INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI®

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

PULSE MICROWAVE MEDIATED SAMPLE CLEAN-UP METHOD TO ANALYSE TRACE METALS, PCBs AND PESTICIDES, AND FOR THE TREATMENT OF ORGANIC WASTES.

Prasad Aysola

A Thesis

in

The Department

of

Chemistry and Biochemsitry

Presented in Partial Fulfilment of the Requirements
for the Masters of Science at
Concordia University
Montreal, Quebec, Canada

August 1998

NOTE TO USERS

Page(s) not included in the original manuscript are unavailable from the author or university. The manuscript was microfilmed as received.

ii

UMI

ABSTRACT

A procedure is described for open vessel pulse microwave wet ashing system. In wet ashing procedure organic matrix in biological tissues is digested in acid. This step must precede trace metal analysis by atomic absorption spectroscopy. Conventional wet ashing procedure using hot plate takes several hours. Digestion of animal and plant tissues by 5.0 ml, 10.0 ml or 20.0 ml of H_2SO_4 (95.0 - 98 %) /HNO_3 (69 - 71%) (v/v) (1:1) acid mixture were effectively achieved by pulse microwave treatment using domestic grade microwave oven. Samples are subjected to 10s (method A) or 6s (method B) microwave heating followed by dormant time of 3 minutes for a total microwave heating time of less than 20 minutes. Data will be presented to show how conditions were chosen. The temperature of digestion mixture was maintained $< 100^0$C. The effect of pulse time variation and acid mixture volume variation was studied in detail. A maximum coefficient of variation of less than 7% for Ca, Cu, Fe, Mn and Zinc in NIST animal, plant reference materials and spiked metals demonstrates the precision of the method. Accuracy of the method is demonstrated by mean recovery of greater than 89% for reference materials and greater than 96% for spiked low (ppb) and higher (ppm) level metals. A one way analysis of variance (ANOVA) was the statistical tool used to analyze the NIST bovine liver data for Cu, Fe and Zn. ANOVA was performed on the data for each element. The reference material showed no significant difference between conventional method, method A (20 ml acid mixture), method B (5.0 ml, 10 ml or 20 ml acid mixture) and pulse time variation for Cu and Fe. ANOVA for Zn showed significant difference between different methods. Tukey's comparison test showed that Zn is sensitive to acid volume and pulse time variation.

The NIST tomato leaves material showed that Ca is sensitive to pulse time variation. Tukey's comparison test showed that Mn is sensitive to acid volume variation. Pulse methods A and B showed matrix interferences effect for Zn in NIST bovine liver. However, student t-test showed that the values obtained from the longer pulse heating times (72s, 108s) are statistically equal to NIST values.

Pulse microwave wet aching procedure was also employed to clean up animal tissue samples contaminated with refractory organic pollutants that are resistant to oxidation. Standard procedures for the clean-up of biological samples for the analysis of polychlorinated biphenyls and pesticides are generally intricate and time consuming (10 - 24 hrs). The selective oxidation of organic matrix contaminated with pesticides was effectively achieved within 20 minutes by method A. Presence of Nujol was critical for quantitative recovery. Hydrophobic Nujol protects hydrophobic pollutants from degradation. Following wet ashing, the samples were extracted with hexane and injected directly into GC or PLC for identification and quantification. Effect of pulse time and Nujol volume variation on recovery rates were studied. Accuracy is demonstrated by mean recovery of greater than 92% for spiked Aroclor 1260 and pesticide isomers BHC. The rates of pesticide and PCB degradation were found to increase as a function of pulse heating time. DDT, DDE and Methoxychlor decomposed completely in 20s of pulse heating time and more 99% of Aroclor 1260 was decomposed in less than 3 hours of pulse treatment procedure. However, the final products were not identified.

Kinetics of two very different reactions in two very different media were studied to understand the effectiveness of pulse microwave heating: a) Hydrolysis of trans$(Coen_2Cl_2)^+$ in a mixture of CH_3OH/H_2O and b) Aromatic nitration of p-nitrobenzoic acid in mixed sulphuric/nitric acid. Both reactions were carried out in vessels open to the atmosphere.

dependence of the reactions were determined using the conventional baths, and the Arrhenius equation (linear fitting with $r > 0.994$) was used to calculate the effective reaction temperature for each of the microwave runs from the observed effective "rate constants". It is apparent from rate constant results from both reactions that superheating occurs in microwave oven under reflux and pulse conditions. Each effective reaction temperature calculated is higher than the maximum temperature measured conventionally at the hottest point in the duty cycle. It is particularly interesting in the context of maximization of the energy efficiency of reaction conditions to consider fully the controlled use of pulsed heating. The pulse microwave allows us to exploit repeated superheating phenomena to accelerate rates to values above average temperatures we can measure by extrapolation technique. This level of overheating will ultimately offer the opportunity to optimize the energy efficiency of microwave heated synthesis.

ACKNOWLEDGEMENT

I would like to express my gratitude to Dr.P.D.Anderson and Dr. C. H. Langford for their support. I am grateful to Dr. O.S. Tee for helpful discussions. I would like to thank my committee members Dr. P. Banks, Dr. L.D. Colebrook and Dr. O.S. Tee. I thank Dr.J . Capobianco, Mr. B. Patterson and Miss M. Posner for their help and support.

Lastly but not least, I am grateful to my wife, Karuna, for her help, support, and understanding. I could not have made it without her encouragement.

LORD VENKATESHWARA

IN MEMORY OF
DR. M. HOGBEN
DIRECTOR OF ECOTOXICOLOGY

DEDICATED

to

Karuna, Kartik, Pooja, Andy, Rao, Bala, Vijay, Yezdani and The memory of My dad,

mom and my brother Raja

TABLE OF CONTENTS

LIST OF TABLES---------- vi

LIST OF FIGURES---------- ix

CHAPTER 1

INTRODUCTION

1.1 TRACE METAL DETECTION IN ENVIRONMENTAL SAMPLES
1.1.1 General---------- 2
1.1.2 Sample clean up methods---------- 3
1.1.2.1 Wet ashing---------- 4
1.1.2.2 Dry ashing---------- 5
1.1.2.3 Standard addition or Spiking method---------- 6
1.1.2.4 Microwave oven based wet ashing 7
1.1.2.4.1 Interaction of microwave radiation with sample 8
1.1.2.4.2 Microwave oven---------- 10
1.1.2.4.3 Wet ashing vessels---------- 13
1.1.3 Direct solid sampling---------- 15
1.1.4 Trace metal detection---------- 16
1.2 REFRACTORY ORGANIC POLLUTANT ANALYSIS IN ENVIRONMENTAL SAMPLES
1.2.1 General 19
1.2.1.1 Polychlorinated Biphenyls (PCBs) ---------- 19
1.2.1.2 Chlorinated hydrocarbon pesticides---------- 21
1.2.2 Sample clean-up methods---------- 22
1.2.2.1 Supercritical fluid extraction (SFE)---------- 23
1.2.2.2 Solid Phase Micro Extraction 24
1.2.2.3 Problems encountered in PCB isolation-------- 26
1.2.2.4 Problems encountered in Pesticide isolation--- 27
1.2.3 Organic pollutant detection 28
1.2.3.1 Electron capture detector---------- 28
1.2.3.2 Gas chromatography/Mass spectrometry------ 29
1.3 PURPOSE OF INVESTIGATION---------- 31

CHAPTER 2

WET ASHING OF BIOLOGICAL SAMPLES IN A MICROWAVE OVEN UNDER PRESSURE USING TEFLON VESSELS.

2.1 INTRODUCTION---------- 33
2.2 EXPERIMENTAL---------- 35
2.2.1 Apparatus---------- 35
2.2.2 Materials---------- 35
2.2.3 Preparation of standard solutions---------- 37
2.2.4 Procedure---------- 38
2.3 RESULTS AND DISCUSSION---------- 40
2.3.1 Effect of wet ashing time---------- 47
2.4 CONCLUSION---------- 48

CHAPTER 3

PULSE MICROWAVE DIGESTION OF ANIMAL TISSUES

3.1 INTRODUCTION---------- 50
3.2 EXPERIMENTAL---------- 51
3.2.1 Apparatus---------- 51
3.2.2 Materials---------- 51
3.2.3 Characterizing optimum conditions---------- 51
3.2.4 Temperature programming---------- 55
3.2.5 Background correction---------- 55
3.2.6 Procedure---------- 57
3.2.6.1 Pulse treatment procedure---------- 57
3.2.6.2 Conventional heating procedure---------- 57
3.2.6.3 Calculations---------- 58
3.3 RESULTS AND DISCUSSION---------- 59
3.4 CONCLUSION---------- 75

CHAPTER 4

DIGESTION OF PLANT SAMPLES BY PULSE MICROWAVES FOR ELEMENTAL ANALYSIS.

4.1 INTRODUCTION---------- 77
4.2 EXPERIMENTAL---------- 78
4.2.1 Apparatus---------- 78
4.2.2 Materials---------- 78
4.2.3 Characterizing optimum conditions---------- 78
4.2.4 Procedure---------- 79
4.2.4.1 Pulse treatment procedure---------- 79
4.2.4.2 Conventional procedure---------- 79
4.2.4.3 Calculations---------- 79
4.3 RESULTS AND DISCUSSION---------- 80
4.3.1 Anova results---------- 90

CHAPTER 5

PULSE MICROWAVE-MEDIATED BIOLOGICAL SAMPLE CLEAN-UP METHOD FOR REFRACTORY ORGANIC ANALYSIS AND DEGRADATION OF PCBs AND PESTICIDES

5.1 INTRODUCTION---------- 100
1.1 Treatment of pesticides and PCB Wastes---------- 101
5.2 EXPERIMENTAL---------- 105
5.2.1 Instrumentation---------- 105
5.2.2 Reagents---------- 105
5.2.3 Preparation of fortified samples---------- 105
5.2.4 Glassware---------- 105
5.2.5 Laboratory clean-up procedure---------- 106
5.2.6 Procedure---------- 106
5.3 RESULTS AND DISCUSSION---------- 107
5.3.1 Extraction efficiency of PCBs---------- 107
5.3.2 Effect of nujol volume and pulse heating time on recovery rates-- 113
5.3.3 PCB recovery from bovine liver---------- 113
5.3.4 PCB recovery from serum---------- 121
5.3.5 PCB recovery from soil---------- 123
5.3.6 Pesticide recovery from bovine liver---------- 126
5.3.7 Molecular structure and recovery rates---------- 127
5.3.8 Nujol's role in the mechanism of refractory pollutant recovery during digestion ---------- 128

5.4 TREATMENT OF PCBs AND PESTICIDES---------- 131
5.4.1 Unknown product formation---------- 131
5.5 CONCLUSION---------- 136
5.5.1 Suggestions for future study---------- 136

CHAPTER 6

MECHANISM OF MICROWAVE REACTIONS UNDER REFLUX CONDITIOND

6.1 INTRODUCTION---------- 139
6.1.1 Hydrolysis of trans$(Co(en)_2Cl_2)^+$.---------- 142
6.1.2 Nitration of p-nitrobenzoic acid---------- 143
6.2 Experimental Section A: Kinetic Studies under Reflux Conditions----- 144
6.2.1 Instruments---------- 144
6.2.2 Apparatus---------- 144
6.2.3 Reagents---------- 145
6.2.4 Procedure---------- 145
6.2.4.1 Preparation of trans $(Co(en)_2Cl_2)Cl$------- 145
6.2.4.2 Hydrolysis of trans$(Co(en)_2Cl_2)^+$--------- 146
A) Water bath---------- 146
B) Microwave oven (reflux)-------- 146
6.2.4.3 Nitration of p-nitrobenzoic acid---------- 146
A) Oil bath---------- 146
B) Microwave oven (reflux)-------- 147
6.3 RESULTS AND DISCUSSION (Section A)---------- 148
6.4 CONCLUSION ---------- 169

APPENDIX A ---------- 170

KINETIC STUDIES BY PULSE SEQUENCE HEATING

A.1 EXPERIMENTAL SECTION---------- 172
C.1.1 Hydrolysis of trans$(Co(en)_2Cl_2)^+$---------- 172
C.1.2 Nitration of p-Nitrobenzoic acid in Microwave oven (pulse heating)-------- 172

A.2 RESULTS AND DISCUSSION ---------- 173
A.3 CONCLUSION---------- 176

REFERENCES---------- 200

LIST OF TABLES

TABLE 2.1	Analytical operating conditions for flame atomic absorption	36
TABLE 2.2a	Certified values of constituent elements for NIST bovine liver 1577a	39
TABLE 2.2b	Noncertified values of constituent elements for NIST bovine liver 1577a	39
TABLE 2.3	Analysis of NIST 1577a bovine liver by flame AAS after wet ashing under pressure in a microwave oven.	42
TABLE 2.4	Effect of wet ashing time: Recovery of copper from NIST 1577a bovine liver by flame AAS after wet ashing under pressure in a microwave oven.	43
TABLE 2.5	Effect of wet ashing time: Recovery of Iron from NIST 1577a bovine liver by flame AAS after wet ashing under pressure in a microwave oven	44
TABLE 2.6	Recoveries of added nickel in fish tissue after wet ashing under pressure in a microwave oven.	45
TABLE 3.1	Analytical operating conditions for flameless atomic absorption (animal tissue samples)	53
TABLE 3.2	Extrapolated temperatures (°C) for acids subjected to 6s and 10s pulses. (Animal tissue samples)	54
TABLE 3.3	A comparison of expected and analyzed values for Copper, Iron and Zinc	60
TABLE 3.4	A comparison of expected and analyzed values for Copper, Iron and Zinc.	61
TABLE 3.5	Effect of acid mixture volume and pulse time variation on recovery of Copper.	62
TABLE 3.6	Effect of acid mixture volume and pulse time variation on recovery of Iron.	63
TABLE 3.7	Effect of acid mixture volume and pulse time variation on recovery of Zinc.	64

TABLE 3.8	Recovery of spiked Cadmium.	73
TABLE 4.1	Comparison of results for Ca determination by different acid mixtures	81
TABLE 4.2	Comparison of expected and analyzed values for Calcium, Manganese and Zinc.	83
TABLE 4.3	Comparison of expected and analyzed values for Calcium, Manganese and Zinc.	84
TABLE 4.4	Comparison of expected and analyzed values for Calcium, Manganese.	85
TABLE 4.5	Effect of acid mixture volume and pulse time variation on recovery of Calcium.	87
TABLE 4.6	Effect of acid mixture volume and pulse time variation on recovery of Manganese.	88
TABLE 4.7	Effect of acid mixture volume and pulse time variation on recovery of Zinc.	89
TABLE 4.8	Recovery of spiked Cu, Cr, Cd, and Pb.	97
TABLE 5.1	Effect of nujol: Recoveries of spiked hexachlorobiphenyl (PCB monomer) from NIST bovine liver.	115
TABLE 5.2	Recoveries of spiked Aroclor 1260 (PCB mixture) from NIST bovine liver.	117
TABLE 5.3	Recovery of spiked Aroclor 1260 from serum	121
TABLE 5.4	Recoveries of spiked Aroclor 1260 (PCB mixture) from soil.	124
TABLE 5.5	Recovery of pesticide BHC from NIST bovine liver.	129
TABLE 5.6	Degradation of PCBs.	132
TABLE 6.1	Percent yield of n-propyl benzoate with conventional and microwave oven heating	140
TABLE 6.2	Experimental conditions used for HPLC analysis	145

TABLE 6.3	Rate constant studies : Hydrolysis of trans$(Co(en)_2Cl_2)^+$ in water bath.	149
TABLE 6.4	Hydrolysis of trans$(Co(en)_2Cl_2)^+$ in microwave oven under reflux conditions	151
TABLE 6.5	Oil bath rate constant studies: Nitration of p-nitrobenzoic acid.	162
TABLE 6.6	Nitration of p-Nitrobenzoic acid in microwave oven under reflux conditions.	166
TABLE A.1	Hydrolysis of trans$(Co(en)_2Cl_2)$ + in microwave oven: Temperature extrapolation Total pulse cycle = 10s	187
TABLE A.2	Hydrolysis of trans$(Co(en)_2Cl_2)^+$ in microwave oven: Temperature extrapolation. Total pulse cycle = 20s	188
TABLE A.3	Microwave oven rate constant studies : Nitration of p-nitrobenzoic acid	198

LIST OF FIGURES

FIGURE 1.1	Electromagnetic spectrum.	9
FIGURE 1.2	The major features of a modern domestic microwave oven.	11
FIGURE 1.3	High pressure vessel for microwave dissolution (Parr Instrument Co).	14
FIGURE 1.4	Microwave digestion unit, Pressure and temperature-sensing equipment, and data acquisition system (CEM Co).	15
FIGURE 1.5	Schematic diagram of Atomic Absorption Spectroscopy.	17
FIGURE 1.6	Cross section of commercial flameless furnace.	18
FIGURE 1.7	Conventional numbering of substituent positions in biphenyl.	19
FIGURE 1.8	Schematic diagram of gas chromatographic system.	29
FIGURE 1.9	Schematic diagram of GC\MS.	30
FIGURE 2.1	Microwave oven set-up for wet ashing with Savillex vessels.	38
FIGURE 2.2	A plot of spiked versus recovered Ni from fish tissue.	46
FIGURE 3.1	Microwave oven set-up for wet ashing with pulse procedure	58
FIGURE 5.1	Structures of dioxin formation.	102
FIGURE 5.2a	Chromatogram of Aroclor 1260 standard (10 ng/1.0 ul) (Standard 1).	109
FIGURE 5.2b	Chromatogram of Aroclor 1260 standard (10 ng/1.0 ul) (Standard 2).	110
FIGURE 5.3a	Extraction efficiency of Aroclor 1260 from acid mixture H_2SO_4/HNO_3 (20 ml) (Sample 1).	111
FIGURE 5.3b	Extraction efficiency of Aroclor 1260 from acid mixture H_2SO_4/HNO_3 (20 ml) (Sample 2).	112

FIGURE 5.4 Chromatogram of (A) hexachlorobiphenyl standard (HCB) (2 ng), (B) recovery of spiked HCB from bovine liver after 60s pulse wet ashing in the presence of 500 ul nujol and C) recovery of spiked HCB from bovine liver after 60s pulse wet ashing in the presence of 1000 ul nujol. 116

FIGURE 5.5 Chromatogram of (A) Aroclor 1260 standard (10ng) and (B) hexane extract from NIST bovine liver (0.1 g) subjected to pulse microwave wet ashing procedure. 118

FIGURE 5.6 Chromatogram of (A) Aroclor 1260 standard (10ng) and (B) hexane extract from NIST bovine liver (0.25 g) subjected to pulse microwave wet ashing procedure. 119

FIGURE 5.7 Chromatogram of (A) Aroclor 1016 standard (10ng) and (B) hexane extract from acid mixture H_2SO_4/HNO3 spiked with Aroclor 1016 and (C) hexane extract from spiked NIST bovine liver subjected to pulse microwave wet ashing procedure. 120

FIGURE 5.8 Chromatogram of (A) Aroclor 1260 standard (10ng) and (B) hexane extract from serum subjected to pulse microwave wet ashing procedure 122

FIGURE 5.9 Chromatogram of (A) Aroclor 1260 standard (2.5 ng) and (B) hexane extract from PEI soil subjected to pulse microwave wet ashing procedure 24s (method B) and 50s (method A). 125

FIGURE 5.10 Chromatogram of (A) DDT, DDE, and methoxychlor and (B) hexane extract from spiked NIST bovine liver subjected to pulse microwave wet ashing procedure. 130

FIGURE 5.11 EC-GC chromatograms showing the hexachlorocyclohexane peak (A) and the response after 60s pulse microwave treatment (B) 133

FIGURE 5.12 EC-GC chromatograms showing the decrease in concentration of Aroclor 1260 as a function of pulse microwave treatment time. Figure A = 10 ppm standard. B = 10,000 ppm after 240 s of pulse heating. C = 600s of pulse heating. 134

FIGURE 5.13 EC-GC chromatograms showing Aroclor 1016 standard and sample after 60s pulse microwave treatment time. 135

FIGURE 6.1	Schematic diagram of modified microwave oven	144
FIGURE 6.2	Arrhenius plot: Hydrolysis of trans$(Co(en)_2Cl_2)^+$ in water bath rate constant studies	150
FIGURE 6.3	Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid at 92 °C (Sample A)	152
FIGURE 6.4	Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid at 92 °C(Sample B)	153
FIGURE 6.5	Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid at 108 °C (Sample A)	154
FIGURE 6.6	Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid at 108 °C (Sample B)	155
FIGURE 6.7	Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid at 108 °C (Sample C)	156
FIGURE 6.8	Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid at 108 °C (Sample D)	157
FIGURE 6.9	Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid at 118 °C (Sample A). Boiling point of acid mixture = 118 °C. Oil bath temperature = 160 °C.	158
FIGURE 6.10	Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid at 118 °C (Sample B). Boiling point of acid mixture = 118 °C. Oil bath temperature = 160 °C.	159
FIGURE 6.11	Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid at 118 °C (Sample C). Boiling point of acid mixture = 118 °C. Oil bath temperature = 160 °C.	160
FIGURE 6.12	Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid at 118 °C (Sample D). Boiling point of acid mixture = 118 °C. Oil bath temperature = 160 °C.	161
FIGURE 6.13	Arrhenius plot: Nitration of p-Nitrobenzoic acid in oil bath	163
FIGURE 6.14	Nitration of p-Nitrobenzoic acid reflux reaction in microwave oven. (Sample A): Boiling point of acid mix = 118 °C.	164

FIGURE 6.15 Nitration of p-Nitrobenzoic acid reflux reaction in microwave oven. (Sample B): Boiling point of acid mix = 118 °C. 165

FIGURE A.1a Solvent temperature extrapolation to t_0 (A) at 50% duty cycle for pulse heating sequence of 10s. 177

FIGURE A.1b Solvent temperature extrapolation to $t_{1/2}$ (B) at 50% duty cycle for pulse heating sequence of 10s. 177

FIGURE A.2a Solvent temperature extrapolation to t_0 (A) at 60% duty cycle for pulse heating sequence of 10s. 178

FIGURE A.2b Solvent temperature extrapolation to $t_{1/2}$ (B) at 60% duty cycle for pulse heating sequence of 10s. 178

FIGURE A.3a Solvent temperature extrapolation to t_0 (A) at 70% duty cycle for pulse heating sequence of 10s. 179

FIGURE A.3b Solvent temperature extrapolation to $t_{1/2}$ (B) at 70% duty cycle for pulse heating sequence of 10s. 179

FIGURE A.4a Solvent temperature extrapolation to t_0 (A) at 80% duty cycle for pulse heating sequence of 10s. 180

FIGURE A.4b Solvent temperature extrapolation to $t_{1/2}$ (B) at 80% duty cycle for pulse heating sequence of 10s. 180

FIGURE A.5a Solvent temperature extrapolation to t_0 (A) at 90% duty cycle for pulse heating sequence of 10s. 181

FIGURE A5b Solvent temperature extrapolation to $t_{1/2}$ (B) at 90% duty cycle for pulse heating sequence of 10s. 181

FIGURE A.6a Solvent temperature extrapolation to t_0 (A) at 40% duty cycle for pulse heating sequence of 20s. 182

FIGURE A.6b Solvent temperature extrapolation to $t_{1/2}$ (B) at 40% duty cycle for pulse heating sequence of 20s. 182

FIGURE A.7a Solvent temperature extrapolation to t_0 (A) at 50% duty cycle for pulse heating sequence of 20s. 183

FIGURE A.7b Solvent temperature extrapolation to $t_{1/2}$ (B) at 50% duty cycle for pulse heating sequence of 20s. 183

FIGURE A.8a Solvent temperature extrapolation to t_0 (A) at 60% duty cycle for pulse heating sequence of 20s. 184

FIGURE A.8b Solvent temperature extrapolation to $t_{1/2}$ (B) at 60% duty cycle for pulse heating sequence of 20s. 184

FIGURE A.9a Solvent temperature extrapolation to t_0 (A) at 70% duty cycle for pulse heating sequence of 20s. 185

FIGURE A.9b Solvent temperature extrapolation to $t_{1/2}$ (B) at 70% duty cycle for pulse heating sequence of 20s. 185

FIGURE A.10a Solvent temperature extrapolation to t_0 (A) at 80% duty cycle for pulse heating sequence of 20s. 186

FIGURE A.10b Solvent temperature extrapolation to $t_{1/2}$ (B) at 80% duty cycle for pulse heating sequence of 20s. 186

FIGURE A.11a Solvent temperature extrapolation to t_0 (A) at 90% duty cycle for pulse heating sequence of 20s. 187

FIGURE A.11b Solvent temperature extrapolation to $t_{1/2}$ (B) at 90% duty cycle for pulse heating sequence of 20s. 187

FIGURE A.12 Pseudo Arrhenius plot (10s): Hydrolysis of trans$(Co(en)_2Cl_2)^+$ in microwave oven. 190

FIGURE A.13 Pseudo Arrhenius plot (20s):Hydrolysis of trans$(Co(en)_2Cl_2)^+$in microwave oven 191

FIGURE A.14 Nitration of p-Nitrobenzoic acid in microwave oven (5s pulse) (Sample A). 192

FIGURE A.15 Nitration of p-Nitrobenzoic acid in microwave oven (5s pulse) (Sample B). 193

FIGURE A.16 Nitration of p-Nitrobenzoic acid in microwave oven (5s pulse) (Sample C). 194

FIGURE A.17 Nitration of p-Nitrobenzoic acid in microwave oven (7s pulse) (Sample A). 195

FIGURE A.18 Nitration of p-Nitrobenzoic acid in microwave oven (10s pulse) (Sample A). 196

FIGURE A.19 Nitration of p-Nitrobenzoic acid in microwave oven (10s pulse) (Sample B) 197

FIGURE A.20 Pseudo Arrhenius plot: Nitration of p-Nitrobenzoic acid. 199

CHAPTER 1

INTRODUCTION

1.1 TRACE METAL DETECTION IN ENVIRONMENTAL SAMPLES

1.1.1 General

The presence of toxic metals in animals, aquatic organisms, fruits and vegetables as trace contaminants has caused considerable concern with respect to their possible effect on the ecological system and eventually on humans. In addition to those metals known to be essential to animal, aquatic and plant life, there are many non-essential metals released into the environment due to industrial activity. Animals, fish and plants showing high levels of any one or more of these contaminants may find their way into the food chain. Pigments containing lead are still used for outdoor purposes because of their bright colours and weather resistant properties. Red lead (Pb_3O_4) is used extensively as a rust proof and primer for structural steel. Contamination of household dust by indoor and outdoor uses of paint containing lead remains a major source of exposure for infants and toddlers (1). Human exposure to cadmium has been and continues to be a major concern, because it has been shown to have effects on a variety of tissues and biological systems and it has been associated with such diverse ailments as hypertension and cancer (2). Inhalation of hexavalant chromium presents an increased risk of lung cancer, and calcium, lead, and zinc chromates are generally accepted as pulmonary carcinogens (3).

