E Spray Chamber 303-0191 with Ultrasonic Nebulizer in Tip Insertion Configuration.
in the side of the rear cap assembly (slot for the arm of the concentric nebulizer), and glued into place. This was to prevent gas leaks from the assembly.

The gas flow schematic for experiments with the ultrasonic nebulizer is shown (Figure 28).

(ii) Rear Insertion into Chamber

1. Spray Chamber Modifications

The inlet hole for the concentric nebulizer in the rear cap assembly (part no. P-E 303-0191) was modified by the author to permit the entire tip of the ultrasonic nebulizer to be inserted into the chamber (Figure 29). The inlet hole was enlarged to 0.625 inch i.d.. The ultrasonic nebulizer was then inserted until the flange was snug against the spray chamber body.

2. Atomic Absorption Measurements for Both Tip and Rear Chamber Insertions

Using the Perkin-Elmer pneumatic concentric nebulizer, the gas flows, burner height, etc., were determined to give optimum signal for a 1 ppm copper solution.

The ultrasonic nebulizer was then inserted into the chamber, either in the tip or the rear insertion mode as described above. The solution of 1 ppm copper was then delivered to the nebulizer at the flow rate of 1.0 ml/min. Flame conditions, gas flow rates were duplicated as nearly as possible to those previously determined using the
Figure 28. Gas Flow Schematic for P-E 303-0191 Spray Chamber with Ultrasonic Nebulizer.
Figure 29. P-E Chamber 303-0191 with Ultrasonic Nebulizer in Front Section Insertion Configuration.
pneumatic nebulizer.

Only a very weak signal was observed. Attempts at improving the signal were not successful.
(iii) Rear Tip Insertion with Cross-Flow Gas Flow

1. Chamber Modifications

A 1/4 inch Swagelok tee was inserted directly beneath the spray chamber neck and sealing compound applied around the exposed threads (Figure 30). Both acetylene and air supply lines were attached to the tee. Auxiliary air still was still supplied through the rear cap assembly (Figure 31).

2. Atomic Absorption Measurements

Optimum conditions were again set for the atomic absorption measurements using a 1.00 ppm copper solution.

The ultrasonic nebulizer was inserted into the chamber and flame conditions reproduced as close as possible to those previously obtained. However, only a weak signal was observed.

3. Observations

With zero gas flow: The aerosol produced by the nebulizer appeared to drift several inches down the chamber and then a significant portion would coalesce on the walls of the chamber.

With increasing gas flow: As the auxiliary gas flow was increased from 0 to 24 L/ min (rotameter setting: 8) the aerosol was entrained and coalesced on the chamber wall were the gas struck.
Figure 30. P-E Spray Chamber with Ultrasonic Nebulizer in Cross-Flow Gas Configuration.
Figure 31. Gas Flow Schematic for P-E Spray Chamber with Gasses in Cross-Flow Configuration.
(iv) A Separated Gas Flow Design

A separated flow design was evaluated in which the nebulizer tip was physically shielded from the auxiliary gas flow. A secondary gas flow entering at the rear of the nebulizer was incorporated (Figure 32).

1. Spray Chamber Modifications

The nebulizer inlet in the rear cap assembly was enlarged by the author to 0.625 inch (diameter of nebulizer horn). The inner side of the cap was then redrilled to 0.750 inch by 0.20 inch deep to accommodate glass tube inserts (Figures 33).

The auxiliary gas inlet was plugged on the inner chamber side with teflon plug. A 3/32 inch hole was then drilled between the auxiliary gas supply inlet and the inner glass insert hole (0.750 inch section).

Glass inserts were cut to fit into the gas port. The glass inserts were 0.750 inch o.d., 0.650 inch i.d. Lengths of 1.00, 1.25, 1.50, 2.60, 3.70, and 4.00 inches were cut.

Glass bents were fabricated by the author as to help direct the air from the rear gas port to move more in a tangential fashion within the chamber. Glass tubing, 0.250 inch o.d., 0.150 inch i.d., was bent by the author by heating it in a Burner burner flame and then shaping to the desired configuration. Bends were made such as to lay along the circumference of the wall. The glass bend lay on approximately a one-quarter turn of the chamber.
Figure 32. Gas Flow Schematic for P-E Spray Chamber 303-0191 with Separated Gas Flow Design.
Figure 33. P-E Spray Chamber 303-0191 with Ultrasonic Nebulizer with Separated Gas Flow Configuration.
2. Purpose

The glass inserts were more to shield the tip of the nebulizer from deleterious gas flows within the chamber. The auxiliary gas flow was to provide some gas flow to move the aerosol away from the tip, and down the length of the inner glass tubing. While the normal air flow was to provide more of a tangential flow pattern within the chamber. The design was an attempt to provide more of a controlled gas flow pattern within the chamber.

3. Observations

This design concept was only partially successful. A true tangential gas flow pattern was not generated by the glass bends. This was apparent by the flow of the aerosol within the chamber during the nebulization studies of water at a flow rate of 1.0 ml/min, and air flow rates varying from 0 to 24 L/min.

One could observe the aerosol being swept up by the gas and then coalescing on the walls of the chamber.

Only the 1.00 and 1.25 inch sections of glass insert allowed the aerosol to be generated and to drift into the chamber. The aerosol would condense inside the longer glass inserts before it could drift out into the chamber.

4. Additional Observations Utilizing a Glass Chamber Body

To better visualize these phenomena, a glass spray chamber body was employed. A glass cylinder with approximately the same dimensions (1.50 inch o.d., 1.380
inch i.d., and 4.10 inches in length) was substituted for the spray chamber body.

The aerodynamical patterns of the aerosol were then clearly observable through the glass. Patterns of flow which were not evident previously were readily apparent as evidenced by the coalescence of the aerosol in different patterns along the glass walls.

Only a small portion of the original aerosol was being transported down the chamber. Most of the aerosol was being directed to the walls within two to three inches of the nebulizer tip. On contact with the walls, the small aerosol droplets were being removed from the nebulization transport system by coalescing into larger drops.

(iv) Summary of Results for Nebulizer Insertion via the Rear Chamber Cap

The transport of the aerosol through the burner chamber for the nebulizer in the rear chamber cap position was not efficient. A significant portion of the aerosol was being lost at the walls with the coalescing of the smaller droplets into larger.

The transport efficiency was highly dependent on the gas flow patterns within the chamber. Attempts to modify and to control the patterns were only partially successful.
(2) Nebulizer Insertion via the Burner Chamber Neck.

In an attempt to increase significantly the total sample throughput, the tip of the nebulizer was inserted into the gas stream directly below the burner head.

(i) Chamber Modifications

A 3/8 inch hole was drilled into the side of the burner chamber neck 1.95 inches from the opening for the burner head. A 1/4 inch long segment of a 3/8 inch plastic Swagelok connector was drilled to 0.340 inch i.d. This piece was then inserted into the hole in the side of the burner chamber neck. This was to serve as a port for the insertion of the tip of the nebulizer into the burner chamber neck.

An O-ring, 0.220 inch i.d. x 0.340 inch o.d., was placed over the tip of the nebulizer. The nebulizer with O-ring was inserted into the port such that the tip of the nebulizer was immediately inside the neck. The O-ring was positioned as a seal to prevent the air/acetylene mixture from inside the chamber from escaping.

(ii) Nebulization Studies

Water was nebulized at a flow rate of 1.0 ml/min from the tip of the nebulizer directly into the gas stream going to the burner head.

The aerosol was observed over a gas flow range of zero to that encountered during the routine operation of an atomic absorption spectrophotometer (28 L/min air).

As the gas flow rate increased the aerosol changed
from a uniform, finely dispersed aerosol cloud to non-uniform pattern. At the high air flow rates large droplets were observed being blown off the tip of the nebulizer.

The nebulization processes taking place on the surface of the nebulizer were obviously being perturbed by the gases flowing by the surface.

(iii) Attempted Atomic Absorption Measurement

Atomic absorption measurements for a 1.00 ppm copper solution at a delivery rate of 1.0 ml/min were attempted. Analysis line was 324.8 nm. Air flow rate: 38 L/min; Acetylene flow rate: 3.5 L/min.

Only one attempt was made using this arrangement and the Perkin-Elmer chamber No. 303-0191.

Immediately following the ignition of the flame, a flashback of the flame into the chamber occurred. There was a subsequent explosion which cracked the burner chamber into several pieces.

The cause of the flashback centered on the O-ring seal around the nebulizer. Immediately upon flashback and prior to the explosion a small flame was evident around the O-ring. This would indicate that the O-ring/chamber port/nebulizer section was not sufficiently sealed. Therefore the gas flow rate across the burner head was too low and there was a resulting flashback.

It was therefore decided to not use the other model 303-0191 available in the laboratory due to the potential
of another explosion.
The Perkin-Elmer spray chamber, model 0040-0144, has several distinct features. It was a stainless steel assembly with a tapered body incorporating a set of flow-spoilers. There was a pressure relief assembly in case of flame flashbacks located directly beneath the spray chamber neck. The chamber body was also coated with an corrosion and acid resistant polymeric compound.

This chamber offered increased safety because of the stainless steel construction and flashback relief assembly.

(i) Spray Chamber Modifications

It was decided that only two modes of sample introduction would be tried with the P-E model 0040-0144 chamber because of information learned from the Varian and P-E model 303-0191 chambers. The two modes would be by insertion through the rear spray chamber cap (part No. 0040-1716), and by insertion through the spray chamber neck directly beneath the burner.

1. Rear Spray Chamber Cap Insertion

Insertion through the rear chamber cap (part No. 0040-1716) was evaluated to determine whether there was any difference due to the this particular spray chamber design. The P-E model 303-0192 had a tapered chamber and a obviously different internal volume, etc., from the two other chambers.
Rear spray chamber cap insertion in the two other chambers had been shown not to be an effective position for nebulization via the ultrasonic nebulizer. However, because aerodynamics and gas flow patterns had been shown to be so critical in aerosol transport, the author wanted to evaluate any differences that might occur due to the new chamber.

A 0.625 inch hole was drilled by the author through the rear cap assembly using the existing concentric nebulizer hole as the center template.

The slot in the rear cap for the concentric nebulizer arm was plugged with pieces of teflon cut from gas chromatographic septums and glued into place.

(ii) Tip and Rear Insertion of Ultrasonic Nebulizer into Chamber

Both tip (Figure 34) and front section insertion (Figure 35) modes were evaluated.

Aerosol transport was studied by observing the aerosol patterns generated by nebulizing water at a rate of 1.0 ml/min.

1. Gas Flow Studies

At zero gas flow rate a fine, uniform aerosol was generated. However, as the gas flow increased from 0 to 45 L/min (rotameter setting: 14) the same phenomena occurred in the model 0040-0144 chamber as had occurred in the two other chambers. The aerosol was entrained by the gasses
Figure 34. P-E Spray Chamber 0040-0144 with Ultrasonic Nebulizer in Tip Insertion Configuration.
Figure 35. P-E Spray Chamber 0040-0144 with Ultrasonic Nebulizer in Front Section Insertion Configuration.
as they passed and a significant portion of the aerosol coalesced on the areas of the chamber walls where the gasses struck.

At gas flow rates typically encountered (45 L/min air) during routine atomic absorption operation only a extremely small fraction of the aerosol was observed exiting from the chamber neck. Essentially all of the aerosol was being deposited on the chamber walls where it coalesced and then ran to the waste drain.

The rear chamber cap assembly approach was therefore not evaluated further since it offered no advantage over any of the other methods.
(3) Side Insertion into Spray Chamber Neck

Insertion of the nebulizer via a port in the side of the burner chamber neck was somewhat successful for nebulization studies utilizing the plastic Perkin-Elmer spray chamber model No. 303-0191. In this configuration the aerosol is generated essentially in the gas stream immediately beneath the burner head.

A major concern for the configuration was safety since there had been a serious flashback with the plastic P-E model. Therefore a separate holder was designed to house the entire nebulizer assembly. This will be described in detail in the following section.

There were several changes made to the spray chamber and also to the gas inlet systems over the course of this investigation. These led to the final design that was used for atomic absorption and metal speciation studies.

(i) Initial Chamber Modifications

A 29/64 inch hole was drilled into the burner chamber neck 1.0 inches below the opening for the burner head. This position was the closest to the burner opening that would allow for the burner holder cap to be screwed on. This hole was to serve as the port for the insertion of the nebulizer holder assembly.

(ii) Nebulizer Holder Assembly

1. Design of the Nebulizer Assembly
A nebulizer holder assembly was constructed by the author to house the entire nebulizer. (Figure 36) The assembly was machined out of teflon. Several of the features are listed below.

The assembly was designed such that the entire nebulizer tip protruded just beyond the end of the holder when the nebulizer was totally inserted.

The front section was machined to fit snugly into the port in the side of the burner chamber neck.

The inside dimensions were such as to provide a tight seal around the flanged portion of the nebulizer. The horn section of the nebulizer was unobstructed to allow optimum nebulization. A teflon seal (1.125 inch o.d., 0.875 i.d., 0.125 thick) was added to seal the rear and to protect the piezoelectric discs from any sample which might accidently get into the holder.

The holder was designed for purging with nitrogen gas (gas trap). A 1/8 inch hole was drilled into the side of the holder for insertion of a piece of teflon tubing. The nitrogen gas was regulated at the nitrogen tank by a regulator with a shutoff valve. The pressure was set at 2-3 psi using the regulator. The flow into the nebulizer assembly was controlled using a Nupro fine metering valve. The effects of gas purging on nebulization will be discussed in detail.

A drain was added to the assembly to provide a outlet for any liquid that were to accidently accumulate in the
holder. A 1 inch section of 3/32 inch o.d. glass tubing was inserted into the bottom of the chamber and affixed with epoxy glue. The positive pressure from the nitrogen gas purge system incorporated into the holder also assisted in the removal of any liquid through the outlet.

The entire holder assembly was held in position on the side of the burner neck by a ring stand clamp that was attached to the spray chamber body.

![Ultrasonic Nebulizer Holder](image)

Figure 36. Ultrasonic Nebulizer Holder

2. Effect of Nitrogen Purge on Nebulization

Because the nebulizer holder was constructed with a gas purge or gas trap system, the effect of nitrogen flow from this system and across the nebulizer tip was studied.

The nebulizer and holder was inserted into the port on the side of the burner chamber neck. Nebulization of water pumped at 1.0 ml/min was optimized using the
oscillator power supply. No flame support gasses were used during this study.

The nitrogen gas flow was set at the tank at 2-3 psi, and the flow rate controlled at the nebulizer holder assembly by a Nupro fine metering valve.

As the nitrogen gas flow was slowly increased from zero flow rate, the droplet size distribution changed. More large droplets were being produced at higher nitrogen flow rates.

On closer observation, small gas bubbles (nitrogen) were forming under the liquid layer on the nebulizer tip. The number of the bubbles increased with increased nitrogen rates.

As the nitrogen flow was increased further, it was observed that the liquid layer was not being allowed to spread across the surface of the nebulizer tip, but was being blown back toward the sample outlet.

It was apparent that a high nitrogen flow in the gas trap system was detrimental to the nebulization process. The nitrogen purge system was used consequently during the startup and flame extinguishing segments of the atomic absorption measurements when the probability of flashbacks is the greatest.

(iii) Atomic Absorption Measurements

The design was evaluated using a 1.00 ppm of copper at a flow rate of 1.0 ml/min. Standard AA operating
procedures for copper analysis as previously described were used.

Initial result were very encouraging. An absorbance of 3.8 percent was obtained. This was approximately 1.5 times that obtained (2.5 %) using the Perkin-Elmer pneumatic concentric nebulizer as a baseline.

It was observed, however, that there were a large number of aerosol droplets that were transversing the flame. This resulted in a large flame flicker (signal noise), and loss of desired analytical signal. Additional spray chamber modification were made to try to capture this loss.

1G. (d) Final Spray Chamber Design

With the observation of the large sample droplets transversing the flame, steps were taken to modify these droplets so that the desolvation and atomization processes of the flame would be more effective.

1. Desolvation

The distance between the nebulizer tip and the flame was approximately 3 inches, and the total volume for desolvation or the formation of any smaller droplets was approximately 50 mls (volume of burner head).

Total or even partial desolvation of any droplets prior to the flame would allow for more effective use of the flame as an "atomizer". A greater proportion of the energy of the flame would be spent on atomization rather
than on desolvation, rather than on the combined processes of desolvation and atomization.

(i) Burner Neck Extensions

A series of extensions that could be used separately or in series with each other were machined. These were designed so that the bottom end would fit directly into the burner head opening on the chamber, and the other end as a receptacle for the burner head.

The extensions (Figure 37) were machined from 1.50 stainless steel cylinders. The neck was 1.00 inch o.d., 0.80 inch i.d., and 0.50 inch in length. Four burner extensions were prepared with lengths of 1.0, 2.0, 3.0 and 4.0 inches.

The burner head end was machined so that the burner head fit snugly, and that other burner extensions could be stacked in series. Two O-rings inserts provided gas tight seals.

The burner extensions were heated by resistive heating. Each segment was wrapped in 1 inch asbestos wrap and then wound with asbestos string. Nichrome wire, 20 guage, was wrapped around the extensions with about 1/8 inch between turns. Asbestos string was then laid over the nichrome wire and a final covering of 1 inch asbestos wrap applied.

The nichrome wire was then attached to the variable voltage supply by a three wire connector. Two wires were
Figure 37. Cross-Section of Burner Neck Extensions.
connected to the variable voltage supply; and the ground wire connected to the body of the burner chamber and grounded through the voltage supply outlet.

The variable voltage supply was set at 22. This gave a flowing air temperature of 170 degrees centigrade with a gas flow of approximately 10 L/min. With a flow rate of water of 1.0 ml/min nebulizing through the extension, this setting gave a temperature of 85-90 degrees for the air/gas mixture.

(ii) Spray Chamber Desolvation

The spray chamber was wrapped in an similar manner. The chamber was wrapped with asbestos wrap and string. Nichrome wire, 20 guage, was wrapped at approximately 1/8 intervals where possible. An overlay of asbestos wrap and string was applied.

The nichrome wire was attached to a variable voltage supply. Two leads went to the voltage supply. A third wire was attached from the burner chamber to the ground on the voltage supply.

The polymeric coating on the insides of the chamber has to be removed during the course of the study. It was found that during the initial phases in which the spray chamber was being heated, the polymeric coating started to melt and blister. Since it would not stand the heat which would be applied during certain portions of this study, it was removed.
The polymeric coating was removed by heating the chamber to approximately 300 degrees centergrade, and then physically scraping the melted coating out. This was repeated several times. The chamber was then scrubbed with stainless steel wool and thoroughly washed with soap and water.

(iii) Gas Flow Patterns and Inlet Redesign Studies

Three basic gas flow patterns were studied with the burner extensions incorporated into the spray chamber system. They were 1) cross-flow with gas entering from directly below the burner head; 2) rear entry with flow spoilers removed; and 3) rear entry with flow spoilers in position.

1. Cross-Flow

A 5/16 inch hole was drilled through the teflon pressure relief plug in the bottom of the chamber. A 5/16 inch Swagelok connector was attached by threading it through the teflon section of the relief plug. A silicone sealant was applied to insure an air-tight seal. The auxiliary air supply was attached.

a. Air flow studies

At a zero auxiliary air flow a fine, uniform aerosol was generated by the nebulizer with the aerosol coalescing on the wall of the burner chamber almost immediately due to the close proximity of nebulizer and wall.

As the auxiliary gas flow was increased from zero to
that typically encountered during routine AA operation (48 L/min), the aerosol became more non-uniform with the appearance of larger droplets in the gas stream. The aerosol was also not uniformly distributed across the burner extension cross-section, nor across the burner head when in position.

(This was analogous to the aerosol loss patterns observed for the rear cap assembly insertion method.)
2. Rear Cap Gas Entry With Flow Spoilers Removed
   a. General Considerations

   Previously glass bends had been incorporated into the spray chambers in attempts to control gas flow patterns. Although these attempts had not been totally successful, they had demonstrated how critical gas flow patterns were to aerosol transport patterns.

   It was postulated that removal of the flow spoilers would result in more of a laminar flow pattern in the spray chamber portion since the flow spoilers are shaped like propellors. Without the flow spoilers removed the air would encounter less obstructions in the chamber.

   b. Gas introduction

      1. Primary gas introduction

      The Perkin-Elmer nebulizer was used to an an introduction port for the primary air.

      As the gas flow was increased from zero to 24 L/min, the aerosol became less uniform. The aerosol was not uniformly dispersed across the burner extension cross-section and there were some larger droplets. However, the aerosol pattern was generally overall good.

      2. Auxiliary gas introduction

      However, as the auxiliary air was introduced through the auxiliary airport in the rear cap, the aerosol pattern severely deteriorated. As the flow was increased from zero to that normally used during routine AA operations, the aerosol became less uniform with increasing numbers of
large droplets.

At 24 L/min of auxiliary air only large aerosol droplets were exiting the burner extensions. On closer observation, it was apparent that the aerosol was being entrained into the gas flow as it was ejected from the tip, and almost immediately it was coalescing on the walls.

3. Rear cap gas entry with flow spoilers in position

a. General Considerations

With the flow spoilers in position, it was postulated that a partial spiral character would be imparted to the gas flow because of the propellor shape of the spoilers.

b. Gas introduction

1. Primary air introduction

The Perkin-Elmer nebulizer was used as the primary air introduction port.

As the air flow rate was increased, the aerosol was observed to take on a slight spiral flow or cyclonic pattern. On observing directly down the extensions during ultrasonic nebulization, a spiral or cyclonical flow pattern was particularly evident at lower gas flow. The pattern was somewhat similiar to the "tangential" flow pattern observed in inductively coupled plasma torch assemblies.

At higher gas flows (around 24 L/min) this pattern was not as observable, but was still evident. The aerosol
appeared to be fairly uniform across the burner extensions though.

2. Auxiliary gas introduction

However as the auxiliary gas flow was increased from zero to normal flow rates typically encountered in AA, the spiral or cyclonic aerosol flow pattern severely deteriorated. At the higher auxiliary air flows, only larger droplets were generally present.

It was apparent that the introduction of the auxiliary air which enters off-center from the primary air flow severely disrupted the aerosol gas pattern.

4. Gas Inlet Redesign

The Perkin-Elmer pneumatic concentric nebulizer was redesigned to so that all of the air necessary to produce flames (> 60 L/min air) typically necessary for routine atomic absorption analysis could be introduced through it. This would allow for the generation of the spiral or cyclonical gas flow pattern by the gasses spiraling past the flow spoilers (propellors) prior to reaching the aerosol.

a. P-E Nebulizer Modification to Serve as Gas Inlet

A P-E pneumatic concentric nebulizer was disassembled and modified by the author. The venturi (303-1810), needle assembly (303-0354) with spring, and the rear washer (303-1802) were removed. A 0.050 inch hole was drilled in the insert piece (303-1812).
A 1.7 inch piece of 1/8 inch o.d. stainless steel tubing (0.050 i.d.) was cut. The tubing was positioned inside the insert piece (303-1812) and Swagelok ferrules (front and rear) attached. The ferrules served to align the tubing in the the insert piece.

A 1/8 inch hole was drilled into the rear knurled knob (303-1460) to accomodate the stainless steel tubing. A rear seal was fashioned from a teflon septum to fix snugly inside the knurled knob and around the 1/8 inch stainless steel tubing.

b. Gas Attachment

The air supply was attached to the 1/8 inch stainless steel tubing. Atleast 60 L/min of air could be supplied by this method.

The acetylene supply was attached to the "normal air inlet" of the P-E nebulizer.

Thus the primary gasses entered in a concentric fashion through one inlet assembly. The air entered through the the inner section (where solution normally passed), and the acetylene entered by the outer concentric passage.

The auxiliary air supply was was left attached to its normal inlet on the side of the rear cap assembly. The auxiliary air was not used during normal atomic absorption determinations but was be used during flame shutoff procedures. During flame shutoff procedures it was desirable to have a high linear air flow across the burner
Figure 38. Gas Inlet for New Nebulization System.

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
head to prevent flashbacks. The auxiliary gas was routinely used to provide additional linear flows (particularly when desolvation was employed).

16.(e) Summary of Burner Chamber Incorporation Design Studies

The final design incorporating the new ultrasonic nebulizer, with holder, extensions for desolvation, and modified gas inlet system (Figure 39) with gas support system (Figure 40) were developed. During the course of these investigation a number of studies were completed and observations made.

(1) The incorporation and development of a new, prototype ultrasonic nebulizer as a means of sample nebulization in atomic absorption spectroscopy and as an interface for high performance liquid chromatographic speciation studies were accomplished.