Routine residue analysis for trace toxic metals has become increasingly important in hazard evaluation programs. Environmental scientists utilize the information gathered from trace metal analysis to ascertain tolerance levels of aquatic and plant species. These levels can then be used to determine acceptable discharge levels of pollutants in lakes and rivers.

1.1.2 Sample Clean-up Methods

The most common analytical techniques applied nowadays in trace element analysis of biological samples normally begin with the dissolution of the substance to be analyzed. Chemical pretreatment of biological samples, as well as rocks, ores, slags, glass, etc., is a critical step during the course of which solid matter is brought into solution by decomposing and destroying the sample matrix.

Despite the importance and widespread applicability of sample dissolution, most conventional digestion procedures are tediously labour-intensive, and a number of them, such as those which use perchloric acid digestion, are potentially hazardous to laboratory personnel.

A number of the sample preparation procedures used today have been in use for more than 100 years. For example, heating samples in open beakers over flames or burners, is still widely used today. The modern hot plate has been added to the list of applicable heating sources. However, the experimental conditions that prevail in open-beaker digestions are, at best, empirical.

Many digestion procedures are available for the destruction of biological material prior to inorganic analysis, but almost all the methods fall into one of the two main classes: wet ashing and dry ashing. Each of these classes has advantages and disadvantages, as do the individual procedures which fall under them.

1.1.2.1 Wet Ashing Method

Wet ashing is widely used as a sample clean up procedure (4). In this procedure, the oxidation of the biological matrix is accomplished by heating the sample in acid solution for extended periods of time. No single procedure for wet digestion will effectively handle a wide range of biological materials. Various techniques have been reported (5) for the determination of heavy metals in fish tissue by atomic absorption spectroscopy after digestion of the sample by different procedures were completed.

The Association of Official Analytical Chemists (AOAC) (6) recommends the use of H_2SO_4/HNO_3(1:1) or $HNO_3/HClO_4$ for the dissolution of most biological samples. The use of $HClO_4$ may increase efficiency at the expense of safety. About 4 hours are required depending on the type of matrix involved. The Analytical Methods Committee (7) recommends that 50% H_2O_2 be used as the oxidation agent in wet digestion procedure of animal tissues. This procedure provides a rapid, but smooth, oxidation with no fumes and produces water as the only side product. Safety equipment is required. Concentrated HNO_3 and H_2SO_4 (1:1 by volume) acid mixtures with vanadium pentoxide as a catalyst has been used as wet ashing agent to oxidize the biological matrix (8). The procedure requires the use of a digestion vessel with a condenser. When the mixture reaches the boiling stage, H_2O_2 (30%) was added dropwise (2-3 drops).

In a wet digestion method of plant tissues by an Official Method of Analysis (9), the sample is boiled gently for 30 - 45 minutes in nitric acid, and then 70% $HClO_4$ is added to the cooled solution. The solution is further boiled for several minutes until dense fumes appear. The method is hazardous (because $HClO_4$ is used), time consuming (2 to 4 hours), and requires expensive and specialized wash-down hoods and temperature-controlled heating units (10,11).

1.1.2.2 Dry Ashing

In dry ashing procedure, the biological matrix is oxidised by the primary heating the sample between 400 °C and 700° C , where atmosphere oxygen serves as the primary oxidising agent. Small amounts of reagents such as $Mg(NO_3)_2$ or H_2SO_4 are added to aid the ashing process (4). Dry ashing is applicable to determine most common metals, usually with the exception of mercury and arsenic, in organic matter. Substances amenable to this method must be charred slowly in a muffle furnace between 400 °C and 700 °C for 16 hours. Loss by volatilisation, or by combination with the material of the container, must be avoided by working at the lowest possible temperature. Sulfuric acid , nitric acid or hydrochloric acid is used as an ashing aid. Particular care must be exercised when large amounts of halogens, either in their covalent or ionic forms, are present. Loss of certain metals, e.g., zinc, tin or antimony, occurs when dry ashing is carried out in the presence of halides; such losses can be minimised by ensuring that an alkaline ash remains.

Dry ashing of plant tissues by an Official Method of Analysis (9) takes a minimum of 4.5 hours heating followed by additional heating of sample on hot plate for several minutes. Dry ashing usually requires little attention. Larger amounts of material can be dealt with more conveniently than can be handled by wet decomposition by repeatedly adding fresh material to already ashed material and re-calcining.

This method avoids the use of large quantities of reagents and the high blank values that can result from sulfuric acid, nitric acid or hydrochloric acid. It is sometimes difficult to obtain complete extraction of the metal being determined from certain residues, such as those obtained from some compound rubbers. Excessive heating also makes certain metallic compounds insoluble, e.g., tin. Certain flour products give a dark melt in which carbon particles are trapped and will not burn. The slow ignition of some organic materials, e.g., rubber and related materials, can cause the evolution of

poisonous fumes, such as hydrocyanic acid and so operations must be carried out in well-ventilated fume cupboards. This method must not be applied to compounds, particularly those of nitrogen, that burn with explosive violence.

1.1.2.3 Standard Addition or Spiking Method:

Incomplete wet ashing results in organic matrix that interferes with metals during trace metal analysis by AAS. It is a common practice to spike the sample and study the recovery rates to check the effectiveness of the method. The wet ashing product of the spiked sample is compared with the metal standards prepared in aqueous matrix to study the completeness of dissolution and chemical interference. The method of standard addition is used to check the chemical interference (12). Perkin-Elmer atomic absorption spectroscopy (AAS) instrument manuel recommends (13) the standard spiking method for unknown matrix samples.

The relative merits of wet and dry ashing oxidations have been discussed in detail (4). In favour of the former are the low temperatures involved and maintenance of liquid conditions, which reduce the chances of retention losses. Furthermore the apparatus required is simple. Disadvantages include the large amounts of reagents added, with the consequent risk of increased blank values and the difficulty of handling large samples. Dry ashing methods require less reagents and the operation is simple. Against these methods must be balanced the lack of knowledge of the interaction between sample constituents, the trace elements and the material of the receptacle, with the consequent risk of loss by volatilisation or retention. High temperatures are required for such reactions, and the equipment is relatively expensive.

1.1.2.4 Microwave Oven Based Wet Ashing

The use of the microwave oven for wet ashing procedures was first demonstrated in 1975 (14). Sample preparation time was reduced significantly in the microwave oven and new applications for microwave heating in closed containers became apparent as a result of high temperatures and pressures reached in a few minutes. The elevated pressure in closed reaction vessels heated by microwaves increased not only the reaction rate in organic syntheses, but the product yield as well (15). Since 1984, there has been renewed interest in microwave-oven based sample dissolution for analytical chemistry. Several papers on the extraction of metals from sediments (16) and biological tissues (17-19) have been presented at various conferences.

Microwaves are nonionizing electromagnetic radiation. Molecules exposed to microwave radiation undergo molecular motion by the migration of ions and the rotation of dipoles without changing the structure. Microwave energy has the frequency range from 300 to 300,000 MHz (Figure 1.1). Most industrial and scientific microwave ovens use four different frequencies: 915 ± 25, 2450 ± 13, 5800 ± 75, and 22,125 ± 125 MHz (20, 21). All domestic grade microwave ovens use a 2450 MHz frequency. The energy output in a domestic microwave system is 600-700 W. Approximately 180.6 kJ will be supplied to the microwave chamber when the sample is heated for 5 minutes.

1.1.2.4.1 Interaction of Microwave Radiation with Sample

When a sample is exposed to microwave energy, the amount of microwave energy absorbed depends on the dissipation factor (tan δ). The ratio of the sample's dielectric loss (ϵ'') to its dielectric constant ($\acute{\epsilon}$) is called the dissipation factor:

$$\tan \delta = \epsilon'' / \acute{\epsilon}$$

The amount of microwave energy that is lost to the sample by being dissipated as heat is called the dielectric loss.

Microwave energy is lost to the sample by ionic conduction and dipole rotation. The migration of dissolved ions in the applied electromagnetic field is called ionic conduction. Resistance to ionic flow during migration results in heat production (I^2R losses). Therefore, heat production during ionic conduction depends on ion concentration, ion mobility and the solution temperature. The alignment of molecules in the samples that have permanent or induced dipole movement when exposed to an electric field is called dipole rotation. Applied microwaves cause molecules to spend slightly more time in one direction. A small amount of energy is associated with this preferred orientation and molecular order. When the microwave field is removed, thermal agitation returns the molecules to disorder and heat energy is released. In a domestic microwave oven, the alignment of molecules followed by their return to disorder occurs 4.9×10^9 times per second and results in rapid heating. Ionic conduction and dipole rotation takes place simultaneously in many practical applications of microwave heating (20,21).

The relative contribution of dipole rotation or ionic conduction energy conversion is determined by the temperature. The dielectric loss to a sample due to the contribution of dipole rotation decreases as the temperature of the sample (water and other small molecules) increases, whereas dielectric loss due to ionic conduction increases as the

sample temperature increases. Therefore, when an ionic sample absorbs microwave radiation, the dielectric loss to the sample is due to dipole rotation; as the temperature increases, it is dominated by ionic conduction (20,21).

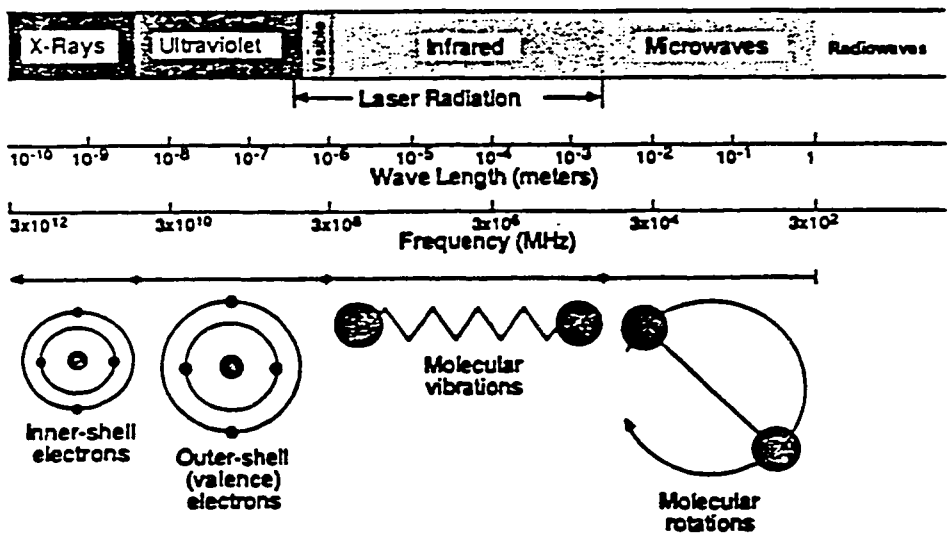


Figure 1.1: Electromagnetic spectrum. Awaiting Copyright Permission.

1.1.2.4.2 Microwave Oven

The principle features of domestic microwave ovens are illustrated in Figure 1.2. The microwave oven used for heating analytical samples consists of the magnetron (microwave generator), the wave guide, the microwave cavity, the mode stirrer, a circular turntable. The microwave radiation produced by the magnetron propagates down the wave guide and enters the microwave cavity. The mode stirrer is made of reflective sheet metal. The reflective walls of the wave guide allow the transmission of microwaves from the magnetron to the microwave cavity. The microwave absorption by the sample is increased because the energy passes through the sample more often and can be partially absorbed on each passage. If the sample load is too small, the energy reflected back into the wave guide will damage the magnetron. When working with small samples, a beaker of water, should always be placed in the cavity along with the sample to absorb excess energy (20,21). To ensure that incoming energy is smoothed out in the cavity, a reflective fan is used. Most microwave ovens are also equipped with a turntable to ensure that the average field experienced by the sample is approximately the same in all directions.

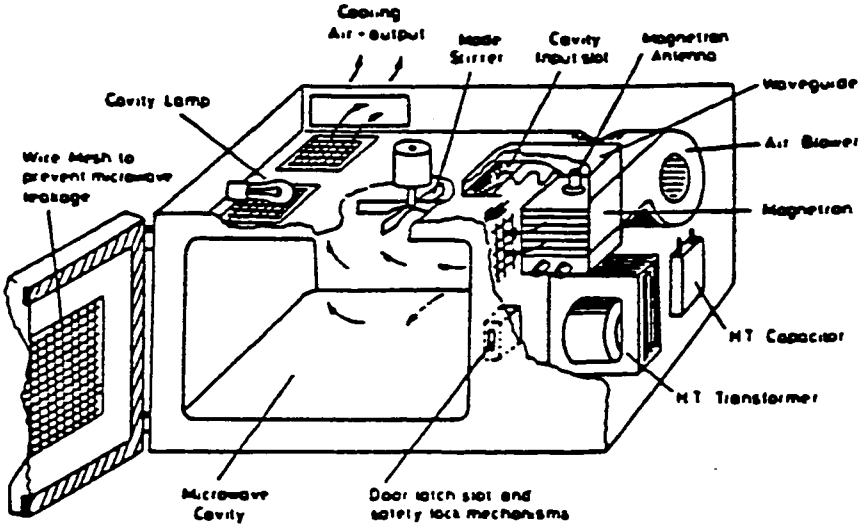


Figure 1.2: The major features of a modern domestic microwave oven. Awaiting Copyright Permission.

The variable power available in domestic ovens is produced by switching the magnetron on and off according to a duty cycle. The microwave power is operated by the control unit within a 32s cycle time base. A 700 W oven can be made to deliver 350 W by switching the magnetron ON and OFF every 16s.

The microwave energy output from the magnetron is generally measured in watts (1 W = 1 joule/s) and is typically 600-700 W in microwave systems used for acid dissolutions. The power output of the magnetron can be indirectly determined by measuring the rise in temperature of a certain quantity of water large enough to absorb essentially all of the energy delivered to the microwave cavity. Ordinarily, the apparent

power output is determined by measuring the rise in temperature, in degrees Kelvin, of 1X 10^{-3} m^3 water heated at full power for 120s. The general relationship used for evaluating the apparent power output is

$$P = C_P K\ m/t\ K' \qquad (1.1)$$

where P is the apparent power absorbed by the sample (joules/s); C_P is the heat capacity in calories ; K is Δ T(change in absolute temperature); m is the mass of the sample (in kg); and t is time (in seconds); and K' the conversion factor (from thermal chemical calories to joules, 4.185J/cal);

Unlike conventional wet ashing and dry ashing methods, dissolution by microwave heating can be completed in a few minutes. The difference between microwave and conventional heating is in the sample heating method. Vessels used in conventional heating are usually poor conductors of heat, and as a result, it takes time to heat the vessel and transfer that heat to the solution. A thermal gradient is established by convection currents because of vaporization at the surface of the liquid, as a result, only a small portion of the fluid is at the temperature of the heat applied to the outside of the vessel. Therefore, in conventional heating, only a small portion of the fluid is above the boiling point temperature of the solution, whereas microwaves heat all of the sample fluid simultaneously for typical analytical sample sizes without heating the vessel, and the solution reaches its boiling point very rapidly.

1.1.2.4.3 Wet Ashing Vessels

Transparent (low loss) materials are used to construct wet ashing vessels so that the microwaves will pass through the vessel to the solution inside. Teflon [poly (tetrafluoroethylene)] and polystyrene are excellent materials for the construction of microwave accessories. Fused quartz, polysulfone and fiberglass-reinforced epoxy, quartz, glass, and plastics, which are transparent to microwave energy and poor conductors of heat, are also good materials for use in a microwave oven (20).

The microwaves in the cavity repeatedly reflect from wall to wall, intercepting samples that absorb microwave radiation. The microwave energy is lost with each interaction until no energy remains in a given wave. When small samples are used, a considerable amount of energy is reflected (unabsorbed). Reflected energy can damage the magnetron; therefore, in analytical work with small samples it is advisable to use a small amount of water to protect the magnetron.

Teflon digestion vessels (Figure 1.3) which can accommodate pressures up to 80 atm and temperatures up to 250 °C were developed by Parr Instruments (20). Teflon has the advantage of being chemically inert and therefore is a suitable containment material for acids, organic, and inorganic solvents. However teflon has a tendency to flow and creep, particularly at temperatures above 150 °C, and it is slightly porous. Therefore, repeated use of the vessel above 150 °C can lead to distortions which reduce the pressure limits of the vessel from its initial 80 atm. The porosity leads to the incorporation of materials, for example organic tars and metal powders, into the walls of the reaction vessel.

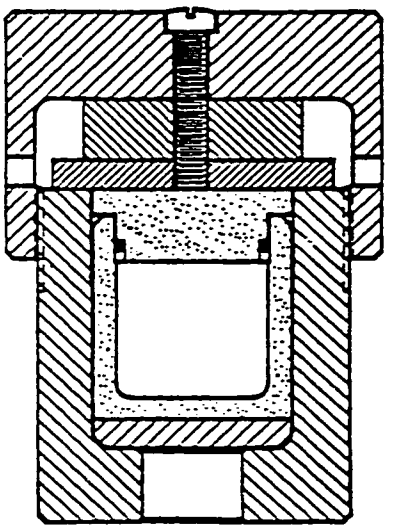


Figure 1.3 High pressure vessel for microwave dissolution (Parr Instrument Co) Awaiting Copyright Permission.

An alternative acid digestion system (20) has been developed by CEM (Figure 1.4). The advantage of this alternative system is that the maximum working pressure is restricted to 1420-1520 kPa. This digestion system has a feedback system that allows constant pressures to be held in the vessels over extended periods of time. A pressure release mechanism is used to ensure that pressure is kept below the specified maximum. The vessel has a much larger volume, which allows larger quantities of inorganic and organic materials to be processed than has previously been possible using other vessels. Although sample preparation time is drastically reduced, pressure vessels need several minutes of cooling time before the sample can be analysed by atomic absorption spectroscopy. Another disadvantage is that a CEM unit costs $30,000.

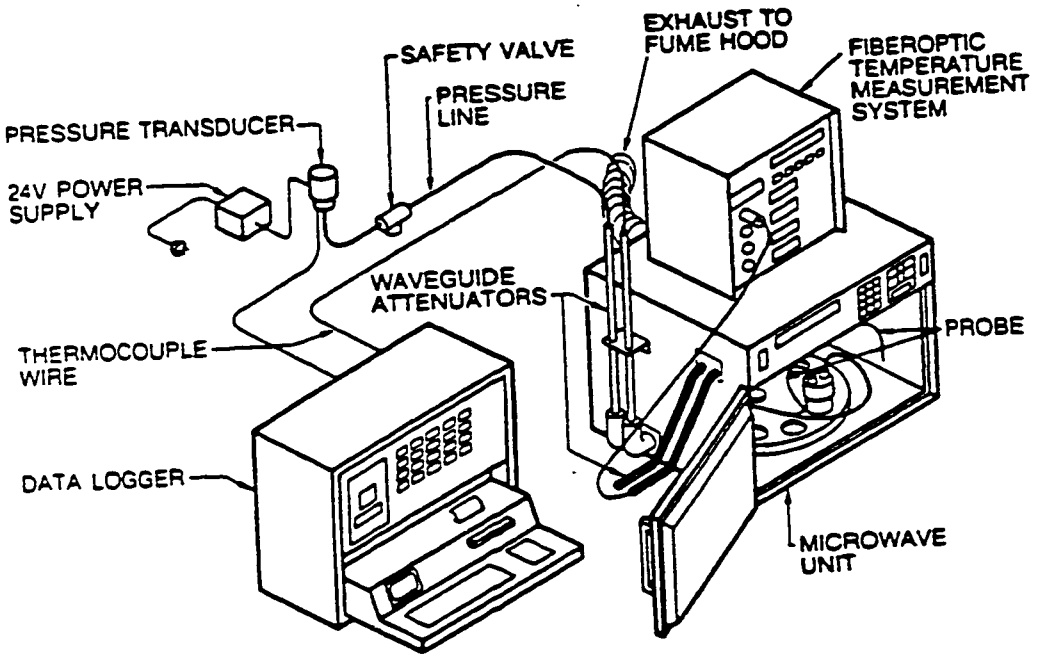


Figure 1.4: Microwave digestion unit, Pressure and temperature-sensing equipment, and data acquisition system (CEM Co). Awaiting Copyright Permission.

1.1.3 Direct Solid Sampling:

Direct sampling was attempted with some success in order to avoid lengthy and cumbersome sample dissolution procedure (22). This method is applicable in the form of powder, soft or hard tissues. Biological fluids are changed into a solid by drying or freeze-drying. This method cannot be used with widely used inexpensive flame AAS. Solid samples are analysed only by expensive electro thermal atomic absorption spectroscopy equipped with a device for background corrector to compensate for

background absorbancies. Only one available instrument, (based on available information) i.e. Grun, is specifically designed for this purpose. This method requires calibration generally made of certified reference materials with similar matrix to the sample or spiking. Direct sampling has some disadvantages. Since the sample is not diluted, high matrix concentrations lead to chemical interferences and high background absorbancies. Smaller sample inhomogeneity may affect precision. Standard deviations ranging from 5 to 30% are generally observed depending on the weight of the sample.

1.1.4 Trace Metal Detection

The importance of trace metal detection in biological materials has grown enormously over the past decade because, it has become clear that even trace and ultra-trace quantities of certain heavy metals can be detrimental to living organisms(1-3). Numerous sophisticated techniques and instruments have been developed in order to analytically detect and measure these metals.

Atomic emission spectrophotometry (AES), including inductively coupled plasma (ICP) and atomic absorption spectrophotometry (AAS) are used frequently in connection with trace analysis of metal ions. AES is normally used to determine alkali metals. Inductively coupled plasma (ICP) is used for nearly all metals, but this refinement of the Atomic emission procedure is relatively new and the instrument is found only in a few laboratories. AAS is widely used to determine concentration of heavy metals in various biological matrices. Analysis by AAS is rapid and cost effective. This analytical method is based on the absorption of ultraviolet or visible light by atoms in the vapour state. In flame atomic absorption spectroscopy, conversion of the sample into an atomic vapour is accomplished by spraying a solution into a flame. The element is not appreciably

excited in the flame, but it is merely dissociated from its chemical bonds and placed in an unexcited ground state. This means that the atom is at a low energy level in which it is capable of absorbing radiation at a very narrow bandwidth corresponding to its nonline spectrum.

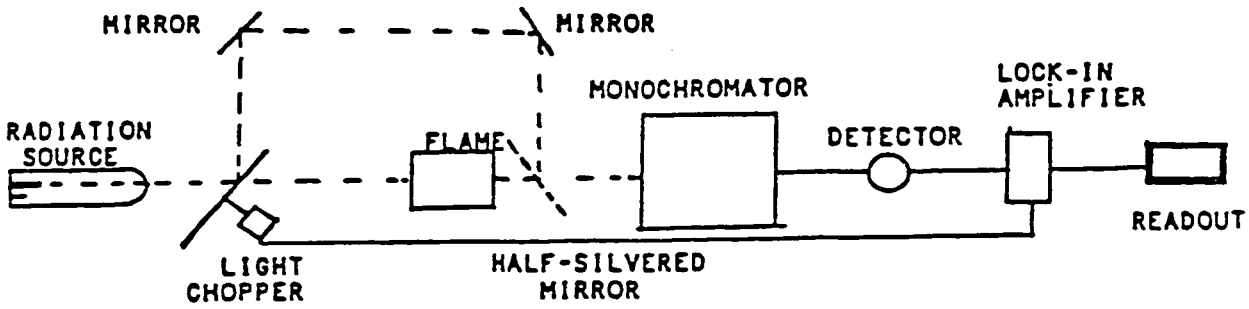


Figure 1.5: Schematic diagram of Atomic Absorption Spectroscopy.

A hollow cathode lamp made of the material to be analysed is used to produce light of a wavelength specific to the kind of metal in the cathode. Thus, if the cathode were made of copper, copper light at predominantly 324.8nm would be emitted by the lamp. When the light from the hollow cathode lamp enters the flame, some of it is absorbed by the ground state copper atoms in the flame, thereby exciting some of these atoms. This absorption results in a net decrease in the intensity of the beam from the lamp. The process is referred to as atomic absorption.