(2) Several modifications made to the original nebulizer to significantly improved nebulization at low sample flow rates (1 ml/min or less). Optimum nebulization of sample at these flow rates is particularly important for the speciation of metals by high performance liquid chromatographic-atomic absorption methods.

(3) The incorporation of this nebulizer into a conventional atomic absorption spectrophotometer
Figure 39. Final Design for New Ultrasonic Nebulizer-Flame Atomizer
Atomic Absorption System.
Figure 40. Gas Flow Schematic for New Ultrasonic Nebulizer-Flame Atomizer Atomic Absorption System.
necessitated the redesign of the entire spray chamber-gas transport system to obtain maximal analytical signal.

(4) Extensive study of aerosol transport in three different spray chambers was conducted for a number of gas flow and chamber designs. Results of these studies were:

(a) The gas flow patterns and aerodynamics of the spray chamber/burner head assembly were critical to aerosol transport.

(b) Turbulent gas flow patterns were detrimental to aerosol transport.

(c) Positioning to the nebulizer with respect to aerosol impaction surfaces was important to aerosol loss and/or aerosol entrainment into the gas flow.

(d) A spiral or cyclonic gas flow significantly improved the overall aerosol transport for the final system described.

(e) A burner head capable of both high linear gas flow and high mass transport was required.
PART I. CHAPTER 2. EVALUATION OF NEW NEBULIZER-FLAME
ATOMIZER SYSTEM FOR ATOMIC ABSORPTION SPECTROSCOPY

3A. PROPOSED METHOD

The new nebulizer-flame atomizer system was evaluated
as an atomic absorption spectrophotometer for the
determination of metals. The proposed method was to
compare spectroscopic results for known concentrations of
a metal for the new system versus the results obtained
using a standard commercial nebulizer and procedures.

Desolvation of the aerosol was used to form smaller
droplets for better atomization in the flame. Desolvation
was achieved by resistively heating the burner extensions.
A small amount of desolvation would also be a result of
the increased desolvation volume provided by the
extensions.

Copper was chosen as the metal to analyze because of
its properties within the flame. Copper exists almost
entirely in the atomic state over the temperature range
of an air/acetylene flame (62). Thus a uniform signal was
observed over the flame profile, and the signal was not a
function of observation at a specific height and
alignment. There were relatively few interferences in the
copper determination of either chemical nature (7) or
spectral (63).

A pump was used to deliver the solution at accurate
rates. Nebulization efficiency will vary for both pneumatic concentric and ultrasonic nebulizer with respect to the type and amount per unit time (rate) for a sample solution (9, 18, 60, 64).

Thus a known concentration (1 ppm) of copper was delivered to the nebulizers by a pump so that absorbance, A, over a range of 0 to 9.9 ml/min (limits of the pump) could be determined. Additional solutions at various concentration were used to determine values for the sensitivity (the concentration that gives 1% absorption) and for the detection limits (the concentration that gives a signal 2X the noise level). Sensitivity and detection limits data serve as general comparisons for the capabilities of one atomic absorption spectrophotometer versus another.

2B. EXPERIMENTAL

The chemicals, equipment and procedures were those developed and described previously (pages 134-40). Since there were a number of spray chamber design changes and gas introduction modifications, those experimental conditions relating to the final design will be described below for clarity.

2B. (a) Chemicals and Solutions

Standard solutions of copper were prepared by
appropriate dilution with deionized water of the 1000 ppm standard stock solution prepared previously.

2B.(b) Equipment and Procedures

(1) Atomic Absorption Spectrophotometer, Perkin-Elmer

305B Settings

(i) Absorbance Mode

(ii) 324.8 nm Copper Line

(iii) Slit: Setting 4; 0.7 nm.

(iv) Copper Demountable Hollow Cathode Lamp: 20 mA

(v) Recorder: 10 mv full scale

(2) Nebulizers

(i) Perkin-Elmer Pneumatic Concentric, Model 0303-0352

The Perkin-Elmer pneumatic concentric nebulizer was interfaced with the pump for sample delivery using the stainless steel tubing-polyethylene tubing assembly previously described.

The P-E nebulizer was positioned into the chamber in the conventional rear cap fashion. The nebulizer was operated in a slight backpressure mode, with signal maximized, as previously described.

(ii) Ultrasonic Nebulizer

The ultrasonic nebulizer was operated in the mode
previously described (page 64). The nebulization was maximized by controlling the power (voltage) to the nebulizer. The optimum nebulization was determined by monitoring the absorbance signal on the strip chart recorder.

Sample was introduced at a constant flow rate by the pump. Sample was delivered to the face of the nebulizer by connecting the outlet of the pump to the teflon tubing insert incorporated into the nebulizer.

(3) New Nebulization System: Spray Chamber/Nebulizer Configuration

The spray chamber/nebulizer configuration was that developed as previously described (pps. 134-40). The nebulizer was inserted into the burner neck through a hole drilled into the side. The nebulizer was contained in an assembly designed to hold the nebulizer in place and to provide a gas seal.

(4) Spray Chamber Extensions

Spray chamber extensions were used in desolvation studies and to help facilitate the induction of a spiral or cyclonical gas flow in the burner neck extensions.

Extensions of 1, 2, 3, and 4 inches, as described previously (pps. 128-32), were constructed so that they could be coupled together to give length extensions of between 1 and 9 inches (although 7 inches was the maximum
length used in this study due to the limitation imposed by the chamber brackets on the atomic absorption spectrophotometer.

Extensions were resistively heated in the desolvation studies.

(5) Burner Head: Perkin-Elmer 3 Slot (303-0401)

(6) Gas Settings and Controls

(i) Pneumatic Concentric Nebulizer
Laboratory Air: 70-80 psig laboratory air regulated to 35 psig by Beckman DB regulator.
Nebulizing Air: Rotameter 9.0 (28 L/min)
Auxiliary Air: Rotameter 9.0 (28 L/min)
Acetylene: 12-13 psig at tank regulated to 10 psig by Beckman DB regulator.
Rotameter: 4.0 during operation (5.5 L/min)

Additional fine adjustments, as recommended by P-E procedure manual, were necessary to obtain optimum sensitivity.

(ii) Ultrasonic Nebulizer
Air (Primary): Rotameter 14.0 for flame ignition (44.5 L/min); Rotameter 18.0 for routine operation (56 L/min).
Air (Auxiliary): Rotameter 0 (zero flow) for routine
operation; Rotameter 14.0 for flame extinguishing (44.5 L/min).

Additional fine adjustments, as recommended by P-E procedure manual, were necessary to obtain optimum sensitivity.
2C. RESULTS AND DISCUSSION

A standard 1.00 ppm copper solution was used to determine the absorbance at various sample flow rates.

Burner neck extensions of 0, 2, 4, 6, and 7 inches were utilized to increase desolvation. The extensions were resistively heated to increase desolvation. Measurement of the air flowing through the extension sections prior to any nebulization and during nebulization (no flame, burner head removed) were done by suspending a thermometer into the center of the extension approximately 1 inch from the mouth.

2C.(a) Desolvation Studies

The burner extensions were heated from fifteen to thirty minutes to reach temperature equilibration. Desolvation was achieved by heat transfer from the hot metal to the sample flowing past in the gas stream. As sample was introduced into the preheated extensions cooling of the extensions occurred until an equilibrium was reached between heat lost from the extensions to that gained by the sample.

For reproducible results for a constant sample rate and concentration, it was necessary to determine the time needed for equilibrium to achieved after sample introduction. Equilibrium would be reached more slowly for the extensions with the greatest mass and highest
temperature differential, i.e., the hottest.

Therefore, the 7 inch extension combination (greatest mass) was used. The temperature at which it was to be operated during the sensitivity and speciation studies was used to determine actual equilibrium time needed for these studies.

The results of the studies for a sample flow rate of 1.00 ml/min (typical HPLC flow rate) for a 1 ppm copper solution are given in Table 6.

<table>
<thead>
<tr>
<th>Cooling Time</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minutes</td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>.240</td>
</tr>
<tr>
<td>3.5</td>
<td>.191</td>
</tr>
<tr>
<td>7.5</td>
<td>.134</td>
</tr>
<tr>
<td>10.5</td>
<td>.133</td>
</tr>
<tr>
<td>12.0</td>
<td>.130</td>
</tr>
</tbody>
</table>

Aqueous copper standard nebulized at flow rate of 1.00 ml/min
Absorbance at the 324.8 nm copper line.
Figure 41. Decrease in Absorbance with Time as Burner Extensions Reach Steady State Heat Transfer.
Discussion of Results: A steady state heat transfer was reached in about 8 minutes for the conditions described (1 ml/min, 7 inch extension). This should represent the greatest time necessary for a constant state heat transfer because the 7 inch extension had the greatest mass of all extensions used, and the operating temperature for this extension was the same or greater than other extensions.

The decrease in signal due to cooling of the walls (less heat transfer) was also observed in previous studies utilizing infrared radiation-heated spray chamber desolvation in atomic absorption analysis (65).

Thus for sensitivity and speciation studies, a burner extension temperature equilibration time of at least 10 minutes (with sample flow rate of at least 1 ml/min) was routinely used.

These studies also indicated that additional signal enhancement would be possible if additional heat transfer was available and desolvation were complete. These results show that desolvation was incomplete as indicated by the decrease in signal from 0.240 A to 0.130 A. Thus an additional increase in absorption of almost twice that obtained at this flow rate (1 ml/min) if the initial high temperature of the burner extension could have been maintained.

2C.(b) Sample Flow/Burner Extension Studies
To assess the performance of the new nebulizer-spray chamber usefulness for atomic absorption measurements, the absorbance for a 1.00 ppm copper solution over a flow range of 0.00 to 9.90 ml/min and for 5 extension lengths was obtained.

The flow rate over a wide range was necessary to give a fair comparison between the new nebulizer and those results that were typically obtained with a conventional pneumatic concentric nebulizer. Concentric nebulizer normally nebulizer at 5-8 ml/min of sample. Therefore when atomic absorption instrument manufacturers quote sensitivity and detection limit data for their products, this represents a signal for a specified flow rate. The greater the flow rate, the greater the signal because of the greater number of free atoms in the flame. The results of these studies are presented in Table 7.
Table 7: Absorbance as a Function of Burner Extension Length and Sample Flow Rate

<table>
<thead>
<tr>
<th>Sample Flow Rate (ml/min)</th>
<th>0 Inch</th>
<th>2 Inch</th>
<th>4 Inch</th>
<th>6 Inch</th>
<th>7 Inch</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>0.038</td>
<td>0.066</td>
<td>0.096</td>
<td>0.068</td>
<td>0.147</td>
</tr>
<tr>
<td>2.00</td>
<td>0.064</td>
<td>0.120</td>
<td>0.162</td>
<td>0.136</td>
<td>0.260</td>
</tr>
<tr>
<td>3.00</td>
<td>0.065</td>
<td>0.196</td>
<td>0.222</td>
<td>0.203</td>
<td>0.325</td>
</tr>
<tr>
<td>4.00</td>
<td>0.073</td>
<td>0.207</td>
<td>0.211</td>
<td>0.220</td>
<td>0.319</td>
</tr>
<tr>
<td>5.00</td>
<td>0.079</td>
<td>0.213</td>
<td>0.212</td>
<td>0.247</td>
<td>0.334</td>
</tr>
<tr>
<td>6.00</td>
<td>0.078</td>
<td>0.192</td>
<td>0.220</td>
<td>0.244</td>
<td>0.334</td>
</tr>
<tr>
<td>7.00</td>
<td>0.086</td>
<td>0.170</td>
<td>0.203</td>
<td>0.239</td>
<td>0.334</td>
</tr>
<tr>
<td>8.00</td>
<td>0.098</td>
<td>0.168</td>
<td>0.208</td>
<td>0.244</td>
<td>0.340</td>
</tr>
<tr>
<td>9.00</td>
<td>0.096</td>
<td>0.174</td>
<td>0.196</td>
<td>0.248</td>
<td>0.246</td>
</tr>
<tr>
<td>9.90</td>
<td>0.101</td>
<td>0.169</td>
<td>0.200</td>
<td>0.260</td>
<td>0.355</td>
</tr>
</tbody>
</table>

- a. No preheating prior to nebulization.
- b. Air temperature prior to nebulization: 130 degrees C; with nebulization: 90 degrees C.
- c. Air temperature prior to nebulization: 150 degrees C; with nebulization: 90 degrees C.
- d. Heated spray chamber: 70 degrees C; air temperature prior to nebulization: 150 degrees C; with nebulization: 90 degrees C.
Discussion of Results: Absorbance generally increased with increasing flow rates and with longer extension. Results are shown in Figure 42.

For comparison, the results obtained with a convention pneumatic concentric nebulizer at flow rates from 0.00 to 9.90 ml/min, and conventional operating conditions. The results for the conventional nebulizer were those typically observed (18). Absorbance increased with flow rate up to about 6 ml/min, at which point it essentially leveled off.
Figure 42. Absorption as a Function of Burner Extension Length and Sample Flow Rate.
Relative Enhancement: The relative enhancement in absorbance for the new nebulization system over conventional atomic absorption system was determined (Table 8).

Table 8: Relative Increase in Absorbance for New Ultrasonic Nebulization System (X Times) versus Perkin-Elmer Pneumatic Concentric Nebulizer

<table>
<thead>
<tr>
<th>HPLC Flow Rate</th>
<th>0 Inch</th>
<th>2 Inch</th>
<th>4 Inch</th>
<th>6 Inch</th>
<th>7 Inch</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>1.5</td>
<td>2.6</td>
<td>3.8</td>
<td>2.7</td>
<td>5.9</td>
</tr>
<tr>
<td>2.00</td>
<td>1.9</td>
<td>3.5</td>
<td>4.8</td>
<td>4.0</td>
<td>7.6</td>
</tr>
<tr>
<td>3.00</td>
<td>1.5</td>
<td>4.7</td>
<td>5.3</td>
<td>4.8</td>
<td>7.7</td>
</tr>
<tr>
<td>4.00</td>
<td>1.6</td>
<td>4.6</td>
<td>4.7</td>
<td>4.9</td>
<td>7.1</td>
</tr>
<tr>
<td>5.00</td>
<td>1.6</td>
<td>4.3</td>
<td>4.3</td>
<td>5.0</td>
<td>6.8</td>
</tr>
<tr>
<td>6.00</td>
<td>1.6</td>
<td>3.8</td>
<td>4.4</td>
<td>4.9</td>
<td>6.7</td>
</tr>
<tr>
<td>7.00</td>
<td>1.7</td>
<td>3.4</td>
<td>4.1</td>
<td>4.8</td>
<td>6.7</td>
</tr>
<tr>
<td>8.00</td>
<td>2.0</td>
<td>3.4</td>
<td>4.2</td>
<td>4.8</td>
<td>6.8</td>
</tr>
<tr>
<td>9.00</td>
<td>2.0</td>
<td>3.7</td>
<td>4.2</td>
<td>5.3</td>
<td>7.4</td>
</tr>
<tr>
<td>9.90</td>
<td>2.1</td>
<td>3.6</td>
<td>4.3</td>
<td>5.5</td>
<td>7.6</td>
</tr>
<tr>
<td>Average</td>
<td>1.8</td>
<td>3.8</td>
<td>4.4</td>
<td>4.7</td>
<td>7.0</td>
</tr>
</tbody>
</table>
Discussion of Results for Burner Extensions:

The absorbance values for the ultrasonic nebulizer at 0 extension (only burner head in place) was significantly greater (an average of 1.8X) that those obtained for the concentric nebulizer. The absorbance patterns were very similar for both nebulizers. This was a result of the similar air flow patterns and aerodynamics for both designs.

It was observed that for the ultrasonic nebulizer that the sample appeared to go up the central portion of the burner head, i.e., it was not evenly dispersed across the burner head. This was a result of the close proximity of the nebulizer tip to the burner head and the short dispersion distance available. Aerosol from a concentric nebulizer is evenly dispersed due to the geometry of the burner chamber, flow spoilers, and the right angle at the end of the chamber before it reaches the burner head.

The increased absorbance values using the 2, 4, and 6 inch extensions were a result of the increased desolvation and the flow pattern imparted to the gas prior to the flame. Greater heat transfer was achieved with increased extension length, with the average increase in relative absorption increasing from 3.8X (2 in) to 4.4X (4 in) to 4.7X (6 in).

Another important feature was the gas flow pattern which had been observed within the longer extensions. The spiral or cyclonical gas flows would mean an increased
sample path length versus a conventional gas flow pattern. By increasing the path length, this effectively increased the desolvation time.

The results for the 7 inch extension were a combination of the increased extension length and preheating the air prior to the nebulizer in addition to heating the extensions. Preheating the gasses prior to the nebulizer appear significantly increased the absorbance. The average absorbance increased 7.0X over the conventional concentric nebulizer. The average absorption increased 67% relative to the 6 inch extension (1 inch shorter) with no preheating. Heating the gasses with only the extensions means that all heating must occur in the space between the nebulizer tip and the burner head. The advantage of preheating the gasses prior to the nebulizer was to increase the time for desolvation to occur.

Disadvantages of preheating the gas were also seen later, though, for nebulization of samples with high solid content. Preheating the gasses resulted in the high solid buildup on the nebulizer tip. This severely affected nebulization. Thus preheating the gasses was not used during speciation studies.

Absorbance increased dramatically up to a flow of about 3 ml/min. At this point the burner extensions were essentially flooded with sample. The extensions could not efficiently desolvate the sample, and thus no significant
increase in signal was observed above 3 ml/min.

Efficiency of ultrasonic nebulizers to nebulizer are a function of the sample flow rate, as previously discussed. At higher flow rates, a greater number of larger droplets are produced. These would not be efficiently desolvated nor atomized by the flame.
2C. (c) Flow Rate-Burner Extensions with Concentric Nebulizer

The influence of the spray chamber extensions in conjunction with a regular Perkin-Elmer pneumatic concentric nebulizer was studied. A significant increase in absorption had been observed for the ultrasonic nebulizer with the addition of the burner extensions. Therefore a study was conducted to see if there would be corresponding increases in absorption through the use of the burner extensions with a concentric nebulizer. Results of this study are given Table 9.

Discussion of Results: Maximum absorbance occurred at a flow rate of 5.0 ml/min. This is a typical value for pneumatic concentric nebulizers, although this may range from 5-10 ml/min depending on the manufacturer and on the individual nebulizer itself (9, 18, 64). In normal atomic absorption measurements the standard procedure calls for the maximization of the particular nebulizer included with the instrument (61). No consistent increase in absorption was noted with the incorporation of the burner extensions. In fact, a slight decrease was noted as the length of the extensions increased, with the most severe decrease noted for the longest extension (7 inches).

Several explanations can account for this. The design of the spray chamber with the flow spoiler in place
removes a significant portion of the aerosol generated by the concentric nebulizer. The aerosol reaching the flame (or the burner extensions) is only the net result of this process. Only the droplets that are the smallest and are the most susceptible to desolvation and atomization within the flame are generally left at this point. However there should be an increase in any case from any desolvation that is available prior to the flame.

However, any desolvation provided by the extensions appeared to be negated through loss of sample to the walls of the extensions. Inspection of the extensions with aerosol flowing through them (burner head removed) showed a significant portion of the aerosol coalescing and evaporating on the walls.

Once again the importance of the aerodynamic design of the chamber was emphasized. The flow patterns used with the concentric nebulizer are turbulent and can lead to a significant loss of sample (and sensitivity) to the walls.
Table 9. Absorbance for Pneumatic Concentric Nebulizer Versus Burner Extension Length

<table>
<thead>
<tr>
<th>HPLC Flow Rate</th>
<th>Burner Neck Extension Length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Inch</td>
</tr>
<tr>
<td>1.00</td>
<td>0.012</td>
</tr>
<tr>
<td>2.00</td>
<td>0.034</td>
</tr>
<tr>
<td>3.00</td>
<td>0.042</td>
</tr>
<tr>
<td>4.00</td>
<td>0.045</td>
</tr>
<tr>
<td>5.00</td>
<td>0.049</td>
</tr>
<tr>
<td>6.00</td>
<td>0.050</td>
</tr>
<tr>
<td>7.00</td>
<td>0.050</td>
</tr>
<tr>
<td>8.00</td>
<td>0.045</td>
</tr>
<tr>
<td>9.00</td>
<td>0.047</td>
</tr>
<tr>
<td>9.90</td>
<td>0.047</td>
</tr>
</tbody>
</table>

Perkin-Elmer Pneumatic Concentric Nebulizer Model No. 0303-0352.
2C. (d) Atomic Absorption Detection Limits

In atomic absorption spectroscopy, the detection limit capabilities of an is often used for comparative purposes. Detection limits is defined as 1) the concentration of a element which can be detected at a 95% certainty. This is the quantity of the element that gives a reading equal to twice the standard deviation of a series of at least 10 determinations at or near the blank level (66). In common laboratory practice it is generally defined to be 2) that concentration of an element that gives a reading equal to twice the noise level (63). The latter definition of 2X the noise level was chosen for this study due to its simplicity and the ease for determination. The first definition usually employs the use of direct digital readouts and statistical data handling equipment. The strip chart recorder used in this study was better suited to the comparison of signal versus noise level. The sample flow rate was 3.00 ml/min because it represented the flow rate at which maximal absorption for a given concentration occurred for the new nebulization system.

Results for a 10 ppb copper solution are shown in Figure 43. The signal obtained exhibited sufficient response for measurement of sample signal and also for noise determination. Direct comparison showed that the signal for the 10 ppb sample concentration was atleast 10
Figure 43. Sensitivity for Copper with New Nebulizer-Flame Atomizer Atomic Absorption System.
times the signal for the noise. Therefore the detection limit would be approximately 2 ppb for copper.

Comparison of the value for the detection limit for copper of 2 ppb to that quoted for this instrument under the best of analytical conditions of 5 ppb (66) indicates an increase of approximately 2.5 fold better in detection limits.

The limiting factor in this determination was the noise level. The new nebulization system showed a considerable increase in signal but also a substantial increase in the noise level. This noise that was present in the signals was not primarily electronic noise, but flame noise due to incomplete desolvation. This was readily apparent on observation of the flame. There was considerably more flame flicker and the traversing of aerosol droplets through the flame was readily observable. This results in increase noise, and hence poorer detection limits.

2C(e) Atomic Absorption Sensitivity Limits

In atomic absorption spectroscopy, sensitivity is defined as that concentration that will produce a 1% absorption (0.0044 absorbance units) (66). Sensitivity values are frequently used to compare various atomic absorption techniques.

To determine the sensitivity for the new nebulization system, solutions of various concentrations were prepared
by appropriate dilution of the stock 1000 ppm copper solution. Direct determination of sensitivity for copper was preferred on extrapolation methods.

The result of this study is shown in Figure 43. A 10 ppb of copper at a flow rate of 3.00 ml/min gave a 1.0 percent absorbance value. Figure 43A is the direct reading; Figure 43B is a 3X scale recorder scale expansion of the determination repeated. The peak at the front on both determinations is an anomaly. It was the result of initial sample buildup on the nebulizer tip prior to cutting the nebulizer on. The signal flowing the initial signal burst is the "true" signal from the aerosol at the specified flow rate.

The value of 5 ppb for copper for the new nebulization system is approximately 15X better than that typically obtained. For the P-E model 305B under the best of experimental conditions, a value of 60 ppb (66) for the sensitivity for copper can be obtained. The Perkin-Elmer value also represents a signal obtained from a sample being nebulized at approximately twice the rate (6 ml vs. 3 ml).

The new nebulization system represents a considerable increase in sensitivity over conventional atomic absorption spectrophotometers.
2D. GENERAL SUMMARY

The incorporation of a prototype ultrasonic nebulizer and the development and construction of a new nebulization system for an atomic absorption spectrophotometer was achieved.

Fundamental studies of the gas flow patterns in the spray chamber-burner assembly were conducted. The aerodynamical properties of the gasses and aerosol droplets were crucial to the transport of the aerosol droplets through the system and to the flame for atomization.

A spiral or cyclonical gas flow pattern in the new nebulization system resulted in a significant increase in the transport of the aerosol to the flame and thus a significant increase in atomic absorption signal.

Desolvation through resistively heating spray chamber extensions resulted in a significant increase in atomic absorption signal. The spray chamber extensions were also important to the formation of the spiral gas which was crucial in the aerosol transport mechanism.

There were significant improvements in both sensitivity and detection limits for the new nebulization system in comparison to a conventional pneumatic nebulizer atomic absorption system. Sensitivity of 5 ppb for copper represents an approximate 15X improvement although a more conservative 10X would be more appropriate until
additional studies are completed; and a detection limit of 2 ppb is an approximate 2.5X improvement.