In flameless atomic absorption spectroscopy, the standard burner head

(Figure 1.5) is replaced with an electrically heated graphite furnace (Figure 1.6). Most metallic elements can be determined with sensitivities and detection limits 20 - 1,000 times better than those obtainable with flame AAS. The samples (20-100 ul) are pipetted into the graphite tube through which the light path of the spectrophotometer passes. The sample tube is heated in three steps: first, a low current dries the sample; second, an intermediate current ashes or chars the sample; third, a high current heats the tube to incandescence and atomizes the sample. This method is called electrothermal atomic absorption spectrophotometry, if the instrument is used in connection with the heated graphite furnace (22).

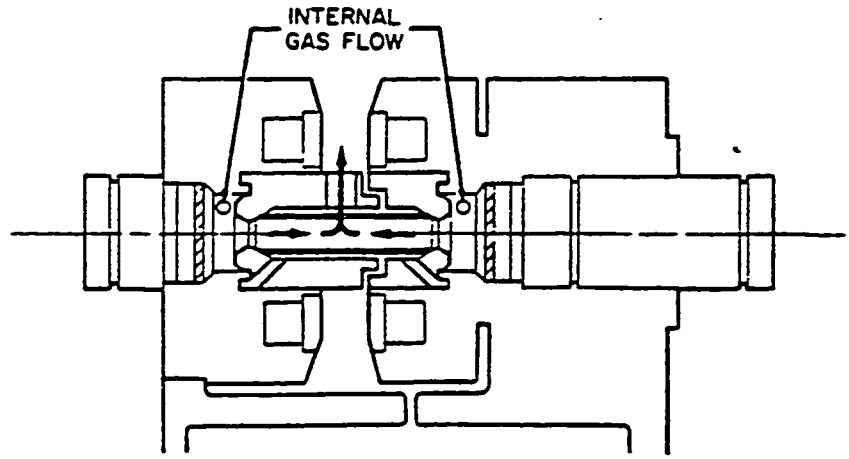


Figure 1.6: Cross section of commercial flameless furnace. Awaiting Copyright Permission.

1.2 REFRACTORY ORGANIC POLLUTANT ANALYSIS IN ENVIRONMENTAL SAMPLES

1.2.1 General

Although chlorinated hydrophobic compounds, including PCBs, were banned more than 15 years ago, they are not banished from our environment. The Great Lakes and the St.Lawrence river are probably the two largest sinks of PCBs and chlorinated hydrocarbon pesticides in Canada (23).

1.2.1.1 Polychlorinated Biphenyls (PCBs).

PCBs are manufactured commercially by the progressive chlorination of biphenyl in the presence of a suitable catalyst. PCBs are known by the trade name Aroclor in Canada and the USA. Individual manufacturers have their own system of identification for their products. In the Aroclor series, a four digit code is used; biphenyls are generally indicated by 12 in the first two positions, while the last two numbers indicate the percentage by weight of chlorine in the mixture. Thus, Aroclor 1260 is a polychlorinated- biphenyl mixture containing 60% chlorine. The conventional numbering of substituent positions is shown in the diagram.

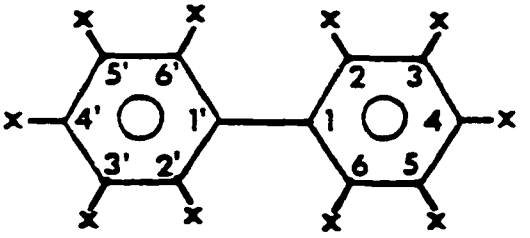


Figure 1.7: Conventional numbering of substituent positions in biphenyl.

PCBs have several industrial applications. They are used as extenders in paints and pesticides, as lubricants in gas turbines, in hydraulic systems, textiles, sealants, carbonless copy paper, air conditioners, TV sets, etc. as insulating materials in electrical equipment, as heat exchange liquids, as plasticisers and for other industrial applications (24). PCBs are chemically stable and resistant to fire, which allowed them to be used in areas where the risk of fire or explosion associated with other coolants was significantly greater.

Hydrophobic polychlorinated biphenyls are insoluble in polar solvents and their hydrophobicity increases with increasing chlorination of the biphenyl rings. The highly hydrophobic nature of these molecules suggests that they should selectively partition into lipid-rich pools. Significantly, the relative partitioning factors of PCBs between aquatic organisms and water is in the order of 10^4 to 10^5 depending on the type of organism and the type of PCB involved (25). In Canada, the average concentration of PCB in human adipose tissue was 0.9 mg/kg (25) . As of yet, no death has ever been attributed to PCBs in Canada. However, an incident in Japan in 1968 involving rice contaminated with PCBs affected about 1000 people. The Japanese Yusho victims experienced severe chloracne, eyelid edema, conjunctival discharge, and various nervous disorders (26,27). The death toll since 1968 is over 100 in Japan.

High chemical inertness of PCBs illustrates that they are difficult to destroy. Complete incineration or combustion of PCBs requires elevated temperatures (1200 ^{o}C to 1400 ^{o}C) and long residence times, while normal incinerators generally operate at lower temperatures (1000 ^{o}C). This latter fact accounts for vaporized PCB residues in the air. Soil and water contamination arise from the dumping of waste PCBs into the environment. PCBs enter the environment via industries that manufacture transformers

and capacitors, during the repair of transformers, after disposal of used or damaged equipment containing PCBs, and of course accidental spills (25).

PCBs stability towards degradation has permitted their identification in environmentally contaminated sinks according to their origin. Not all PCBs in environmental systems remain as such. These can be chemically transformed to dioxins, and some chlorinated substituents may become hydroxylated. This transformation depends on the chlorine content and the position of the chlorine on the biphenyl ring. However, safe disposal of waste PCBs still remains a problem due to the very nature of halogenated hydrocarbons, i.e. high stability to chemical and biological degradation. There were about $6.8X10^8$ kilograms of PCBs produced or imported to the United States between 1929 and 1977 (28). A Canadian task force showed that 10% of this amount (i.e. $6.8X10^8$) was distributed in the environment (25). PCBs were banned in late 1970s because of their persistence in the environment and as a major source of ecological problems including toxic effects on humans, animals and vegetation (29).

1.2.1.2 Chlorinated Hydrocarbon Pesticides.

The introduction of persistent chlorinated hydrocarbon insecticides (CHI) and 1,1,1-Trichloro-2,2-bis(p-chlorophenyl) ethane (DDT) in particular began in the 1940s and accelerated greatly in the 1950s. Dr. Muller received the Nobel prize in Medicine for introducing DDT as an effective pesticide. DDT, Methoxychlor, Aldrin, and Dieldrin are used for controlling insects in agriculture. More than 4 billion pounds of DDT have been disseminated in the environment since its introduction with 80% used in agriculture and the balance in public health programs to control insect vectors (30,31). The use of DDT was restricted in 1973 and procedures against other chlorinated

insecticides soon followed. Hexachlorobenzene is used as a pesticide, a herbicide, and a fungicide. Studies in animals noted an association of liver and kidney carcinoma with hexachlorobenzene. Hexachlorocyclohexane (Lindane) was banned in North America in 1976. Lindane is currently used as a scabicide for humans and animals. It has also been used on fruit and vegetable crops and animal treatment. General toxicity of chlorinated hydrocarbon pesticides is stimulation or depression of central nervous system depending on the dosage and the compound (32).

Routine analysis of these pollutants has become increasingly important in hazard evaluation programs since the discovery that some chemicals, such as lindane, hexachlorobenzene, dichlorodiphenylbischloroethylene (DDE), and polychlorinated biphenyls (PCBs) pose a greater threat to consumers of aquatic food than to the aquatic population. The threat of these hydrophobic pollutants is due to the fact that exposure of aquatic organisms, such as fish, to concentrations that are apparently safe, even permitting normal reproduction, can result in the accumulation of residues in the fish tissue up to concentrations that pose a hazard to consumers. Thus, it is hoped that any undesirable concentration of a pollutant can be recognized and appropriate action taken before any detrimental effects occur.

1.2.2 Sample Clean-up Methods

Residue analysis of the persistent pesticides and PCBs found in many different types of environmental samples is lengthy and cumbersome and requires technical knowledge and skill. The major difference among the analytical techniques is in sample preparation. Isolation of a residue from substances that interfere with and/or prevent specific detection is the most critical component of analytical procedures. Clean-up procedures must be designed to prevent substantive loss and/or alteration of sample.

Most analytical methods consist of 3 steps: (1) Extraction of pollutant of interest from the sample matrix together with fat, with non polar solvents such as petroleum ether or hexane (33); (2) "Clean up of extract, i.e., isolation of analytes of interest from interfering compounds present in the extract" (34-36). Most of the clean-up methods used for pollutant separation employ adsorption column chromatography and constitute the most difficult and time consuming step in residue analysis. Some of the problems are associated with adsorption column clean-up methods, such as "the procedure for adsorbent preparation, the large quantity of glassware required, and the errors that may be introduced by the way of adsorptive loss of residues on the column and from interfering substances present on the adsorbent or in the eluting solvent" (34-36) and; (3) Qualitative and quantitative instrumental analysis by various analytical techniques. The objectives of the above procedures are to extract, separate, identify, and quantitate the compound(s) of interest (34-36). However, in all sampling and analytical procedures, there is a great risk of human error.

1.2.2.1 Supercritical Fluid Extraction (SFE):

Supercritical fluid extraction (SFE) is a very important method for sample preparation, especially for chromatography. This method is rapid, less labour-intensive, and gives cleaner extraction than conventional liquid extraction.

A substance above its critical temperature and pressure, where the distinction between gases and liquid disappears, is called supercritical fluid. In SPE, the solubilities are pressure and density dependent. This property is used to extract the solutes selectively. The supercritical fluid penetrates the sample matrix rapidly to extract the analyte of interest. Carbon dioxide is the most widely used compound in SFE because

it has convenient critical parameters (T_c = 31 °C and P_c = 73 atm and it is inexpensive, nonexplosive, nontoxic, and environmentally friendly (37).

In SFE, CO_2 is classified as nonpolar. It can also be used to extract some polar solutes. For the extraction of other polar solutes, small quantities of modifiers, such as alcohol, acetone, and acetonitrile, are added to carbon dioxide. A procedure needs to be developed for each application on the basis of the good understanding of extraction process. In a typical SFE extraction procedure, 50% of analyte is extracted in a few minutes, but it may be few hours before quantitative extraction occurs. Quantitative extraction of some polymers takes about 80 hours (37).

Three factors affect SFE: solubilities, diffusion and matrix. The solute must be sufficiently soluble for quantitative extraction. The solubility depends on the temperature, density and nature of the fluid. Analyte of interest is extracted under various conditions to determine if the solubility is sufficient. In SFE, the analyte must be transported rapidly from the interior of the matrix. Although the precise mechanism is not known, a transport process similar to diffusion occurs. Affect of sample matrix is least understood at present. Matrix effects include the following examples: adsorption of solutes on surface sites; trapping of molecules in polymer chains; and the need for analyte molecules to penetrate cell walls of plant and animal tissue matrices. Spiking technique is used to check the quantitative extraction of analyte, although spiked sample may not be a true representative of real sample for plant and animal tissues.

1.2.2.2 Solid Phase Micro Extraction

Solid phase micro extraction (SPME) is a new sample preparation technique (37). The SPME unit consists of fused silica fibre coated with phase such as polyacrylate. This method is used to concentrate volatile and nonvolatile compounds by exposing the

fibre to the matrix. SPME is economical, fast and versatile. Some typical applications for SPME are environmental analysis of water samples, flavor analysis and head space analysis of trace impurities of polymers. This method can be used with GC or GC/MS.

1.2.2.3 Problems Encountered in PCB Isolation

The analytical problems encountered in dealing with PCBs have been studied by Cairns and Siegmund (38 - 40). The complications induced by analysing mixtures of PCBs rather than any single specific isomer have probably contributed to the most serious impediment in both identification and quantification. Although there are about 209 possible congeners, the actual number of major components in Aroclor 1254, for example, by capillary GC was only 69 and studies using packed columns have demonstrated elution profiles with less than 20. In analytical terms, this problem can be summed up as having to deal with a potential group of compounds within the mol wt range 188 to 494 daltons possessing vastly different chemical and physical properties. The most serious problem in identifying PCB residues from environmental samples is the inability to identify the congeners with a known reference standard. EC/GC elution profiles obtained from samples containing PCBs do not always directly match with reference standards. Although a number of reasons have been advanced for this phenomenon, the challenge experienced by the analytical chemist is often solved by employing a supplementary technique, such as GC\MS or HPLC\MS. These methods assist in selecting reference standards that closely resemble the actual sample. A high degree of skill is required. The analyst in this arena must clearly develop a high degree of skill to interpret elution profiles correctly, particularly if quantitation is required. The analytical methods ensure that PCB residues have been separated from most of the other organo chlorine residue that interfere during GC or GC\MS analysis. Reliability of PCB quantitative results by ECD improved considerably by interlaboratory study utilizing a peak-by-peak area comparison. This approach, suggested by Webb and McCall (39), was highly dependant on properly characterized reference materials (i.e., weight percentage by peak) and gives improved precision and accuracy over existing methods.

1.2.2.4 Problems Encountered in Pesticide Isolation

Routine analysis of pesticides is tedious, especially when the results are commonly at or below the limit of detection. In the analysis of organochlorine pesticides contaminated with polychlorinated biphenyls (PCBs), there are several possibilities of confusing the identity of individual residues (40). In addition, unless the appropriate GLC columns are chosen for analysis, it is possible to get confused between the following pairs of contaminants: DDE and dieldrin, hexachlorobenzene and x- or y-BHC, op' -DDT and pp' -DDD, op' -DDT and endrin, heptachlor epoxide and dibutyl phthalate, sulphur and aldrin, and various PCBs with DDE, DDD, DDT and dieldrin (41). Jensen's breakthrough in 1966 (41) of the first confirmed report of PCBs in fish and wildlife was made after repeated and somewhat frequent encounters with similar GC elution patterns while routinely analysing for DDT and other chlorinated pesticides. The earlier failures to properly recognize this PCB interference must surely have contributed to the overestimation of DDT and TDE in the environment. Since Jensen's historical discovery of PCB contamination, emphasis shifted to PCBs and its residues were then described to have interferences from a wide variety of organochlorine pesticides.

Finally, the cost of isolation is rarely mentioned in the planning of monitoring programs. For a limited number of organochlorine analyses, including for example, the DDT group, two or three other organochlorine pesticides and the PCB group, the overall cost of analyses per sample is usually in the order of $200. The sampling operation, transport of samples, clerical time and computer time after analysis involve additional expenditures. For a program involving 25 samples from each of two populations of a species, and from each of ten areas, a total of five hundred analyses, costing 100,000

Canadian dollars for the analysis is involved, and the overall cost is probably two or three times greater. This is only for one group of pollutants, and although some analytical processes may be much cheaper, the total programs would cost several million dollars annually.

1.2.3 Organic Pollutant Detection

The pesticide chemist is becoming more reliant on a battery of analytical procedures for identification of pollutants and makes most extensive use of specialized detectors. This is probably best evidenced by noting that the pesticide chemist has actually led the development of specialized detectors. For specific analysis, he has found the GC/CD and GC/MS/Computer an invaluable tool for unique compound identification.

1.2.3.1 Electron Capture Detector

Organochlorine insecticides and PCBs are most often analysed by gas chromatographic (GC) techniques that utilize electron capture (EC) detection of nanogram amounts or less. Column effluent enters the detector chamber which has a radioactive foil usually containing ^{63}Ni. The ion current in the detector is kept constant. When an electron capturing substance enters the detector cell, the pulse frequency is changed in a closed loop circuit in order to maintain a constant current. Here, the basis of quantitative measurement is the relationship between the change of pulse frequency and the concentration of the electron capturing substance. The EC detector is very sensitive to compounds containing halogens, sulphur, anhydrides, or peroxides, but is virtually insensitive to hydrocarbons.

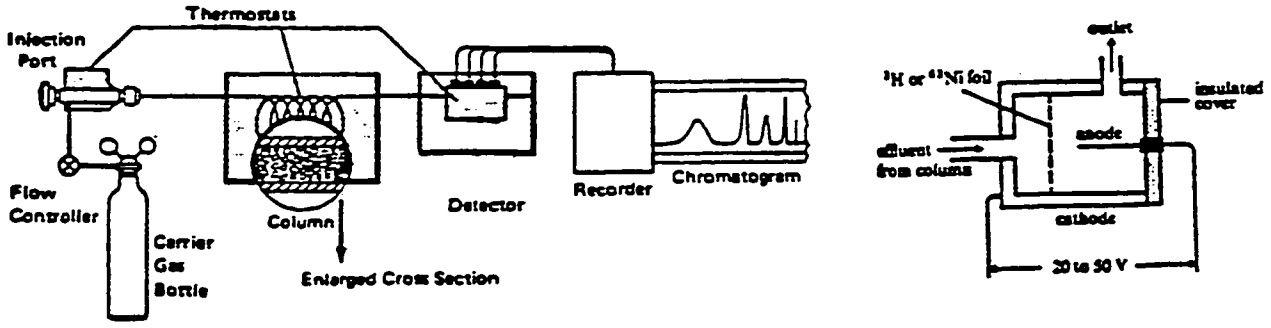


Figure 1.8: Schematic diagram of gas chromatographic system.

1.2.3.2 Gas Chromatography/Mass Spectrometry

In mass spectrometry, when sample molecules in gaseous or vapour state are subjected to high voltage, electric current can be made to lose electrons and form positively charged ions (cations). These cations can be accelerated and deflected by magnetic and\or electrical fields. The deflection of an ion depends on its mass, charge and velocity. If the charge, velocity, and deflecting force are constant, the deflection is less for a heavy particle and more for a light one. Mass spectrometry is very useful to measure the abundance of a given isotope, the mass of an atom or to elucidate organic structure. In GC/MS, organic pollutant mixture is separated into individual components by gas chromatography and mass spectrometry is used as detector. Unknown samples are identified by scan or sim mode runs. Scan mode run gives several

ion fragmentations. This method is less sensitive. Sim method results in detection levels which can be 100 times lower than a SCAN analysis. Detector monitors specific ions rather than scanning a continuous mass range. Thus, signal to noise ratio is greater than for scan and can obtain much greater sensitivity. The mass spectrometer spends a specific time (Dwell -Time) at the analyser setting required for transmission of an ion of a particular m/z.

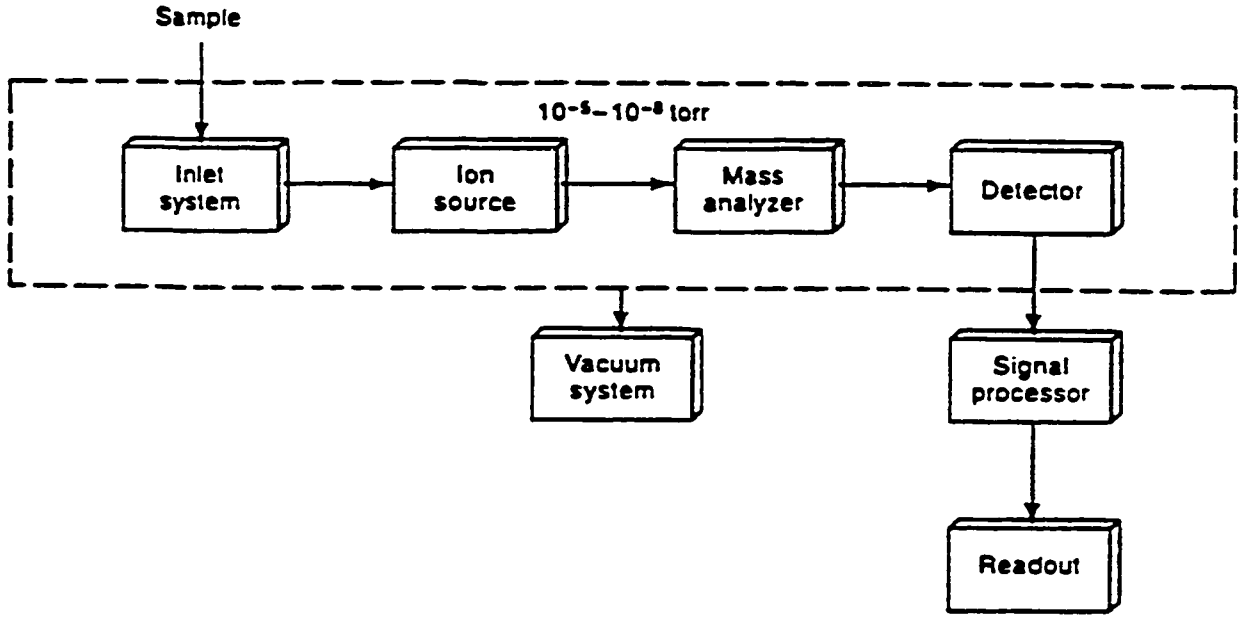


Figure 1.9: Schematic diagram of GC\MS. Awaiting Copyright Permission.

1.3 PURPOSE OF INVESTIGATION

The purpose of investigation is to re-evaluate the issue of domestic microwave oven for sample clean-up methods because commercial microwave ovens are too expensive for laboratory use. Wet ashing of biological samples in domestic microwave oven will be evaluated

Organic pollutants such as PCBs, lindane, and hexachloro benzene are persistent in the environment due to their resistance to oxidation. In wet ashing, organic matrix is oxidised to analyse trace metals in biological samples. The fate of these persistent (refractory organics) pollutants during wet ashing procedure is not known. An investigation of wet ashing treatment of biological samples contaminated with refractory organics is, therefore, warranted. Biological samples contaminated with persistent pollutants subjected to wet ashing , will be analysed by electron capture gas chromatography. The results will be useful to classify these pollutants into three groups: A) Quantitative analysis: If the compounds under investigation are not affected by wet ashing procedure; B) Qualitative analysis : If the compounds under investigation are partially degraded ; and C) Treatment: If the compounds under investigation decompose completely.

Recent reviews and publications argue that the acceleration of reactions achieved with microwave heating is the result of rise of boiling temperatures resulting from increase of pressure. The basis of this conclusion is a small number of observations, some only semi-quantitative. This thesis will reexamine the issue by using extensive kinetic studies of two very different, slow and fast, reactions in two very different media: an aromatic nitration in mixed H_2SO_4/HNO_3 (1:1) and the hydrolysis of $trans(Coen_2Cl_2)^+$ in a mixture of CH_3OH/H_2O.

CHAPTER 2

WET ASHING OF BIOLOGICAL SAMPLES IN A MICROWAVE OVEN UNDER PRESSURE USING TEFLON VESSELS. [1]

---

[1] Published in Anal.Chem,.1582- 83 (1987).

2.1 INTRODUCTION

Atomic absorption spectroscopy is widely used to determine the concentration of heavy metals in various biological matrices. Sample clean-up procedure is the limiting factor to determine trace metals in biological samples (Chapter 1). Some clean-up methods used by other workers specific to matrix mentioned in this work are given here. Paus (42) used teflon bombs in a conventional oven. However, the heating time was not given for the dissolution of fish tissue samples in H_2SO_4/HNO_3 mixture to analyze Cd, Cu, Hg and Zn. A teflon bomb takes 1-2 hours to cool to room temperature. Some workers (43) heated fish tissue on a hot plate in $HClO_4$ for "complete dissolution". $HClO_4$ is efficient but several hazards associated with its use have been described in Chapter 1. Adrien (44) used nalgene bottles with polypropylene screw caps as pressure digestion vessels for wet ashing of animal tissue samples. Four different volumes of $HClO_4$,H_2SO_4,HNO_3 were used. The samples were predigested overnight followed by 2 to 3 hours of heating.

Several workers attempted to use wet ashing procedures in unmodified microwave ovens. Koirtyohann et al.(45) and Barrett et al.(46) modified microwave ovens by adding an exhaust port. Nadkarni (47) exploited an unmodified microwave oven by using a pyrex desiccator as a pressurized vessel and reported significant losses of Cu (26%) and Pb (20%). Matts et al (48) tried polycarbonate pressurized vessels, but the plastic quickly became opaque and brittle. Disadvantages are associated with generation of acid fumes which promote metal loss and can contribute to corrosion of oven circuitry including safety switches. Attempts to overcome fume related problems have involved the use of sealed reaction vessels. These in turn are subject to high internal pressures during microwave treatment and to avoid explosions, costly exhaust systems are employed(Chapter 1).

The prospects for use of an unmodified microwave oven with pressurized vessels were reevaluated. Pyrex vessels gained heat in the glass quickly. Polycarbonate vessels were substituted by teflon TFA vessels, and were found to have superior chemical and mechanical properties. These semi sealed reaction vessels were enclosed in an outer secondary chamber to contain any vapors that were produced. This approach has the advantage of using an unmodified microwave oven. This simple internal venting system has been applied safely in our laboratory to a wide range of biological materials.

2.2 EXPERIMENTAL

2.2.1 Apparatus:

The microwave oven was a 700-W commercial model with variable power capacity available locally. The teflon PFA containers were Savillex Corp. (Minneton, MN) 60 mL vessels 0.28m thick. The outer vessel was a 3 L, wide mouth microwave oven-proof plastic container. The glassware was soaked overnight in Acationex detergent and cleaned with tap water followed by deionized water for flame atomic absorption experiments. A Perkin Elmer model number 503 AA equipped with 0.1016 m burner was used. Atomic spectra were recorded with flame atomic absorption spectroscopy.

2.2.2 Materials: Water was double deionized. H_2SO_4,HNO_3 "trace metal grade" was purchased from Fisher Scientific Co. Metal standards were prepared from certified grade salts from Fisher Scientific. Bovine liver samples (National Institute of Standards and Technology, NIST,1577) were used as such or spiked. Samples were spiked with Fisher Scientific certified grade 1000 ppm metal standards. For example, 100 ul Cd standard in Eppendorf pipette was injected into the sample to get 100 ug spiking. The sample was dried in a slow stream of nitrogen. Whole fish samples (freeze dried) were Brachydaniorerio cultured in our laboratory.

Table 2.1

Analytical operating conditions for flame atomic absorption

Instrument: Perkin - Elmer Model 503 Atomic Absorption Spectrophotometer

Radiation source: Hollow cathode lamp

Element	Cd	Cu	Fe	Pb	Zn
Line (nm)	228	324.8	248.3	283.3	213.9
Slit (nm)	0.7	0.7	0.5	0.7	0.7
Detection limits(ppm)	0.03	0.03	0.11	0.15	0.003

Readout: 3 - seconds integration

Readout: 3 - seconds integration

Burner: 0.1m, single slot

Fuel: Acetylene - 20 gauge units

Oxidant: Air - 40 gauge units

Flame: Oxidizing (Lean blue)

Water: Double - deionized water

Sample size: Continuous flow

2.2.3 Preparation of Standard Solutions:

1) All standard solutions were prepared from a 1000.0 ± 10 ppm. Supplied by Fisher Scientific Company Limited.

2. Dilution of Standard stock solution was carried out as follows:

A) 10.00 ± 0.06 ml of 1000.0 ± 10 ppm standard solution pipetted into a 100.00 ± 0.08 volumetric flask and diluted to the mark to make 100.0 ± 1.2ppm.

B) 100.0 ± 1.2 standard solution was further diluted to desired AA standard concentration with acid mixture to get same acid matrix concentration as samples.

2.2.4 Procedure:

Samples of 0.25 g were placed in Savillex vessels along with 1.5 mL of H_2SO_4 and 1.5 mL of HNO_3. The cap was screwed on finger tight. The sample was then placed in a wide mouth plastic container which was closed with a screw cap. A small beaker containing 20 mL of water was placed in the oven along with the sample container to avoid damage to the magnetron. Each sample was heated for various time periods (ranging from 30s to 420s) at the maximum power setting of the oven. The container was removed and cooled in an ice bath for 5 minutes. The contents were then diluted to 25 mL volume with deionized water. Conventional flame AAS procedures were followed. An acid blank containing the same amount of H_2SO_4 and HNO_3 was used.

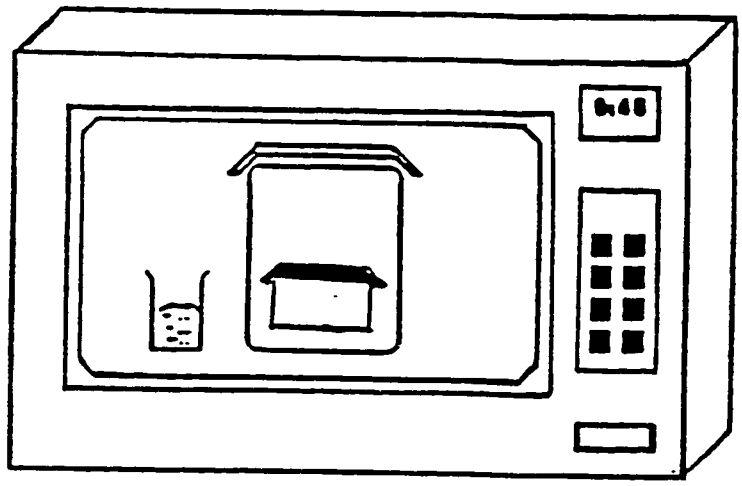


Figure 2.1: Microwave oven set-up for wet ashing with Savillex vessels.

TABLE 2.2a. Certified values of constituent elements for NIST bovine liver 1577a

Element	Concentration (ug/g)	Element	Concentration (ug/g)
Ar	0.047 ± 0.006	Hg	0.004 ± 0.002
Cd	0.44 ± 0.06	Mo	3.5 ± 0.5
Ca	120 ± 7	Ru	12.5 ± 0.1
Co	0.21 ± 0.05	Se	0.71 ± 0.07
Cu	158 ± 7	Ag	0.04 ± 0.01
Fe	194 ± 20	Sr	0.138 ± 0.003
Pb	0.135 ± 0.015	U	0.00071 ± 0.00003
Mg	600 ± 15	V	0.099 ± 0.008
Mn	9.9 ± 0.8	Zn	123 ± 8

TABLE 2.2b. Noncertified values of constituent elements for NIST bovine liver 1577a

Element	Concentration (ug/g)	Element	Concentration (ug/g)
Al	2	Br	9
Sb	0.003	Tl	0.003

The certified values for the constituent elements are based on the results obtained by two or more independent analytical methods. Noncertified values are given for information only. The following analytical methods are used for certified values:

A. Atomic absorption spectroscopy
B. Isotope dilution mass spectroscopy
C. Isotope dilution spark source mass spectroscopy
D. Kjeldahl method for nitrogen
E. Neutron activation
F. Nuclear track technique
G. Optical emission spectroscopy
H. Spectrophotometry
I. Polarography.

2.3 RESULTS AND DISCUSSION:

Wet ashing procedure under pressure in a microwave oven was used to analyse several animal tissue samples. Throughout, recovery was satisfactory for Cu and Fe for NIST bovine liver and spiked Ni. Recovery of Zn from reference material is not satisfactory. There is no apparent matrix effect. Table 2.3 reports data for determination of Cu, Fe, and Zn for NIST bovine liver 1577a. Effects of wet ashing time on the recovery of Cu and Fe from NIST bovine liver are given in Tables 2.4 and 2.5 respectively. Table 2.6 compares recoveries of added Ni in fish liver, muscle, and kidney tissues. Table 2.6 values for spiked Ni are plotted in Figure 2.1.

A maximum coefficient of variation of less than 4% and mean recovery of greater than 100% for Cu and Fe for NIST reference materials (Table 2.3) demonstrates the precision and accuracy of the method. The student t-test (equation 2.1) was used to determine whether the values obtained from the savillex method are statistically equal to the accepted NIST bovine liver values for Cu, Fe, and Zn.

$$\pm t = (x - u)\sqrt{N}/s \qquad (2.1)$$

t stands for student t value, u represents true value...

The student t value for Cu: $\pm t = (158 - 158)\sqrt{3}/3 = 0$

Fe: $\pm t = (202 - 194)\sqrt{3}/8 = 1.7$

Zn: $\pm t = (133 - 123)\sqrt{3}/3 = 5.7$

For two degrees of freedom, the table value of t at the 95% confidence level is 4.303.

Since calculated values for Cu and Fe is less than this, savillex method gives statistically correct value for Cu and Fe at 95% confidence level. The calculated value for Zn is greater than 4.303, there is a 95% probability that the difference between the savillex data for Zn and the reference value is not due to chance, and there is a determinate error in the method[2]. Since atomic absorption spectroscopy results have very good precision, the mean metal values should be closer to NIST values to get acceptable t values (Table 2.6 and Figure 2.2).

Efficiency of savillex method was also studied by comparing recoveries of added Ni in control fish liver, muscle, and kidney tissues. Ni is absent in control fish tissue samples. Unspiked fish tissue sample readings are zeroed in AAS. A slope value of 0.99 for a plot of recovered vs spiked Ni concentration from fish liver shows that there was no apparent matrix effect for spiked metals.

Tables 2.a and 2.b give certified and non certified values for different elements. The standard deviation values in NIST reference materials is calculated from the mean values of different methods used. This results in larger standard deviation. Individual method used by NIST may have a better standard deviation than Table 2.3 values.

---

[2] Note that as the precision is improved, the calculated t becomes larger and the mean result of the test method must be closer to the reference value for the discrepancy to be due to random differences.

TABLE 2.3

Analysis of NIST 1577a bovine liver by flame AAS after wet ashing under pressure in a microwave oven.[a]

Acid mixture :3 mL H_2SO_4\HNO_3 (1:1)

Wet ashing time: 60s

Element	Expect. conc,ug/g	Conc. found,ug/g
Copper	158 ± 7	158 ± 3
Iron	194 ± 20	202 ± 8
Zinc	123 ± 8	133 ± 3

[a] Analyzed values are the mean of three replicates.

TABLE 2.4

Effect of wet ashing time: Recovery of copper from NIST 1577a bovine liver by flame AAS after wet ashing under pressure in a microwave oven[a,b]

Acid mixture : 3.0 mL H_2SO_4\HNO_3 (1:1)

Expected copper concentration (conc,ug/g) : 158 ± 7

Wet ashing time, s	Cu conc. found, ug/g
30	144
60	154 ± 3[b]
90	145
120	140
150	129
180	130

[a] Analyzed one sample only.

[b] Analyzed values are the mean of three replicates.

TABLE 2.5

Effect of wet ashing time: Recovery of Iron from NIST 1577a bovine liver by flame AAS after wet ashing under pressure in a microwave oven[a,b]

Acid mixture :3.0 mL H_2SO_4\HNO_3 (1:1)

Expected iron concentration conc (ug/g) : 194 ± 20

Wet ashing time,s	Fe conc.found, ug/g
30	169
60	202 ± 8[b]
90	194
120	190
150	180
180	185

[a] Analyzed one sample only.

[b] Analyzed values are the mean of three replicates.

TABLE 2.6

Recoveries of added nickel in fish tissue after wet ashing under pressure in a microwave oven [a]

Acid : 3.0 mL H_2SO_4\HNO_3 (1:1)

Sample	Amt. added, ug	Amt. recovered, ug
Liver	10.0	9.6 ± 0.4
	30.0	29.5 ± 0.8
	50.0	49.7 ± 0.6
Muscle	10.0	9.9 ± 0.5
	30.0	29.9 ± 1.1
Kidney	10.0	9.2 ± 0.3
	30.0	29.1 ± 0.8

[a] Analyzed values are the mean of three replicates.

[b] Freeze dried fish samples (control) (Brachydaniorerio) cultured in our laboratory were used. Ni is absent in control fish samples.

Linear regression analysis:

Slope = 1.0025

Intercept = -0.475

Correlation coefficient = 0.999

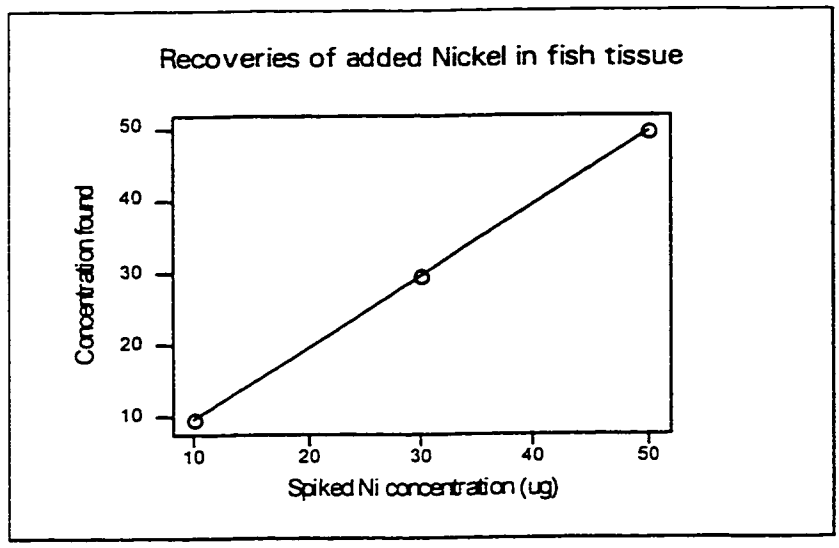


FIGURE 2.2 A plot of recovered versus spiked Ni from fish tissue

2.3.1. Effect of Wet Ashing Time:

Recovery rates of Cu and Fe from NIST bovine liver (Table 2.4) show that losses gradually increased for longer wet ashing times to a maximum loss of 18% for Cu and 8% for Fe when subjected to 180s of wet ashing time. Since only one sample was analysed for each longer wet ashing time, it is not statistically possible to determine the optimum wet ashing time. However, student t test shows that 60s wet ashing time is optimum for the determination of trace metals with the exception of Zn.

When biological samples spiked with Aroclor 1260 were subjected to wet ashing procedure in Savillex vessels, Aroclor 1260 decomposed completely (Figure A.1) . The objective was to analyse persistent pollutants that are resistant to wet ashing oxidation. Several conditions were studied including shorter wet ashing times and periodic heating to reduce drastic conditions . This process developed into open vessel pulse microwave wet ashing procedure described in next chapter.

2.4 CONCLUSION:

Results of this study show that wet ashing in Savillex vessels in domestic microwave ovens is a viable alternative for digestion of biological samples for trace metal analysis by atomic absorption spectroscopy. Some of the shortcomings of microwave treatment- exorbitant cost of commercial microwave ovens and loss of volatile metals - are avoided. The minimal fumes which do arise from longer heating times are contained by the outer loosely sealed vessel. Zn value for NIST reference material is not satisfactory by this method.

CHAPTER 3

PULSE MICROWAVE DIGESTION OF ANIMAL TISSUES[1]

---

[1] Partly published in Analytical letters, 21(11),2003-2010 (1988).
Accepted for publication by Intl. Journal of Environmental Analytical Chemistry
Partly presented at SETAC, Arlington, Virginia (1988).
Partly presented at SETAC, Toronto (1989).

3.1 INTRODUCTION

Microwave heating to enhance acid digestion has been explored in recent years (Chapter 1). Use of teflon vessels in an unmodified microwave oven was demonstrated in Chapter 2. The work presented here is part of a method developed to efficiently clean biological samples by heating them in acid solution in a microwave oven. It was found that microwaves in pulses of 10 seconds or less separated by dormant time of 180 seconds maintained the temperature of acid mixture below boiling point. This technique allows tissue samples to be treated in open reaction vessels which, in turn, are held in a sealed container. Volatile metals that would normally be lost in open systems are retained in this pulse method. The pulse method eliminates the possibility of hazardous conditions because the temperature is maintained below the boiling point of the acid mixture in an open vessel at 1atm.

The present study compares method A (10s pulse) and method B (6s pulse) on a wide range of animal tissues representing major types of matrices which included fish tissue, NIST standard bovine liver, and cream cheese. (The unavailability of high fat reference materials led to the use of cream cheese to represent high fat material in a study of spike recoveries.) The temperature of digestion mixture (H_2SO_4/HNO_3 or HNO_3) was maintained <110 °C for method A and <80 °C for method B. The effects of such parameters as pulse time and acid mixture volume variation are examined.

3.2 EXPERIMENTAL SECTION

3.2.1 Apparatus:

Microwave oven and flame atomic absorption spectroscopy are described in Chapter 2. Flameless AAS was PE 503 equipped with graphite furnace model 2100. Table 2.1 and Table 3.1 provides details specific to each metal used for flame and flameless conditions respectively.

3.2.2 Materials:

In addition to materials mentioned in Chapter 2, cream cheese was Philadelphia cream cheese light (21% fat). Freeze dried whole fish tissue samples were Brachdaniorerio.

3.2.3 Characterizing Optimum Conditions

The objective in the pulse method is to choose an appropriate pulse time followed by a dormant time giving a minimum temperature for achieving complete digestion and recovery in a reasonable total time. The temperature reached by acid mixture (H_2SO_4 (95.0 - 98.0 %)/HNO_3(69.0 - 71.0%) (1:1)(v/v) or HNO_3(69.0 - 71.0%) in a given microwave oven depends on the available microwave power, which in turn, depends on the power setting and duty cycle. The power absorbed by the substance (power density) in the microwave cavity may be expressed by equation 1.1 (Chapter 1). The sample's temperature can be predicted by measuring the length of exposure time and available power. The power absorbed by water at 100% duty cycle is used to calibrate all microwave ovens that have power outputs between 500 and 800 W. The calibration is achieved by measuring the temperature rise of 1 kg of water after heating in a microwave oven for a fixed period of time. In a homogeneous microwave field, 1 kg of water absorbs approximately same amount of power in one container as

it does when it is equally divided between two or five containers. The actual power delivered by magnetron must be determined so that the absolute power settings can be interchanged from one microwave unit to another. Different volumes of (5 mL, 10 mL, 20 mL) H_2SO_4 (95.0 - 98 %)/HNO_3(69 - 71%) (1:1)(v/v) or HNO_3(69.0 - 71%) and HNO_3 (5 mL and 20 mL) were subjected to different pulse lengths followed by dormant time of 180s. Immediately after pulse treatment, a thermometer was inserted into the sample mixture and the temperature was recorded at different time intervals. The temperature extrapolated to time t_o was taken as the temperature reached by the acid mixture immediately after the last pulse treatment (Table 3.2).

The microwave power is operated by the control unit within a 32s cycle time base. In order to maintain temperature below 110 °C, two sequences were studied: (1) Pulse procedure A (10s): 10s pulse followed by 180s dormant time. This step was repeated. (2) Pulse procedure B (6s): 6s pulse followed by 180s dormant time. This procedure was repeated. 6s corresponds to duty cycle at the low power setting of the oven.

Table 3.1

Analytical operating conditions for flameless atomic absorption (animal tissue samples)

Instrument: Perkin - Elmer Model 503
Flameless furnace model # 2100
Atomic Absorption Spectrophotometer

Radiation source: Hollow cathode lamp

Element	Cd	Mn	Pb
Line (nm)	228.8	279.5	283.3
Slit (nm)	0.7	0.5	0.7
Drying temp (°C)	100	100	100
Time (s)	40	40	40
Ramp (s)	15	15	15
Ashing temp (°C)	500	500	500
Time (s)	30	30	30
Ramp (s)	10	10	10
Atomizing temp (°C)	2100	2700	2300
Time (s)	7	7	6
Final temp (°C)	2700	2700	2700
Time (sec)	5	5	5

Table 3.2

Extrapolated temperatures (°C) for acids subjected to 6s and 10s pulses.[a, b, c] (animal tissue samples)

Acid	Volume	Pulse time	
		6s Proc.B	10s Proc.A
H_2SO_4/HNO_3[d]	5.0 mL	79.0 ±3 °C	108.0 ±4 °C
"	10.0 mL	76.0 ±3 °C	109.0 ±3 °C
"	20.0 mL	68.0 ±2 °C	95.0 ±3 °C
HNO_3[e]	20 .0mL	53.0 °C	96.0 ±3 °C

[a] Temperature may fluctuate slightly for other MWO.

[b] Analyzed values are the mean of three replicates.

[c] Starting temperature of acid mixture and HNO_3 is 22 °C

[d] Boiling point of H_2SO_4 / HNO_3 (1:1) (v/v) mixture is 118 °C

[e] Boiling point of conc HNO_3 is 120 °C

3.2.4 Temperature Programming:

There are four stages of temperature programming (Table 3.1): drying (to remove solvent), charring (to remove traces of organics), atomizing, and a final stage of heating at high temperature to remove from the graphite tube all traces of sample in order to prevent memory effects.

The rate of temperature rise in the graphite furnace, together with residence time at each temperature, can be programmed and reproduced by controls. In automatic feature, reproducibility can be maintained and settings repeated to match requirements of samples according to their different characteristics, both physical and chemical.

The purpose of gas flow is to increase retention time of the analyte vapour in the optical path and also to reduce background signal. Fumes produced during ashing process deposit on the cooler ends of the tube and the deposited material is then atomized when the heat is increased, adding to the background signal. A stop flow device is used to stop the flow of purge gas momentarily to increase sensitivity.

3.2.5 Background Correction:

The excited radiation in the fume is scattered by some molecular species, salt particles, and smoke particles and results in increased absorption. Without background correction, this increased absorption will cause high results, and it cannot be eliminated by conventional double-beam arrangement of the optics or by an AC excitation source. Deuterium or hydrogen lamp is normally used for background correction. Correction is satisfactory for most of the conditions.

The background correction works in the following manner. A continuum beam

from the deuterium lamp is inserted into the optical path by the chopper, which produces a rapid alternation of the deuterium and exciting beams at a frequency to which the amplifier is tuned. After both beams pass through the vapour in the graphite tube (or the flame) and reach the detector, the deuterium signal is subtracted electronically from the exciting beam signal, which contains the sum of background and atomic absorption signals.

3.2.6 PROCEDURE

3.2.6.1 Pulse Treatment Procedure:

Samples of 0.25 g pulverized whole fish tissue (freeze dried), bovine liver (powder) or cream cheese were placed in 250 mL Erlenmeyer flasks along with 5.0 mL, 10.0 mL or 20.0 mL of H_2SO_4/HNO_3 (1:1) or 20.0 mL HNO_3. Each flask was placed in a wide mouth, microwave oven proof plastic container. The outer vessel was firmly closed with a screw cap. The container was then placed in the microwave oven along with a beaker containing about 10 mL of water as a protection against damage to the magnetron during operation. Microwaves were applied in pulses of 10s separated by a dormant time of 180s for method A. Pulsing was repeated 6 times (6 × 10s) for a total time of 60s pulse time plus 18 minutes of cooling time. For method B, microwaves were applied in pulses of 6s separated by a dormant time of 180s. This was repeated 6,12 or 18 times. Note that samples may be exchanged during the dormant time to increase the throughput. At the end, the contents were diluted to 100 mL with doubly deionized water. Diluted samples were centrifuged, when required. Conventional flame and flameless atomic absorption procedures were followed. Acid blanks and standards containing the same amount of acid were used.

3.2.6.2 Conventional Heating Procedure:

0.25 g of NIST bovine liver samples were placed in 250 mL Erlenmeyer flasks along with 20 mL of (1:1) acid mixture. Samples were heated on hot plate at low heat set-up for 5 hours. The contents were diluted to 100 mL with doubly deionized water. Conventional flame atomic absorption procedures were followed. Acid blanks and standards containing the same amount of acid were used.

3.2.6.3 Calculations

Regression lines were determined for each element from standards run in both concentration ranges (ppm or ppb). Metal concentrations in each sample were calculated from the corresponding regression lines and dilution factors.

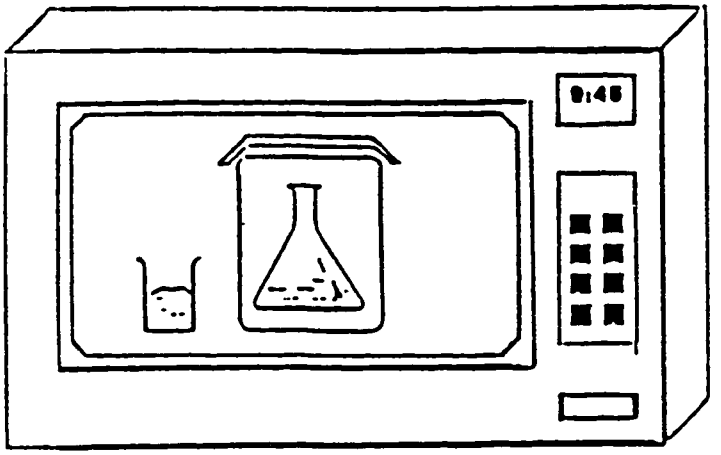


Figure 3.1: Microwave oven set-up for wet ashing with pulse procedure.

3.3 RESULTS AND DISCUSSION

NIST reference material bovine liver, fish tissue, and cream cheese were selected to study the efficiency of our method. Due to unavailability of high fat reference materials, cream cheese was used to represent relatively high fat tissue. Fish tissue samples are easy to digest, bovine liver samples represent an intermediate matrix, and cream cheese represents a difficult matrix to digest. The bovine liver sample plays a central role since NIST reference material is available.

Tables 3.3 and 3.4 show data for determination of Cu, Fe, and Zn for NIST bovine liver 1577a by method A and method B respectively. Effects of acid mixture volume and pulse time variation on recovery of Cu, Fe, and Zn from NIST bovine liver are given in Tables 3.5 to 3.7 respectively. Table 3.8 compares recoveries of added Cd in fish liver for method A, B (5.0 mL) and method B (20.0 mL). Table 3.8 values for spiked Cd are plotted in Figure 3.2

The student t-test (equation 2.1) was used to determine whether the values obtained from methods A and B are statistically equal to the accepted NIST bovine liver values for Cu, Fe, and Zn .

Student's t values for method A and method B

Element	Method A	Method B
Copper	1.6	2.7
Iron	4.5	2.7
Zinc	2.7	2.5

For three degrees of freedom, the table value of t at the 95% confidence level is 3.182. Since calculated values for Cu and Zn are less than this, methods A and B give statistically correct value for Cu and Zn at 95% confidence level. Since the

calculated value for Fe for method A is greater than 3.182, there is a 95% probability that the difference between the method A data and reference value for Fe is not due to chance and there is a determinate error in the method[2].

TABLE 3.3

A comparison of expected and analyzed values for Copper, Iron and Zinc[a,b,c,d,e].

Sample: NIST bovine liver

Acid: 20 mL H_2SO_4/HNO_3 (1:1)

Procedure: Wet ashing by open vessel pulse microwave method A

Temperature: 95 °C

Element	Expect. Conc. ug/g	Conc. Found, ug/g
Copper	158 ± 7	165 ± 9 (162 ± 11)
Iron	194 ± 20	210 ± 7 (198 ± 9)
Zinc	123 ± 8	127 ± 3 (123 ±4)

[a] Analysed values are the mean of four replicates.
[b] Sample size 0.25 g for all the tables.
[c] Analyzed by flame A.A.
[d] Results in parenthesis are for hot plate wet ashing.
[e] Please see Table 2.1 for experimental set-up.

[2] Note that as the precision is improved, the calculated t becomes larger and the mean result of the test method must be closer to the reference value for the discrepancy to be due to random differences.