More importantly these studies indicate that greater sensitivity and detection limits are possible through redesign and refinement of the basic nebulization system and principles developed in this study.
PART II. CHAPTER 1. SPECIATION OF ORGANOLEAD COMPOUNDS
BY HPLC-AA AND TLC-COLORIMETRY

1A. GENERAL INTRODUCTION

The determination of a specific metal specie in environmental, industrial, and physiological samples is extremely important to the elucidation of the role which that metal may play. The health or physiological effects of a metal is not only dependent upon the concentration, but its chemical form. Elemental analysis, such as the determination of the total concentration of lead or mercury in a sample by atomic absorption, provides only part of the information needed to help determine the role that a metal may have, either to its toxicity, or to its usefulness. Therefore the importance of identification and determination, i.e., speciation, cannot be overemphasized.

Speciation is very important in determining the fate and toxicity of lead compounds in the environment and man. For example, inorganic lead and alkyllead compounds have very different toxic properties. Even though the use of leaded gasoline is decreasing, the use of the alkyllead tetraethyllead as an octane booster is still in wide use. The effects that the lead from leaded gasoline has on the environment and on human have been the subject of world-wide studies (67, 68, 69) with a recommendation of
an additional reduction of 28 percent in the use of lead in gasoline over the next 8 years (70).

A major threat of lead pollution has been the possibility of spills of large amounts of alkyllead compounds that are transported in very large amounts by rail or by sea. Concern regarding the fate of these compounds in the environment has increased following their accidental release (71). At least three such accidents have occurred, with the largest being in 1974 when a ship with 325 tons of lead alkyl compounds sunk off Otranto Cape in the Adriatic Sea. Most of the TEL was recovered, but 7 percent was unaccounted for. Following this accident a number of extensive investigations (72, 73) were conducted to try to determine fate of alkyllead compounds in sea water.

TEL and other alkyllead compounds are more toxic than inorganic lead compounds. It has been proposed that tetraethyllead decomposes in blood and water by the following mechanism (74, 75, 76):

\[
\text{PbEt}_4 \xrightarrow{\text{fast}} \text{PbEt}_3\text{Cl} \xrightarrow{\text{slow}} \text{PbEt}_2\text{Cl}_2 \rightarrow \text{Pb}^{2+} (\text{Cl}_2, 0)
\]

It has been shown that inhalation of trialkyllead of tetraalkyllead compounds resulted in the formation of trialkyllead in the fluids and tissue of rats (74), humans (77), and fish (78). The toxic effects of tetraalkyllead to mammals have been attributed to the
trialkyl species (79), which is more toxic than the
tetraethyllead. The conversion of tetraalkyllead to the
trialkyleads occurs rapidly in liver homogenates from rats
and rabbits (74, 80). Acute toxicities of the
tetraalkyleads and the trialkyllead salts are similar
(77, 79) and are at least an order of magnitude greater
than dialkyllead compounds or inorganic lead salts (74,
81). Dialkyllead compounds cause symptoms of toxicity
similar to those produced by inorganic salts and they have
an affinity for thiol compounds (74). Trialkyllead
compounds inhibit oxidative phosphorylation and bind to
proteins (75, 82).

Thus the conversion or stability of the alkylleads in
under environmental and physiological conditions is of
extreme importance as to the effect that specific
alkyllead would have. It is important to determine the
specific form that the lead is present in at a given time;
and whether there is complete conversion to one form,
I.e., lead oxide or if an equilibrium exists between
different or all forms.

To investigate the conversion of tetraethyllead in sea
water, or alkylleads in environmental or under
physiological conditions, an analytical method was needed
that was accurate, highly sensitive, and capable of
determination of the specific metal species that may be
present or formed.
IB. ANALYTICAL DETERMINATION AND SPECIATION

IB.(a) General Analytical Determinations

Methods available for the determination of alkyllead ions include spectrophotometric measurements of their dithizonates in chloroform at different wavelengths for dialkyl- and trialkyl-lead (83). Trialkyllead compounds have been separated from rat blood, urine, brain, liver, and kidney via a laborious multiple extraction separation procedure with final dithozone determination complexation of the decomposed organoleads and subsequent colorimetric determination of the lead dithizone complex (74, 84, 85). Trialkyllead can be determined colorimetrically as the dialkyllead complex with 4-(2-pyridylazo)resorcinol (PAR) following conversion of the trialkyllead to dialkyllead with iodine monochloride (86). Enhanced sensitivity for a number of cations, including Pb(2+), have been observed using a PAR-ZnEDTA reagent mixture, but it has not yet been applied to the determination of the alkyllead ions.

Recently, two electrochemical methods have been developed. Anodic stripping voltammetry at different plating potentials was used to determine the alkyllead species after extraction from biological materials (87). The dialkyllead and trialkyllead is determined as a total. The second method employs differential plating together with selective extraction to achieve separation and identification of R4Pb, R3Pb(+), P2Pb(2+), and Pb(2+)
(R=methyl or ethyl) in water (88). This latter procedure is lengthy and involves many steps of calculation by difference. None of these methods have achieved quantitative extraction of all of the ionic alkyllead species.

Trialkyllead ion has been separated as the benzoate derivative from liver and identification by infrared spectroscopy (89).

Trialkyllead ion has been extracted from street dust (90), and from normal human brain (77) with subsequent measurement by atomic absorption spectroscopy.

Atomic absorption offers the advantages of being highly sensitive, and a very specific means of determination of the lead present. However atomic absorption itself does not separate or identify the specific lead species present. Therefore a method that would combine the advantages of separation, in as much, specificity, for the individual species present with that of a spectroscopic method with high sensitivity would be of tremendous value in the speciation of desired compounds.
IB. (b) Speciation Methods

(1) Gas Chromatographic Methods

Hayakawa first described the determination of trialkyllead chloride at biological and environmental trace levels with gas chromatography (91). Gas chromatographic methods coupled with atomic absorption spectroscopy were later developed to separate a number of alkylleads in gasoline (92), and for the direct determination of tetraethyllead (TEL), and triethyllead chloride in sea water (93). However inorganic leads (2+), and diethyllead dichloride could not be chromatographed directly on this column, and the speciation of other organoleads, such as triphenyllead (1+), or tetraphenyllead, was not attempted (94).

The direct quantitative gas chromatographic determination of trimethyllead chloride or triethyllead chloride suffers from two major difficulties. 1) Both compounds are thermally unstable and tend to decompose even at the lowest temperature possible injection port temperatures, 160-170 °C, required to give complete and rapid volatilization. 2) Both compounds are very chemically reactive even with the most inert gas chromatographic column available, resulting in some tailing of the chromatographic peaks (95, 96, 97). Diethyllead dichloride is also not stable at the temperature needed for volatilization into the gas chromatograph, but readily decomposes. Thus, direct quantitative gas chromatographic
measurement of these compounds is difficult, if not nearly impossible.

Gas chromatographic-atomic absorption methods for the simultaneous determination of tetraalkylleads (Me₄Pb, Me₃EtPb, Me₂Et₂Pb, MeEt₃Pb, Et₄Pb), ionic alkylleads (Me₂Pb (2+), Et₂Pb (2+), Me₃Pb (+), Et₃Pb (+)), and lead (2+) has been developed (97, 98). However, the method is not a direct one. The leads are first extracted with diethyldithiocarbamate, followed by n-butylation to their corresponding tetraalkyl forms for chromatographic separation. The average recovery varied from 71% to 101% depending on the particular alkyllead. Recovery was also highly dependent upon the matrix.

This GC-AA method was adopted from a gas chromatographic method with detection via atmospheric pressure microwave induced plasma emission for the two trialkyleads, trimethyllead chloride and triethyllead chloride (95). The compounds were extracted into benzene from an aqueous solution saturated with sodium chloride. The trialkyleads were then derivatized into n-butyltrialkyllead by reaction with an n-butyl Grignard reagent. The extraction efficiency ranged from 5.7 ± 0.6% for trimethyllead chloride to 93 ± 12% for the triethyllead chloride.

The low extraction efficiencies was in line with the extraction data for a number of solvent extraction systems used in the determination of trialkyleads by graphite
furnace atomic absorption spectroscopy (99). These values ranged for timethyllead from 14% in carbon tetrachloride to 75% in chloroform, and for triethyllead from 81% in hexane to 100% in diisopropyl ether.

Another GC separation methods for alkyllead salts \((R \text{Pb} (+), \text{and } R \text{Pb} (2+))\) employed extraction with dithizone followed by phenylation and speciation by capillary column GC with electron capture detection (100).

A batch GC method for \(\text{Pb} (2+)\) involved extraction of the analyte into chloroform as the dithiocarbamate complex followed by solvent evaporation and methylation of the residue with methyllithium to form tetraalkyllead (101). The analyte vapor was then trapped on a GC packing material from which it was eluted into a quartz furnace atomic absorption detector.

GC-AA has been widely used for the determination of tetraalkylleads in gasoline (102, 103, 104). Other gas chromatographic methods have employed flame photometry (105), microwave plasma wavelength modulation (106), and hydrogen atmospheric flame ionization (107). Gas chromatography has also been combined with on-line electron capture (108), thermal conductivity (109), and mass spectroscopy (110, 111).
(2) Liquid Chromatography Methods

Liquid chromatography has the advantage of high efficiency of separation, operation at room temperature, and the versatility of a wide selection of column packing and possible solvent systems. Therefore, liquid chromatography offers real potential for separation of compounds such as the alkyllead halides, and in particular, diethyllead dichloride, which are not volatile or thermally stable.

Speciation of lead compounds by liquid chromatography essentially limited to the tetraalkylleads, not the alkyllead halides. The tetraalkylleads in gasoline, i.e., tetramethyllead (TML), trimethylethyllead (TMEL), dimethyldiethyllead (DMDEL), methyltriethyllead (MTEL), and tetraethyllead (TEL), were separated by reversed-phase ODS column, acetonitrile-water eluent, with detection by atomic absorption (112). A reversed-phase ODS column with methanol-water eluent was used to separate TML and TEL with AA detection (113); and TML, TMEL, DMDEL, MTEL, and TEL in gasoline by fraction collection and analysis by GFAA (114).

Chromatographic separation of four of the five tetraalkylleads in gasoline was achieved on a silica gel column, 75% ethanol as eluent, and detection by inductively coupled plasma (115); and a methanolic eluent was used for the separation of TML and TEL with atomic absorption (116).
The only report of separation of alkyllead halides was for trimethyllead (+) and triethyllead (+) on an Lichrosorb NH2 column; 40 % methanol/water and 0.06 molar in ammonium acetate, pH 5.5; with electrochemical detection (117).

Lead (2+) has been separated from other divalent cations, for example, on ion-exchange columns using ethylenediamine, pH 4.0 tartrate buffer as eluent, and detection by conductivity (118).

(3) Other Chromatographic Methods

There are two reports on the separation of alkylleads by paper chromatography. The formation of complexes between diethyllead (2+) and triethyllead (1+) cations and chloride ligands in concentrated aqueous HCl and LiCl aqueous solutions was studied by anion exchange paper chromatography (119). The following mixtures were separated by paper chromatography (120): Pb(2+), dimethyllead(2+), trimethyllead(+), lead(2+), diethyllead(2+), triethyllead(+); and lead(2+), diphenyllead(2+), triphenyllead(+), tetraphenyllead. Resolution was poor; and detection was by spraying the paper with 6N ammonium hydroxide, heating for 30 minutes at 100 C to decompose the compound, and then spraying with KSCN.

Triethyllead ion in natural water has been determined by silica gel TLC (121). Trimethyllead chloride,
triethyllead dichloride, dimethyllead dichloride, and
diethyllead didichloride were separated on silica gel TLC
plates using an acetone/hexane/propionic mixture as the
mobile phase.
1C. OBJECTIVES OF RESEARCH

The objective was to speciate a number of physiologically important organolead compounds, particularly diethyllead dichloride, directly without going through complexation, extraction, and/or derivatization which would offer significant advantages over current methods that employ these techniques. Complexation, extraction, derivatization, etc., are generally not quantitative (88, 89, 90, 95, 99) and can result in artifacts, etc., and multiple products which can affect the accuracy of the determination.

The objective of this research project, therefore, was to develop complementary methods of separation and direct determination, i.e., speciation, for the physiologically important organoleads. High performance liquid chromatography with a lead specific detector (atomic absorption spectrophotometry) was chosen as the primary speciation method; and thin layer chromatography with colorimetric detection was chosen as the verification method. Thus, two complementary chromatographic methods were employed. HPLC exhibits high chromatographic efficiency and resolution, particularly for the compounds eluting first; and TLC has good separatory powers and is particularly good for the detection of compounds that do not migrate (or that do not elute in HPLC). Two independent detection methods were selected: atomic absorption spectroscopy as the metal
specific detector; and classical colorimetry reagents which were highly sensitive and well-documented for the compounds of study.

The studies performed utilizing this new method were as follows:

1. Conversion of tetraethyllead in sea water to other alkyllead compounds.

2. Decomposition of solid dialkyllead dichloride

From the studies, it was hoped that a better understanding regarding the behavior of TEL in sea water would be achieved. Perhaps more importantly, a speciation method was developed that can separate and determine directly a number of environmentally and physiologically important organolead compounds. Therefore application of this speciation technique to other sample types would offer a means of gaining significant information about the role of such metal species.
ID. EXPERIMENTAL:

ID.(a) Instrumentation and Supplies

(1) High Performance Liquid Chromatographs

Two high performance liquid chromatographs were used. A Waters Associates Model 6000, and a Laboratory Data Control Constametrics II. Flow rate range for the Waters Associates Model 6000 was 0.0 to 9.9 ml/min.; and for the LDC Constametric II, the range was 0.00 to 10.00 ml/min. Both were capable of solvent delivery backpressure of up to 6000 psi.

(2) High Performance Liquid Chromatography Columns and Accessories

(i) Columns: Whatman Partisil-10, Reverse Phase C-18, ODS-2, 25 cm.; Whatman Partisil-10, Reverse Phase C-8, 25 cm.; LDC-LiChrosorb, Reverse Phase RP-8, 10 micron, 25 cm.; and LDC Spherisorb Silica, 10 micron, 25 cm.

(ii) Guard Columns: Laboratory constructed by the author from 1/4 o.d. stainless steel tubing: 5 cm. length, 2 mm. i.d. Outlet was Swagelok reducing union (1/4 inch to 1/16 inch). A 2 micron stainless steel frit was the outlet plug; glass wool was inlet plug.
(iii) Guard Column Packing: The guard columns were packed with similar packing as the analytical HPLC column. Packings: Whatman Reverse Phase Co:Pell ODS; Applied Science Laboratories, Reverse Phase Pellicular C-8; and Pellicular Silica, Applied Science Laboratories.

(iv) Injector: Reeve Angel, Septum Type, 1000 psi. The injector was a septum type rated to 1000 psi. The range was extended to 2500 psi by the author by using a series of two teflon septa (10 mm diameter x 3 mm thick) punched from gas chromatographic septa (13 mm diameter, Tek Lab, Baton Rouge, LA).

(v) Syringes: Hamilton 0-10 and 0-50 microliter syringes.

(3) High Performance Liquid Chromatograph-Atomic Absorption Spectrophotometer

The HPLC-AA was described in detail in Chapter I, Experimental. The operation of the atomic absorption spectrophotometer, nebulizer, and HPLC units were as described.

(i) Sample Injection: The only minor difference in the operation of the HPLC-AA between the speciation studies and the nebulizer development studies was that the HPLC
had to be turned off momentarily so that the samples be injected onto the column.

(ii) Hollow Cathode Lamps

1. Copper: The copper hollow cathode lamps and operating conditions were described in detail in Chapter I, Experimental.

2. Lead: The lead hollow cathode lamps and operating conditions were described in detail in Chapter I, Experimental.

(4) Thin Layer Chromatography Supplies

(i) TLC Plates: 1. Reverse Phase Plates: Whatman KC-18, without fluorescent indicator, Cat. No. 4801-600 (5 X 20 cm.) and Cat. No. 4803-600 (10 X 10 cm.); EM Reagents, HP-TLC, RP-8, Cat. No. 13725 and HP-TLC, RP-18, Cat. No. 13724.

2. Silica Gel Plates: Whatman with K5 silica gel formulation, LK5, Cat. No. 4855-620 (5 X 20 cm.) and LK5D, Cat. No. 4855-821 (20 X 20 cm.); EM Reagents, Silica Gel 60, Cat. No. 5763.

(ii) Chambers: Glass chambers, 17 cm. X 8 cm. X 17 (LxWxH) and 21 cm. X 5 cm. X 27 cm (LxWxH).

(iii) Spotting Capillaries: 1, 2, 5, 10 ul Drummond
Microcaps

(iv) Air Gun: Variable temperature and air flow.

(v) Glass Tray: A 30 X 40 X 4 cm (W X L X H) glass tray for dipping of TLC plates.

(5) Mass Spectrophotometer: Hewlett-Packard 5985A

(6) Melting Point Apparatus

1D. (b) Chemicals and Solutions

(1) Chemicals

All chemicals were analytical reagent grade or the highest grade commercially available. The suppliers for some of the more specialty chemicals and specific reagents are given.

Acetonitrile (ACN): J. T. Baker
Chloroform: J. T. Baker
Diphenythiocarbazone (Dithizone): J. T. Baker
Ethanol (EtOH): AAPEL Alcohol and Chemical Co
(Ethylenedinitrilo)-tetraacetic acid disodium dihydrate salt (EDTA): Matheson, Coleman & Bell
Lead Compounds:
Diethyllead dichloride: Pfaltz & Bauer, Alfa
Lead chloride: Allied Chemical
Tetraethyllead (TEL): Ethyl Corporation
Tetraphenyl lead: Peninsular Chemical Research
Triethyllead acetate: Alfa
Triethyllead chloride: Alfa
Trimethyllead acetate: Alfa
Methanol (MeOH): J. T. Baker
4-(2-Pyridyazo)resorcinal monosodium salt monohydrate (PAR): Aldrich
Sodium bromide: Matheson, Coleman & Bell
Sodium chloride: Matheson, Coleman & Bell
Tetrahydrofuran (THF): J. T. Baker

(2) Solutions

Dithizone: 0.05% (w/v) in chloroform. Dissolve 0.250 g of dithizone in 500 ml of chloroform.

PAR: 0.002 Molar. Dissolve 0.4744 g of PAR in water and dilute to 1 liter.

Buffer pH 9: Adjust 0.1 M ammonium nitrate to pH 9 with ammonia solution

PAR Buffered pH 9 Solution: Add 5 mls of 0.002 M PAR to 95 mls of pH 9 ammonium nitrate buffer. EDTA: 0.001 M. Dissolve 0.372 of disodium EDTA dihydrate in 1 liter of water.

Sea water: collected in October, 1982, in Panama City, Florida, from the Gulf of Mexico.
1D.(c) Experimental Procedure

(1) Development of HPLC System for the Speciation of Lead Compounds

A series of solvent systems (Tables I, II, III, IV) were used to determine which systems separated the compounds under study. High performance liquid chromatography and thin layer chromatography were in conjunction to develop methods for separation. Although they complement each other, both HPLC and TLC exhibit a number of the unique advantages and properties of their own.

The method employed for the preparation of the solvent systems was because of the behavior characteristic of mixed organic solvent systems, particularly those containing inorganic salts.

A number of the system evaluated were close to the point where the inorganic salt used, either sodium chloride or sodium bromide, would "salt-out" if there was a slight change in either the organic solvent or the salt concentration. Another problem encountered in the preparation of mixed organic-water solvent systems is that the final volume measured after mixing an organic solvent with water is frequently less that the sum of the two individual volumes. Therefore a consistent method was used throughout this study to prepare the solutions.
An aqueous solution, either 1.0 molar or 2.0 molar, in sodium chloride or sodium bromide was used to adjust the systems to the desired salt concentration.

For example, to prepare the 50 ACN/50 Water/.10 M NaBr system, 50 ml of acetonitrile was pipetted into a 100 ml volumetric flask, approximately 25 ml of water was added (to prevent "salting-out" of the NaBr if the NaBr was added directly), then 10 ml of 1.0 molar sodium bromide was added. The solution was then brought to 100 ml total with water. Thus this solution would be denoted as 50 parts ACN, 50 parts water (V/V), and 0.10 molar in sodium bromide.

(2) TLC Plate Development and Colorimetry

(i) Application of Sample to Plate

Samples were applied to the plate using Drummond Microcap spotting capillaries. Sample volumes were 1, 2, 5, or 10 uls.

The sample zones were dried using a warm air stream from the air gun.

The exception to this procedure was for TEL. Because of the volatility of TEL, the sample was allowed to air dry. The sample was then developed as quickly as possible.

(ii) TLC Plate Development
The thin layer chromatography developing chamber was lined with 10 cm filter paper. Approximately 25 milliliters of developing solvent was added. The system was then equilibrated for at least 15 minutes.

The TLC plate was placed into the chamber. The plate was allowed to fully develop. The plate was then removed from the chamber.

The solvent was removed from the plate with the air gun by directing a warm stream of air across the plate.

The exception to this was for TEL. Because TEL is so volatile, the plate was allowed to air dry, and the color development process carried out as quickly as possible.

(iii) Sample Detection by TLC Colorimetry

1. Dithizone Colorimetry

Approximately 50 mls of dithizone solution (0.05%) in chloroform was poured into the glass tray.

The TLC plate was laid sample side down into the the dithizone solution, and immediately removed. This motion was done as quickly and as evenly as possible to give uniform color development.

The plate was then dried using a very gentle stream of warm air from the air gun. Color development was recorded.

2. PAR Colorimetry

Approximately 50 mls of PAR pH 9 Buffered Solution was poured into the glass tray.
The TLC plate was laid sample side down into the PAR solution, and immediately removed. This motion was done as quickly and as evenly as possible to give uniform color development.

The plate was then dried using a stream of warm air from the air gun. Color development was recorded.

3. PAR Solution Colorimetry

To check for the purity of diethyllead dichloride, a qualitative colorimetric determination was made according to the following procedure. Approximately 10 mg of sample was placed in a test tube. Add 5 ml of PAR pH 9 Buffered Solution. Then add 5 ml of EDTA solution (0.001 M). Note any color change.

(3) Conversion of TEL in Sea Water over a Long Time Period

Samples were prepared in centrifuge tubes with screw tops with hole 1/8 inch in diameter drilled through the top. A Teflon septum was used to prevent the TEL from escaping. Teflon septa were necessary because TEL decomposes rubber septa (94).

The sea water was filtered through a medium porosity glass frit to remove suspended matter. A portion of the sea water was boiled in order to kill any microorganisms that might be present. Studies employing boiled and unboiled sea water samples were used to monitor the effect
that any microorganism originally present in the sea water might have on the conversion of TEL.

(i) Unboiled Sea Water

Specially designed values were used to stopper small bottles that held approximately 33 milliliters when completely filled. TEL, 200 ul, was added to 25 milliliter of unboiled sea water. This ratio of TEL and sea water was comparable to the ratio used in previous studies. Light was excluded by covering the containers with aluminum foil. The sample was manually shaken for 20 minutes and stored in the dark. Aliquots were taken periodically over a period of a year and analyzed by HPLC-AA and by reverse-phase TLC-Colorimetry.

(ii) Boiled Sea Water

TEL, 200 ul, was added to 25 milliliters of boiled sea water in the small bottles. Light was excluded by covering with aluminum foil. The sample was manually shaken for 20 minutes and stored in the dark. Aliquots were taken periodically over a period of a year and analyzed by HPLC-AA and by reverse phase TLC-colorimetry.

(4) Decomposition of Solid Diethyllead Dichloride

The decomposition of solid diethyllead dichloride was monitored by HPLC-AA and reverse-phase TLC-Colorimetry. Decomposition was confirmed by qualitatively by solution
PAR colorimetry and by mass spectroscopy.
1E. RESULTS AND DISCUSSION

1E. (a) Results of HPLC and TLC System Development for the Speciation of Lead Compounds

High performance liquid chromatography and thin layer chromatography used in conjunction proved very successful in the development of systems for the speciation of lead by HPLC-atomic absorption spectroscopy. Separations achieved on Whatman KC18 reversed phase plates had been translated to Whatman reversed phase HPLC columns for a number of polyaromatic hydrocarbons (122). The combination of HPLC-AA and TLC-colorimetry for the organolead compounds also were ammendable to this treatment.

The systems developed for the separation of the organoleads were new and unique. The systems were developed using a combination of two chromatographic separation techniques: 1) the solvent-selectivity triangle and 2) "ion-pair" liquid chromatography.