TABLE 3.4

A comparison of expected and analyzed values for Copper, Iron and Zinc[a,b,c]

Sample: NIST bovine liver

Acid: 20 mL H_2SO_4/HNO_3 (1:1)

Procedure: Wet ashing by open vessel pulse microwave method B

Temperature: 70 °C

Element	Expect. Conc. ug/g	Conc. Found, ug/g
Copper	158 ± 7	166 ± 6
Iron	194 ± 20	210 ± 12
Zinc	123 ± 8	118 ± 4

[a] Analyzed values are the mean of four replicates.

[b] Sample size 0.25 g for all the tables.

[c] Analyzed by flame A.A.

TABLE 3.5

Effect of acid mixture volume and pulse time variation on recovery of Copper.[a,b,c]

Sample: NIST bovine liver

Acid: H_2SO_4/HNO_3 (1:1)

Procedure: Wet ashing by open vessel pulse microwave method B

Element	Acid mix	Pulse time, s	Expect. conc., ug/g	Conc. found,ug/g
Copper	5.0 mL	36	158 ± 7	162 ± 5
	10.0 mL	36		158 ± 9
	20.0 mL	36		166 ± 6
	20.0 mL	72		164 ± 6
	20.0 mL	108		172 ± 11

[a] Analyzed values are the mean of four replicates.

[b] Analyzed by flame AAS.

[c] Regression lines were determined for Cu from standards run in 5%, 10%, and 20% acid solutions and metal concentrations were calculated from the corresponding regression lines.

TABLE 3.6

Effect of acid mixture volume and pulse time variation on recovery of Iron.[a,b,c]

Sample: NIST bovine liver

Acid: H_2SO_4/HNO_3 (1:1)

Procedure: Wet ashing by open vessel pulse microwave method B

Element	Acid mix	Pulse time, s	Expect. conc., ug/g	Conc. found, ug\g
Iron	5.0 mL	36	194 ± 20	210 ± 15
	10.0 mL	36	"	191 ± 9
	20.0 mL	36	"	210 ± 12
	20 .0mL	72	"	204 ± 11
	20 .0mL	108	"	213 ± 10

[a] Analyzed values are the mean of four replicates.

[b] Analyzed by flame AAS.

[c] Regression lines were determined for Fe from standards run in 5%, 10%, and 20% acid solutions and metal concentrations were calculated from the corresponding regression lines.

TABLE 3.7

Effect of acid mixture volume and pulse time variation on recovery of Zinc.[a,b,c]

Sample: NIST bovine liver

Acid: H_2SO_4/HNO_3 (1:1)

Procedure: Wet ashing by open vessel pulse microwave method B

Element	Acid mix	Pulse time, s	Expect. conc. ug/g	Conc. found, ug/g
Zinc	5.0 mL	36	123 ± 8	135 ± 4
	10.0 mL	36		133 ± 3
	20.0 mL	36		118 ± 4
	20.0 mL	72		122 ± 3
	20.0 mL	108		119 ± 2

[a] Analyzed values are the mean of four replicates.

[b] Analyzed by flame AAS.

[c] Regression lines were determined for Zn from standards run in 5%, 10%, and 20% acid solutions and metal concentrations were calculated from the corresponding regression lines.

ANOVA RESULTS

One way analysis of variance (ANOVA) was the tool used to compare the means of all the methods used for Cu, Fe[3], and Zn including pulse time and acid volume variation. To perform a hypothesis test on the value of mean (σ), the following hypothesis was set up:

The null hypothesis, H_o: There is no significant difference between different methods used.

The alternate hypothesis, H_1: There is a significant difference between methods used.

The ANOVA was performed on the data for each element. The reference material showed no significant differences between all the methods used for Cu and Fe.

Methods used for analysis of variance for Fe:

Level	Method used
1	Conventional hot plate wet ashing method
2	Wet ashing by open vessel pulse microwave method B Acid mixture volume: 5.0 mL H_2SO_4/HNO_3 (1:1) Pulse heating time : 6 × 10s pulse /180s dormant
3	Wet ashing by open vessel pulse microwave method B Acid mixture volume: 10.0 mL H_2SO_4/HNO_3 (1:1) Pulse heating time : 6 × 6s pulse /180s dormant
4	Wet ashing by open vessel pulse microwave method B Acid mixture volume: 20.0 mL H_2SO_4/HNO_3 (1:1) Pulse heating time : 6 × 6s pulse /180s dormant
5	Wet ashing by open vessel pulse microwave method B Acid mixture volume: 20.0 mL H_2SO_4/HNO_3 (1:1) Pulse heating time : 6 × 12s pulse /180s dormant
6	Wet ashing by open vessel pulse microwave method B Acid mixture volume: 20.0 mL H_2SO_4/HNO_3 (1:1) Pulse heating time : 6 × 18s pulse /180s dormant

[3] Fe values for method A are not included due to high student t values

Methods used for analysis of variance for Cu and Zn:

Level	Method used
1	Conventional hot plate wet ashing method
2	Wet ashing by open vessel pulse microwave method A Acid mixture volume: 20.0 mL H_2SO_4/HNO_3 (1:1) Pulse heating time : 6 × 10s pulse /180s dormant
3	Wet ashing by open vessel pulse microwave method B Acid mixture volume: 5.0 mL H_2SO_4/HNO_3 (1:1) Pulse heating time : 6 × 10s pulse /180s dormant
4	Wet ashing by open vessel pulse microwave method B Acid mixture volume: 10.0 mL H_2SO_4/HNO_3 (1:1) Pulse heating time : 6 × 6s pulse /180s dormant
5	Wet ashing by open vessel pulse microwave method B Acid mixture volume: 20.0 mL H_2SO_4/HNO_3 (1:1) Pulse heating time : 6 × 6s pulse /180s dormant
6	Wet ashing by open vessel pulse microwave method B Acid mixture volume: 20.0 mL H_2SO_4/HNO_3 (1:1) Pulse heating time : 6 × 12s pulse /180s dormant
7	Wet ashing by open vessel pulse microwave method B Acid mixture volume: 20.0 mL H_2SO_4/HNO_3 (1:1) Pulse heating time : 6 × 18s pulse /180s dormant

Anova for Cu : Analysis of variance on C1

Source	DF	SS	MS	F	p
C2	6	395.2	65.9	0.98	0.467
Eeeor	18	1210.7	67.3		
Total	24	1605.9			

Individual 95% CI'S for mean
Based on pooled stdev

Level	N	Mean	Stdev	--- + --------- + --------- + --------- + ---
1	3	162.33	10.50	(---------*---------)
2	4	164.50	9.00	(--------*-------)
3	3	161.63	4.97	(---------*---------)
4	3	157.50	8.60	(---------*--------)
5	4	165.45	5.80	(-------*--------)
6	4	163.92	6.05	(--------*--------)
7	4	171.68	10.63	(--------*-------)

Pooled Stdev = 8.20 --- + --------- + --------- + --------- + --- 150 160 170 180

An ANOVA table includes four components:

A) The F test result 0.98, and corresponding p- value, 0.467.The null hypothesis is not rejected at the 0.05 level.

B) A diagram of the individual 95% confidence interval for each of the methods, based on the pooled standard deviation. The * represents the sample mean and the () the 95% confidence limits.

C) Pooled standard deviation: In this case 8.2

D) A descriptive summary of each method including sample size, the sample mean, and the sample standard deviation.

When the p-value is greater than the chosen level of significance, null hypothesis is not rejected. In this case, the p-value of 0.467 is greater than 0.05, so it was concluded that there was no significant difference between different methods used for Cu.

Anova for Fe:

Analysis of variance on C1

Source	DF	SS	MS	F	p
C2	5	1419	284	2.18	0.112
Error	15	1956	130		
Total	20	3376			

Individual 95% CI'S for mean

Based on pooled stdev

Level	N	Mean	Stdev	---+---------+---------+---------+---
1	3	198.13	9.47	(--------*--------)
2	3	210.00	15.00	(--------*--------)
3	3	190.67	8.95	(--------*--------)
4	4	212.50	12.12	(-------*-------)
5	4	203.50	11.00	(-------*-------)
6	4	214.50	11.00	(-------*-------)

---+---------+---------+---------+---

Pooled stdev = 11.42 180 195 210 225

The p-value of 0.112 is greater than 0.05, so it was concluded that there was no significant difference between different methods used for Iron.

Anova for Zn:

Analysis of variance on C1

Source	DF	SS	MS	F	p
C2	6	841.6	140.3	13.60	0.000
Error	16	165.0	10.3		
Total	22	1006.6			

Level	N	Mean	Stdev	Individual 95% CI'S for mean Based on pooled stdev
1	4	122.50	3.79	(----*----)
2	3	124.93	2.20	(----*-----)
3	3	135.33	4.04	(----*-----)
4	3	132.67	2.89	(-----*----)
5	4	118.00	3.46	(----*---)
6	3	122.40	3.08	(-----*----)
7	3	119.00	2.00	(-----*-----)

Pooled stdev = 3.21 — 119.0 — 126.0 — 133.0

ANOVA for Zn showed significant difference between different methods. The F test result 13.6, and corresponding p- value, 0.00. The null hypothesis is rejected at the 0.05 level.

The null hypothesis that the two means are equal is rejected whenever the confidence interval for the difference in the means does not contain 0. Tukey's comparison test shows that Zinc is sensitive to acid volume and pulse time variation

Tukey's pairwise comparisons

Family error rate = 0.0500

Individual error rate = 0.00405

Critical value = 4.74

Intervals for (column level mean) - (row level mean)

	1	2	3	4	5	6
2	-10.654 5.787					
3	-21.054 -4.613	-19.188 -1.612				
4	-18.387 -1.946	-16.522 1.055	-6.122 11.455			
5	-3.111 12.111	-1.287 15.154	9.113 25.554	6.446 22.887		
6	-8.121 8.321	-6.255 11.322	4.145 21.722	1.478 19.055	-12.621 3.821	
7	-4.721 11.721	-2.855 14.722	7.545 25.122	4.878 22.455	-9.221 7.221	-5.388 12.188

Null hypothesis that the two means are equal is rejected whenever the confidence interval for the difference in the means does not contain 0. Tukey's test shows that Zn is sensitive to longer pulse time variation and low acid mixture volumes.

A maximum coefficient of variation of less than 6% for Cu and Zn in NIST reference materials and maximum coefficient of variation of less than 12% for spiked low and less than 3% for spiked high level Cd demonstrate the precision of the method A (Table 3.3). Method A's accuracy is demonstrated by mean recovery of greater than 104% for NIST reference materials and greater than 89% for spiked low and high level metals.

The acid mixture H_2SO_4/HNO_3(1:1) used in our extraction procedure was found not to be suitable for the extraction of low concentration of lead by method A. Difficulties encountered in measuring Pb in 1:1 acid mixture have been documented by other researchers (4). This is probably due to the formation of insoluble lead sulphate during digestion. HNO_3 alone gave satisfactory results for fish tissue and cream cheese[4]. Reoptimization of flameless conditions were required for low level Cadmium analysis. The acid mixture gave high background signal during atomization cycle for Cd, when charring temperature of 250 °C was used. A charring temperature of 500 °C with ramp of 10s was found optimum for the matrices used in flameless atomic absorption analysis.

The precision of method B is demonstrated by a maximum coefficient of variation of less than 7% for Cu, Fe, and Zn in NIST reference materials (Tables 3.4 - 3.7) and maximum coefficient of variation of less than 2% for spiked Cd, Pb and Mn[4] . Accuracy is demonstrated by mean recovery of greater than 97% for NIST reference materials and greater than 96% for spiked high level metals (Tables 3.8). Accuracy and precision of low level recovery was demonstrated by mean recovery of 96% and coefficient of

variation of 5% for Mn from NIST bovine liver by 5.0 mL acid mixture[4] .

---

[4] Reported only spiked cadmium results.

Results of this study show that pulse procedure A and procedure B are viable alternatives for digestion of bovine liver and fish tissue samples for recovery of high and low level trace metals.

A 5.0 mL acid mixture is sufficient for satisfactory extraction of trace metals by method B. However, recovery of low level spiked metals by method B was rather unsatisfactory for cream cheese using a 20.0 mL acid mixture (Tables 3.10 - 3.11). Precision of the method showed a maximum coefficient of variation of 27% with a mean recovery of 69% for Cd and a maximum coefficient of variation of 21% with a mean recovery of 75% for Mn from cream cheese. The standards and samples were subjected to the same atomic absorption procedure and poor recoveries are traceable to the sample extraction procedure. The loss may be explained by retention of Cd and Mn by fatty matter that was incompletely digested under these mild conditions.

Absence of matrix interference in methods A and B was shown by application of the standard addition method to the fish tissue matrix. A slope comparative test was established between the regression line corresponding to a series of 25 ug, 50 ug, and 75 ug spiked cadmium in the matrix under study for method A and method B (5.0 mL and 20.0 mL) (Table 3.8). ANOVA showed that there was no significant difference between all the methods used.

TABLE 3.8

Recovery of spiked Cadmium.[a,b,c]

Sample: Fish tissue and cream cheese

Acid: 5.0 mL and 20.0 mL H_2SO_4/HNO_3 (1:1)

Procedure: Wet ashing by open vessel pulse microwave methods A and B

Element	Sample	Amt. Added, ug	Amt. Recovered, ug Method A	Conc. found, ug/g Method B (5.0 mL)	Amt. Recovered, ug Method B (20.0 mL)
Cadmium	Fish tissue	25.0 ± 0.4	23.7 ± 0.6[b]	23.9 ± 0.9	25.2 ± 0.7 [b]
		50.0 ± 0.8	49.6 ± 1.2[b]	50.1 ± 0.9	51.1 ± 1.1 [b]
		75.0 ± 1.2	76.7 ± 1.1[b]	74.8 ± 1.3	78.2 ± 0.6 [b]
		0.75 ± 0.03	0.69 ± 0.08[c]	-	0.52 ± 0.14 [c]
	Cream cheese	0.75 ± 0.03	0.74 ± 0.04[c]	-	-
		2.0 ± 0.05	1.92 ± 0.16[c]	-	-

[a] Analyzed values are the mean of 3 replicate spikes.

[b] Analyzed by flame AAS

[c] Analyzed by flameless AAS

It is difficult to provide specific detection limits. Low concentration samples are chosen to represent realistic low level separation. Acid blanks are normally used to determine the detection limits of the methods (44). Detection limits with acid blanks for our methods are included in the experimental section.

The use of the term "digestion " is not intended to imply complete digestion but digestion only to the extent the cations sought can be extracted into acid mixture. Studies on the fate of hydrophobic refractory organics such as PCBs, DDT, DDE, Dieldrin, Methoxychlor, lindane when subjected to pulse procedure A in H_2SO_4/HNO_3 (1:1) (Chapter 5 and Appendix B) suggest that the procedure is most likely a partial digestion.

3.4 CONCLUSION

The pulse method is deemed to circumvent the traditional shortcomings of microwave treatment: risk of explosion in pressurized vessels, risk of corrosion of oven parts including safety circuits, high cost of laboratory ovens designed for wet ashing under pressure, and loss of volatile metals. The efficiency of microwave treatment in tissue clean - up is retained. 24 samples could be digested within 1 hour under the conditions described and manual sample exchange. The pulse method eliminates the possibility of hazardous conditions because the temperature is maintained below the boiling point of the acid mixture in an " open" vessel at 1 atm atmosphere pressure. Because of the pulse application of microwaves, the generation of acid fumes is restricted and moderated during intermittent cooling periods. The maximum temperature reached during wet ashing was below 110 °C for procedure A and less than 80 °C for procedure B. Volatile metals that would normally be lost in open systems are retained in pulse method. Preliminary results on Hg analysis in egg and fish tissue samples by cold vapor technique using this pulse procedure show promise. The minimal fumes which do arise in procedure A are contained by the outer loosely sealed vessel.

CHAPTER 4

DIGESTION OF PLANT SAMPLES BY PULSE MICROWAVES FOR ELEMENTAL ANALYSIS[1]

[1] Partly presented at CIC conference, Toronto, 1988.

4.1 INTRODUCTION

Inorganic ions in plant tissue are first converted to a soluble form either by extracting the tissue with a suitable solvent or by removing the organic fraction by wet or dry ashing digestion techniques (50). In the wet digestion method of plant tissues by an Official Method of Analysis (9), $HClO_4$ was used. Several hazards associated with its use are described in Chapter 1. To reduce the hazards of explosion due to $HClO_4$, Allen (51) recommended the use of only 10 mg of plant tissue. In the context of standard sample distribution, NIST stipulates that a minimum of 0.5 g of plant material is acceptable for trace metal determination (52).

Pulse microwave technique was developed in our work to digest animal tissue samples (Chapter 3). In continuing studies into the wet ashing of biological samples, it was of interest to examine the effect of the pulse microwave digestion method on the efficiency of trace metal recovery from plant tissue samples. The present study compares method A (10s pulse) and method B (6s pulse) for a wide range of plant tissues, such as NIST tomato leaves, pine needles and margarine. Due to the non-availability of high fat reference materials, margarine was used to represent high fat in order to study spiked recoveries to estimate the accuracy of the pulse method. The temperature of digestion mixture was maintained <110 °C (Method A) and <80 °C K (Method B) for 10s and 6s pulse methods respectively.

4.2 EXPERIMENTAL

4.2.1 Apparatus:

Description of microwave oven and atomic absorption is given in Chapter 2. Table 3.1 provides details specific to each metal used for flame A.A.S..

4.2.2 Materials:

H_2SO_4 and HNO_3 "trace metal grade" and 30% H_2O_2 were purchased from Fisher Scientific Co. Metal standards were prepared from certified grade salts from Fisher Scientific Co. Tomato leaves (1573) and pine needles (1575) were NIST standards. The metals (Ca, Mn, Zn) for which the samples are certified, were used unmodified. The same matrices were used for Cu, Cr, Pb, and Cd by appropriate spiking. For the second group of samples, lettuce and radish leaves were oven dried for 3 days at 70 °C and ground in a Wyllie mill. These were then spiked with Fisher Scientific certified grade 1000 ppm metal standards. For example, 200 ul Cu standard in Eppendorf pipette was injected into the sample to get 200 ug spiking. The sample was dried in a slow stream of N_2

4.2.3 Characterizing Optimum Conditions:

Different volumes (5.0 mL, 10.0 mL and 20.0 mL) of acid mixtures were subjected to different pulse lengths followed by dormant time of 180s at various power settings. The extrapolated temperatures were determined (Table 3.2) as described in Chapter 3.

4.2.4 Procedure:

4.2.4.1 Pulse treatment procedure:

Please see section 3.2.6.1

4.2.4.2 Conventional Procedure:

Please see section 3.2.6.2

4.2.4.3 Calculations:

Regression lines were determined for each element from standard runs. Metal concentrations in each sample were calculated from the corresponding regression lines and dilution factors.

4.3 RESULTS AND DISCUSSION

NIST tomato leaves, NIST pine needles, lettuce leaves, radish roots and margarine were selected to study the efficiency of our method. Due to the un-availability of high fat reference materials, margarine was used to represent high fat to study spiked recoveries to estimate the accuracy of method B.

Table 4.1 reports the digestion efficiency of different acid mixture(s) for Ca recovery from NIST tomato leaves. Tables 4.2 and 4.3 show data for determination of Ca, Mn, and Zn for NIST tomato leaves by method A and method B respectively. Table 4.4 demonstrates the recovery of Ca and Mn from NIST pine needles by method B. Effect of acid mixture volume and pulse time variation on the recovery of Ca, Mn, and Zn from NIST tomato leaves are given in Tables 4.5 to 4.7 respectively. Table 4.8 gives the recovery of spiked volatile metals from NIST tomato leaves, NIST pine needles, lettuce and radish roots.

Table 4.1

Comparison of results for Ca determination by different acid mixture

Sample : NIST 1573 tomato leaves

Acid: 1) 20.0 mL H_2SO_4/H_2O_2 (1:1

2) 20.0 mL H_2SO_4/HNO_3 (1:1)

3) 20.0 mL $H_2SO_4/HNO_3/H_2O_2$ (7.5 : 7.5 : 5)

Procedure: Wet ashing by open vessel pulse microwave method A

Acid mixture	Expected Conc(%)	Found (%)	Recovery(%)	Coefficient of variation (%)
1	3.10 ± 0.03	2.58 ± 0.22	86	8.5
2	3.10 ± 0.03	2.72 ± 0.21	91	7.7
3	3.10 ± 0.03	3.06 ± 0.06	99	1.9
3[b]	3.10 ± 0.03	3.11 ± 0.10	104	3.2

[a] Reported values are the mean of 3 replicates.

[b] Followed pulse procedure B

The student t-test (equation 2.1) was used to determine whether the values obtained from different acid mixtures are statistically equal to the accepted NIST values for Ca, Mn, and Zn .

Student's t values for different acid mixtures (Table 4.1).

Element	Acid mixture 1	Acid mixture 2	Acid mixture 3	Acid mixture 3[2]
Ca	3.13	4.09	1.15	0.73

For two degrees of freedom, the table value of t at the 95% confidence level is 4.303. Since calculated values for Ca is less than this, all acid mixtures give statistically correct value at 95% confidence level. Acid mixture $H_2SO_4/HNO_3/H_2O_2$ was chosen for this work because of its better precision and closer mean value to NIST reference material value.

[2] Procedure B

TABLE 4.2

Comparison of expected and analyzed values for Calcium, Manganese and Zinc [a,b]

Sample : NIST 1573 tomato leaves

Acid: 20.0 mL $H_2SO_4/HNO_3/H_2O_2$ (7.5 : 7.5 : 5.0)

Procedure: Wet ashing by open vessel pulse microwave method A

Temperature: 95 °C

Element	Expected conc.	Conc. found.
Ca	3.10 ± 0.03%	3.06 ± 0.06% (2.82 ±0.03)
Mn	238 ± 7 ug/g	236 ± 4 ug/g (245 " 6)
Zn	62 ± 6 ug/g	63 ± 3 ug/g (59 ± 3)

[a] Reported values are the mean of 3 replicates.

[b] Values in parenthesis are for hot plate wet ashing

TABLE 4.3

Comparison of expected and analyzed values for Calcium, Manganese and Zinc [a,b]

Sample: NIST 1573 tomato leaves

Acid: 20.0 mL $H_2SO_4/HNO_3/H_2O_2$ (7.5 : 7.5 : 5.0)

Procedure: Wet ashing by open vessel pulse microwave method B

Temperature: 80 °C

Element	Expected conc.	Conc. found, ug/g
Ca	3.10 ± 0.03 %	3.11 ± 0.10 %
Mn	238 ± 7 ug/g	259 ± 7 ug/g
Zn	62 ± 6 ug/g	69 ± 2 ug/g

[a] Reported values are the mean of 3 replicates.

[b] 0.25 g

TABLE 4.4

Comparison of expected and analyzed values for Calcium, Manganese [a,b]

Sample: NIST 1575 pine needles

Acid: 20.0 mL $H_2SO_4/HNO_3/H_2O_2$ (7.5 : 7.5 :5.0)

Procedure: Wet ashing by open vessel pulse microwave method B

Temperature: 95 °C

Element	Expected conc. ug/g	Conc. found, ug/g
Ca	0.41 ± 0.02	0.40 ± 0.01
Mn	675 ± 15	703 ± 12

[a] Reported values are the mean of 3 replicates.

[b] 0.25 g

Student's t values for NIST reference materials (Table 4.2 - 4.4).

Element	NIST tomato leaves (method A)	NIST tomato leaves (method B)	NIST Pine needles
Ca	1.15	0.173	1.73
Mn	0.87	0.6	4.04
Zn	0.6	6.06	—

For two degrees of freedom, the table value of t at the 95% confidence level is 4.303. Since calculated values for Ca is less than this, methods A and B give statistically correct values at 95% confidence level for Ca and Mn. Since the calculated value for Zn for method B is greater than 3.182, there is a 95% probability that the difference between method A data and reference value for Zn is not due to chance and there is a determinate error in the method[3].

[3] Note that as the precision is improved, the calculated t becomes larger and the mean result of the test method must be closer to the reference value for the discrepancy to be due to random differences.

TABLE 4.5

Effect of acid mixture volume and pulse time variation on recovery of Calcium[a,b,c]

Sample: NIST tomato leaves

Acid: HNO_3/H_2O_2 (7.5 : 7.5 : 5.0)

Procedure: Wet ashing by open vessel pulse microwave method B

Element	Acid mix (vol)	Pulse time (s)	Expect. conc. ug/g	Conc found ug/g
Ca	5.0 mL	36	3.10 ± 0.03	3.12 ± 0.14
	10.0 mL	36		2.95 ± 0.02
	10.0 mL	36		3.11 ± 0.1
	10.0 mL	72		2.94 ± 0.12
	10.0 mL	108		2.67 ± 0.38

[a] Reported values are the mean of 3 replicates.

[b] 0.25 g

[c] Regression lines were determined for Ca from standards run in 5%, 10% and 20% acid solutions and metal concentrations were calculated from the corresponding regression lines.

TABLE 4.6

Effect of acid mixture volume and pulse time variation on recovery of Manganese[a,b,c]

Sample: NIST tomato leaves

Acid: HNO_3/H_2O_2 (7.5 : 7.5 : 5)

Procedure: Wet ashing by open vessel pulse microwave method B

Element	Acid mix (vol)	Pulse time (s)	Expect.conc (ug/g)	Conc. found ug/g
Mn	5.0 mL	36	238 ± 7	211 ± 10
	10.0 mL	36		224 ± 5
	20.0 mL	36		243 ± 17
	20.0 mL	72		243 ± 6
	20.0 mL	108		230 ± 10

[a] Reported values are the mean of 3 replicates.