The solvent-selectivity triangle is a method developed for optimizing the mobile phase in liquid chromatography developed by J. J. Kirkland and J. L. Glajch (123). Their first step was to use the Synder selectivity triangle (124).

In this triangle, the most common HPLC solvents were divided into eight major groups, each of which has different selectivity in a separation. Solvent groups were
placed within the triangle on the basis of the relative strength as proton acceptors, proton donors, or dipole interaction.

In making the choice of three solvents for optimizing selectivity, solvents were chosen from groups nearest to the three vertices of the triangle, so as to produce the largest differences in the solvent action.

Finally, the weak or strength adjusting solvent - water or hexane - was chosen. Therefore four solvents are recommended for carry out the optimization route.

In developing the 4-solvent technique, Kirkland and Glajch (123) used a triangle to define the entire selectivity space of a separation. This triangle may be plotted within the confines of several isocratic experiments using a combination of the four solvents selected.

To optimize the separation, first use a mixture of two of the four solvents, for example, methanol-water, to define one vertex of the triangle. The remaining two vertices are defined through the use of the THF-water and ACN-water solvents. By observing the chromatographic behavior in each of the solvent systems, a blend of the 4 systems can be achieved that will give an optimum separation.

Unfortunately, in the laboratory this becomes rather complicated both in theory and in practice. DuPont has commercialized this technique though through the use of a
computer analysis and graphic display. However the HPLC had to be designed also to have 4 solvent capability.

A number of other factors affect the selectivity factor in liquid chromatography besides the mobile-phase composition. Although not inclusive, these include stationary-phase composition, temperature, pH or other ionic effects, and secondary equilibria (125). The effect of a number of these are not fully understood. Thus, although greater separation through the use of greater number of solvent systems is possible, optimization becomes increasing more difficult unless a method is available for data analysis and handling.

A simplified strategy was used by J. C. Hodgin (126) for the separation of a number of pharmaceuticals. This method employed using binary mixtures of ACN-water and THF-water to outline a separation. By this method a series of solutions are prepared. A solution with a specific composition (for example, 60% ACN/40% water) that separated the front peaks but not the more retained, was combined with a solvent mixture (for example, 60% THF/40% water) that separated the later peaks but not the front peaks. By ratioing the solvent compositions, an initial tertiary mixture was made separated all the compounds reasonably well. This tertiary mixture was then adjusted using data from previous separations.

The second chromatographic technique used to optimize the separation of the organolead compounds was "ion-pair"
chromatography. The exact model for separation in ion-pair chromatography has been debated for quite some time as shown by the many names given to the technique. This technique has been called soap chromatography (127), solvent-generated (dynamic) ion-exchange chromatography (128, 129), hetereric chromatography (130), paired-ion chromatography (131), detergent-based cation-exchange chromatography (132), solvophobic ion-chromatography (133), surfactant chromatography (134), and most recently, ion-interaction chromatography (135, 136). As the large number of names suggest, a number of hypotheses have been proposed as to the separation mechanism.

The ion-interaction model (135, 136) is the only hypothesis that acknowledges the multiple forces that have been experimentally shown to affect separation in ion-pair liquid chromatography, particularly those systems that employed simple inorganic salts such as sodium chloride as the ion-pairing reagent.

The implications of this model were important to the design of solvent systems for the separation of the organolead compounds. The binding of the layer on the reversed phase TLC KC18 was disrupted for solvent mixtures containing greater than 40% water. To counter this effect, ion-pairing salts or ordinary sodium chloride are recommended in concentrations of 0.1 M (minimum) to 0.5 M ideal (137). This insured the physical stability of the layer.
The addition of the sodium chloride to the solvent mixture was not supposed to affect the separation according to the TLC manufacturer literature (137). This appeared to hold for the aqueous ethanol/sodium bromide systems (Table 12) in which the Rf of the organolead compounds did not change appreciable with sodium bromide concentration over the range of 0.10 to 0.50 molar.

However dramatic changes in Rf’s were evident for the aqueous acetonitrile/sodium bromide systems. As the sodium bromide concentration was increased the Rf’s increased.

There were also dramatic differences in mobility noted for sodium bromide versus sodium chloride at the same concentration. The Rf’s of the organoleads were greater for the solvent systems containing sodium chloride than for those with the same concentration of sodium bromide.

The ion-interaction model employs multiple electrostatic and adsorptive (lipophilic) forces as the basis of separation in ion pair chromatography. Additional support for the ion-interaction model has come from a number of recent reports (138, 139). Pietrzyk and Iskandarani (139) showed a relationship between the capacity factor and the inverse of the eluent ionic strength for several quaternary ammonium compounds.

Because ionic strength is an important factor in controlling the separation, it is necessary to specify ionic strength or ion pairing reagent concentrations when
reporting ion-pair systems. When attempting to reproduce an ion-pair system, attention should be given to using the same ionic strength with the same salt (140). The differences in separation results between sodium bromide and sodium chloride emphasizes this fact.

Therefore combination of both techniques, mixed solvent systems and ion pair chromatography, were used for the separation of the organolead compounds in this study. Binary mixtures of ACN-water, THF-water, and ethanol-water were chosen initially as the principle systems to be developed for separation of the organolead compounds. Coupled with this mixed solvent system technique was that of ion pair chromatography. The ion pair chromatography allowed for the utilization of another chromatographic separation technique and for the use of reverse phase TLC to complement the the screening and for investigative results.

Other systems were also evaluated because of the diversity of the samples (from TEL to the inorganic lead chloride). These were the tertiary systems of ACN-THF-water. Results from these systems proved useful in separation some of the organolead compounds and for "gradient elution" of TEL after the separation of the organolead halides.

Of the many HPLC and TLC solvent systems evaluated and developed simultaneously (Tables 10 I, II, III, IV), three solvent systems proved to be the most useful and effective
Table 10. Systems Evaluated for Separation of Organolead Compounds

I. ETHANOL SYSTEMS

A. Ethanol/Water/NaBr Systems

1. 90 Ethanol/10 Water
2. 90 Ethanol/10 Water/.10 M NaBr
3. 90 Ethanol/10 Water/.20 M NaBr
4. 90 Ethanol/10 Water/.50 M NaBr

5. 85 Ethanol/15 Water
6. 80 Ethanol/20 Water
7. 80 Ethanol/20 Water/.10 M NaBr
8. 80 Ethanol/20 Water/.50 M NaBr

9. 75 Ethanol/25 Water
10. 75 Ethanol/25 Water/.10 M NaBr
11. 75 Ethanol/25 Water/.20 M NaBr
12. 75 Ethanol/25 Water/.50 M NaBr

13. 60 Ethanol/40 Water
14. 60 Ethanol/40 Water/.10 M NaBr
15. 60 Ethanol/40 Water/.20 M NaBr
16. 60 Ethanol/40 Water/.50 M NaBr

B. Ethanol/Water/NaCl Systems

17. 60 Ethanol/40 Water/.10 M NaCl
18. 25 Ethanol/75 Water/1.0 M NaCl

90 Ethanol/10 Water/.10 M NaBr is a system that is 90 parts ethanol, 10 parts water (v/v), and 0.10 M in sodium bromide.
II. ACETONITRILE (ACN) SYSTEMS

A. Acetonitrile/Water/NaBr Systems

19. 90 ACN/10 Water
20. 90 ACN/10 Water/.05 M NaBr
21. 90 ACN/10 Water/.10 M NaBr
22. 85 ACN/15 Water
23. 80 ACN/20 Water
24. 80 ACN/20 Water/.10 M NaBr
25. 80 ACN/20 Water/.15 M NaBr
26. 70 ACN/30 Water
27. 70 ACN/30 Water/.10 M NaBr
28. 70 ACN/30 Water/.20 M NaBr
29. 60 ACN/40 Water/.10 M NaBr
30. 60 ACN/40 Water/.50 M NaBr
31. 55 ACN/45 Water/.43 M NaBr
32. 53 ACN/47 Water/.50 M NaBr
33. 50 ACN/50 Water
34. 50 ACN/50 Water/.05 M NaBr
35. 50 ACN/50 Water/.10 M NaBr
36. 50 ACN/50 Water/.25 M NaBr
37. 50 ACN/50 Water/.50 M NaBr
38. 50 ACN/50 Water/.75 M NaBr
39. 42 ACN/58 Water/1.1 M NaBr
40. 25 ACN/75 Water/.75 M NaBr
41. 25 ACN/75 Water/1.0 M NaBr

B. Acetonitrile/Water/NaCl Systems

42. 75 ACN/25 Water/.10 M NaCl
43. 55 ACN/45 Water/.10 M NaCl
44. 55 ACN/45 Water/.25 M NaCl
45. 55 ACN/45 Water/.38 M NaCl
46. 25 ACN/75 Water/1.0 M NaCl

90 ACN/10 Water/.05 M NaBr is a system that is 90 parts ACN, 10 parts water (v/v) and 0.05 molar in sodium bromide.
III. MIXED ACN/THF/METHANOL/ETHANOL/NaBr SYSTEMS

47. 90 ACN/10 Methanol
48. 80 ACN/20 Methanol
49. 80 ACN/20 Methanol/.15 M NaBr
50. 70 ACN/30 Methanol
51. 60 ACN/40 Methanol
52. 50 ACN/50 THF
53. 45 ACN/45 THF/10 Water
54. 45 ACN/45 THF/10 Water/.10 M NaBr
55. 45 ACN/45 THF/10 Methanol
56. 45 ACN/40 THF/15 Water/.15 M NaBr
57. 40 ACN/40 THF/20 Ethanol
58. 40 ACN/40 THF/10 Methanol/10 Water
59. 35 ACN/35 THF/30 Water
60. 35 ACN/35 THF/30 Water/.10 M NaBr
61. 35 ACN/35 THF/30 Water/.25 M NaBr
62. 25 ACN/35 THF/35 Water/.35 M NaBr

IV. TETRAHYDROFURAN (THF) SYSTEMS

63. 100 THF
64. 90 THF/10 Water
65. 90 THF/10 Methanol
66. 80 THF/20 Methanol
67. 70 THF/30 Water
68. 50 THF/50 Water

90 ACN/10 Methanol is a system that is 90 parts acetonitrile and 10 parts methanol (v/v).
Table 11. Retention Factors (Rf) for Organolead Compounds in Ethanol Based System

<table>
<thead>
<tr>
<th>System</th>
<th>Me₃Pb OAc</th>
<th>Et₃Pb Cl, OAc</th>
<th>Ph₃Pb OAc</th>
<th>Pb Ph₄Pb Cl₂</th>
<th>TEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Aqueous Ethanol/NaBr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90 EtOH/.10 M NaBr:</td>
<td>.84</td>
<td>.79</td>
<td>.79</td>
<td>.75</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>90 EtOH/.20 M NaBr:</td>
<td>.84</td>
<td>.79</td>
<td>.79</td>
<td>.71</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>90 EtOH/.50 M NaBr:</td>
<td>.86</td>
<td>.81</td>
<td>.81</td>
<td>.75</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>75 EtOH/.10 M NaBr:</td>
<td>.80</td>
<td>.73</td>
<td>.73</td>
<td>.55</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>75 EtOH/.20 M NaBr:</td>
<td>.79</td>
<td>.75</td>
<td>.75</td>
<td>.56</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>75 EtOH/.50 M NaBr:</td>
<td>.81</td>
<td>.74</td>
<td>.74</td>
<td>.55</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>60 EtOH/.10 M NaBr:</td>
<td>.73</td>
<td>.59</td>
<td>.59</td>
<td>.25</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>60 EtOH/.20 M NaBr:</td>
<td>.75</td>
<td>.62</td>
<td>.62</td>
<td>.30</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>60 EtOH/.50 M NaBr:</td>
<td>.74</td>
<td>.61</td>
<td>.61</td>
<td>.31</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>25 EtOH/1.0 M NaBr:</td>
<td>.37</td>
<td>.06</td>
<td>.06</td>
<td>.01</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>(b) Aqueous Ethanol/NaCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 EtOH/.10 M NaCl:</td>
<td>.67</td>
<td>.63</td>
<td>.63</td>
<td>.29</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>25 EtOH/1.0 M NaCl:</td>
<td>.50</td>
<td>.14</td>
<td>.14</td>
<td>.02</td>
<td>&lt;.02</td>
</tr>
</tbody>
</table>

a. 90 EtOH/.10 M NaBr means a system that is 90 parts ethanol, 10 parts water (v/v) and .10 molar in sodium bromide.

* Triethyllead specie in tetraethyllead sample easily detected.
Table 12. Retention Factors (Rf) for Organolead Compounds in Acetonitrile Based System

<table>
<thead>
<tr>
<th>System</th>
<th>Me₃Pb Cl₂</th>
<th>Et₃Pb Cl₂</th>
<th>Ph₃Pb Cl₂</th>
<th>Pb Cl₂</th>
<th>TEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Aqueous ACN/NaBr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>85 ACN/.15 M NaBr:</td>
<td>.92</td>
<td>.81</td>
<td>.81</td>
<td>.62</td>
<td>.02</td>
</tr>
<tr>
<td>80 ACN/.00 M NaBr:</td>
<td>.24</td>
<td>.55</td>
<td>.55</td>
<td>.54</td>
<td>.02</td>
</tr>
<tr>
<td>80 ACN/.10 M NaBr:</td>
<td>.94</td>
<td>.81</td>
<td>.81</td>
<td>.61</td>
<td>.08</td>
</tr>
<tr>
<td>55 ACN/.43 M NaBr:</td>
<td>.61</td>
<td>.38</td>
<td>.38</td>
<td>.16</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>25 ACN/.75 M NaBr:</td>
<td>.04</td>
<td>.04</td>
<td>.04</td>
<td>.00</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>25 ACN/1.0 M NaBr:</td>
<td>.23</td>
<td>.04</td>
<td>.04</td>
<td>.01</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>(b) Aqueous ACN/NaCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>55 ACN/.10 M NaCl:</td>
<td>.69</td>
<td>.50</td>
<td>.50</td>
<td>.17</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>55 ACN/.25 M NaCl:</td>
<td>.77</td>
<td>.56</td>
<td>.56</td>
<td>.21</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>(c) ACN/Methanol/NaBr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80 ACN/20 MeOH :</td>
<td>.04</td>
<td>.10</td>
<td>.10</td>
<td>.11</td>
<td>.58</td>
</tr>
<tr>
<td>80 ACN/20 MeOH/ .15 M NaBr</td>
<td>.91</td>
<td>.77</td>
<td>.77</td>
<td>.62</td>
<td>.08</td>
</tr>
</tbody>
</table>

a. 85 ACN/.15 M NaBr means a system that is 85 parts acetonitrile, 15 parts water (v/v) and 0.14 molar in sodium bromide.

Table 13. Systems For Separation of Diethyllead Dichloride

<table>
<thead>
<tr>
<th>System</th>
<th>Et₂Pb Cl₂</th>
<th>Me₃Pb OAc</th>
<th>Et₃Pb Cl₁</th>
<th>Ph₃Pb OAc</th>
<th>Ph₄Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 EtOH/.10 M NaBr:</td>
<td>.85</td>
<td>.80</td>
<td>.73</td>
<td>.47</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>25 ACN/ .10 M NaCl:</td>
<td>.81</td>
<td>.60</td>
<td>.50</td>
<td>.17</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>55 ACN/ .43 M NaBr:</td>
<td>.73</td>
<td>.61</td>
<td>.38</td>
<td>.38</td>
<td>&lt;.02</td>
</tr>
</tbody>
</table>

a. 75 Ethanol/.10 M NaBr means a system that is 75 parts ethanol, 25 parts water (v/v), and is .10 molar in sodium bromide.
for the speciation of the compounds of interest. The three systems were as follows:

1) 75 parts ethanol/25 parts water (v/v) and 0.10 molar in sodium bromide

2) 55 parts acetonitrile/45 parts water (v/v) and 0.43 molar in sodium bromide

3) 55 parts acetonitrile/45 parts water (v/v) and 0.10 molar in sodium chloride

Each of these systems will be described as to its particular advantage.

(1) 75 Ethanol/25 Water/0.10 Molar NaBr

The speciation by HPLC-AA of three organoleads, trimethyllead acetate, triethyllead chloride, and triphenyllead acetate with detection at the 283.3 nm lead line is shown in Figure 44. At least one additional impurity which eluted near the solvent front was also detected. The peak(s) was also present in varying amount in the three standards. No attempt was made at identification of this minor component, but separation of this specie serves to illustrate the potential of speciation using HPLC-AA.

The separation of these compounds by TLC with detection using dithizone is shown next to the HPLC-AA chromatogram (Figure 45). The order of elution is the same for both the reverse phase thin layer and for the
Figure 44. Speciation of Organoleads by HPLC-AA by Reversed-Phase Chromatography. Mobile Phase: 75 Ethanol/25 Water/0.10 Molar NaBP.
Figure 45. Comparison of TLC-Colorimetry and HPLC-AA Separations of Organolead Compounds. Mobile Phase: 75 Ethanol/25 Water/0.10 Molar NaBr.
HPLC.

Tetraphenyllead standard remained at the origin on the TLC plate. Several attempts to chromatograph tetraphenyllead on the HPLC-AA showed no elution of the sample. The tetraphenyllead was apparently retained on the guard column.

The most significant disadvantage of this system was the poorer resolution of the trimethyllead specie from the triethyllead specie as compared to the two other chromatographic systems which will be described.

The utility of this system is illustrated in the speciation of triethyllead specie from a tetraethyllead standard (Figure 46). At least two other lead compounds eluting close to the solvent front were detected. No attempts were made to identify these compounds. However, they serve to illustrate the potential of speciation by HPLC-AA.
Figure 46. Speciation of Triethyllead Chloride and TEL by HPLC-AA. Mobile Phase: 75 Ethanol/25 Water/0.10 Molar NaBr.
The HPLC-AA of standards of trimethyllead acetate and triethyllead chloride are shown in Figures 47A and 47B, respectively. A mixture of the two standards is shown in Figure 47C.

Based upon the retention time and the spiking of the sample, one of the small peaks in the triethyllead chloride sample was apparently due to a trace of trimethyllead impurity. The identity of this peak was also confirmed by retention factor and color comparison by TLC.

At least one additional trace impurity was detected by HPLC-AA in the triethyllead sample. This impurity was not identified, but it was postulated to be the diethyllead \( (2+) \) specie based upon subsequent TLC observations.

The 55 acetonitrile/45 water/0.43 molar sodium bromide eluent gave excellently shaped chromatography peaks, which are highly desirable for speciation. Unfortunately, the high salt content (0.43 molar) lead to a salt build-up on the nebulizer. The build-up of the salt rapidly decreased the performance of the nebulizer. The salt was also very corrosive to the tip of the aluminum nebulizer and some pitting of the surface was noted after even a short period of use.

The solution proved to be the best for separation of
Figure 47. Speciation of Alkylleads by Reversed-Phase HPLC-AA. Mobile Phase: 55ACN/45 Water/0.43 Molar NaBr.
the organoleads under study via thin layer chromatography with detection with dithizone (Figure 48). Excellent separation of the organoleads, along with a number of impurities in each of the standards, was achieved. This system provided the best resolution of the diethyllead specie from the other standards. The diethyllead was also not at the solvent front as was noted in a number of other systems.

Tetraphenyllead, tetraethyllead, and inorganic lead (2+) species did not migrate with this system, but remained at the origin. Thus this system was most ideally suited for the separation of the organoleads (1+, 2+) and not the saturated organolead or the inorganic leads.

Sensitivity was also excellent. Levels of 10 nanograms and less were routinely detected. This was due to a couple of reasons. The first was the high resolution and very compact and thus, concentrated zones observed. The second was the unique color development. Upon reaction with the dithizone, intense yellow dithizone-organolead complexes were formed on the TLC plates. However, equally important, the TLC plates changed to a dark brilliant blue, which provided an intense contrasting background for the bright yellow dithizone-lead complexes.

(3) 55 Acetonitrile/45 Water/0.10 Molar Sodium Chloride
Figure 48. Separation of Organoleads by Reversed-Phase TLC-Dithizone Colorimetry.
A solution of 55 acetonitrile/45 water/0.10 molar sodium chloride was selected as a compromise so that both samples could be speciated by HPLC-AA and by TLC-colorimetry. Resolution of the organoleads by HPLC-AA was just slightly less using this system than with the 55 ACN/45 Water/0.43 Molar NaBr. However, the lower salt content of 0.10 molar versus 0.43 molar of the former allowed for the nebulizer to function without clogging or severe salt build-up that is deleterious to nebulization.

The HPLC-AA of diethyllead, trimethyllead, triethyllead, and triphenyllead is shown in Figure 49. The ability to speciate all four in a single sample is presented in Figure 50.

The diethyllead sample used in this initial separation was impure (Figure 49A). A significant amount of triethyllead was found present. This lead to an investigation of the decomposition of diethyllead dichloride in the solid form.

The separation of triethyllead chloride from tetraethyllead is clearly illustrated the usefulness of speciation by HPLC-AA. (Figure 51). The separation of the triethyllead chloride was achieved using the 55 ACN/45 water/0.10 Molar NaCl system. The tetraethyllead was eluted by switching the HPLC eluent to 80 ACN/20 methanol (v/v).

The excellent capacity for speciation by HPLC-AA was
Figure 49. Separation of Organoleads by HPLC-AA. Mobile Phase: 55 ACN/45 Water/0.10 Molar NaCl.
Figure 50. Speciation of Oganoleads by HPLC-AA. Mobile Phase: 55ACN/45 Water/0.10 Molar NaCl.
Figure 51. Speciation of Triethyllead Ion from TEL by Gradient Elution HPLC-AA.
clearly illustrated in the separation of three extremely physiologically important alklyleads species: diethyllead (2+), trimethyllead (1+), and triethyllead (1+) (Figure 52). The diethyllead dichloride used for this study was a freshly prepared standard from Pfaltz and Bauer. The sample was prepared by Pfaltz and Bauer upon our request because the diethyllead dichloride in our stock was not pure. Upon checking their supply, they found that their supply had decomposed (verbal communication). This necessitated their preparing a new, pure standard.

All of the organolead compounds of interest in these investigations were easily and rapidly separated using this solvent system (Figure 53). Ten (10) centimeter developed was from 7 to 10 minutes, depending on the chromatographic conditions in the chamber.

It is recommended that freshly prepared solvent be added to the chamber approximately 15 minutes prior to plate development. This is recommended because the diethyllead dichloride runs very close to the solvent front. The diethyllead dichloride ran at or closer to the front when the solvent had been left in the TLC tank. This is due presumably to evaporation of some of the solvent over time which lead to poorer resolution of the diethyllead dichloride from the other lead compounds, and the tendency of the diethyllead dichloride to run at or near the solvent front.
Figure 52. Speciation of Alkylleads by HPLC-AA. Mobile Phase: 55% ACN/45 Water/0.10 Molar NaCl.
Figure 53. Separation of Organoleads by TLC-Dithizone Colorimetry. Mobile Phase: 55ACN/45 Water/0.10 Molar NaCl.
IE. (b) Results of Conversion of TEL in Sea Water Over a Long Time Period Study

Tetraethyllead (TEL) had been shown previously by GC-AA to convert to triethyllead chloride in sea water (94). The triethyllead chloride peak was very broad, indicating decomposition and poorer chromatographic resolution than by HPLC-AA. Also diethyllead dichloride and lead(2+) compounds could not be chromatographed by GC. Thus GC-AA did not allow for the determination of any of the dialkyllead or inorganic lead species.

The experimental set-up used for the conversion of TEL in sea water in HPLC-AA speciation studies were identical to the ones previously used in these laboratories for the conversion monitored by GC-AA. The allowed for the studies to be complementary. The GC-AA studies fully documented the conversion of TEL in sea water over a six month period (94).

The HPLC-AA study was to address two major questions:
1) Is the triethyllead species still present after longer times? The continued presence of triethyllead would indicate that the potential toxic effects of releases of TEL into the environment is more severe than if the TEL were more rapidly converted to less toxic forms, i.e., diethyllead or inorganic lead species. 2) Can diethyllead or inorganic lead species be detected or monitored? The presence of the species would confirm the previously proposed mechanism of TEL conversion, and
possibly allow for the rates of conversion to be studied.

The samples were analyzed periodically, primarily by TLC-colorimetry, over a period of 12 months in contrast to the GC-AA studies that were conducted over a six month period. HPLC-AA was used for the final analysis at the end of the 12 month period. TLC-colorimetry was used over the entire 12 month period because of the ease of use (entire plate chromatography and color development in less than 20 minutes) and its capability to detect all of the species desired including compounds remaining at the origin (these would also not elute in HPLC).