[b] 0.25 g

[c] Regression lines were determined for Mn from standards run in 5%, 10% and 20% acid solutions and metal concentrations were calculated from the corresponding regression lines.

TABLE 4.7

Effect of acid mixture volume and pulse time variation on recovery of Zinc[a,b,c].

Sample: NIST tomato leaves

Acid: HNO_3/H_2O_2 (7.5 : 7.5 : 5)

Procedure: Wet ashing by open vessel pulse microwave method B

Element	Acid mix vol	Pulse time (s)	Expect.conc. ug/g	Conc. found ug/g
Zn	5.0 mL	36	62 ± 6	41 ± 3
	10.0 mL	36		54 ± 2
	20.0 mL	36		69 ± 2
	20.0 mL	72		59 ± 3
	20.0 mL	108		55 ± 6

[a] Reported values are the mean of 3 replicates.

[b] 0.25 g

[c] Regression lines were determined for Zn from standards run in 5%, 10% and 20% acid solutions and metal concentrations were calculated from the corresponding regression lines.

A maximum coefficient of variation of less than 5 % for Ca, Mn, and Zn in NIST reference materials demonstrates the precision of method A (Table 4.2). Method A accuracy is demonstrated by mean recovery of greater than 98% for NIST reference materials. Acid mixture volume and time variation (5.0 mL and 10.0 mL) on the extraction efficiency for procedure A was not studied due to high temperatures (>100 °C) reached by the acid mixture.

ANOVA RESULTS:

A one way analysis of variance (ANOVA) was the tool used to compare the means of all the methods used for Ca, Mn and Zn. To perform a hypothesis test on the value of mean (σ), the following hypothesis was set up:

The null hypothesis, H_o: There is no significant difference between different methods used.

The alternate hypothesis, H_1: There is a significant difference between methods used.

The ANOVA was performed on the data for each element. Methods used for analysis of variance: Conventional method, method A, method B (5.0 mL, 10.0 mL and 20.0 mL 1:1 acid mixture) and pulse time variation (36s, 72s and 108s).

Methods used for analysis of variance:

Level	Method used
1	Wet ashing by open vessel pulse microwave method A Acid mixture volume:20.0 mL $H_2SO_4/HNO_3/H_2O_2$ Pulse heating time : 6 X 10s pulse /180s dormant
2	Wet ashing by open vessel pulse microwave method B Acid mixture volume:20.0 mL $H_2SO_4/HNO_3/H_2O_2$ Pulse heating time : 6 X 10s pulse /180s dormant
3	Wet ashing by open vessel pulse microwave method B Acid mixture volume:5.0 mL $H_2SO_4/HNO_3/H_2O_2$ Pulse heating time : 6 X 6s pulse /180s dormant
4	Wet ashing by open vessel pulse microwave method B Acid mixture volume:10.0 mL $H_2SO_4/HNO_3/H_2O_2$ Pulse heating time : 6 X 6s pulse /180s dormant
5	Wet ashing by open vessel pulse microwave method B Acid mixture volume:20.0 mL $H_2SO_4/HNO_3/H_2O_2$ Pulse heating time : 6 X 12s pulse /180s dormant
6	Wet ashing by open vessel pulse microwave method B Acid mixture volume:20.0 mL $H_2SO_4/HNO_3/H_2O_2$ Pulse heating time : 6 X 18s pulse /180s dormant
7	Conventional hot plate wet ashing method

Analysis of variance for Ca :

Analysis of variance on C1

Source	DF	SS	MS	F	p
C2	6	0.5207	0.0868	4.02	0.011
Error	17	0.3667	0.0216		
Total	23	0.8874			

Individual 95% CI'S for mean

Based on pooled stdev

Level	N	Mean	Stdev	-+---------+---------+---------+-----
1	4	3.0575	0.0562	(-----*-----)
2	3	3.1100	0.0000	(------*-------)
3	3	3.1167	0.1447	(------*------)
4	4	2.9550	0.0173	(-----*-----)
5	3	2.9367	0.1206	(------*-------)
6	3	2.6667	0.3761	(------*------)
7	4	2.8150	0.0289	(------*-----)

-+---------+---------+---------+-----

Pooled stdev = 0.1469 2.50 2.75 3.00 3.25

When the p-value is greater than the chosen level of significance, null hypothesis is rejected. Calcium p-value of 0.0011 is less than 0.05, so it was concluded that some methods were significantly different. Tukey's pairwise comparison test was used to determine which methods differ from one other.

Tukey's pairwise comparisons

Family error rate = 0.0500

Individual error rate = 0.00402

Critical value = 4.70

Intervals for (column level mean) - (row level mean)

	1	2	3	4	5	6
2	-0.4253					
	0.3203					
3	-0.4320	-0.4052				
	0.3136	0.3919				
4	-0.2426	-0.2178	-0.2111			
	0.4476	0.5278	0.5345			
5	-0.2520	-0.2252	-0.2185	-0.3545		
	0.4936	0.5719	0.5785	0.3911		
6	0.0180	0.0448	0.0515	-0.0845	-0.1285	
	0.7636	0.8419	0.8485	0.6611	0.6685	
7	-0.1026	-0.0778	-0.0711	-0.2051	-0.2511	-0.5211
	0.5876	0.6678	0.6745	0.4851	0.4945	0.2245

Tukey's pair comparison test showed that longer pulse time (method B, 108s) is significantly different from other methods.

Anova for manganese:

Analysis of variance on C1

Source	DF	SS	MS	F	p
C2	6	3530.8	588.5	13.66	0.000
Error	18	775.4	43.1		
Total	24	4306.2			

Individual 95% CI'S for mean

Based on pooled stdev

Level	N	Mean	Stdev	----+---------+---------+---------+--
1	4	236.00	4.00	(---*----)
2	3	243.00	0.00	(----*----)
3	4	211.00	10.00	(----*---)
4	4	223.75	4.50	(---*----)
5	3	243.33	5.77	(----*-----)
6	3	230.00	10.00	(----*-----)
7	4	245.00	5.77	(---*----)

----+---------+---------+---------+--

Pooled stdev = 6.56 210 225 240 255

Manganese p-value of 0.000 is less than 0.05, so it was concluded that some methods were significantly different. Tukey's pairwise comparison test was used to determine which methods differ from one other.

Tukey's pairwise comparisons:

Family error rate = 0.0500

Individual error rate = 0.00396

Critical value = 4.67

Intervals for (column level mean) - (row level mean)

	1	2	3	4	5	6
2	-23.55					
	9.55					
3	9.67	15.45				
	40.33	48.55				
4	-3.08	2.70	-28.08			
	27.58	35.80	2.58			
5	-23.89	-18.03	-48.89	-36.14		
	9.22	17.36	-15.78	-3.03		
6	-10.55	-4.70	-35.55	-22.80	-4.36	
	22.55	30.70	-2.45	10.30	31.03	
7	-24.33	-18.55	-49.33	-36.58	-18.22	-31.55
	6.33	14.55	-18.67	-5.92	14.89	1.55

Tukey's pair comparison test showed that manganese is sensitive to acid volume variation.

Gorsuch (4) found that H_2SO_4 decreases the extraction efficiency due to the formation of insoluble $CaSO_4$. H_2SO4 in our extraction mixture may react with Ca to form $CaSO_4$. Quantitative extraction of Ca in our method may be due to the solubilization of $CaSO_4$ by HNO_3 and the use of the same proportion of acid mixture in control and standards. However, there is a gradual decrease in Ca recovery for longer pulse times (Table 4.9). For 108s pulse sequence, mean Ca recovery was 86% with coefficient of variaton of 14%.

Method B showed matrix interference for Zn recovery from NIST tomato leaves. Zn is sensitive to acid volume variation. However, student's t test showed that the values obtained from longer pulse times (72s, 108s) are statistically equal to the accepted NIST values.

Precision of method B is demonstrated by a maximum coefficient of variation of less than 7% for Ca, Mn in NIST reference materials (Tables 4.3 & 4.4) and maximum coefficient of variation of less than 4% for spiked metals. Accuracy is demonstrated

by mean recovery of greater than 89% for NIST reference materials and greater than 96% for spiked high level metals .

Several problems are associated with recovery of volatile metals (53) Recovery of volatile metals such as Cr, Cd and Pb is satisfactory in our study and there is no apparent matrix effect and no loss due to volatilization. This is probably due to the low temperatures used in our extraction procedure.

TABLE 4.8

Recovery of spiked Cu, Cr, Cd, and Pb.[a,b]

Acid : 20.0 mL $H_2SO_4/HNO_3/H_2O_2$ (7.5 : 7.5 : 5)

Procedure : Wet ashing by open vessel pulse microwave method B

Element	Amt. added, ug	Amt. recovered, ug (Nist tomato leaves)	Amt. recovered,ug (NIST Pine needles)	Amt recovered, ug (Lettuce leaves)	Amt. recovered, ug (Radish roots)
Copper	200 ± 4	215 ± 8	211 ± 4	210 ± 4	204 ± 2
Chromium	100 ± 2	96 ± 2	101 ± 5	97 ± 3	105 ± 1
Cadmium	100 ± 2	109 ± 2	103 ± 1	100 ± 3	99 ± 1
Lead	500 ± 7	503 ± 36	514 ± 25	493 ± 37	535 ± 15

[a] Reported values are the mean of 4 replicates samples.

[b] 0.25 g

Method A and method B (20.0 mL) pulse procedure is suitable for most of the plant tissue samples, such as vegetables and leaves. It is clear that further study on the extraction efficiency of varying sample weights would be useful. It is not known if pulse procedure can be used for plant and tree samples with high concentrations of cellulose and lignin. These samples require a greater amount of energy to breakdown their high molecular weight compounds for complete dissolution. American standard testing materials (ASTM) (54) recommends the use of a separate procedure to break down high molecular weight biological polymers.

Results of this study show that pulse methods A and B are viable alternatives for digestion of samples for high and low level recovery of trace metals. Based on this study, 24 samples could be digested within 1 hour under the conditions described. The maximum temperature reached during wet ashing was below 100 °C for procedure A and less than 80 °C for procedure B. Volatile metals that would normally be lost in open systems are retained in pulse method. Pulse sequence method B is insensitive to acid mixture volumes and pulse time variations for Mn recovery from NIST tomato leaves. Ca and Zn recovery is sensitive to acid mixture volume and pulse time variation).

CHAPTER 5

PULSE MICROWAVE-MEDIATED BIOLOGICAL SAMPLE CLEAN-UP METHOD FOR REFRACTORY ORGANIC ANALYSIS AND DEGRADATION OF PCBs AND PESTICIDES[1]

---

[1]Presented (refractory organics in tissues) at SETAC, Arlington, Virginia. Nov , 1988.

Presented (PCBs in soil) at SETAC, Toronto, Ontario, Nov, 1989.

Received two patents for organic waste Treatment: 1) US Patent Number:4,980,039

2) US Patent Number 5,118,429.

5.1 INTRODUCTION

The use of gas-liquid chromatography (GLC) with electron capture detection for the analysis of chlorinated hydrocarbon residues requires the prior separation of these residues from interfering biological substances. General clean up method and some of the problems associated with clean-up methods are discussed in Chapter 1. Some clean- up methods used by regulatory agencies specific to the matrices used in this thesis (tissue, serum, and soil) are given in this section.

In the case of tissue sample analysis, the officially adopted method by the regulatory agencies involved petroleum ether extraction of the sample followed by residue partitioning into acetonitrile, dilution with water, re-extraction into petroleum ether, and finally column chromatography on Florisil with 6% ethyl ether in petroleum ether. Additional clean-up procedures have also been devised to permit separation of DDT and these procedures can be flawed by various experimental factors which cause variability in column preparation, and hence in recoveries. Additionally, those congeners with the lowest chlorination were held on the silicic acid column and could only be eluted by a more polar solvent than petroleum ether (55). Stalling et al. (56) used a semi-automated gel permeation system to remove lipids from fish tissue extracts, but insufficient clean-up was obtained with some samples.

H_2SO_4 is used as a clean-up agent (57,58). Others used adsorbent columns followed by clean-up wash with H_2SO_4, but their methods are subject to many of the problems listed in Chapter 1.

Ten different laboratories followed Environmental Protection Agency's (EPA) (59) procedure to analyze three samples of sediments, environmentally contaminated with PCBs, by using uniform calibration standards and standardized procedures. In procedure A, the samples were air dried for four days, followed by soxlet extraction for 16 hrs. The concentrated extract was eluted on floricil column and the eluate was analyzed by GC-ECD. In procedure B, the samples were soxlet extracted in 2-propanol for 16 hrs, followed by 16 hrs more of extraction with dichloromethane. The concentrated dichloromethane extract was eluted on floricil column and the eluate was analyzed by GC-ECD. Inspite of written standardized procedures, results showing large differences were reported. For ECD data, the total relative standard deviation of measurements of total Aroclor concentrations was 30%; for MS data deviation was 38%.

5.1.1 Treatment of Pesticide and PCB Wastes

Safe disposal of waste PCBs and halogenated hydrocarbon pesticides still remains a problem due to the inherent nature of halogenated hydrocarbons, i.e., high stability to chemical and biological degradation. A number of methods have been proposed to decompose polychlorinated biphenyls and other halogenated hydrocarbons. Some of the methods employ a high temperature treatment and therefore carry the risk of air pollution due to the emission of noxious fumes and vapours to the environment. PCBs produce highly toxic dioxins when they are heated to temperatures ranging from 300 °C to 900° C in the presence of air. Also, incineration as a way to dispose of hazardous chemicals in general has a notable drawback in that it requires substantial energy consumption.

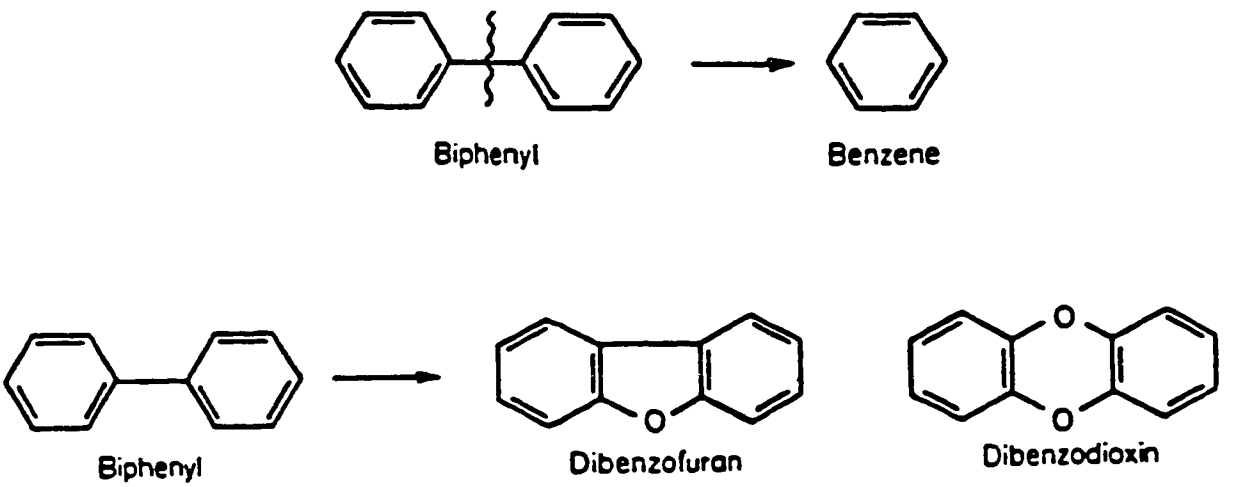


Figure 5.1: Structures of dioxin formation.

It has been found that certain decomposition reactions of halogenated organic compounds can be stimulated by the use of radiation, e.g. UV or solar energy and microwave. Wan (US. Pat. No. 4,345,98) demonstrates a process in which a chlorinated hydrocarbon is brought into contact with iron powder in the presence of high-intensity microwave radiation. Tundo (US. Pat. No. 4,632,742) discloses a method in which a halogenated organic compound is reacted with a mixture of reactants

including a polyethylene glycol, a base, and a source of free radicals such as peroxide, persalt or metals of high valence. The reactions were carried out in the presence of electric fields, and ultrasounds. Solar energy has been proposed in some studies (US. Pat. No. 4,432,344). EPA scientists (60) received an award for treating PCB wastes efficiently with CaO. It was later found that PCBs did not degrade but evaporated when treated with CaO (61). There is no safe and efficient process for the decomposition of halogenated or polyhalogenated hydrocarbons where the risks associated with high-temperature treatment are eliminated. Most of the patented procedures to degrade PCBs were not exploited commercially.

This chapter evaluates the rapid, efficient clean-up procedure which results from pulse wet ashing treatment of NIST bovine liver, serum, and soil samples fortified with refractory pesticides and PCBs. Toxic metals and organic pollutants are transported to liver by blood (serum) for detoxification. Analysis of residue organic pollutants in liver and serum is important in hazard evaluation programs. Organic matter in soils consists of decayed plant and animal tissue products and their wastes. The organic products can be subdivided into two categories: (a) non humic substances and (b) humic substances. The bulk of the organic matter in soils and waters exists as humic substances. The humic substances are responsible for binding metals and organic pollutants and transporting them to lakes and rivers. It has therefore been suggested that the determination of environmental fate of pollutants should also been performed in the presence of humic acids (62).

The clean-up method is based on the microwave pulse wet ashing procedure (Chapter 2-4). In wet ashing procedure, organic matrix that interferes in atomic absorption spectroscopy is oxidised for trace metal determination. This procedure is exploited to analyze refractory organic pollutants that are resistant to oxidation. The procedure is based on a separation with destruction strategy. It was found that addition of nujol (mineral oil) is critical for quantitative recovery of pollutants.

In addition to quantitative recovery of some pesticides and PCBs, it was found unexpectedly that these compounds can be decomposed by using longer pulse heating times. In this work, quantitative recovery and destruction of some PCBs and pesticides will be demonstrated by monitoring recovery rates by electron capture gas chromatograph.

5.2 EXPERIMENTAL

5.2.1 Instrumentation:

The gas chromatography/data system used was a Perkin-Elmer Sigma series equipped with a Ni detector. A 1.83 m by 2 mm (i.d.) glass gas chromatography column packed with 2% OV/1.3% QF on Chromosorb W, AW was used.

5.2.2 Reagents:

Pesticide grade Hexane, H_2SO_4, and HNO_3 were ACS grade from Fisher Scientific Co. Bovine liver (1577a) samples were purchased from NIST. Pesticide and PCB samples were purchased from Supelco Company. Serum samples were from Brachydaniorerio cultured in our laboratory. Soil was from Armadale PEI.

5.2.3 Preparation of Fortified Samples :

Samples were fortified with given concentration of pesticide or PCB standard in hexane and mixed thoroughly. The hexane solvent was evaporated under a slow stream of N_2. For decomposition studies, pesticide or PCB standard was taken in Erlenmeyer flask and the hexane solvent was evaporated under slow stream of N_2.

5.2.4 Glassware:

The glassware was washed with detergent 3 times, rinsed with deionized water, and dried with acetone. Finally, the glassware was rinsed three times with pesticide grade hexane and dried in an oven. Optimum number of washings required to clean the glassware free from PCBs was determined as follows: New Erlenmeyer flasks were cleaned several times with soap, water, and acetone. The flask was rinsed with small amount of hexane and the hexane extract was injected into ECD - GC to make sure that no PCBs were present. The flasks were spiked with 1000 ug of PCB

mixture. Erlenmeyer flask was cleaned once with acationex soap, water, and acetone. The dried flask was rinsed with hexane and the hexane extract was injected into ECD-GC. It was found that when the flask was cleaned three times each with soap, water, acetone and hexane, no traces of PCBs were found.

5.2.5 Laboratory Clean - up Procedure:

Electron capture gas chromatograph instrument is extremely sensitive to chlorinated and polar solvents. Chloroform in an open beaker will make the instrument unstable for several hours. All the polar solvents are stored under the fume hood. Care was taken to ensure that the counters were cleaned regularly and the laboratory floor was cleaned with tap water only.

5.2.6 Procedure:

Fortified samples in Erlenmeyer flask were mixed with 20.0 ml of H_2SO_4/HNO_3 (1:1). Nujol was added as required. Erlenmeyer flask was then placed in a wide-mouth plastic container which was closed with a screw cap. Each sample was heated for 10s (method A) or 6s (method B) followed by a dormant time of 180s. This procedure was repeated. Samples were exchanged during the dormant time to increase the throughput. The container was removed and cooled to room temperature in an ice bath. Exactly 100.0 ml or 50.0 ml of pesticide grade hexane was added depending on the pesticide used. The stoppered solution contents were stirred for 20 min with a magnetic stirrer. The supernatant hexane solution was collected and rinsed first with water and then with saturated $NaHCO_3$ and dried in Na_2SO_4 . The hexane layer was injected directly into gas chromatograph.

5.3. RESULTS AND DISCUSSION:

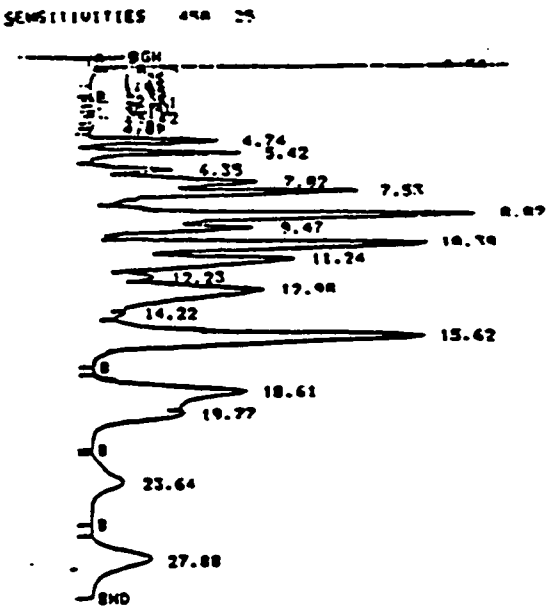
Recoveries of fortified PCBs and pesticides from bovine liver and recoveries of PCBs from serum and soil samples were studied to examine the efficiency of the method. The presence of nujol is critical for quantitative recovery of pesticides and PCBs.

Figures 5.2a and 5.2b show chromatogram of Arochlor 1260 standards (10 ng/1.0 ul) in hexane solvent. Figures 5.3a and 5.3b show the extraction efficiency of extraction efficiency of Aroclor 1260 from acid mixture $H_2SO_4/HNO_3$for two samples. Table 5.1 and Figure 5.4 show the effect of nujol on the recoveries of spiked hexachlorobiphenyl (PCB monomer) from NIST bovine liver. Table 5.2 shows the effect of sample weight, nujol volume and pulse time variation on the recoveries of spiked Aroclor 1260 (PCB mixture) from NIST bovine liver. Table 5.3, Figure 5.8 and Table 5.4, Figure 5.9 demonstrate the recovery of Aroclor 1260 from serum and soil samples respectively. Table 5.5 and Figure 5.10 reports the recovery of some pesticides. Table 5.6 show the effect of pulse heating time on the degration of PCB congener, Aroclor 1260 and Aroclor 1016.

5.3.1. Extraction Efficiency of PCBs:

Mean peak area from retention time 4 minutes to 28 minutes (Figures 5.2a and 5.2b) was used to determine the extraction efficiency of fortified Aroclor from acid mixture (Figures 5.3a and 5.3b).The average peak area for 10 ng of standard was 344 units with coefficient of variation of 1%. The extraction efficiency of Aroclor from acid mixture (Figures 5.3a & 5.3b) was greater than 103 %. Extreme precaution was taken to keep the laboratory clean (5.24 and 5.25). The coefficient of variation is less than

to keep the laboratory clean (5.24 and 5.25). The coefficient of variation is less than 5% for day to day operation. Chromatogram of acid extract was unstable up to retention time 120s. This may be due to ultra trace quantities of acid in the hexane extract.