The sample storage conditions were stationary, and with light excluded. These two conditions were chosen because at the bottom of the ocean there is slow water movement (maximal movement or wave action is generally confined to essentially the surface); and because light penetrates only the first 20 of so feet of water in the ocean. Two variable conditions chosen were boiling and non-boiling of the sea water. There have been reports of methylation of lead by microorganisms in the environment. Thus one sample was boiled to kill any microorganisms present. Both boiled and non-boiled samples would be monitored to determine if there were any differences over the course of the study.

The results of the study for both the boiled and non-boiled were essentially the same over the course of the study. The chromatograph (Figure 54) shows both
Figure 54. Conversion of TEL in Seawater Over a Long Time Period (50 Weeks). Detection of Triethyllead Chloride by HPLC-AA.
boiled and non-boiled sea water samples spiked with TEL at the end of the 12 month study. Essentially no difference was detected indicating that either microbiological conversion did not play a role in the conversion process for these samples. This does not mean that microbiological conversion is not important in the environment. In these samples the relative amount of TEL is high, and may exert too great a toxic effect on the original microorganisms present. In the environment there is a greater opportunity for the microorganism to replenish itself.

What is significant is that triethyllead is still present in high concentrations even after one year. This shows that triethyllead species are very stable in environment. Thus the potential toxic effects from release of TEL may persist for a long time period. Results of Grove indicated that triethyllead dichloride and lead ion were very slow in forming for sea water spiked with TEL (141). These experimental conditions were considerably different than in this report, and analysis was colorimetric versus the direct method here. These results were supported by GC-AA analysis of TEL and triethyllead in seawater previously studied (94).

Equally important was that no diethyllead dichloride was detected. However this does not mean that diethyllead is not formed in the conversion process. The non-detection of diethyllead dichloride in the sample may
mean that the conversion of triethyllead to diethyllead dichloride is very slow and the conversion of diethyllead dichloride to the inorganic lead species is very fast. Thus the concentration at any one time is very low, essentially below the limits of detection of this technique. Diethyllead dichloride has been shown to decompose rapidly in water (142, 143), however not sea water. It is also known to be unstable even in solid form, which is discussed in even more detail in the following section.

Since the inorganic lead was not chromatographed on this isocratic system and thus was not detected, the complementary TLC-colorimetric system was used to simultaneously monitor the conversion process. The TLC of standard triethyllead chloride and the 12 month old sea water sample spiked with TEL is shown in Figure 54. The presence of a large amount of triethyllead species was confirmed by comparison of the Rf and visual color comparison.

No diethyllead dichloride was detectable in the sea water sample at the loading spotted. However, increasing amount of dithizone positive species were detected at the origin. Inorganic leads remain at the origin in the chromatographic system. This confirms that inorganic lead(2+) is one of the end products of the conversion process of TEL in sea water.

Addition studies of the conversion process of TEL in
Figure 55. Conversion of TEL in Seawater Over a Long Time Period (50 Weeks). Detection of Triethylead Chloride by TLC-Dithizone Colorimetry.
sea water would be readily possible with the right equipment. These studies were done isocratically because the HPLC did not have the capability of gradient elution. Therefore the solvent system was designed for maximum resolution of the alkyllead species, which was the primary focus. Gradient elution would allow for the initial separation of the alkylleads and then the chromatographic elution and detection of the inorganic lead.

IE.(c) Results of Studies on the Decomposition of Solid Diethyllead Dichloride

The study of the decomposition of the solid diethyllead dichloride was prompted by observations during the initial stages of development of the solvent system for the separation of the alkylleads. A bottle of diethyllead dichloride (Alfa Chemicals, lot 57126) was being used to prepare standard solutions used in the chromatography. This sample had been recently purchased (less than one year old) for use by another research group in the department.

On TLC this sample generally gave three major zones. The first zone had an Rf identical to that of the triethyllead chloride, the second zone was at the origin, and the third zone generally migrated slightly ahead of the triethyllead zone. This indicated that the sample was not pure but had decomposed considerably.
Diethyllead dichloride is a white compound that decomposes at 220 °C. Visual inspection of the sample showed discoloration of part of the sample. A melting point was taken on the sample. The sample started melting/decomposing from 170 °C - 220 °C.

Therefore a mass spectra were obtained for the diethyllead dichloride sample, triethyllead chloride, and for tetraethyllead. The mass spectra for TEL is in Figure 56. This shows the standard fragmentation pattern for TEL. It is interesting to note that no triethyllead chloride is apparent in the mass spectrum, although triethyllead was easily separated and detected by HPLC-AA. This is due to the volatility of the TEL versus the triethyllead chloride. TEL is very volatile; triethyllead has a much lower volatility. Thus the mass spectrum shows only the much more volatile tetraethyllead.

Mass spectra for both solid and aqueous solutions of the diethyllead dichloride sample and the triethyllead chloride were obtained. The mass spectra for the diethyllead dichloride solid is shown in Figure 57, and for aqueous solution in Figure 58. No parent peak for diethyllead dichloride was obtained. The fragmentation pattern was that for triethyllead chloride. The mass spectra for standard triethyllead chloride, solid and in aqueous solution, are given in Figures 59 and 60, respectively.

The mass spectra were obtained 125 °C for the solid
Figure 56. Mass Spectrum of TEL.
Table 14. Mass Spectral Data for TEL.

<table>
<thead>
<tr>
<th>MASS</th>
<th>ABUND</th>
</tr>
</thead>
<tbody>
<tr>
<td>51</td>
<td>.2</td>
</tr>
<tr>
<td>53</td>
<td>.4</td>
</tr>
<tr>
<td>55</td>
<td>1.7</td>
</tr>
<tr>
<td>57</td>
<td>3.2</td>
</tr>
<tr>
<td>58</td>
<td>.1</td>
</tr>
<tr>
<td>59</td>
<td>.2</td>
</tr>
<tr>
<td>67</td>
<td>.2</td>
</tr>
<tr>
<td>69</td>
<td>.6</td>
</tr>
<tr>
<td>70</td>
<td>.2</td>
</tr>
<tr>
<td>71</td>
<td>1.1</td>
</tr>
<tr>
<td>73</td>
<td>.2</td>
</tr>
<tr>
<td>77</td>
<td>.2</td>
</tr>
<tr>
<td>83</td>
<td>.6</td>
</tr>
<tr>
<td>85</td>
<td>.7</td>
</tr>
<tr>
<td>87</td>
<td>.3</td>
</tr>
<tr>
<td>89</td>
<td>.2</td>
</tr>
<tr>
<td>91</td>
<td>.2</td>
</tr>
<tr>
<td>98</td>
<td>1.0</td>
</tr>
<tr>
<td>99</td>
<td>1.2</td>
</tr>
<tr>
<td>101</td>
<td>.2</td>
</tr>
<tr>
<td>115</td>
<td>.4</td>
</tr>
<tr>
<td>127</td>
<td>.3</td>
</tr>
<tr>
<td>128</td>
<td>.5</td>
</tr>
<tr>
<td>129</td>
<td>.6</td>
</tr>
<tr>
<td>131</td>
<td>.2</td>
</tr>
<tr>
<td>141</td>
<td>1.2</td>
</tr>
<tr>
<td>142</td>
<td>.2</td>
</tr>
<tr>
<td>151</td>
<td>.2</td>
</tr>
<tr>
<td>152</td>
<td>.2</td>
</tr>
<tr>
<td>153</td>
<td>.2</td>
</tr>
<tr>
<td>155</td>
<td>.3</td>
</tr>
<tr>
<td>156</td>
<td>.5</td>
</tr>
<tr>
<td>157</td>
<td>.3</td>
</tr>
<tr>
<td>169</td>
<td>.2</td>
</tr>
<tr>
<td>198</td>
<td>.3</td>
</tr>
<tr>
<td>204</td>
<td>1.0</td>
</tr>
<tr>
<td>206</td>
<td>17.9</td>
</tr>
<tr>
<td>207</td>
<td>33.5</td>
</tr>
<tr>
<td>208</td>
<td>55.7</td>
</tr>
<tr>
<td>209</td>
<td>36.1</td>
</tr>
<tr>
<td>220</td>
<td>.4</td>
</tr>
<tr>
<td>221</td>
<td>.6</td>
</tr>
</tbody>
</table>

FRN 6017 SPECTRUM 12 RET. TIME = .5

<table>
<thead>
<tr>
<th>MASS</th>
<th>ABUND</th>
</tr>
</thead>
<tbody>
<tr>
<td>222</td>
<td>.7</td>
</tr>
<tr>
<td>223</td>
<td>.4</td>
</tr>
<tr>
<td>231</td>
<td>.3</td>
</tr>
<tr>
<td>233</td>
<td>3.7</td>
</tr>
<tr>
<td>235</td>
<td>43.0</td>
</tr>
<tr>
<td>236</td>
<td>44.4</td>
</tr>
<tr>
<td>238</td>
<td>2.6</td>
</tr>
<tr>
<td>249</td>
<td>.2</td>
</tr>
<tr>
<td>250</td>
<td>.2</td>
</tr>
<tr>
<td>251</td>
<td>.4</td>
</tr>
<tr>
<td>253</td>
<td>.2</td>
</tr>
<tr>
<td>261</td>
<td>.5</td>
</tr>
<tr>
<td>263</td>
<td>6.2</td>
</tr>
<tr>
<td>264</td>
<td>12.0</td>
</tr>
<tr>
<td>265</td>
<td>23.9</td>
</tr>
<tr>
<td>266</td>
<td>17.5</td>
</tr>
<tr>
<td>267</td>
<td>10.3</td>
</tr>
<tr>
<td>268</td>
<td>.4</td>
</tr>
<tr>
<td>270</td>
<td>.4</td>
</tr>
<tr>
<td>291</td>
<td>2.2</td>
</tr>
<tr>
<td>293</td>
<td>29.7</td>
</tr>
<tr>
<td>294</td>
<td>31.7</td>
</tr>
<tr>
<td>295</td>
<td>66.3</td>
</tr>
<tr>
<td>296</td>
<td>4.3</td>
</tr>
</tbody>
</table>
Figure 57. Mass Spectrum of Decomposed Diethyllead Dichloride Standard.
Table 15. Mass Spectral Data For Decomposed Diethyllead Dichloride Standard.

<table>
<thead>
<tr>
<th>MASS</th>
<th>ABUND</th>
<th>MASS</th>
<th>ABUND</th>
<th>MASS</th>
<th>ABUND</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>3.9</td>
<td>238</td>
<td>2.4</td>
<td>289</td>
<td>1.5</td>
</tr>
<tr>
<td>41</td>
<td>1.0</td>
<td>239</td>
<td>1.9</td>
<td>293</td>
<td>11.4</td>
</tr>
<tr>
<td>43</td>
<td>1.2</td>
<td>241</td>
<td>29.1</td>
<td>294</td>
<td>11.4</td>
</tr>
<tr>
<td>44</td>
<td>1.7</td>
<td>242</td>
<td>26.6</td>
<td>295</td>
<td>26.2</td>
</tr>
<tr>
<td>45</td>
<td>1.1</td>
<td>243</td>
<td>68.4</td>
<td>296</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>297</td>
<td>1.1</td>
</tr>
<tr>
<td>55</td>
<td>1.1</td>
<td>244</td>
<td>9.6</td>
<td>299</td>
<td>17.5</td>
</tr>
<tr>
<td>57</td>
<td>1.3</td>
<td>245</td>
<td>20.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>0.7</td>
<td>263</td>
<td>0.8</td>
<td>301</td>
<td>44.3</td>
</tr>
<tr>
<td>71</td>
<td>0.8</td>
<td>264</td>
<td>3.8</td>
<td>302</td>
<td>8.3</td>
</tr>
<tr>
<td>149</td>
<td>1.2</td>
<td>266</td>
<td>9.7</td>
<td>303</td>
<td>11.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>267</td>
<td>2.4</td>
<td>304</td>
<td>0.3</td>
</tr>
<tr>
<td>204</td>
<td>1.7</td>
<td>269</td>
<td>1.0</td>
<td>328</td>
<td>1.2</td>
</tr>
<tr>
<td>206</td>
<td>20.0</td>
<td>270</td>
<td>2.7</td>
<td>329</td>
<td>1.2</td>
</tr>
<tr>
<td>207</td>
<td>43.3</td>
<td>271</td>
<td>5.3</td>
<td>330</td>
<td>2.5</td>
</tr>
<tr>
<td>208</td>
<td>80.5</td>
<td>331</td>
<td>1.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>209</td>
<td>29.7</td>
<td>272</td>
<td>7.2</td>
<td>332</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>273</td>
<td>5.7</td>
<td>333</td>
<td>0.8</td>
</tr>
<tr>
<td>221</td>
<td>1.0</td>
<td>274</td>
<td>2.4</td>
<td>334</td>
<td>0.8</td>
</tr>
<tr>
<td>222</td>
<td>1.0</td>
<td>275</td>
<td>1.5</td>
<td>335</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>285</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>233</td>
<td>3.9</td>
<td>286</td>
<td>1.0</td>
<td>345</td>
<td>1.5</td>
</tr>
<tr>
<td>235</td>
<td>44.9</td>
<td>287</td>
<td>2.4</td>
<td>347</td>
<td>0.8</td>
</tr>
<tr>
<td>236</td>
<td>39.0</td>
<td>288</td>
<td>2.4</td>
<td>393</td>
<td>1.7</td>
</tr>
<tr>
<td>237</td>
<td>100.0</td>
<td>289</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 58. Mass Spectrum of Decomposed Diethyllead Dichloride, Aqueous Solution.

<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</td>
<td>89</td>
<td>.3</td>
<td>241</td>
<td>8.1</td>
<td>291</td>
<td>.5</td>
</tr>
<tr>
<td>36</td>
<td>1.0</td>
<td>95</td>
<td>.4</td>
<td>242</td>
<td>25.8</td>
<td>293</td>
<td>9.6</td>
</tr>
<tr>
<td>38</td>
<td>1.5</td>
<td>97</td>
<td>.5</td>
<td>243</td>
<td>64.1</td>
<td>294</td>
<td>9.2</td>
</tr>
<tr>
<td>40</td>
<td>3.7</td>
<td></td>
<td></td>
<td>295</td>
<td>19.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>1.4</td>
<td>109</td>
<td>.3</td>
<td>244</td>
<td>3.0</td>
<td>296</td>
<td>1.5</td>
</tr>
<tr>
<td>42</td>
<td>.9</td>
<td></td>
<td></td>
<td>245</td>
<td>19.5</td>
<td>297</td>
<td>.8</td>
</tr>
<tr>
<td>43</td>
<td>1.6</td>
<td></td>
<td></td>
<td>246</td>
<td>.4</td>
<td>299</td>
<td>13.2</td>
</tr>
<tr>
<td>44</td>
<td>3.2</td>
<td>149</td>
<td>.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>5.6</td>
<td></td>
<td></td>
<td>263</td>
<td>6.6</td>
<td>300</td>
<td>12.6</td>
</tr>
<tr>
<td>55</td>
<td>1.1</td>
<td>206</td>
<td>35.0</td>
<td>264</td>
<td>3.8</td>
<td>301</td>
<td>34.2</td>
</tr>
<tr>
<td>56</td>
<td>1.4</td>
<td>207</td>
<td>47.9</td>
<td>265</td>
<td>5.0</td>
<td>302</td>
<td>6.0</td>
</tr>
<tr>
<td>57</td>
<td>1.4</td>
<td>208</td>
<td>30.7</td>
<td>266</td>
<td>7.7</td>
<td>303</td>
<td>9.9</td>
</tr>
<tr>
<td>58</td>
<td>1.3</td>
<td>209</td>
<td>34.6</td>
<td>267</td>
<td>1.6</td>
<td>304</td>
<td>.5</td>
</tr>
<tr>
<td>59</td>
<td>1.4</td>
<td>270</td>
<td>2.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>3.3</td>
<td>220</td>
<td>6.6</td>
<td>271</td>
<td>3.9</td>
<td>316</td>
<td>.4</td>
</tr>
<tr>
<td>61</td>
<td>7.7</td>
<td>222</td>
<td>1.1</td>
<td>272</td>
<td>6.5</td>
<td>318</td>
<td>.4</td>
</tr>
<tr>
<td>62</td>
<td>1.7</td>
<td>231</td>
<td>.5</td>
<td>274</td>
<td>1.7</td>
<td>328</td>
<td>.9</td>
</tr>
<tr>
<td>66</td>
<td>1.9</td>
<td>232</td>
<td>.4</td>
<td>275</td>
<td>1.1</td>
<td>329</td>
<td>.8</td>
</tr>
<tr>
<td>71</td>
<td>1.6</td>
<td>233</td>
<td>4.1</td>
<td>285</td>
<td>1.4</td>
<td>330</td>
<td>1.9</td>
</tr>
<tr>
<td>73</td>
<td>.4</td>
<td>235</td>
<td>44.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>81</td>
<td>.5</td>
<td>237</td>
<td>100.0</td>
<td>287</td>
<td>3.9</td>
<td>333</td>
<td>.5</td>
</tr>
<tr>
<td>83</td>
<td>.4</td>
<td>238</td>
<td>2.5</td>
<td>288</td>
<td>1.2</td>
<td>334</td>
<td>.5</td>
</tr>
<tr>
<td>85</td>
<td>.5</td>
<td>239</td>
<td>1.6</td>
<td>289</td>
<td>2.2</td>
<td>335</td>
<td>1.3</td>
</tr>
</tbody>
</table>

(CONT)
Figure 59. Mass Spectrum of Triethyllead Chloride Standard.
Table 17. Mass Spectral Data for Triethyllead Chloride Standard.

<table>
<thead>
<tr>
<th>MASS</th>
<th>ABUND</th>
<th>MASS</th>
<th>ABUND</th>
<th>MASS</th>
<th>ABUND</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>1.2</td>
<td>244</td>
<td>10.4</td>
<td>296</td>
<td>1.7</td>
</tr>
<tr>
<td>41</td>
<td>4</td>
<td>245</td>
<td>20.8</td>
<td>297</td>
<td>1.4</td>
</tr>
<tr>
<td>43</td>
<td>4</td>
<td>246</td>
<td>4</td>
<td>299</td>
<td>10.4</td>
</tr>
<tr>
<td>204</td>
<td>1.7</td>
<td>263</td>
<td>1.0</td>
<td>300</td>
<td>21.0</td>
</tr>
<tr>
<td>206</td>
<td>29.4</td>
<td>264</td>
<td>4.4</td>
<td>301</td>
<td>52.2</td>
</tr>
<tr>
<td>207</td>
<td>40.7</td>
<td>265</td>
<td>6.1</td>
<td>302</td>
<td>9.2</td>
</tr>
<tr>
<td>208</td>
<td>77.1</td>
<td>266</td>
<td>10.4</td>
<td>303</td>
<td>14.4</td>
</tr>
<tr>
<td>209</td>
<td>27.1</td>
<td>267</td>
<td>2.4</td>
<td>304</td>
<td>0.7</td>
</tr>
<tr>
<td>210</td>
<td>27.1</td>
<td>269</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>220</td>
<td>0.7</td>
<td>270</td>
<td>2.8</td>
<td>317</td>
<td>0.4</td>
</tr>
<tr>
<td>221</td>
<td>0.8</td>
<td>271</td>
<td>4.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>222</td>
<td>0.8</td>
<td>272</td>
<td>7.6</td>
<td>329</td>
<td>0.9</td>
</tr>
<tr>
<td>223</td>
<td>3.8</td>
<td>273</td>
<td>5.9</td>
<td>330</td>
<td>2.3</td>
</tr>
<tr>
<td>231</td>
<td>6.6</td>
<td>274</td>
<td>2.0</td>
<td>331</td>
<td>0.5</td>
</tr>
<tr>
<td>232</td>
<td>5.5</td>
<td>275</td>
<td>1.4</td>
<td>332</td>
<td>0.7</td>
</tr>
<tr>
<td>233</td>
<td>3.8</td>
<td>285</td>
<td>6</td>
<td>335</td>
<td>0.5</td>
</tr>
<tr>
<td>235</td>
<td>45.3</td>
<td>286</td>
<td>6</td>
<td>343</td>
<td>0.4</td>
</tr>
<tr>
<td>236</td>
<td>40.7</td>
<td>287</td>
<td>1.6</td>
<td>344</td>
<td>0.5</td>
</tr>
<tr>
<td>237</td>
<td>100.0</td>
<td>288</td>
<td>1.1</td>
<td>345</td>
<td>1.0</td>
</tr>
<tr>
<td>238</td>
<td>2.5</td>
<td>289</td>
<td>1.1</td>
<td>346</td>
<td>0.4</td>
</tr>
<tr>
<td>239</td>
<td>1.9</td>
<td>291</td>
<td>0.7</td>
<td>347</td>
<td>0.6</td>
</tr>
<tr>
<td>241</td>
<td>29.4</td>
<td>293</td>
<td>12.3</td>
<td>PAUSE</td>
<td></td>
</tr>
<tr>
<td>242</td>
<td>28.1</td>
<td>294</td>
<td>12.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>243</td>
<td>67.7</td>
<td>295</td>
<td>25.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Figure 60.** Mass Spectrum of Triethyllead Chloride, Aqueous Solution.

<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>.2</td>
<td>73</td>
<td>.5</td>
<td>106</td>
<td>.2</td>
<td>151</td>
<td>.2</td>
</tr>
<tr>
<td>36</td>
<td>.2</td>
<td>75</td>
<td>.3</td>
<td>107</td>
<td>.2</td>
<td>157</td>
<td>.2</td>
</tr>
<tr>
<td>39</td>
<td>.5</td>
<td>80</td>
<td>.6</td>
<td>111</td>
<td>.6</td>
<td>165</td>
<td>.3</td>
</tr>
<tr>
<td>40</td>
<td>3.2</td>
<td>89</td>
<td>.5</td>
<td>119</td>
<td>.6</td>
<td>163</td>
<td>.2</td>
</tr>
<tr>
<td>41</td>
<td>1.9</td>
<td>90</td>
<td>.2</td>
<td>122</td>
<td>.2</td>
<td>167</td>
<td>.2</td>
</tr>
<tr>
<td>42</td>
<td>.5</td>
<td>81</td>
<td>.5</td>
<td>112</td>
<td>.2</td>
<td>171</td>
<td>.2</td>
</tr>
<tr>
<td>43</td>
<td>2.3</td>
<td>82</td>
<td>.4</td>
<td>113</td>
<td>.2</td>
<td>167</td>
<td>.2</td>
</tr>
<tr>
<td>44</td>
<td>1.2</td>
<td>83</td>
<td>.2</td>
<td>115</td>
<td>.2</td>
<td>179</td>
<td>.2</td>
</tr>
<tr>
<td>45</td>
<td>2.8</td>
<td>84</td>
<td>.9</td>
<td>119</td>
<td>.3</td>
<td>177</td>
<td>.2</td>
</tr>
<tr>
<td>46</td>
<td>2.8</td>
<td>85</td>
<td>.3</td>
<td>121</td>
<td>.2</td>
<td>179</td>
<td>.2</td>
</tr>
<tr>
<td>53</td>
<td>.2</td>
<td>87</td>
<td>.3</td>
<td>122</td>
<td>.2</td>
<td>204</td>
<td>2.1</td>
</tr>
<tr>
<td>54</td>
<td>1.8</td>
<td>89</td>
<td>.2</td>
<td>124</td>
<td>.2</td>
<td>206</td>
<td>3.9</td>
</tr>
<tr>
<td>55</td>
<td>1.9</td>
<td>90</td>
<td>.3</td>
<td>125</td>
<td>.3</td>
<td>207</td>
<td>43.8</td>
</tr>
<tr>
<td>56</td>
<td>1.8</td>
<td>91</td>
<td>.3</td>
<td>127</td>
<td>.2</td>
<td>208</td>
<td>87.8</td>
</tr>
<tr>
<td>57</td>
<td>1.8</td>
<td>93</td>
<td>.3</td>
<td>128</td>
<td>.2</td>
<td>209</td>
<td>31.7</td>
</tr>
<tr>
<td>58</td>
<td>4.4</td>
<td>94</td>
<td>.2</td>
<td>129</td>
<td>.2</td>
<td>218</td>
<td>.2</td>
</tr>
<tr>
<td>59</td>
<td>4.4</td>
<td>95</td>
<td>.1</td>
<td>130</td>
<td>.2</td>
<td>219</td>
<td>.3</td>
</tr>
<tr>
<td>60</td>
<td>.5</td>
<td>96</td>
<td>.3</td>
<td>133</td>
<td>.2</td>
<td>220</td>
<td>.2</td>
</tr>
<tr>
<td>61</td>
<td>.5</td>
<td>97</td>
<td>.2</td>
<td>133</td>
<td>.2</td>
<td>222</td>
<td>.3</td>
</tr>
<tr>
<td>62</td>
<td>2.6</td>
<td>99</td>
<td>.3</td>
<td>139</td>
<td>.2</td>
<td>223</td>
<td>.4</td>
</tr>
<tr>
<td>63</td>
<td>.7</td>
<td>100</td>
<td>.5</td>
<td>141</td>
<td>.2</td>
<td>223</td>
<td>.4</td>
</tr>
<tr>
<td>64</td>
<td>1.3</td>
<td>101</td>
<td>.3</td>
<td>149</td>
<td>.9</td>
<td>231</td>
<td>.5</td>
</tr>
<tr>
<td>65</td>
<td>4.4</td>
<td>102</td>
<td>.2</td>
<td>150</td>
<td>.2</td>
<td>233</td>
<td>3.8</td>
</tr>
</tbody>
</table>

<CONT>
samples and at 50 °C for the aqueous samples. This are well below the decompositon temperature for diethyllead dichloride. Therefore it is apparent that the mass spectra for the diethyllead dichloride sample reflects the results from the more volatile triethyllead chloride present. Thus the mass spectra indicated that the diethyllead dichloride had decomposed, and that triethyllead chloride was one of the decomposition products.