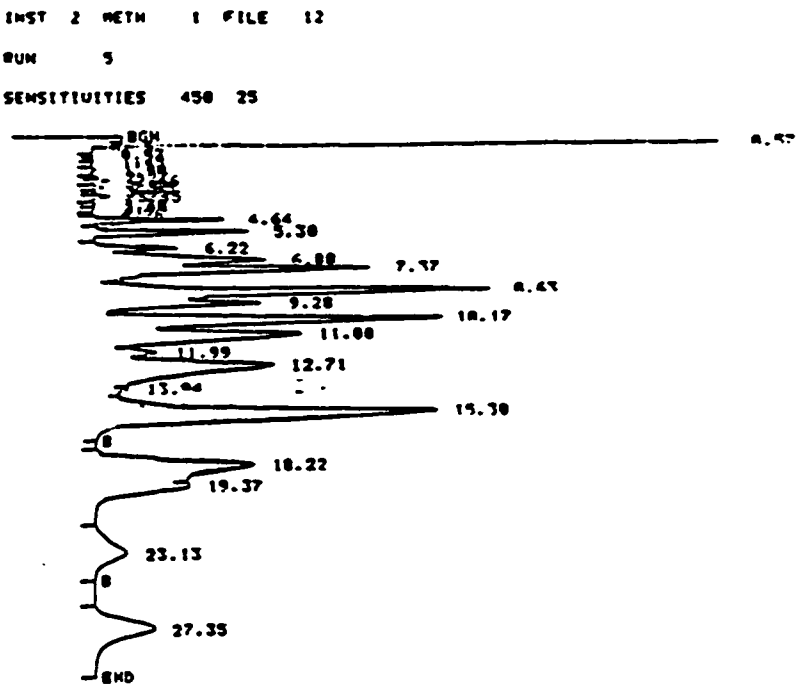
INST 2 METH 1 FILE 14

RUN A 72 : 31.1 A / A / A

SENSITIVITIES 450 25

TIME	AREA	BC	PRT	PF	P	AREA
0.58	6.7382	U	0.058	0.000	----------	*
0.75	0.0000	U	0.075	0.000	----------	*
1.25	0.0411	U	0.125	0.000	----------	*
1.48	0.0279	U	0.148	0.000	----------	*
1.63	0.0125	U	0.163	0.000	----------	*
1.95	0.1926		0.195	0.000	----------	*
2.32	0.1633	U	0.232	0.000	----------	*
2.51	0.6652	U	0.251	0.000	----------	*
2.91	0.4574	U	0.291	0.000	----------	*
3.11	0.1092	U	0.311	0.000	----------	*
3.42	0.0836	U	0.342	0.000	----------	*
3.86	0.1443	U	0.386	0.000	----------	*
4.07	0.0966	U	0.407	0.000	----------	*
4.74	9.3382	U	0.474	0.000	----------	*
5.42	13.6486	U	0.542	0.000	----------	*
6.35	4.5649	U	0.635	0.000	----------	*
7.92	11.9328	U	0.792	0.000	----------	*
7.53	16.8454	U	0.753	0.000	----------	*
8.82	38.8812	U	0.882	0.000	----------	*
9.47	9.9305	U	0.947	0.000	----------	*
10.39	42.9849	U	1.039	0.000	----------	*
11.24	28.9748	U	1.124	0.000	----------	*
12.23	2.1889	U	1.223	0.000	----------	*
12.98	34.0380	U	1.298	0.000	----------	*
14.22	0.2939	U	1.422	0.000	----------	*
15.62	79.9667		1.562	0.000	----------	*
18.61	27.0272	U	1.861	0.000	----------	*
19.77	0.0000		1.977	0.000	----------	*
23.64	14.1798		2.364	0.000	----------	*
27.88	25.4310		2.788	0.000	----------	*

Figure 5.2 a . Chromatogram of Aroclor 1260 standard (10 ng/1.0 ul) (Standard 1). Mean peak area from retention time 4 min to 28 min was used to determine the extraction efficiency of fortified Aroclor from acid mixture.



INST 2 METH 1 FILE 12

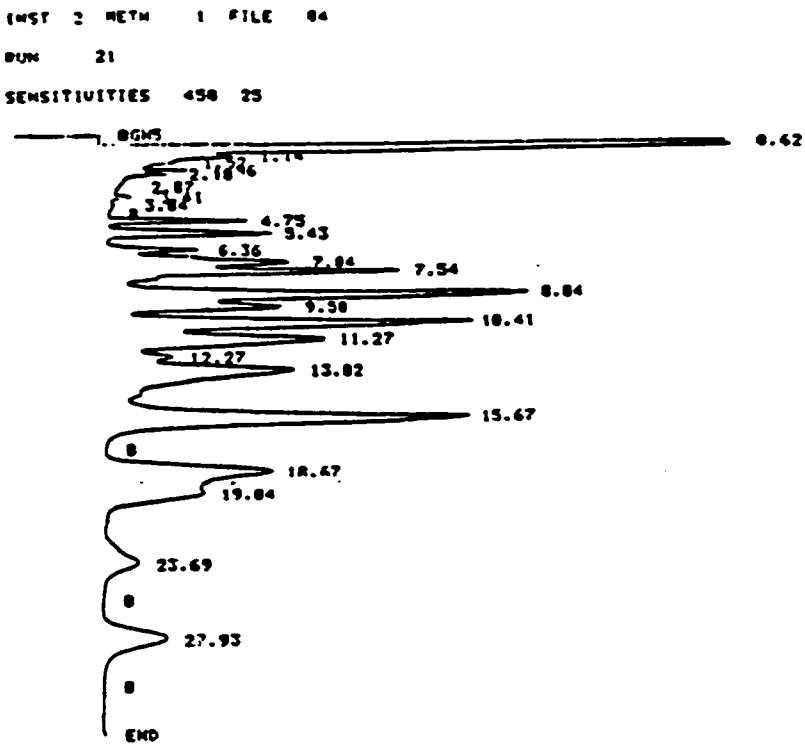
RUN 5 4 : 39.3 0 / 0 / 0

SENSITIVITIES 450 25

TIME	AREA	BC	PRT	PF	C	NAME
0.57	7.1760	U	0.057	0.000	----------	
0.97	0.8166	U	0.097	0.000	----------	
1.24	0.0674	U	0.124	0.000	----------	
1.43	0.0915	U	0.143	0.000	----------	
1.90	0.2399	U	0.190	0.000	----------	
2.27	0.1666	U	0.227	0.000	----------	
2.46	0.6093	U	0.246	0.000	----------	
2.85	0.4570	U	0.285	0.000	----------	
3.05	0.1091	U	0.305	0.000	----------	
3.35	0.9844	U	0.335	0.000	----------	
3.79	0.1405	U	0.379	0.000	----------	
3.99	0.1165	U	0.399	0.000	----------	
4.26	0.0129	U	0.426	0.000	----------	
4.64	9.5126	U	0.464	0.000	----------	
5.30	13.7932	U	0.530	0.000	----------	
6.22	4.5689	U	0.622	0.000	----------	
6.88	11.7894	U	0.688	0.000	----------	
7.37	16.9273	U	0.737	0.000	----------	
8.63	39.2793	U	0.863	0.000	----------	
9.28	9.9939	U	0.928	0.000	----------	
10.17	43.2742	U	1.017	0.000	----------	
11.00	28.6976	U	1.100	0.000	----------	
11.99	2.8952	U	1.199	0.000	----------	
12.71	14.7758	U	1.271	0.000	----------	
13.94	0.2748	U	1.394	0.000	----------	
15.30	98.5555		1.530	0.000	----------	
18.22	24.1158	U	1.822	0.000	----------	
19.37	0.0000	U	1.937	0.000	----------	
23.13	14.1468		2.313	0.000	----------	
27.35	24.2937					

PERKIN ELMER

Figure 5.2 b . Chromatogram of Aroclor 1260 standard (10 ng/1.0 ul) (Standard 2). Mean peak area from retention time 4 min to 28 min was used to determine the extraction efficiency of fortified aroclor from acid mixture.



INST 2 METH 1 FILE 04

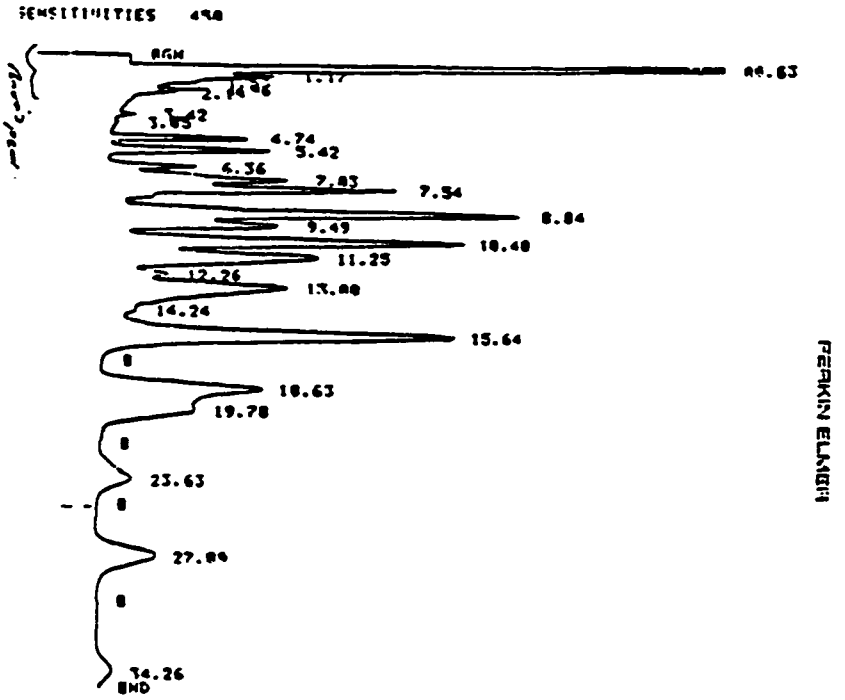
RUN 21 3 : 45.8 2 / 9 / 0

SENSITIVITIES 450 25

TIME	AREA	RC	RRT	RF	C	NAME
0.62	209.0393	U	0.862	1.000	34.5666	
1.14	1.6296	U	0.114	1.000	0.2695	
1.52	0.0000	U	0.152	1.000	0.0000	
1.96	1.7424	U	0.196	1.000	0.2815	
2.18	0.0000	U	0.218	1.000	0.0000	
2.87	0.0387	U	0.287	1.000	0.0064	
3.41	1.0007	U	0.341	1.000	0.1655	
3.84	0.3155		0.384	1.000	0.0522	
4.75	9.0364	U	0.475	1.000	1.6266	
5.43	14.4102	U	0.543	1.000	2.3829	
6.36	4.9992	U	0.636	1.000	0.8267	
7.84	12.6227	U	0.784	1.000	2.0873	
7.54	18.1830	U	0.754	1.000	3.0067	
8.84	43.2128	U	0.884	1.000	7.1456	
9.50	10.5766	U	0.950	1.000	1.7489	
10.41	46.8198	U	1.041	1.000	7.7421	
11.27	30.9952	U	1.127	1.000	5.1253	
12.27	2.2441	U	1.227	1.000	0.3711	
13.82	38.9516	U	1.382	1.000	6.4418	
15.67	86.3590		1.567	1.000	14.2803	
18.47	28.9625	U	1.847	1.000	4.7892	
19.84	0.0000	U	1.984	1.000	0.0000	
23.69	15.2512		2.369	1.000	2.5219	
27.93	27.5929		2.793	1.000	4.5628	

PERKIN ELMER

Figure 5.3 a. Extraction efficiency of Aroclor 1260 from acid mixture H_2SO_4/HNO_3 (20 ml) (Sample 1): Chromatogram of hexane extract. Expected concentration is 10 ng/1.0 ul. ECD for acid extracts was unstable up to retention time of 2 minutes.



INST 2 METH 1 FILE 85

RUN 20 3 : 4.5 2 / 9 / 0

SENSITIVITIES 450 25

TIME	AREA	BC	RRT	RF	C	NAME
0.63	204.1344	U	0.063	1.000	34.1533	
1.17	3.5408	U	0.117	1.000	0.5924	
1.53	0.0000	U	0.153	1.000	0.0000	
1.96	1.8220	U	0.196	1.000	0.3050	
2.19	0.0000	U	0.219	1.000	0.0000	
3.42	24.5715	U	0.342	1.000	4.1125	
3.85	0.3147	U	0.385	1.000	0.0527	
4.74	9.8614	U	0.474	1.000	1.6499	
5.42	14.4243	U	0.542	1.000	2.4133	
6.36	4.9504	U	0.636	1.000	0.8282	
7.83	12.4960	U	0.783	1.000	2.0907	
7.54	18.2560	U	0.754	1.000	3.0544	
8.84	42.6086	U	0.884	1.000	7.1288	
9.49	10.5920	U	0.949	1.000	1.7721	
10.40	46.2156	U	1.040	1.000	7.7322	
11.25	30.4819	U	1.125	1.000	5.0999	
12.26	2.1826	U	1.226	1.000	0.3652	
13.08	35.0892	U	1.308	1.000	5.9912	
14.24	0.3214	U	1.424	1.000	0.0538	
15.64	84.0691		1.564	1.000	14.1993	
18.63	27.1946	U	1.863	1.000	4.5482	
19.78	0.0000		1.978	1.000	0.0000	
23.63	14.3212		2.363	1.000	2.3961	
27.89	26.0462		2.789	1.000	4.4347	
34.26	5.4761		3.426	1.000	0.9764	

Figure 5.3b: Extraction efficiency of Aroclor 1260 from acid mixture H_2SO_4/HNO_3 (2.0×10^{-5} m^3) (Sample 2): Chromatogram of hexane extract. Expected concentration is 10 ng/1.0 ul. Chromatogram of acid extract was unstable up to retention time 120s.

5.3.2. Effect of Nujol Volume and Pulse Heating Time on Recovery Rates:

PCB isomer hexachlorobiphenyl was selected to study the effect of nujol volume and pulse time variation on the recovery rates (Table 5.1). Presence of nujol is critical for the quantitative recovery. ECD-GC chromatogram of hexane extract showed no response for HCB when nujol was absent or for lower nujol concentration(100ul). Figure 5.4 illustrates that recovery rates gradually increased to 97.7% when the nujol volume was increased from 0.1 ml to 1.0 ml. Nujol volume of 1.0 ml was found to be optimum for the quantitative recovery of HCB. The optimum total pulse heating time for quantitative recovery of spiked HCB is 60s. Longer pulse heating time (120s) decreased HCB recovery to 34.3%. PCB's recovery rate from spiked bovine liver and serum samples was less than 23% with coefficient of variation greater than 16% when nujol was absent. It is difficult to explain the loss of 2/3 of the hexachlorobiphenyl with simple doubling of heating time without extensive rate constant studies.

5.3.3. PCB Recovery from Bovine Liver:

The precision of the method used is demonstrated by a maximum coefficient of variation of less than 4.6% for liver samples and accuracy of the method is demonstrated by mean recovery of greater than 83% (Table 5.2). Figures 5.5 and 5.6 illustrate that total pulse heating time of 40s was optimum for quantitative recovery of fortified Aroclor 1260 from 0.1g and 0.25g bovine liver samples respectively. Mean recovery from 0.1g liver sample was greater than 95% with coefficient of variation less than 2.8 % for a total pulse heating time of 40s. Mean recovery rate was reduced to 92% with coefficient of variation less than 5% for a 60s total pulse heating time. Similar pattern was found for 0.25 g samples but the overall recovery rates were slightly lower.

Similar pattern was found for 0.25 g samples but the overall recovery rates were slightly lower.

Pulse microwave wet ashing procedure is not suitable for Aroclor 1016 analysis. Figure 5.7 shows the hexane extract from Aroclor spiked NIST bovine liver subjected to wet ashing procedure.

TABLE 5.1

Effect of nujol: Recoveries of spiked hexachlorobiphenyl (PCB monomer) from NIST bovine liver.

NIST Bovine liver: 0.25g

Acid mixture vol: 25.0 ml H_2SO_4/HNO_3 (1:1)

HCB spiked conc : 50 ug

Heating method : A

Nujol volume added	**Time**	**% Recovery**
-	60s	Absent[a]
0.1 X 0.1 ml	60s	Absent
0.5 X 0.5 ml	36s[e]	51.5 ± 10[b]
0.5 X 0.5 ml	60s	57.9 ± 6.4[c]
1.0 X 1.0 ml	60s	97.7 ± 10.4[d]
1.0 X 1.0 ml	120s[f]	34.3[b]

[a] Analyzed value is for 1 run.
[b] Analyzed values are the mean of 3 replicates.
[c] Analyzed values are the mean of 4 replicates.
[d] Analyzed values are the mean of 2 replicates.
[e] 6 × (6s pulse heating / 180s dormant time).
[f] 12 × 10s pulse heating / 180s dormant time.

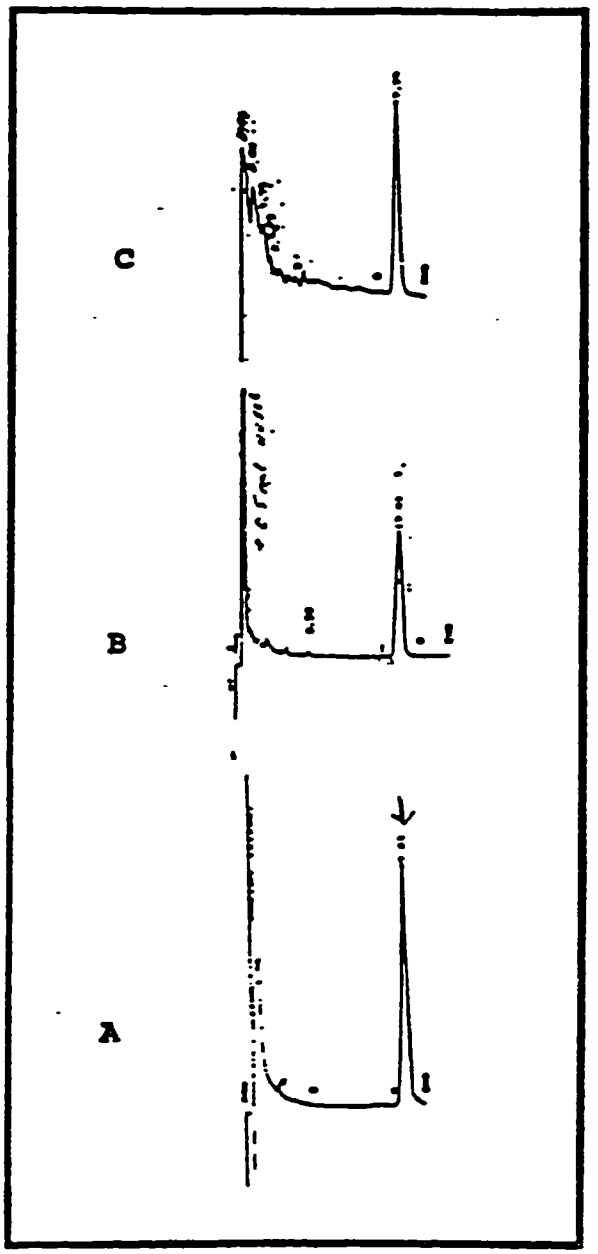


Figure 5.4:
Chromatogram of (A) hexachlorobiphenyl standard (HCB) (2ng), (B) recovery of spiked HCB from bovine liver after 60s pulse wet ashing in the presence of 500 ul nujol and (C) recovery of spiked HCB from bovine liver after 60s pulse wet ashing in the presence of 1000 ul nujol. Expected concentration of HCB is 2 ng.

TABLE 5.2

Recoveries of spiked Aroclor 1260 (PCB mixture) from NIST bovine liver.[a,b,c]

Acid mixture vol : 20.0 ml H_2SO_4/HNO_3 (1:1)

Aroclor 1260 spiked conc: 1000 ug

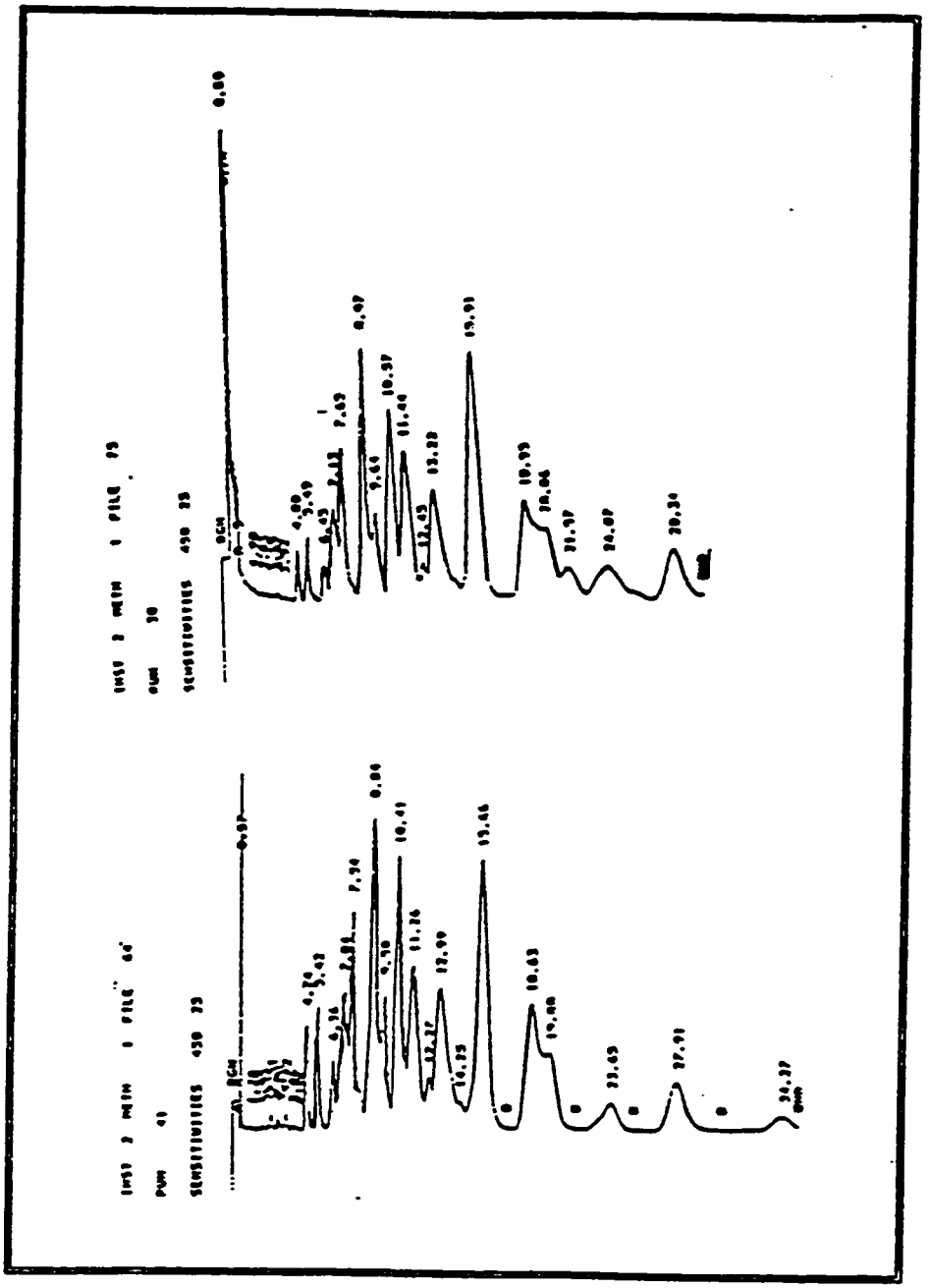
Heating method : A

Sample weight	Nujol volume added	Pulse time (s)	% Recovery
0.25 g	-	40	20.3 ± 5[a]
0.25 g	0.1 ml	40	91.5 ± 0.5[a]
0.25 g	0.2 ml	40	90[b]
0.25 g	0.1 ml	60	83.3 ± 1.5[a]
0.1 g	0.1 ml	40	95.2 ± 2.7[c]
0.1 g	0.1 ml	60	92.1 ± 4.2[a]

[a] Analyzed values are the mean of 3 replicates.

[b] Analyzed value is from single run.

[c] Analyzed values are the mean of 2 replicates.

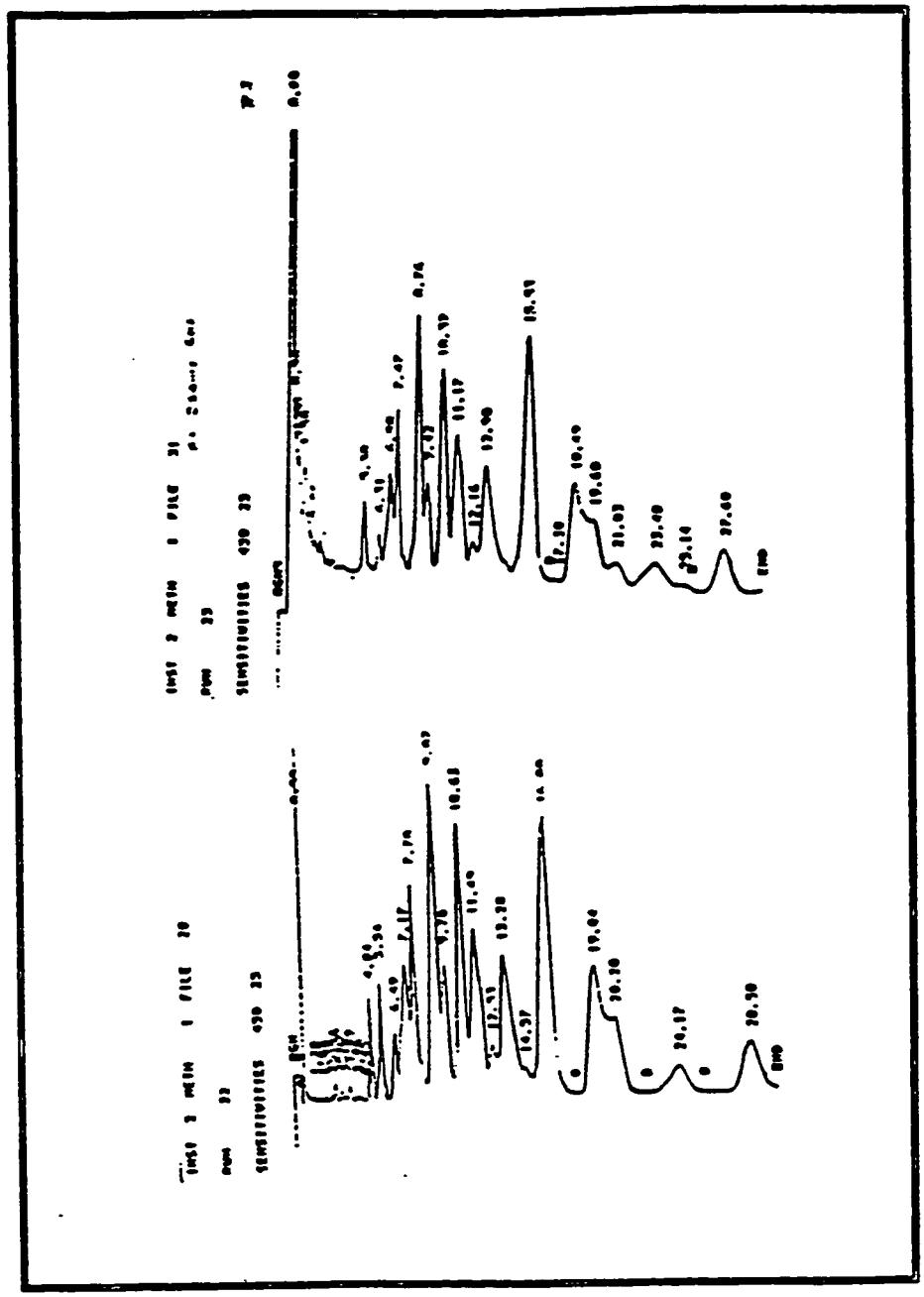


B

A

Figure 5.5: Chromatogram of (A) Aroclor 1260 standard (10ng) and (B) hexane extract from NIST bovine liver (0.1 g) subjected to pulse microwave wet ashing procedure. Expected concentration is 10 ng.