Alfa Chemicals was contacted. Their subsequent analysis of the "house sample" taken from this lot confirmed that the sample had decomposed (verbal communication).

However, subsequent HPLC-AA analysis of this sample showed that it had only partially decomposed (Figure 49A). The sample did have a large portion of triethyllead chloride present. The presence of trialkyllead and lead(2+) impurities due to sample instability in commercially supplied dialkyllead compounds have been reported previously (98, 99). These analyses, however, were not direct, but either colorimetric determination, or conversion of the trialkyllead to dialkyllead with ICI followed by extraction and subsequent analysis by atomic absorption.

These initial results prompted a study of the decomposition of diethyllead dichloride by HPLC- AA, and TLC-colorimetry. The HPLC- AA offered separation and
specific metal detection by AA; the TLC-colorimetry offered complementary analysis and detection via standard colorimetric reagents.

New diethyllead dichloride was prepared especially upon our request to Pfaltz and Bauer Chemicals. This new standard sample was monitored by HPLC-AA and by TLC-colorimetry.

The HPLC-AA of this material (Pfaltz and Bauer, lot 01800) over a 10 month period is given in Figure 61. The newly prepared material was primarily diethyllead dichloride. However, a slight trace of triethyllead chloride is detected. After approximately 6 months, the sample has partially decomposed with a significant amount of triethyllead chloride now present. At the end of approximately 10 months triethyllead chloride was the main species detectable in the sample by HPLC-AA.

Since the presence of inorganic lead species could not be detected by HPLC-AA, the complementary TLC-colorimetry was used to check for their presence. Two colorimetric reagents, dithizone and PAR, was used for detection of the lead species on the TLC plates. An example of their usefulness is given in Figure 62. As the diethyllead decomposed, the parent diethyllead dichloride, triethyllead chloride, and inorganic lead(2+) compounds could be monitored with dithizone as the colorimetric reagent. Figure 62 depicts some of the results obtained for the partially decomposed diethyllead dichloride
Figure 61. Decomposition of Diethyllead Dichloride in Solid Form. Speciation of Triethyllead Chloride by HPLC-AA.
Figure 62. TLC-Colorimetry of Decomposed Diethyllead Dichloride.
sample.

Since PAR reacts only with dialkyllead(2+) and lead(2+) species, and not trialkyllead species, the second chromatogram depicts the result from dipping a duplicate plate into a PAR developing solution. Only the diethyllead dichloride species and the inorganic lead(2+) compounds at the origin are visualized. Unfortunately, the PAR reagent is much less sensitive as a visualization dip for TLC chromatography than dithizone.

Although the results were qualitative, TLC-colorimetry did confirm the decomposition and loss of diethyllead dichloride over the course of this study and the formation of trialkyllead and inorganic lead (2+) compounds. The method was rapid and extremely useful. Moreover, with the use of a scanning TLC spectrodensitometer quantitative results might be achieved.

1F. SUMMARY OF RESULTS

A HPLC-AA speciation method was developed that separates and determines directly a number of organolead species, including diethyllead dichloride, triethyllead(+), trimethyllead(+), and triphenyllead(+) species. These compounds have significant toxicological properties and are important in enviromental and physiological terms.

The HPLC-AA method was applied to the study of the conversion of TEL in enviromental samples to the more
toxic triethyllead species, and to decomposition of diethyllead dichloride in solid form.

Supplementary TLC-colorimetry methods using either dithizone or PAR as the colorimetric reagent allowed for a rapid, qualitative monitoring of the organolead species under investigation.
PART II. CHAPTER 2. SPECIATION OF ORGANOMERCURIALS BY HPLC-AA AND TLC-COLORIMETRY

2A. GENERAL INTRODUCTION

Many mercury compounds have proven very beneficial to mankind and have thus been widely used for industrial and medicinal purposes. However, the toxicity of mercury compounds have also been the subject of worldwide study for a number of years. The toxicity of mercury compounds and their effects on mankind and his environment is highly documented (144, 145, 146).

The toxicity of mercury depends on both its concentration, and its chemical form. The chemical form determines the physiological properties as to the degree of absorption, transport, biotransformation, retention and excretion modes. Alkyl mercurials are many times more toxic than aryl or inorganic mercurials. This difference is attributed to its chemical form.

Methylmercury ion, for example, is a better inhibitor of membrane-bound enzymes than inorganic mercuric ion (147), and can penetrate the hydrophobic core of some proteins better than the mercuric ion (148).

The chemical form determines to a large extent the degree of absorption by the human body. It has been estimated that more than 70% of methylmercury chloride that is ingested is eventually absorbed by the human body, in contrast to only 5% absorption of ingested mercuric
chloride (146).

The chemical and toxicological properties of dimethylmercury have been recently reviewed (149, 150). Dimethylmercury is readily absorbed and is found concentrated almost entirely in the lipids. Its toxicological properties vastly increase with its ionization to the methylmercury(+) specie.

The toxicity potential of elemental mercury is greatly enhanced when it is in gaseous form. As a vapor it is a non-polar, highly diffusible gas which is soluble in lipids. This allows for rapid transfer through the tissues of the lungs into the bloodstream, and consequently across the blood-brain barrier (151, 152).

The mechanisms by which mercury compounds exert their toxic effects are areas of intense investigation (144). For example, the effects of methylmercury exposure on the concentration of the brain biogenic amines, and on the activities of important regulating enzymes involved in synthesis and degradation of the monoamines have been studied (153, 154). Partial characterization of a low-molecular weight methylmercury complex in the rat brain has been investigated (155).

Since the effects of mercury is dependent not only on its concentration but its chemical form, analytical techniques are needed that can speciate the different mercury compounds.
2B. ANALYTICAL DETERMINATION AND SPECIATION

2B(a) General Analytical Techniques

Several techniques have been used to determine various mercury compounds. These include isotope exchange methods for inorganic mercury in the presence of organic mercury in biological systems (156). Methylmercury and mercury in digested hair samples have been determined by substiochiometric isotope dilution (157).

Volatile mercury compounds hand elemental mercury vapor in air have been determined through the use of selective adsorption tubes (58).

Cold-vapor atomic absorption spectroscopy have been used to selectively analyze for different mercury compounds (159 – 163). By controlling the reducing conditions during analysis, the different forms of mercury could be selectively released from the sample solution.

The techniques generally employ elaborate chemical pretreatment of the sample including digestion, clean-up, separation, extraction, and concentration steps. Extreme caution must be exercised during such pretreatment steps to avoid contamination of the sample, loss of the analytes either through volatization or to the container, or changes in the chemical nature of the species present.

Differences in volatization from a heated platinum loop or glass rod with subsequent detection by atomic absorption spectroscopy have been used to differentiate a
number of mercury compounds and complexes (164). Unfortunately the peaks were rather broad and additional development work necessary for control of the volatization parameters.

A two-stage atomic absorption spectrophotometer has been used to investigate mercury species based upon their volatization (165). Profiles of mercuric salts of the acetate, bromide, chloride, and sulfate were obtained. This method was not applied, however, to the organomercurials.

2B.(b) Methods of Speciation Analysis

Speciation methods that employ a chromatographic separation prior to detection offers a number of significant advantages over other techniques. The most obvious advantage is that the compound of interest can been separated physically from similiar organomercurials and inorganic mercury species and often from a number of possible spectral and physically matrix interferences contained in the original sample.

Techniques that employ chromatography in the determination of mercurials have been reviewed (166, 167). These techniques have met with varying degrees of success in the differentiation of alkyl, aryl, and inorganic mercurials.

(1) Gas Chromatographic Methods

The use of gas chromatography in the speciation of
mercurials has been reviewed (166 - 167). Methylmercury chloride was determined in seawater by gas chromatography with detection by atmospheric pressure helium microwave-induced plasma emission spectroscopy (168). Analysis was performed after a preconcentration involving a number of extraction and back-extraction procedures with an estimated efficiency of approximately 48%. This procedure was based on the Westoo procedure (169, 170, 171) which involves the formation of a water-soluble adduct of methylmercury and cysteine, its extraction into water, acidification, and finally the extraction of the liberated CH$_3$HgX with an aromatic solvent (benzene or toluene). The CH$_3$HgX is then determined by GC with electron capture detection (GC/ECD). Electron capture detection is highly sensitive but is not a mercury specific detector.

Seven diorganomercury compounds were separated by GC with detection by atmospheric pressure active nitrogen (APAN) afterglow (172). This method was applied only to aqueous mercurial standards.

The speciation of inorganic mercury and alkylmercurials in human biological fluids (sweat, urine) by GC with detection by atomic absorption is currently being investigated (173). The procedure involves an attempt to separate the mercurials directly (without prior extraction, derivatization, or preconcentration) and employs a mercury specific detector (atomic absorption spectroscopy).
However, for all the gas chromatographic techniques to be successful requires that the mercury specie of interest be both volatile and thermally stable at the temperature at which the gas chromatographic separation takes place. Therefore speciation of mercurials by GC methods generally are limited to the very volatile species, i.e., inorganic mercury, dimethylmercury, methylmercury, and ethylmercury. Speciation of physiologically bound mercury, such as that bound to protein, aminoacids, etc., by gas chromatographic techniques has not success. Even the analysis of the volatile mercurials employs the digestion, and numerous extraction procedures which can introduce significant errors. Also, investigations with GC-mass spectroscopy and labeled organomercury compounds have shown that several organomercurials are thermally unstable, decompose, or are converted into other organomercury compounds in the GC column (174, 175, 176).

(2) Liquid Chromatographic Methods

Liquid chromatography, on the other hand, offer the ability to separate compounds at moderate temperature. The solvent system can also be chosen that are very compatible to sample conditions.

A number of liquid chromatographic systems have been reviewed (166, 167). However this review generally covered liquid chromatography prior to what is currently regarded
as high performance liquid chromatography (HPLC).
Consequently many of the separations are poor in comparison to separations achieved recently. Also since reversed-phase columns were not available, separations are generally normal-phase silica gel separations of the mercury dithizonates, or are low performance ion exchange.

Separation of mercuric, methylmercuric, ethylmercuric, and phenylmercuric ions was achieved on a reversed-phase ODS column (40% methanol/water containing 0.06 mole/L ammonium acetate, pH 5.5 and 5 millimole/L 2-mercaptoethanol) with electrochemical detection (117). This same HPLC system was used to speciate methylmercuric, ethylmercuric, and phenylmercuric ions with detection using differential pulse electrochemistry (177). This method was used to determine the methylmercury content of fish samples. However the electrochemical detection methods employed in these techniques is not totally metal specific, but dependent upon the electrochemical behavior of other metals present.

Mercuric, methylmercuric, and ethylmercuric ions were separated on a reversed-phase C8 column (0.01 M ammonium acetate in 4% methanol/water containing 25 ppm 2-mercaptoethanol) with detection by flameless atomic absorption detection of the column fractions (178).

A reversed-phase C8 column (40% acetonitrile/water containing 0.1 M sodium bromide, pH 3.5) was used to separate mercuric, methylmercuric, ethylmercuric,
propylmercuric, and 3-chlorophenylmercuric ions. The column fractions were derivatized with dithizone with subsequent colorimetric analysis. This method proved applicable to a test analysis of homogenized fish spiked with methylmercury and ethylmercury at a level of 2 ppm (178).

Only one example of the direct speciation of alkylmercury compounds with a metal specific detector was found in the literature survey. Mercuric, methylmercury, ethylmercury, and propylmercury were separated on a silica column (33% ethanol/water, 0.03 M NaBr, pH 3.0) with detection by inductively coupled argon plasma emission spectroscopy (180). The authors noted that the use of the organic solvent normally used in normal-phase silica gel chromatography limited their applications by the ICP. Another potential problem is that Gast and Kraak (115) observed decomposition of diphenylmercury into phenylmercury chloride in an almost identical system; but did not observe decomposition by reversed-phase chromatography.

(3) Other Chromatographic Methods

Other chromatographic techniques applied to the separation of mercury compounds include thin layer, paper, gel filtration, electrophoresis, and others (166, 167).

Thin-layer chromatography (TLC) has been the most extensively used of these. The mercurials are generally
separated as the dithizonate complex (166, 167) on silica gel plates.

One unique method for detection of mercuric chloride on TLC employed succinate dehydrogenase inhibition using 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride and N-methylphenazonium methosulfate as the chromogenic reagent (180). The detection limit was 50 ng. This technique was applied to the determination of mercuric chloride in fresh and sea water without cleanup.

Methylmercury, separated as the dithizonate on TLC, was quantitated by scraping the zone from the plate into an ignition tube, heating the tube to volatilize the mercury into an absorption cell, and detection by atomic absorption spectroscopy (181). This method was applied to the analysis of methylmercury in fish and sewage.

It was noted during the survey of the literature only one mention of a reversed-phase TLC system was reported (182). This system was not today's commercially available support (C8 or C18), but cornstarch as the chromatographic support coated with liquid parrafin.

Thus the separatory potential of reversed-phase C8 or C18 had not been utilized for the separation of inorganic mercury compounds and the organomercurials.
2C. OBJECTIVES OF RESEARCH

The objective was to develop a method for the speciation of a number physiologically important organomercurials by reversed-phase HPLC with metal specific detection. Reversed-phase HPLC was specifically chosen because compounds can be separated at room temperature, and the aqueous based solvent systems used for reversed-phase separations permit direct separation without the laborious extractions necessary in normal-phase chromatography. The HPLC was to be interfaced directly to a mercury specific detector (atomic absorption spectrophotometer).

Reversed-phase thin layer chromatography with colorimetric detection of the mercurials using dithizone was to be used to predict HPLC conditions and as a verification method for future studies.

Thus two complementary systems were to be developed. HPLC exhibits high chromatographic efficiency and resolution (particularly for compounds eluting first); and TLC has good separatory power and is particularly suited for the detection compounds that do not migrate (i.e., would not migrate in HPLC).

Two independent detection methods were selected: atomic absorption spectroscopy as the metal specific detector; and the classical colorimetric reagent dithizone which is highly sensitive and whose use for the detection of these compounds is well-documented.
2D. EXPERIMENTAL

2D.(a) Instrumentation and Supplies


The HPLC-AA was identical, with exception to the mercury hollow cathode lamps, to that used for the speciation of organoleads and has been described in detail in Part II, Chapter 1, Experimental.

(2) Mercury Hollow Cathode Lamps

Both a laboratory constructed demountable hollow cathode lamp and a commercially available lamp were used. The mercury cathode for the laboratory constructed lamp was prepared by amalgamating mercury with copper foil, or a copper tube of the proper size to fit in the cathode brass cup. A d.c. current of 3-5 mA was used. The commercial lamp was a Perkin-Elmer Intensitron lamp, #303-6044. A d.c. current of 6-10 mA was used.

The 253.6 nm resonance line was used for both lamps.

(3) HPLC and TLC Supplies

All chromatography supplies, columns, plates, chambers, etc., were the same as used for the separation of the organoleads.
2D. (b) Chemicals and Solutions

(1) Chemicals

All chemicals and solvents were analytical reagent grade or the highest grade commercially available. The suppliers for some of the more specialty chemicals and reagents, not given previously, are given:

Mercury Compounds
- Diphenylmercury: Alfa
- Ethylmercury chloride: Alfa
- p-Hydroxy-phenylmercuric chloride: Eastman
- Mercury(II) chloride: Matheson, Coleman & Bell
- Methylmercuric bromide: Alfa
- Methylmercuric chloride: Alfa
- Methylmercuric iodide: Alfa
- Phenylmercuric acetate: Matheson, Coleman & Bell
- Phenylmercuric chloride: Matheson, Coleman & Bell

(2) Solutions

Dithizone: 0.05% (w/v) in chloroform. Dissolve 0.250 grams of dithizone (J. T. Baker) in 500 ml of chloroform.

2D. (c) Experimental Procedure

(1) Development of HPLC-AA System for the Speciation of Mercury and Organomercurial compounds
The development of the systems for the speciation of mercuric ions and organomercury compounds were done simultaneously with system development for the organoleads (Part II, Chapter 1, Experimental). The organolead compounds and the mercury compounds were spotted and developed on the same TLC plates. The systems evaluated were, therefore, the same as given previously for the organoleads (Tables 10 I, II, III, IV).

(2) TLC Plate Development and Colorimetry

(i) Application of Sample to Plate

Samples were applied to the plate using Drummond Microcap spotting capillaries. Samples volumes were 1, 2, 5, and 10 uls. The sample zones were dried using a warm air stream from the air gun.

(ii) TLC Plate Development

The TLC chamber was lined with 10 cm filter paper. Approximately 25 milliliters of developing solvent was added. The system was then equilibrated for at least 15 minutes. The TLC plate was placed into the chamber. The plate was allowed to develop (10 cm). The plate was then removed from the chamber.

The solvent was removed from the plate with the air gun by directing a warm stream of air across the plate.
(iii) Sample Detection by TLC Dithizone Colorimetry

Approximately 50 mls of dithizone solution (0.05%) in chloroform was poured into the glass tray.

The TLC plate was laid sample side down into the dithizone solution, and immediately removed. This motion was done as quickly and as evenly as possible to give uniform color development.

The plate was then dried using a very gentle stream of luke warm air for the air gun. Brightly colored mercury-dithizionate zones appeared on the deep royal-blue background of the plate (Whatman C18 reversed-phase). Mark zones on plate with a pencil and record Rf.
2E. RESULTS AND DISCUSSION

2E. (a) Results of HPLC and TLC System Development for the Speciation of Mercury Compounds

High performance liquid chromatography and thin layer chromatography used in conjunction with each other proved very successful in the development of systems for the speciation of mercury by both HPLC-AA and TLC-colorimetry. The characteristics of several unique systems for the speciation of mercury compounds will be discussed. The systems were developed simultaneously with those for the organoleads using the same strategy: 1) the solvent-selectivity triangle and 2) ion-pair chromatography (Part II, Chapter 1). Since the background for the use of these were discussed in detail previously, only the results will be presented in this section.

(1) Results of TLC-Colorimetry Speciation of Mercury Compounds

The separation of a number of mercury compounds in several select solvent systems is given in Tables 19 and 20. These systems gave the best separation of the multitude of systems tested. They can be broken into two general types: ethanol based, and acetonitrile based.

(i) Ethanol Based Solvent Systems
The mercuric ion was not retained in the ethanol based systems, but migrated with the solvent front ($R_f = 1.00$). The methylmercury and ethylmercury could thus be separated from the mercuric ion. However, resolution of the methylmercury from the ethylmercury was only adequate.

The arylmercurials, p-hydroxy-phenylmercuric chloride, phenylmercuric chloride, and diphenylmercury, were easily resolved in the ethanol based systems. The more ionic the specie, the greater the migration.

(ii) Acetonitrile Based Solvent Systems

These systems were preferred over the ethanol based systems for the separation of the mercury compounds. This was for several reasons: 1) all the mercurials studied could be resolved on one plate, 2) zones on the TLC were well defined, and 3) color contrast for the mercury dithizonates versus the TLC plate background was better.

Two acetonitrile based solvent systems were used throughout this study for the speciation of mercuric and organomercuric compounds. The two systems were:

1) 55 parts acetonitrile/45 parts water (v/v) and 0.10 molar in sodium chloride.
2) 55 parts acetonitrile/45 parts water (v/v) and 0.43 molar in sodium bromide.

Representative TLC separations for these two systems will be presented with the results of the HPLC-AA speciation of the mercury compounds. This method of presentation was chosen since the systems were developed...
Table 19. Retention Factor (Rf) for Mercury Compounds in Ethanol Based System

<table>
<thead>
<tr>
<th>System</th>
<th>Methyl Hg</th>
<th>Ethyl Hg</th>
<th>OH-Ph</th>
<th>PhHg</th>
<th>Ph₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HgCl₂</td>
<td>Cl, Br, I</td>
<td>HgCl</td>
<td>HgCl</td>
<td>Hg</td>
</tr>
</tbody>
</table>

(a) Aqueous Ethanol/NaBr
75 EtOH/1.0M NaBr: 1.00 .69, .69, .69 .63 .74 .46 .24
25 EtOH/1.0M NaBr: 1.00 .43, .43, .43 .20 .14 .00 .00

(b) Aqueous Ethanol/NaCl
60 EtOH/.1M NaCl: 1.00 .64, .64, .64 .59 .69 .44 .07
25 EtOH/1.0M NaCl: 1.00 .46, .46, .46 .28 .24 .00 .00

a. 75 EtOH/1.0M NaBr means a system that is 75 parts ethanol, 25 parts water (v/v), and 1 molar in sodium bromide.

Table 20. Retention Factor (Rf) for Mercury Compounds in Acetonitrile Based Systems

<table>
<thead>
<tr>
<th>System</th>
<th>Methyl Hg</th>
<th>Ethyl Hg</th>
<th>OH-Ph</th>
<th>PhHg</th>
<th>Ph₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HgCl₂</td>
<td>Cl, Br, I</td>
<td>HgCl</td>
<td>HgCl</td>
<td>Hg</td>
</tr>
</tbody>
</table>

(a) Aqueous ACN/NaBr
55 ACN/.43M NaBr: .74 .46, .46, .46 .36 .57 .30 .11
25 ACN/1.0M NaBr: .67 .24, .24, .24 .10 .08 .00 .00

(b) Aqueous ACN/NaCl
55 ACN/.25M NaCl: .93 .57, .57, .57 .47 .57 .34 .13
55 ACN/.38M NaCl: .98 .78, .78, .78 .71 .67 .67 .37
25 ACN/1.0M NaCl: .86 .33, .33, .33 .17 .14 .00 .00

a. 55 ACN/.43 M Na/Br means a system that is 55 parts acetonitrile, 45 parts water (v/v), and .43 molar in sodium bromide.
together and separations patterns can be correlated.

(2) HPLC-AA Speciation of Mercury Compounds

(a) 55 Acetonitrile/45 Water/0.10 Molar NaCl

The speciation by HPLC-AA of standard solutions of m ercuric chloride, methylmercury chloride, and ethylmercury chloride is shown in Figure 63. The 253.6 nm mercury resonance line was used for detection.

A trace amount of methylmercury is evident in the ethylmercury standard. This was confirmed by HPLC-AA spiking studies and from retention indexes (Rf’s) from high loading of the ethylmercury standard on TLC.

A chromatogram of the three compounds is presented in Figure 64. Good resolution of all three compounds was achieved.

A representative thin layer chromatogram with the separation of mercuric, methylmercuric, ethylmercuric, p-hydroxy-phenylmercuric, phenylmercuric ions and diphenylmercury is presented in Figure 65. The order of migration for the mercuric, methylmercuric and ethylmercuric ions on the TLC are the same as the order of elution on HPLC-AA.

Speciation of the arylmercurials by HPLC-AA was not done using this system, although they were easily separated using the TLC system. The potential for the HPLC-AA speciation of such compounds will be amply demonstrated in the following section.
Figure 63. HPLC-AA of Mercury Compounds. Mobile Phase: 55\% ACN/45 Water/0.10 Molar NaCl.
Figure 64. Speciation of Mercury Compounds by HPLC-AA. Mobile Phase: 55ACN/45 Water/0.10 Molar NaCl.
Figure 65. Speciation of Mercury Compounds by TLC-Dithizone Colorimetry. Mobile Phase: 55 ACN/45 Water/0.10 Molar NaCl.
Gast and Kraak (179) used a similar system for the separation of six organomercuric compounds. They used a reversed-phase LiChrosorb RP-8 column with an eluent, 50 acetonitrile/50 water and 0.05 molar in sodium chloride, to separate the following: methylmercury, ethylmercury, phenylmercury, 3-chlorophenylmercury, 2-bromophenylmercury, and diphenylmercury. These results confirmed the order of elution of methylmercury, ethylmercury, phenylmercury and then diphenylmercury. However, peak broadening due to mixing of the dithizone with the column eluent was noted and colorimetric interference from cysteine used in their extraction method were experienced. These difficulties are not present in the HPLC-AA system.
(ii) 55 Acetonitrile/45 Water/0.43 Molar NaBr

A representative thin layer chromatogram with the separation of the six test mercury compounds (mercuric, methylmercuric, ethylmercuric, p-hydroxyl-phenylmercuric, phenylmercuric ions and diphenylmercury) is presented in Figure 66. Excellent separation of all six compounds were achieved and easily distinguished on the TLC. The TLC zones for the mercury-dithizonates were well defined which is indicative of a good separation system. The brightly colored (yellow to orange) mercury-dithizonates were readily detected against the brilliant royal-blue of the dipped TLC plate.

The three major physiologically important compounds, mercury (II), methylmercury, and ethylmercury were well resolved. It was noted for this solvent system that p-hydroxy-phenylmercury chloride exhibited an retention factor (Rf) close to that of methylmercury. Therefore all four compounds were chosen initially to test the resolution capability of the HPLC. Standard test solutions for each compound is presented in Figure 67. Once again, the ethylmercury chromatogram (Figure 67D) exhibits a trace of methylmercury as indicated in the previous HPLC-AA studies.

The p-hydroxy-phenylmercury contains a trace amount of mercury (II). This is based upon the retention time for the trace peak in the HPLC-AA; and detection of a trace zone with an Rf identical to the mercuric ion on TLC.
Figure 66. Speciation of Mercury Compounds by TLC-Dithizone Colorimetry. Mobile Phase: 55 ACN/45 Water/0.43 Molar NaBr.
Figure 67. HPLC-AA of Mercury Compounds. Mobile Phase: 55 ACN/45 Water/0.43 Molar NaBr.
The capability of HPLC-AA as a speciation technique is demonstrated in Figure 68. All four compounds were easily separated and resolved.

Figure 68. Speciation of Mercury Compounds by HPLC-AA. Mobile Phase: 55ACN/45 Water/0.43 Molar NaBr.
The potential for separating arylmercury compounds was clearly demonstrated with the separation of the three test arylmercury compounds. The HPLC-AA chromatogram for the separation of p-hydroxy-phenylmercuric chloride, phenylmercuric chloride, and diphenylmercury is presented (Figure 69). Traces of mercury (II) and additional impurities were observed. A test mixture containing all three compounds is shown in Figure 70.

Gast and Kraak (179) used a similar system for the separation of six organomercury compounds. A reversed-phase LiChrosorb RP-8 column with an eluent (50 acetonitrile/50 water and 0.10 molar in sodium bromide) to separate the following: methylmercury, ethylmercury, 3-chlorophenylmercury, 2-bromophenylmercury, and diphenylmercury. Detection was by dithizone colorimetry of the eluent. Their results confirmed the order of elution for the methylmercury, ethylmercury, phenylmercury and diphenylmercury.

Analysis of the data from Gast and Kraak indicated poorer resolution of the compounds. This could be attributed to three factors: 1) peak broadening due to mixing of the column with the dithizone detection reagent, 2) the solvent system was not optimized for separation and 3) different chromatographic conditions. The solvent system of Gast and Kraak was evaluated by TLC, both C8 and C18 plates, for the test compounds in this study, and poorer resolution was noted. Differences from lot to lot in
Figure 69. Separation of Arylmercury Compounds by HPLC-AA. Mobile Phase: 55 ACN/45 Water/0.43 Molar NaBr.
Figure 70. Speciation of Arylmercury Compounds by HPLC-AA. Mobile Phase: 55 ACN/45 Water/0.43 Molar NaBr.
columns, solvent purity, etc., often occurs and can affect chromatographic behavior. Gast and Kraak used a C8 reversed-phase column. A C18 reversed-phase column was used in these laboratories for the HPLC-AA.

Based upon retention times from the HPLC-AA, all six of the test mercury compounds could be separated. However, this was not done.

2F. SUMMARY OF RESULTS

Speciation methods by HPLC-AA for a number of mercury compounds including inorganic mercury, alkylmercurials, and arylmercurials was developed. These compounds have significant toxicological properties and are important in environment and physiological terms.

Supplementary TLC-colorimetric methods using dithizone as the colorimetric reagent were developed. These are rapid, highly sensitive qualitative methods that separate a number of mercury compounds.

Although no physiological or environmental samples were analyzed, such methods should be readily adapted to these analyses. Initial investigations to check the feasibility of using these methods for application to the speciation of mercury in human fluids is currently being conducted by another member of this research group. If successful, HPLC-AA would provide a method for speciation of mercury directly without derivatization, extraction, or possible artifacts due to thermal degradation.
PART II. CHAPTER 3. SPECIATION OF AMINOGlyCOSIDE-METAL COMPLEXES BY HPLC-AA AND TLC-COLORIMETRY

3A. INTRODUCTION

3A.(a) General Introduction

Aminoglycoside antibiotics are a class of antimicrobial agents that are in wide use for the treatment of a number of life-threatening bacterial infections, especially psuemoneous and gram-negative varieties. Inorganic ions have been shown to exhibit a marked effect on the therapeutic value of the aminoglycosides. Extensive studies have demonstrated of inorganic ions on a wide range of other antibacterial agents, including the polypeptide antibiotics. However the effect of inorganic ions and metals on aminoglycoside is by far the best documented.

Beggs (183) recently concluded a review on the effect of cations on aminoglycoside. Two excellent review on the effect of a number of agents, including cations and anions, on aminoglycosides and their uptake and mode of action have appeared (184, 185). More than one mechanism appears involved in the anatognestic effect of inorganic cations on the biological activity of these antibiotics. This is not unexpected. Biological systems are not simple one step functional entities. Biological systems are in fact interplays of many systems into one another.
As early as the late 1940's, both nonvalent and divalent cations were shown to interfere with the biological activity of streptomycin (186, 187). The search for broader and more effective antibiotics lead to a wide array of agents, including the kanamycins, tobramycin, gentamicins, amikacin, and sisomicin (Figures 71, 72, 73).

Early workers soon recongnized problems in susceptibility testing of the aminoglycosides. An increase in the level of sodium chloride in the medium was shown to decrease the minimum inhibitory concentration (MIC) for gentamicin against a number of gram-negative bacilli. Waitz and Weinstein had observed that four different simi-solid media gave four different results for gentamicin MIC against P. aeruginosa (188). Similiar observations were made by Traub (189) and Washington (190). By now the importnce of magnesium(2+) and calcium(2+) concentrations for the susceptibility testeing of gentamicin was evident (191 - 197).

The effects of salts on aminoglycosies was shown to be very species dependent. The degree of antagonism varied from microorganism specie to specie (191, 193, 195, 196). Davis and Iannetta have shown also that physiological concentration of calcium in serum can inhibit the activities of the polypeptide polymyxin B antibacterial (198). Calcium and magnesium ions have been shown to increase the MIC of polymyxin B in susceptibility tests to Pseudomonas species (196).
Figure 71. Structures of Kanamycin A, B, and Amikacin.
Figure 72. Structures of the Gentamicins.
Figure 73. Structures of Neomycin B and C.
Several mechanisms have been proposed to explain the effects of cations on aminoglycoside antibiotics. In fact, from the scientific evidence, more than one mechanism is probably occurring at the physiological level. The proposed mechanisms are given as follows:

(1) Changes in the Cell Wall

The induction of a major outer membrane protein, H1, in Pseudomonas aeruginosa resulted in decreased susceptibility to gentamicin and streptomycin. Magnesium ion at a concentration of 1 millimolar inhibited the aminoglycoside-mediated outer permeabilization of a lysozyme. Therefore, it was proposed (199) that the uptake and killing by gentamicin and streptomycin required interaction with the magnesium ion binding site at the outer membrane which permitted the aminoglycoside uptake into the periplasm.

Similarly, the introduction of a major outer membrane protein in P. aeruginosa also gave resistance to polymyxins and ethylenediaminetetraacetic acid (EDTA) (200). A decrease in the magnesium concentration in the cell wall was found. This indicated that a site in the outer membrane was involved which was protected by the
proteins.

Ramirez-Ronda, et. al, (201) suggested that the mechanism was the competition between the aminoglycoside and cation for a common binding site on the cell wall. They found that the binding of 3H-labelled gentamicin to P. aeruginosa was enhanced in the absence of divalent cations. In contrast, the binding of the 3H-labelled gentamicin to E. coli and bacterial activity against E. coli did not vary significantly in the presence or absence of divalent cation.

Several workers found that calcium, magnesium, zinc and other cations are present in appreciable amounts in the wall of P. aeruginosa and are necessary for structural integrity (193, 195, 201). The importance of magnesium and calcium ions to the structural integrity of the bacterial surface was suggested by the reaction of P. aeruginosa to EDTA, which resulted in the release of lipopolysaccharides from the cell wall (202, 203, 204). Chelation of divalent cations from the surface of P. aeruginosa can release autolysin (205). Brown suggested that divalent cations stiffen the lipoprotein membrane thus preventing disruption of the membrane (206). Other researchers have suggested that divalent cations may link the polysaccharide subunits of the cellular surface (207 - 210).

The EDTA-induced removal of cell wall cation increased the bacterial permeability of the wall to a
variety of unrelated molecules (211). \textit{P. aeruginosa} which is normally resistant to penicillin and ampicillin became fully sensitive after exposure to EDTA. The effect of EDTA was reversed by addition of magnesium and calcium ions to the media (212).

However, Beggs and Andrew (183) showed that strotium chloride and barium chloride, two metals that are non-essential in the cell wall of \textit{M. smegnatis}, exhibited the same antagonistic effects as magnesium chloride and calcium chloride against dihydrostreptomycin. These finding were confirmed (213) in experiments which involved viability determination.

It is doubtful that strotium (2+) can play a significant role in the structures of bacterial cell walls, yet strotium (2+), was as effective as magnesium(2+) and calcium(2+) in antagonism. This would indicate that in \textit{M. smegnatis}, at least, that this was a nonspecific phenomenon dependent more on the doubly charged nature of the cation than to the specific metal ion specie (183).

However Bryan and Van der Elzen (214) have argued that divalent cation antagonism to \textit{P. aeruginosa} and \textit{E. coli} was nto the result of a structural defect in the cell wall.

(2) Ionic Strength Effects

\textit{Medeiros, et. al.}, illustrated the ability of monovalent cations to antagonize the effect of gentamicin
to *P. aeruginosa* (191). *P. aeruginosa* was one of many organisms exhibiting the salt effect. The cation antagonism on gentamicin against a number of gram-negative bacilli is well documented (191, 193, 215).

Beggs and Andrews showed that protection of *E. coli* from dihydrostreptomycin or gentamicin by magnesium chloride, sodium chloride, or sodium sulfate was attributed to ionic strength alone (216). The degree of protection increased with ionic strength and was independent of the particular salt used within a monovalent or divalent series.

For *M. smegnatis* with dihydrostreptomycin, the specific divalent cation and ionic strength effects were evident. Divalent cations exhibited the most dramatic effect, while monovalent sodium antagonism was a function of ionic strength (217). These results would support the hypothesis that more than one mechanism is involved in the effect of metal cations on the therapeutic value for aminoglycoside.

(3) Binding Studies

(i) Cell surface

Beggs and Andrew's experiment with 3H-labelled dihydrostreptomycin uptake by *M. smegnatis* was similar to that by *M. tuberculosis* (218), *E. coli* (219) and *P. aeruginosa* (214). It was found that after apparent instantaneous binding of the drug, there was a plateau
followed by two hours of time-dependent linear uptake (213). The initial binding apparently involved a weak electrostatic binding of the dihydrostreptomycin at the cell wall surface. Up to 90 of the drug bound initially can be removed by washing with a salt solution. Dihydrostreptomycin uptake during the next two hours was more firmly bound. Both sodium chloride and divalent cation salts strongly inhibited the instantaneous reaction to an extent that correlated well with the secondary uptake and reduced bacterial activity.

(ii) Binding to Protein

The binding of an antibiotic to protein can reduce the effective concentration of the antibiotic, and decrease tissue pharmacokinetics or toxicity (220).

Most studies have shown little or no binding of aminoglycosides to serum protein under normal physiological conditions (221, 22, 223).

Scholtan, et. al., concluded that the binding of sisomicin and streptomycin in the absence of calcium(2+) was primarily by ionic bonds, not hydrophobic (224). Hydrophobic bonds appear to be the dominant bond in protein binding for most drugs (225). Rosendrang calculated the binding affinities, binding constants, and the maximum number of aminoglycosides that can be bound by a molecule of albumin in the absence of calcium ion (224).

Ramirez-Ronda, et. al., (222) studied the binding of
sisomycin, kanamycin, gentamicin, tobramycin, and amikacin to human serum. They concluded that a maximal binding was approximately 70% in the absence of devalent cations. However, no significant binding of aminoglycosides to plasma proteins occurred under normal magnesium(2+) and physiological levels. They speculated that binding could occur under pathological condition in man where abnormal low serum cation concentrations might exist.

Gordan, et. al., (225) found that no serum binding of gentamicin, tobramycin, or kanamycin, whereas streptomycin was 35% bound in normal physiological serum. Ultrafiltration was used to separate the serum fractions.

(iii) Binding to Transport Protein

Whether the aminoglycoside compete for the same site on a specific transport protein (226, 227) would appear unlikely based on the previous protein binding studies.

(iv) Binding to Ribosomes

The primary mode of action of aminoglycosides is the inhibition of protein synthesis at the ribosomal level (228-232). Mager, et. al., reversed the inhibition of protein synthesis by streptomycin in cell-free preparations by the addition of magnesium ion (233). Chang and Flaks (232) found that optimum binding of 3H-dihydrostreptomycin occurred at 0.01 M magnesium(2+), but higher concentrations reducing binding. Levels of
potassium chloride above 0.07 molar reduced ribosomal
3H-dihydrostreptomycin binding.

Beggs argued that competition for the same site on the
bacterial ribosomes at the intracellular level was very
unlikely based on the large marked effect on the initial
drug-cell wall interaction which drastically reduced the
uptake of the drug into the cell (183).

(v) Binding found in Cellulose, Clays, and Fecal
Material

Wagman, et. al., studied the binding of five
aminoglycosides, entamicin, sisomicin, neomycin, kanamycin
and tobramycin, to cellulose powder, diatomaceous earth,
and Seitz filter sheets (234). From 30 to 100% of these
aminoglycosides antibiotics were adsorbed to the cellulose
and diatomaceous earth depending on the ratio of antibiotic
to adsorbant. Acid washes were not able to remove all of
the bound material.

These observations were correlated with the binding of
aminoglycosides to feces since fecal matter contains large
quantities of cellulose-like material (235). From 44 to
90% of gentamicin, N-acetyl gentamicin, neomycin, and
paromomycin were absorbed to the fecal material. An acid
wash only partially removed the bound drugs.

McGinty and Hill studied the adsorption or neomycin to
attapulgite and montmorillonite clays, and desorption by
sodium and magnesium ions (236). Magnesium ions were
shown to be more efficient in the desorption of neomycin from the clays. It was proposed that ions present in the gastointestinal tract could increase the bioactivity of neomycin in antidiarrheal suspensions.

(4) Complexation and Stereochemistry

Aminoglycosides have a number of functional groups which could easily facilitate complexation under the proper conditions. Streptomycin complexes with nickel (2+), copper (2+), magnesium (2+), and cobalt (2+) readily (237).

Cobalt ions were involved in the biotransformations of the gentamicins during fermentation (238). Whether this was strictly a nutritional requirement for the fermentation microorganism, or whether it was a cobalt-gentamicin complex with stereochemical specificity was not established.

Complexations with tetramminecopper (II) sulfate, ammonical cuprous chloride, and Cupra B are used extensively for conformational analysis (239, 240), including the gentamicins (241), kanamycins (241, 242, 243), and neomycins (245). The conditions for the conformational analyses (very high ammonical concentrations) certainly do not approach the conditions found under normal physiological circumstances, but do illustrate the potential under specific conditions.

Complexation of kanamycin A and ribostamycin with
copper (II) was studied using carbon 13 nuclear magnetic resonance (NMR) (246). The copper (II) concentration was varied over a wide range. This technique was a very useful tool for studying the structural and stereochemical features of the aminoglycoside antibiotics.

A colorimetric assay was developed for neomycin which utilized complexation with copper (II) and sodium potassium tartrate as an auxiliary ligand (247). Complexation was found to occur from pH 5.2 to pH 10.0, with the maximal complexation occurring at the higher pH.

Complexation has been illustrated to the greatest extent in the chemical transformation of a number of aminoglycosides. The selective acylation of kanamycin A was achieved by complexation of the suitably disposed vicinal amino alcohol functional groups as the copper (II) chelates, and subsequent acylation of the unbound amino group(s) with a variety of acylating groups (246).

This was extended in the patent literature to cover the aminocyclitol-aminoglycoside transformations. Divalent salts of copper, nickel, cobalt, and cadmium were used for the preparing complexes with a large number of aminoglycosides including sisomicin, kanamycins, and the gentamicins (248). The reactions proceeded best in polar, aprotic organic solvents, particularly dimethylformamide and dimethylsulfoxide. Polar protic solvents such as lower alcohols, particularly methanol and ethanol, could also be used. Weaker complexes were shown to be formed in
protic solvents.

Anhydrous solvents were preferred in order to obtain maximum yields (248). Water (up to 25%) could be used provided that additional divalent transition metal salt was employed since water, being a protic solvent, weakened the metal complex intermediates. An equilibrium was proposed for complexes having at least one available neighboring amino/hydroxy group pair [A], the transition metal salt (e.g. cupric acetate, Cu(OAc)₂) and the solvent which was indicated as follows:

\[
\begin{align*}
[A] + \text{Cu(OAc)}_2 + \text{solvent} & \rightarrow \text{A-Cu(OAc)}_2\text{-solvent} + \text{Cu(OAc)}_2\text{-solvent} + \text{solvent} \\
\text{protic solvent} & \rightarrow \text{aprotic solvent} \\
[A] + \text{Cu(OAc}2) + \text{solvent} & \rightarrow \text{A-Cu(OAc}2\text{-solvent} - \text{Cu(OAc)}_2\text{-solvent} + \text{solvent} \\
\text{protic solvent} & \rightarrow \text{aprotic solvent}
\end{align*}
\]

With an aprotic solvent, the equilibrium favored the formation of the transition metal-aminoglycoside complex, while an organic protic solvent or the presence of water in an organic aprotic solvent favored decomplexation.

It was shown that calcium did not chelate with gentamicin in normal human serum or in phosphate buffered pH 7.4 saline (249). An ion-specific calcium electrode was used to determine the influence of increasing gentamicin
concentrations on the ionized calcium concentration in physiological solutions.

2A.(c) Summary

Aminoglycosides are a major class of therapeutically active antibiotics used in the treatment of a number of life-threatening diseases. Metal cations have been shown to significantly affect the therapeutic value of these agents. Some of the mechanisms that have been proposed to explain this include 1) changes in the cell wall, 2) binding of the aminoglycoside to various physiological components, and 3) complexation and stereochemistry. However, it is most likely that several different mechanisms may be taking place depending on the bacterial specie involved and the chemical nature of the metal ion involved.

Although several aminoglycoside-metal complexes have been prepared in reaction vessels, and studied using a number of spectroscopic and electrochemical techniques, no reports were found regarding the separation and identification of the complex from either the parent metal or aminoglycoside ligand.

Due to the tremendous importance of the agents to human healthcare, the development of a method that would facilitate the elucidation interaction of metal ions with aminoglycosides would be extremely significant.
3B. CHROMATOGRAPHY OF AMINOGLYCOSIDES

3B.(a) General Introduction

A review of analytical methods for aminoglycoside analysis and related antibiotics is published biannually in Analytical Chemistry (250). Extensive reviews on the analysis of aminoglycoside antibiotics were compiled recently (251, 252) and a comprehensive comparison of the methods has also been published (253).

Although only two aminoglycoside antibiotics, kanamycin A and amikacin, were studied extensively in these laboratories, the chromatographic methods for aminoglycosides in general will be reviewed. These reviews will be grouped according to the specific antibiotic.

(1) Neomycins

Reviews of analytical methods for the neomycins have appeared recently (250, 251, 152).

Tsuji developed an HPLC method for quantifying neomycin sulfate powders and ointments by derivatization with 1-fluoro-2,4-dinitrobenzene and then chromatographing isocratically on a normal-phase system. Neomycin B, C, neamine, and several other factors were separated (254).

Several other methods have been reported that separate neomycin B and C. These include paper chromatography (255), and ion-exchange (256, 257). A gas chromatographic
method (258, 259) is normally used for routine analyses, but it is difficult to perform (260).

Thin layer chromatography (TLC) of neomycin was reviewed recently (250, 251, 252). Brodasky described a TLC procedure that separated the neomycins. However, it was on carbon black and there were difficulties encountered in reproducibility, plate preparation, and quantitation (261).

Other TLC methods have not resolved neomycin B and C satisfactory. Vickers obtained some resolution of neomycin B and C, but with considerable streaking and overlapping of zones (262). Chatterjee reported a better resolution of the sulfoxides of B and C on layers of cellulose. Neamine (neomycin A) and a number of closely related antibiotics were not resolved (263). This method did not lend itself to quantitation.

(2) Gentamicins

Analytical reviews for the analysis of gentamicin have appeared recently (251, 252).

The method of Tsuji for neomycin was applied to gentamicin (254). Freeman, et al., used pre-column derivatization with o-phthalaldehyde/thioglycollic acid and then ion-paired chromatography on reversed-phase with UV detection (264). Maitra, et al., separated the antibiotic in serum by passage through a silica gel column, derivatized with o-phthalaldehyde and eluted with
ethanol. The derivatized genamicin was then separated by reversed-phase chromatography and quantified by flurometry (265).

Peng, et. al., derivatized with dansylchloride, extracted, and chromatographed on a reversed-phase column with fluorometric detection (266).

Anhalt and coworkers separated the major gentamicin components Cl, C1a, C2, and several minor components by ion-paired HPLC on a reversed-phase column, then derivatized post-column with o-phthalaldehyde (267, 268).

Thin layer chromatographic (TLC) methods for gentamicin was reviewed (251).

Wilson, et. al., quantitated the gentamicin components Cl, C1a, and C2 by TLC densitometry. The factors were separated on silica gel with a methanol-chloroform-ammonia solution, with detection with ninhydrin (269).

Kabasakalian, et. al., used the TLC procedure of Wilson for separation of the gentamicin factors and then derivatized them with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazone (NBD-C1) for fluorometric scanning. This method was used to quantitate gentamicin in fermentation broths (270).

Gentamicin Cl, C1a, and C2 were separated on silica gel using a methanol-chloroform-ammonia mobile phase. The zones were detected following derivatization with fluorescamine (271).
(3) Kanamycins

The analytical methods for kanamycin have been reviewed extensively (250 - 252), particularly in Analytical Profiles of Drug Substances (272).

The methods of Tsuji for neomycin was applied to the kanamycins (254).

May, et. al., (273) resolved the kanamycins on a pellicular strong-cation exchange column and then quantitated by post-column detection of the fluorescent o-phthalaldehyde or fluorescamine derivatives. Less than 0.35 ug of kanamycin B could be detected in 7 ugs of kanamycin A. The limit of detection for kanamycin A was 20 ngs.

Recently, a TLC methodc was developed for the detection of kanamycin B in tobramycin using a methanol-chloroform-ammonia solvent system on silica gel (274). Several TLC systems have been used to separate separate minor aminoglycoside factors of the nebramycin fermentation. One of these systems included the use of an aqueous 1.5 molar sodium acetate/10% t-butyl alcohol solvent system on silica gel (275).

(4) Amikacin

Amikacin is a newly introduced aminoglycoside antibiotic with significant therapeutic applications.

Amikacin was assayed in serum after extraction employing a CM-sephadex column. The extract was then
chromatographed by reversed-phase with post-column derivatization with o-phthalaldehyde and fluorescent detection (276).

Maitra, et. al., analyzed amikacin in serum by adsorption of the antibiotic on silica gel. The adsorbed drug was then derivatized with o-phthalaldehyde, eluted, and separated on a reversed-phase column with fluorescence detection (277). This method has been adapted to separate four isomers of amikacin (278).

Amikacin was separated from gentamicin, kanamycin, neomycin, and tobramycin by TLC on silica gel using a methanol-chloroform-ammonia mobile phase. The zones were detected using ninhydrin or fluorescamine derivatization (279).

3B.(b) Summary

Analysis of aminoglycosides by liquid chromatography has proved to be very difficult. This is due in part to the difficult encountered in detection since aminoglycoside do not possess chromophores that absorb in the ultraviolet region. This has necessitated derivatization with a UV adsorbing or with a fluorescence reagent either pre- or post-column. A significant portion of the methods have employed derivatization prior to chromatographic separation.

Liquid chromatographic methods that employ post-column
derivatization have generally used an ion-pairing reagents, such as pentane sulfonic acid, to modify the mobile phase. The mobile phases that have been employed are generally mixed aqueous organic.

Thin layer chromatography (TLC) have proven very useful in separating a number of aminoglycosides. However, TLC methods generally do not exhibit high resolution for closely related aminoglycosides. The mobile phases employed for separated of the aminoglycosides have been to the great extent mixed methanol-chloroform-ammonia solutions.

3C. OBJECTIVES OF RESEARCH

The objective of this research project was to develop a method to separate and aminoglycoside-complexes either from the parent metal ion or from the aminoglycoside ligand. Chromatographic methods previously used were not suitable because they either involved chemical modification of the aminoglycoside prior to chromatographic separation, or the mobile phase was such that the stability of the metal complex would be questionable. The system would ideally be aqueous based such that some correlation might be inferred as to the presence of the metal complexes under physiological conditions.
3D. EXPERIMENTAL

3D.(a) Instrumentation and Supplies

(1) High Performance Liquid Chromatograph-Atomic Absorption Spectrophotometer (HPLC-AA)

The HPLC-AA was identical, except for the hollow cathode lamps, to that used for the speciation of the organoleads and mercury compounds, and has been described in detail in Part II, Chapter 1, Experimental.

(2) Hollow Cathode Lamps

(i) Cobalt Hollow Cathode Lamp

A commercial Perkin-Elmer Intensitron multielement Fe-Cu-Co-Mn-Cr hollow cathode lamp, # 303-6103, was used. A current of 30-35 mA was applied.

(ii) Copper Hollow Cathode Lamps

Both commercial and laboratory constructed demountable hollow cathode lamps were used. These were described in detail previously (Part I, Experimental).

(3) HPLC and TLC Supplies

All chromatography supplies, columns, plates, chambers, etc., were described previously.

3D.(b) Chemicals and Solutions

(1) Chemicals

All chemicals and solvents were analytical reagent
grade or the highest grade commercially available. Some of the more specialty chemicals and the suppliers are given below:

Barium chloride: Baker
Cadmium iodide: Baker
Cesium chloride: Matheson, Coleman & Bell
Calcium chloride: Baker
Cobalt chloride: Mallinckrodt
Copper chloride: Merck
Magnesium chloride: Mallinckrodt
Potassium chloride: Baker
Sodium chloride: Matheson, Coleman & Bell
Sodium hypochlorite solution (NaOCl) (5%): Clorox
Starch: Baker

(2) Aminoglycosides

The aminoglycoside standards were generously supplied by the following:

Amikacin: Bristol Laboratories
Gentamicin Sulfate: Schering Corporation
  Gentamicin C1
  Gentamicin C1a
  Gentamicin C2
Kanamycin A: Bristol Laboratories
Kanamycin B: Bristol Laboratories
Neomycin Sulfate: Burroughs Wellcome Co.
Neomycin Sulfate: The Upjohn Co.
Neomycin B: Burroughs Wellcome Co.
Neomycin C: Burroughs Wellcome Co.
Neomycin A: The Upjohn Co.

(3) Solutions

(i) Sodium Hypochlorite (NaOCl) Reagent, 0.5%: Mix 10 ml of 5% NaOCl solution with 90 ml of water.
(ii) Cadmium Iodide-Starch Reagent
Dissolve 11 grams of cadmium iodide in 1000 ml of water. Boil 15 minutes. Slowly add 15 grams of starch. Filter the solution through a No. 1 Whatman filter. Solution is stable for months.
(iii) Dithizone: 0.05% (w/v) in chloroform. Dissolve 0.250 grams dithizone in 500 ml of chloroform.

3D.(c) Experimental Procedure:

(1) Development of HPLC-AA System for the Speciation of Aminoglycoside-Metal Complexes.

Both normal-phase and reversed-phase systems were employed in the development of a system for the separation of aminoglycoside-metal complexes. A list of systems evaluated is given in the Discussion of Results.

(2) TLC Plate Development and Colorimetry
(i) Application of Sample to Plate

Samples were applied to the plate using Drummond Microcap spotting capillaries. Samples volumes were 1, 2, 5, and 10 uls.

The sample zones were dried using a warm air stream from the air gun.

(ii) TLC Plate Development

Approximately 25 milliliters of developing solvent was added to the TLC chamber. The system was equilibrated for at least 8 hours prior to plate development. The plate was placed into the chamber and allowed to develop (either 10 or 20 cms). The plate was then removed from the chamber. The solvent was removed from the plate with the air gun by directing a warm stream of air across the plate.

(iii) Sample Detection

1. Aminoglycoside Detection

Spray the plate uniformly with the NaOCl reagent. Dry the plate with lukewarm air stream from the air gun. Spray the plate evenly and lightly with 95% ethanol and dry the plate again with lukewarm air stream from the air gun. Spray the plate evenly and lightly with the cadmium iodide-starch reagent until intense, dark blue colored zones appear. Cover the plate with another glass plate to prevent color fading of the zones.
2. Metal and Metal-Aminoglycoside Complex Detection

Pour about 50 milliliters of dithizone solution (0.05%) in the glass tray. Lay the TLC plate sample side down into the dithizone solution and immediately remove. This motion should be done as quickly and as evenly as possible to give uniform color development. Dry the plate with a gentle stream of luke warm air from the air gun. Brightly colored zones for the metal-dithizonates and for the aminoglycoside-metal-dithizones develop.

(3) Preparation of Aminoglycoside Complexes

Aminoglycoside-metal complexes were prepared in various molar ratio quantities. Depending upon on the design of the experiment complexes were prepared as follows:

(i) Addition of Metal to Aminoglycoside

Stock aqueous solutions of both the aminoglycoside and metal were prepared such that when mixed the desired ratio of metal:aminoglycoside would be prepared. To the aminoglycoside solution add the specified amount of metal ion. Adjust pH to 7.4 (physiological pH) with either 0.01 molar sodium hydroxide or 0.01 molar sulfuric acid.

(ii) Addition of Aminoglycoside to Metal

Stock aqueous solutions of both the aminoglycoside and metal were prepared such that when mixed the desired ratio of metal:aminoglycoside would be prepared. To the metal solution add the specified amount of aminoglycoside.
Adjust the pH to 7.4 (physiological pH) with either 0.01 molar sodium hydroxide or 0.01 sulfuric acid.
3E. RESULTS AND DISCUSSION

3E. (a) Results of HPLC and TLC Solvent System Development for the Speciation of Aminoglycoside-Metal Complexes

The development of a system that would separate the aminoglycoside-metal complex from either the parent aminoglycoside ligand or from the metal ion was difficult in design. First the system would have to be able to separate the desired species. Second the system would have to maintain the integrity (stability) of the complex. Third the system would need to be aqueous-based so that a correlation, at least to a first degree approximation, could be made to the extent of complexation in physiological solution.

Through the generosity of several pharmaceutical companies, a number of aminoglycoside antibiotics were provided for study. In the initial chromatographic studies, all the compounds were utilized to check for separation, resolution.

However, two were selected for the aminoglycoside-metal complex studies. They were kanamycin and amikacin. Kanamycin was selected because it was one of the first major aminoglycoside antibiotics and its chemistry has been extensively studied. Amikacin was chosen because it is a major new antibiotic and there is a need to gather addition data on its behavior.
HPLC and TLC systems were developed simultaneously as in previous studies. Systems developed previously for the separation of aminoglycosides were not acceptable for use in these studies. They either necessitated derivatization of the aminoglycoside prior to development, or utilized mobile phases, such as methanol-chloroform-ammonia mixtures, that allowed no correlation to physiological conditions. What was desired essentially was a column and system with reduced activation (but not deactivated) that would not disrupt the complex, but could also separate the desired species.

A totally new system would be needed. The approach was multifold. Both reversed-phase and "normal-phase" (silica gel) would be used. Modifiers would be added to the system that would have minimal effect on the complex, but could change the activity of the chromatographic support. Silica gel, although typically regarded as "normal-phase" has a number of chromatographic behaviors, including ion-exchange and capabilities typical of what is normally considered "reversed-phase" (280). Therefore silica gel was not ruled out as a possible support.

(1) Results of Silica Gel Chromatography

(i) Water as the Mobile Phase

The initial approach was to try water or a saline solution to try to achieve separation by either "normal-phase" (silica gel), or reversed-phase, C18. However, neither solution gave any mobility of the test
aminoglycosides.

(ii) Addition of Monovalent Salts as Mobile Phase Modifiers

1. Sodium Chloride as a Modifier

Therefore it was necessary to add modifiers to the systems. Sodium chloride was chosen as the solvent modifier. First, sodium chloride has been widely used in reversed-phase systems as an ion-pairing reagent. It also serves to suppress the active sites on the support.

Second, it is very unlikely that sodium ion complexes with the aminoglycoside moiety for several reasons. At physiological pH 7.4, most aminoglycosides exhibit a number of cationic sites (224). Divalent calcium does not complex with gentamicin in physiological solutions (249), it is therefore more unlikely that monovalent sodium ion would complex.

Sodium chloride was added over a concentration range of 0.5 - 2.5 molar. It was found that all the test aminoglycosides could be separated using this method. Mobility (Rf) of the aminoglycoside increased with increasing sodium chloride concentration. Separation of all the test aminoglycoside compounds was achieved.

2. Potassium Chloride as a Modifier

Potassium chloride was added as a mobile phase modifier over a concentration range of 0.5 to 2.5 molar. Analogous results were achieved with potassium chloride, except that mobilities (Rf’s) were less than with sodium
chloride.

3. Cesium chloride as a Modifier

Cesium chloride was added as a mobile phase modifier over a concentration range of 0.5 to 2.5 molar. Analogous results were achieved as with sodium and potassium chlorides, except the mobilities of the test compounds were less.

A comparison of the mobilities (Rf's) of the test aminoglycosides at 2.50 molar salt concentration is given in Figure 74.

4. Mobilities in Mixed Salt Solutions

Mixtures of sodium chloride and potassium chloride were prepared at varying concentrations and the mobilities of the test aminoglycoside compounds were measured. It was found that the mobility of the aminoglycoside was sum of the value expected for the individual salt concentrations.

(iii) Addition of Divalent Salts as Mobile Phase Modifiers

Divalent salts of calcium, magnesium, and barium chloride were investigated as mobile phase modifiers. This investigation was conducted because of the success of the monovalent cations as modifiers. Complexation of these divalent cations did not seem likely under these conditions, calcium had already been shown not to complex gentamicin under physiological conditions (249).

1. Calcium Chloride and Barium Chloride as Mobile
Figure 74. Retention Factors (Rf's) for Aminoglycosides on Silica Gel Plate for Three Monovalent Cationic Salt Solutions.
Phase Modifiers

Attempts to use either calcium chloride or barium chloride as mobile phase modifiers were not successful. Reproductable results on TLC were not achieved because the TLC coating tended to separate from the glass backing.

2. Magnesium Chloride as a Mobile Phase Modifier

Magnesium chloride was added as a mobile phase modifier over a concentration range of 0.10 molar to 4.50 molar. Significantly greater Rf’s were observed for equivalent concentrations of magnesium chloride versus any of the monovalent cationic salts.

The results of these studies are shown in Figure 75. Up to a concentration of 1.00 molar magnesium chloride the mobilities of the test aminoglycosides increased. Above 1.00 molar magnesium chloride significant changes occurred in the chromatographic behavior for the gentamicins. Amikacin and kanamycin remained close to the solvent front. No additional studies were done to explain this behavior.

(2) Results of TLC Reversed-Phase C18 Chromatography

The chromatographic behavior of aminoglycosides on reversed-phase C18 TLC plates (Whatman) could not be studied using the NaOCl-cadmium iodide-starch detection spray used in this study. The spray reacted instantaneously with the plate upon spraying and changed to a blue-black color. Therefore the aminoglycosides
Figure 75. Retention Factors for Aminoglycosides on Silica Gel Plates as a Function of Magnesium Chloride Concentration (0.10 - 4.50 Molar).
could not be detected on reversed-phase TLC plates, in contrast to results on silica gel TLC plates.

However, the metal moiety, both complexed and uncomplexed, could be detected on the reversed-phase TLC plates using dithizone. Thus complexation could be investigated using the reversed-phase TLC plates, but only via the metal portion.
Table 21. Solvent Systems Useful for the Separation of Aminoglycoside-Metal Complexes

<table>
<thead>
<tr>
<th>Chromatographic Solvent System</th>
<th>Support</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 M NaCl</td>
<td>K5, C18</td>
<td>Mobility increases with increasing salt content</td>
</tr>
<tr>
<td>2.5 M NaCl</td>
<td>K5, C18</td>
<td>Mobility in mixed salt system is sum of the two single systems</td>
</tr>
<tr>
<td>2.5 M KCl</td>
<td>K5, C18</td>
<td>Mobility in mixed salt system is sum of the two single systems</td>
</tr>
<tr>
<td>1.0 M NaCl/1.5 M KCl</td>
<td>K5, C18</td>
<td>Mobility increases with increasing salt content</td>
</tr>
<tr>
<td>0.5 M MgCl₂</td>
<td>K5, C18</td>
<td>Recommended for amikacin and kanamycin on C18. Poor K5 TLC plate surface stability</td>
</tr>
<tr>
<td>1.0 M MgCl₂</td>
<td>K5, C18</td>
<td>Recommended for amikacin and kanamycin</td>
</tr>
</tbody>
</table>

K5 is silica gel formulation from Whatman. C18 is reversed-phase ODS support from Whatman.
3E.(b) Speciation of Aminoglycoside-Metal Complexes by HPLC-AA and TLC-Colorimetry

(1) Introduction

Three divalent cations were chosen for investigation to see whether any complexes could be separated. The three were copper(2+), cobalt(2+), and zinc(2+). Copper was chosen because copper complexes of aminoglycosides have been prepared previously (but not under these conditions). Cobalt complexes have been used in the chemical transformation of aminoglycosides (246, 248). Zinc is present in serum at an average level of 1.2 ppm (63).

(2) Separation of Aminoglycoside Complexes by TLC-Colorimetry

(i) Silica Gel Chromatographic Support Separations

1. Complexation Studies with Copper(2+), Cobalt(2+), and Zinc(2+)

Solutions containing copper(2+), cobalt(2+), and zinc(2+) were prepared with a metal to aminoglycoside ratio of 10:1. The solutions and the aminoglycoside standard were spotted on a silica gel TLC and chromatographed in a 2.50 molar sodium chloride solution. The aminoglycoside fraction was detected using the NaOCl-cadmium iodide-starch solution (Figures 76).

Both uncomplexed and complexed amikacin and kanamycin
Figure 76. Separation of Uncomplexed and Complexed Aminoglycosides by TLC. Detection of Aminoglycoside Moiety.
were detected for the copper and cobalt solutions. Only uncomplexed aminoglycoside was detected for the zinc solution.

A second plate prepared and developed identically to the first plate was then dipped into the dithizone solution to visualize the metal moiety (Figure 77). Copper, both standard and complexed, was at the origin. For cobalt, two zones were detected in the aminoglycoside solutions. The first was for the uncomplexed cobalt, and the second for the complexed cobalt which was very near the origin. For the zinc solutions, only the uncomplexed zinc was detected, which confirmed the results of the aminoglycoside detection system.

Only copper and cobalt, not zinc, appeared to form complexes under these conditions. Unfortunately, the complexes were at or very close to the origin.
Figure 77. Detection of Uncomplexed and Complexed Metal-Aminoglycoside by TLC. Detection of Metal Portion with Dithizone.
2. Additional Studies with Complexation with Copper (2+)

To check the versatility of this system for the separation and qualitative detection of complexed, solutions of amikacin and kanamycin with copper to aminoglycoside ratios 0, 1, 2, 3, 5, and 10 were prepared (Figures 78 and 79).

The solutions were spotted and chromatographed. The aminoglycoside moiety was detected using the NaOCl-cadmium iodide-starch spray.

As the ratio of copper was increased, the amount of uncomplexed (free) aminoglycoside decreased and a corresponding increased was noted for the complex zone.

Duplicate plates were developed and dipped in dithizone for detection of the metal portion. Only a single zone was detected for copper since both the complexed and uncomplexed copper remained at the origin.

This system though amply demonstrated the use of TLC-colorimetry for the separation and qualitative detection of both the complexed and uncomplexed aminoglycoside. This represents a significant increase over current methods. For example, in the patent literature covering the use of metal complexes for the chemical transformation of aminoglycosides, chromatographic separations were achieved only for the initial and final aminoglycoside products (not the complexes!).
Figure 78. Separation of Uncomplexed and Complexed Amikacin as a Function of Ratio of Copper to Amikacin.
Figure 79. Separation of Uncomplexed and Complexed Kanamycin as a Function of Ratio of Copper to Kanamycin.
3E.(c) Reversed-Phase TLC-Colorimetry of Metals

Although the Whatman reversed-phase TLC could not be used for the detection of the aminoglycoside with the reagent, the plates were very amendable to the detection of the copper(2+), cobalt(2+) and zinc(2+).

Analogous results were achieved for the separation of the complexed and uncomplexed divalent cations using the same system as for the silica gel TLC plates. (Figure 80)

Best results were achieved however for a system that was 0.5 molar in magnesium chloride. Excellent separation of the three divalent cations used for the complexation studies plus several additional ones. The TLC-dithizone colorimetry of 5 divalent cations is shown in Figure 81.

3E.(d) HPLC-AA Studies of Aminoglycoside-Metal Complexation

Although the aminoglycoside complexes formed were not mobile in any of the system evaluted, either silica gel or C18, HPLC-AA investigations of the extent of any complexation is possible for cases in which the uncomplexed metal is mobile. For example, the extent of complexation of cobalt with one of the aminoglycoside would lend itself to study.

Cobalt complexation with kanamycin was chosen because cobalt complexation had been shown on TLC, and because its
Figure 80. Complexation of Metal with Amikacin and Kanamycin. Separation by TLC. Detection of Metal with Dithizone.
Figure 81. Separation of Some Transition Metal Cations by Reversed-Phase TLC. Detection with Dithizone.
potential in chemical transformation of a number of aminoglycosides has been extensively utilized.

Solutions of cobalt with kanamycin with ratios of kanamycin to cobalt of 0, 1, 2, 4, and 9. The HPLC-AA of three of the solutions is shown in Figure 82. As the ratio of kanamycin is increased the amount of uncomplexed (free) cobalt in solution decreased. The results are plotted in Figure 83.
Figure 82. HPLC-AA of Cobalt(2+) Not Complexed by Kanamycin A.
Figure 83. Plot of Cobalt(2+) Not Complexed by Kanamycin A as a Function of Ratio of Kanamycin A to Cobalt(2+).
3F. SUMMARY

A comprehensive study was conducted of new and unique class of solvent systems for the separation of aminoglycosides and their metal complexes. Separations were achieved on both silica gel and C18 reversed phase supports using monovalent and divalent salt solutions.

The complexation of aminoglycoside with copper(2+), cobalt(2+), and zinc(2+) was investigated on TLC by using both a colorimetric methods to detect the aminoglycoside (free and complexed) and dithizone to detect the metal cation (free and complexed).

The potential of HPLC-AA for speciation was illustrated once again in the detection of the uncomplexed cobalt in a series of kanamycin-cobalt solutions.
REFERENCES


3. J. E. Poldoski, ibid,(1979) 13, 701


42. P. Hulmston, Analyst, (1982) 107, 166.


C. D. Stevens, C. J. Feldhake and R. A. Kehoe, J.


137. "How to Use the KC18/KC18F Reversed Phase TLC Plate", Whatman, Clifton, New Jersey.


158. R. Braman and D. Johnson, Environ. Sci. Tech.,


173. J. W. Robinson and J. C. Wu, Louisiana State University, (private communication).


268. J. P. Anhalt, F. D. Sancilio, and T. McCorkle, J.
ABSTRACT

Part I. Development of a new nebulizer-flame atomizer atomic absorption system designed for metal speciation.

A new flame atomic absorption system has been designed and developed. A new, highly efficient ultrasonic nebulizer was used for sample introduction and aerosol generation. Extensive evaluations and modifications of commercially available atomic absorption spray chambers and burner heads were conducted. Observations made during these studies centered on some of the fundamental properties of aerosol transport within the atomic absorption nebulization system.

The new flame atomic absorption system consisted of the ultrasonic nebulizer incorporated into a modified spray chamber. The aerodynamic properties of the chamber were designed to give maximal aerosol transport. Burner extensions were incorporated into the chamber and could be resistively heated to aid in desolvation of the aerosol.

This system exhibited an increase in sensitivity,
depending on the conditions and flow rates, of approximately 5 to 7 fold, and an increase in detection limits of approximately 2 to 4 fold over that reported for commercial flame atomizers.

Part II. The speciation of organoleads, mercury compounds, and metal complexes of aminoglycosides. A number of physiologically and environmentally important organoleads and mercurials were speciated by high performance liquid chromatography-atomic absorption (HPLC-AA). HPLC systems were developed to separate these compounds by using complementary thin layer chromatographic (TLC) methods with colorimetry detection. These systems were used to study the conversion of tetraethyllead in seawater over a long period of time and the decomposition of diethyllead dichloride. Results showed that the triethyllead chloride was stable in seawater for at least one year, and that diethyllead chloride decomposes into triethyllead chloride and lead(2+) compounds.

Metal complexation with a class antibiotics, aminoglycosides, was investigated both by HPLC-AA and TLC-colorimetry. New HPLC and TLC systems were developed to separate the metal/complexes or the uncomplexed aminoglycoside. The formation of the copper and cobalt complexes was shown to be a function of the ratio of metal to aminoglycoside.
Edmund D. Boothe received a B.S. in Chemistry in 1970 and a M.S. in Chemistry in 1974 from the University of Alabama.

He joined Eli Lilly and Company in 1973 as an Analytical Chemist in the Biochemical Division with responsibilities for analytical and process development. In 1977 he was named Quality Control Representative for Clinton Laboratories, a major production facility of Eli Lilly and Company. At Clinton Laboratories, he had responsibility for the quality control and technical services functions.

During this time he was actively involved in a number of scientific and community activities. He was elected Treasurer and Executive Committeeman for the Indiana Section of the American Chemical Society. He served on the Executive Committee for three years and was named Membership Committee Chairman for the ACS. He was also active in the Society for Applied Spectroscopy.

He also served as Institutional Representative for Eli Lilly to the Boy Scouts and was an advisor to an Explorer Post.

In 1980 he came to Louisiana State University to pursue the Ph.D. degree. During this time he was served as graduate student representative to the Department of Chemistry Graduate Studies Committee. He was also active
in Alpha Chi Sigma, and a member of Phi Lambda Upsilon. He was active in local scientific organizations. He served as a Minuteman for the Baton Rouge Section of the American Chemical Society for three years, and as a member of the Executive Committee. He was named the first Chairman of the Publicity Committee for the local ACS. He has also served as Secretary of the Louisiana Chapter for the Society for Applied Spectroscopy for the past 2 years. He is also a member of American Association for the Advancement of Science.

He is married and has two children. The youngest was born while at Louisiana State University.

Following graduation he will be employed as Supervisor of Analytical Services and Development with G. D. Searle & Company. He will have responsibilities for analytical development at a new fermentation facility incorporating the latest in biotechnology and genetic engineering. He will also have responsibility for laboratory computer needs at the plant site.
EXAMINATION AND THESIS REPORT

Candidate: Edmund D. Boothe

Major Field: Analytical Chemistry

Title of Thesis: Speciation Studies of Biologically Active Compounds

Approved:

[Signature]
Major Professor and Chairman

[Signature]
Dean of the Graduate School

EXAMINING COMMITTEE:

Norman Blake

William R. Lee

[Signature]

[Signature]

Date of Examination:

July 10, 1984