B



A

Figure 5.6: Chromatogram of (A) Aroclor 1260 standard (10ng) and (B) hexane extract from NIST bovine liver (0.25 g) subjected to pulse microwave wet ashing procedure. Expected concentration is 10 ng for 1.0 ul of extract. PCB isomer at retention time 290s decomposed completely.

C

B

A

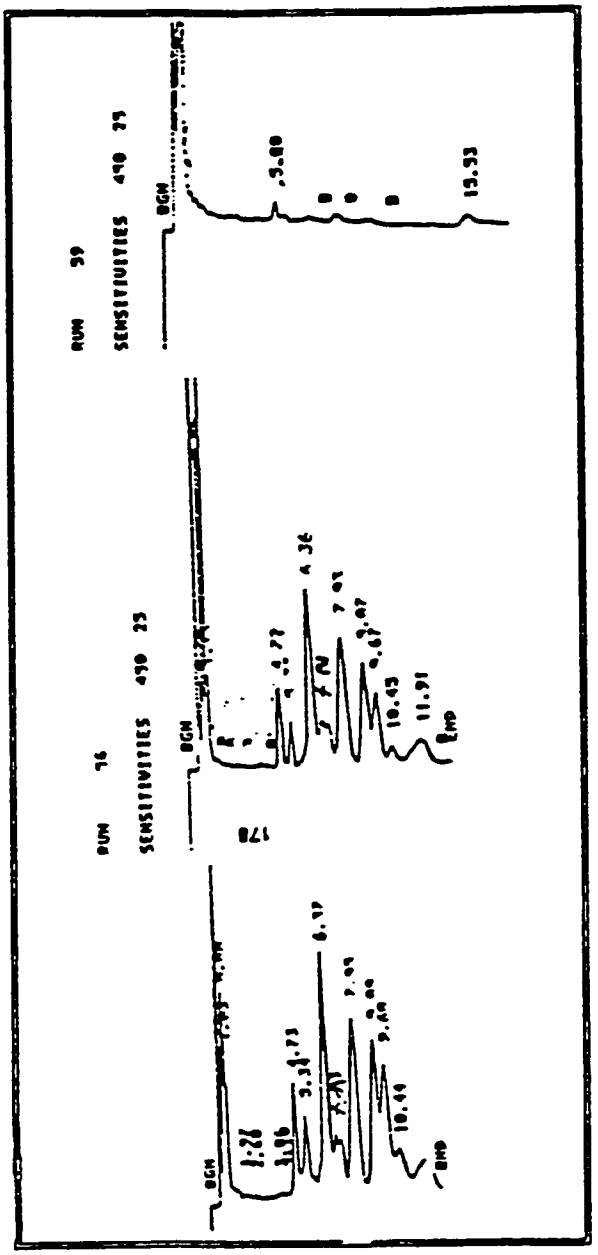


Figure 5.7: Chromatogram of (A) Aroclor 1016 standard (10ng) and (B) hexane extract from acid mixture H_2SO_4/HNO_3(20.0 ml) spiked with Aroclor 1016 and (C) hexane extract from spiked NIST bovine liver subjected to pulse microwave wet ashing procedure. Expected concentration 10 ug/1.0 ul.

5.3.4 PCB Recovery from Serum:

Precision of the method is demonstrated by the maximum coefficient of variation of less than 2.7% for serum sample and accuracy of the method is demonstrated by mean recovery of greater than 92% (Table 5.3 and Figure 5.8).

TABLE 5.3

Recovery of spiked Aroclor 1260 from serum

Acid mixture: 20.0 ml H_2SO_4/HNO_3 (1:1)

Serum weight: 2.0 g

Pulse time: 4 x (10s pulse /180s dormant)

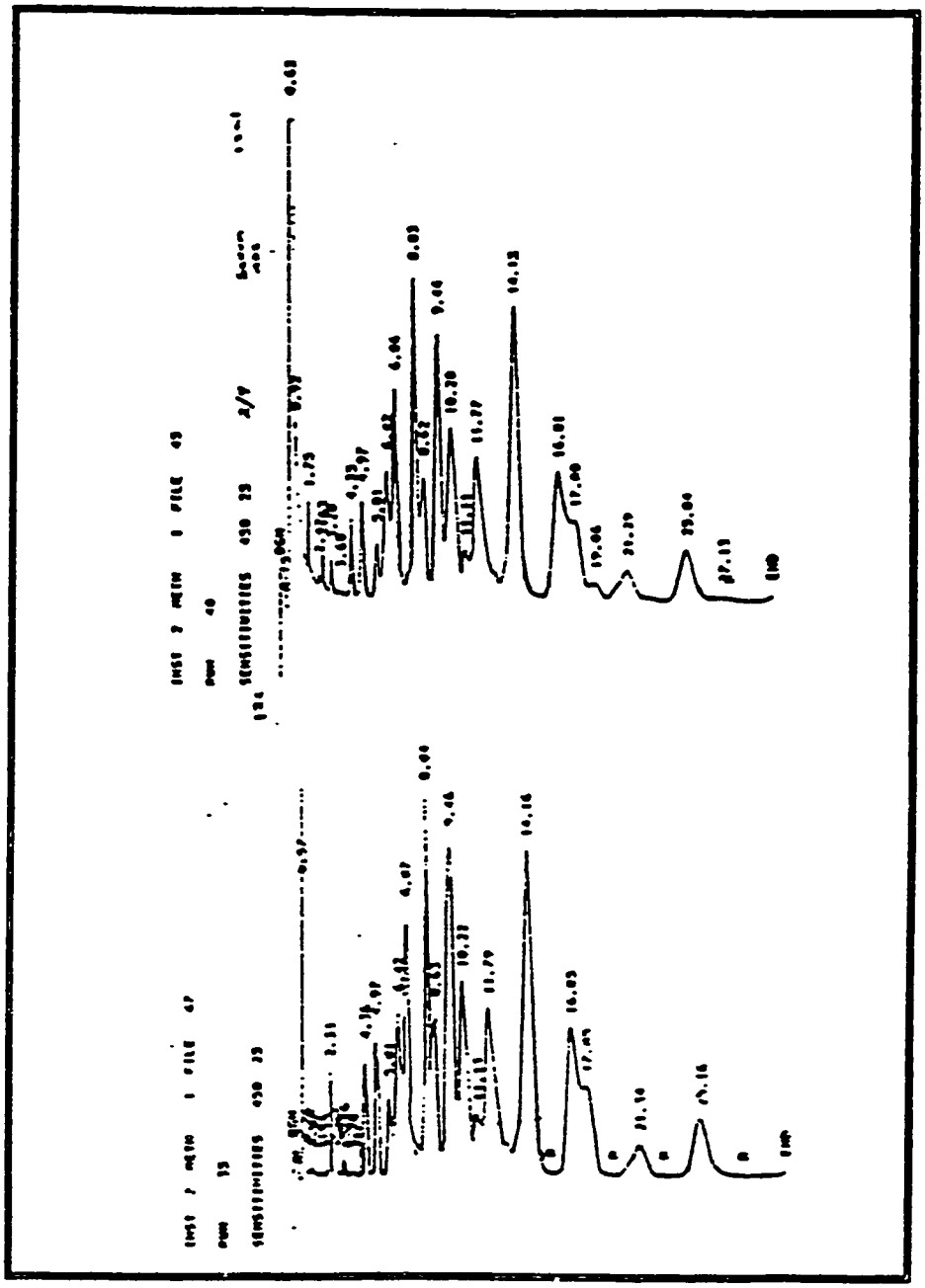
Method: A

Nujol volume added	Amt spiked	% Recovered	% Coefficient of variation
-	1000 ug	22.5	15.6[a]
1.0 ml	"	92.3	2.7[b]

[a] Analyzed values are the mean of 2 replicates.

[b] Analyzed values are the mean of 3 replicates.

B



A

Figure 5.8. Chromatogram of (A) Aroclor 1260 standard (10ng) and (B) hexane extract from serum subjected to pulse microwave wet ashing procedure. Expected concentration is 10 ng for 1.0 ul of extract.

5.3.5 PCB Recovery from Soil:

Extraction efficiency was not satisfactory for soil samples fortified with 50 ug of Aroclor 1260 (Table 5.4). Maximum mean recovery rate was only 63% for total pulse treatment time of 24s (4 × 6s pulse/180s dormant) and decreased to 28% for 50s pulse time. Recovery was 95% when the soil sample was fortified with 500 ug of Aroclor 1260 (Figure 5.9). Humic and fulvic acids possess functional groups such as phenolic hydroxyl, alcoholic hydroxyl , and hydrophobic sites. The absorption of some hydrophobic pollutants such as DDT may depend upon three chlorine atoms attached to one carbon atom of DDT and physical absorption occurs on the surface of humic and fulvic acids by van der Waals forces. The chlorine atom on the ethyl group of DDT molecule may have a residual negative charge strong enough to be attracted to positively charged sites. The adsorption of PCBs on humic substances increases as the number of chlorine atoms in the congeners increases. The Freundlich constant for different soils, which is an indirect measure of extent of adsorption constant k, consistently increases from dichloro congener to hexachloro congener. For an individual PCB congener, the adsorption rate depends on total organic content of soil, hydrophobic nature, and surface area of humic acids.

TABLE 5.4

Recoveries of spiked Aroclor 1260 (PCB mixture) from soil.[a,b,c]

Acid mixture vol: 20.0 ml H_2SO_4/HNO_3 (1:1)

Weight: 2.0 g

Amount of Aroclor spiked (ug)	Nujol volume added	Method	Pulse time (sec)	% Recovery	% Coefficient of variation
50	-	B	24	〈10[a]	-
50	1.0 ml	B	24	63	6.3[b]
	1.0 ml	B	30	45	2.2[b]
	1.0 ml	A	50	28	21.4[c]
500	1.0 ml	A	60	95[a]	-

[a] Analyzed 1 sample

[b] Analyzed values are the mean of 2 replicates

[c] Analyzed values are the mean of 3 replicates

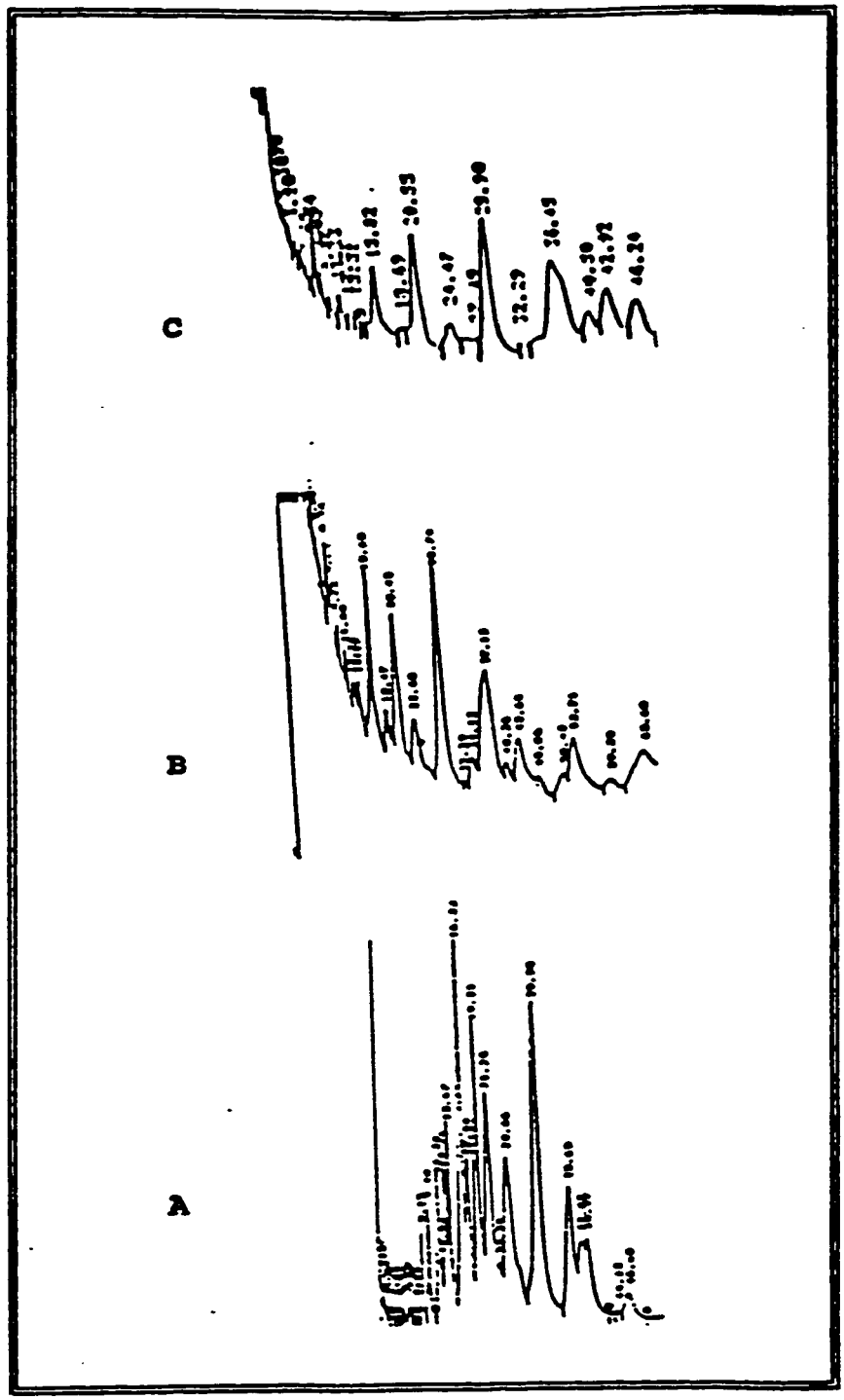
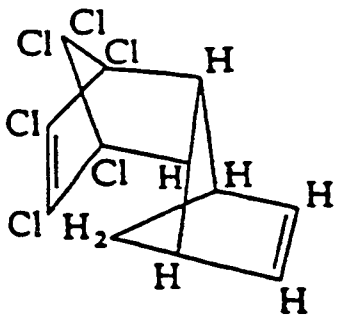
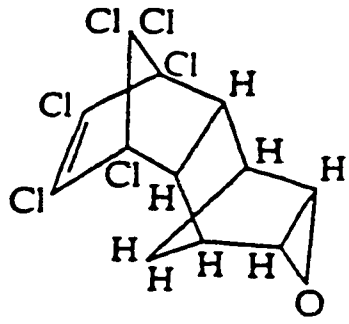


Figure 5.9 Chromatogram of (A) Aroclor 1260 standard (2.5 ng) and (B) hexane extract from PEI soil subjected to pulse microwave wet ashing procedure 24s (method B) and 50s (method A)

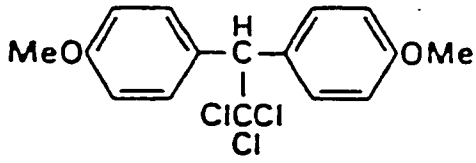
PESTICIDE STRUCTURES



ALDRIN



DIELDRIN

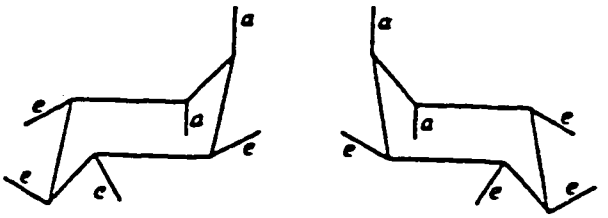


methoxychlor

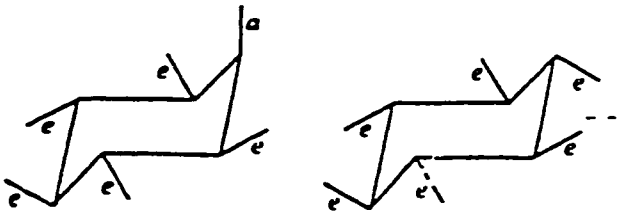
5.3.6 Pesticide Recovery from Bovine Liver:

Only BHC isomers survived the pulse wet ashing procedure. One of the most widely used pesticides, gama isomer of BHC, is lindane. The precision of method for BHC isomers is demonstrated by a maximum coefficient of variation of less than 15% and accuracy of the method is demonstrated by mean recovery of greater than 98% (Table 5.6). Pesticides aldrin, dieldrin, DDT, DDE, and methoxychlor completely decomposed in less than 20s of pulse treatment time (Figure 5.10).

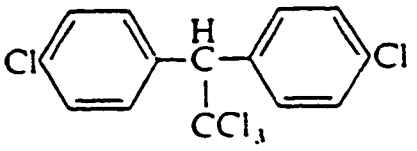
PESTICIDE STRUCTURES



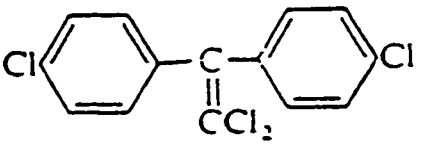
α MIRROR PAIR



δ-ISOMER β-ISOMER



DDT



DDE

5.3.7 Molecular Structure and Recovery Rates: Recovery rates of fortified PCBs and pesticides correlate favourably with the molecular structure of the pollutant. Hexachloro cyclohexane (pesticide lindane) and BHC isomers are saturated cyclic chlorinated compounds which are highly resistant to oxidation. Recovery rate for BHC isomers was quantitative (Table 5.5). Presence of unsaturated bond in aldrin, dieldrin, and DDE, and electron donating methoxy group in methoxychlor makes them easy targets for oxidation.

The pattern of PCB isomer retention time is proportional to M.W. i.e. high molecular weight PCBs elute at longer retention times. Low molecular weight PCBs have more sites for attack by the oxidising agent. Recovery of low molecular weight Aroclor 1016 was less than 5%. As expected, the recovery of early eluting peaks were lower compared to longer retention time peaks for Aroclor 1260. It is important to note that less stable persistent pollutants such as Aldrin, Dieldrin, DDT, DDE, and methoxychlor elute along with PCBs during extraction and separation by adsorption columns. Until 1966, PCB peaks were mistaken for other pesticides and the concentrations of DDT and DDE were over estimated due to mistaken identity (Chapter 1). Further separation of PCBs from interfering pesticides is required; these issues were discussed in chapter 1. In pulse procedure, less stable pesticides that interfere with PCBs are completely destroyed in 20s of total pulse time.

5.3.8 Nujol's function:

Mechanism of pollutant extraction can be speculated by careful observation of recovery rates obtained for different matrices, effect of nujol, and effect of pulse heating time. Mean recovery of fortified pollutants for all the matrices studied gradually increased to optimum level when the total pulse heating time was 40s and recovery rates decreased when the total pulse time was longer than 60s. Refractory organic pollutants are bound to hydrophobic sites of lipids and other biomolecules. In the case of soil samples, the pollutants are bound to hydrophobic sites in humin, fulvic, and humic acids. During wet ashing procedure, the bound pollutants are released into the solution when the hydrophobic sites are disturbed. Nujol, in the acid solution, solubilises the hydrophobic pollutants and protects them to some extent during the initial stages of oxidation by acid mixture.

TABLE 5.5

Recovery of pesticide BHC from NIST bovine liver[a,b,c,d]

Sample weight :0.25 g

Nujol volume added : 1.0 ml

Pulse time : 6 × 10s pulse/180s dormant time

Pesticide	Retention time	Standard peak area	Sample peak area	% Recovery
BHC	2.08	14.9 ± 0.9	15.3 ± 1.2	102 ± 8.1
	2.44[d]	29.1 ± 0.3	28.7 ± 2.5	98.7 ± 8.6
	2.76	4.7 ± 0.4	5.17 ± 0.8	109.9 ± 15.9

[a] Amount spiked 125 μg.

[b] Analyzed values are the mean of two replicates.

[c] Pesticides aldrin, dieldrin, DDT, DDE, and methoxychlor did not give any respose.

[d] This isomer is pesticide lindane.

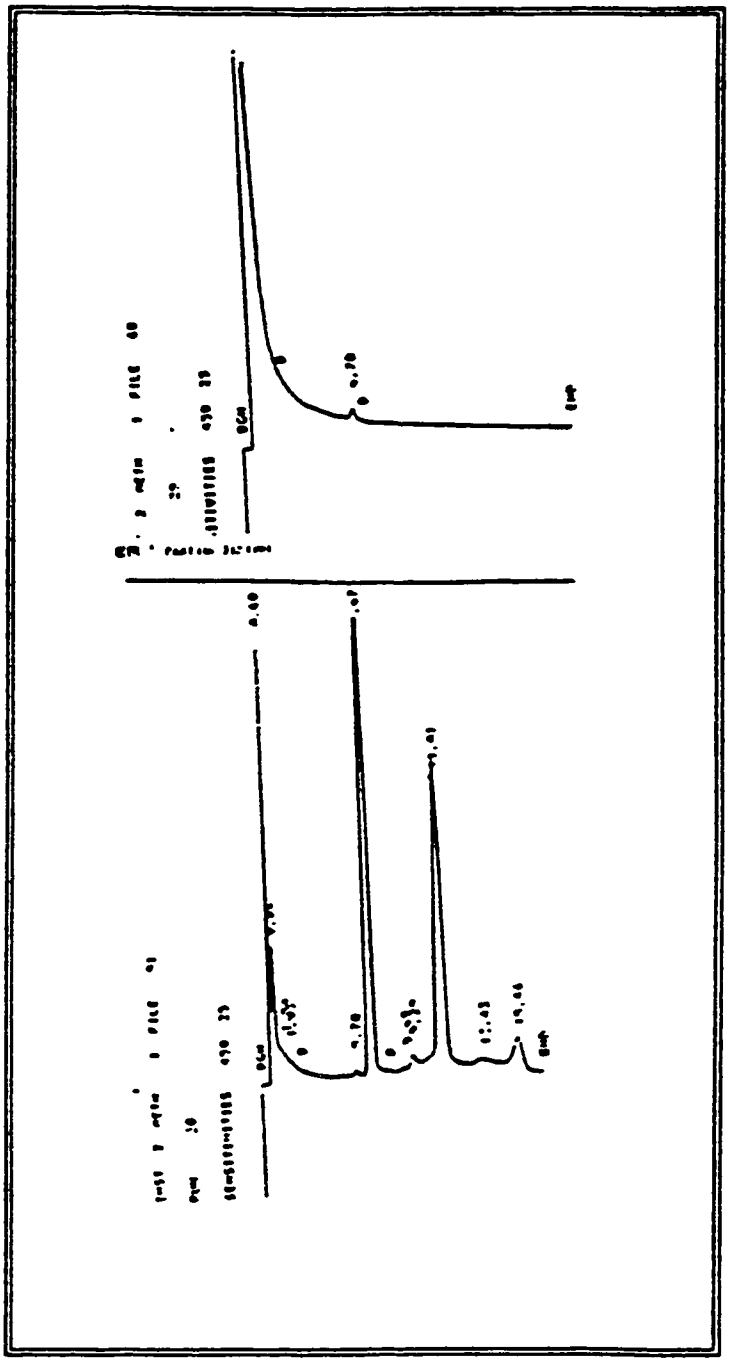


Figure 5.10 Chromatogram of (A) DDT, DDE and methoxychlor and (B) hexane extract from spiked NIST bovine liver subjected to pulse microwave wet ashing procedure.

5.4 TREATMENT OF PCBs AND PESTICIDES :

In pulse treatment of matrices contaminated with PCBs, recovery rates decreased for longer pulse heating times. Degradation of PCBs and pesticides accelerated when nujol was absent. Hexachloro biphenyl (HCB) decomposed in less than 60s of pulse treatment time in the absence of nujol (Figure 5.11). Recoveries of PCBs decreased with treatment time and about 99.99% PCBs were destroyed in 10 min of treatment time (total time = 3 hrs and 10 min) (Figure 5.12). ECD gas chromatograph showed some peaks at longer retention times; attempts to identify the peaks by GC\MS were unsuccessful. However, HCl was detected as one of the byproducts.[2] Table 5.6 shows the degradation of hexachlorobiphenyl, PCB 1016, and effect of time variation on PCB 1260 degradation. Aroclor 1016 was completely destroyed in 60s of pulse treatment time in the presence and absence of nujol (Figure 5.13). However extraction efficiency studies (Figure 5.6 B) showed that Aroclor 1016 was stable in H_2SO_4/HNO_3

[2]The author thanks Dr.Z.Wan for detecting HCl as one of the byproducts.

Table 5.6

Degradation of PCBs[a,b]

Sample	Amount	Pulse treatment time (s)[b]	Total treatment time	Concentration found after treatment	% Degradation
HCB (PCB monomer)	50 ug	60	19 min	0.5 ug	99
Aroclor 1260	1000 ug	240	1.5 hrs	150 ± 10 ug	85
	"	600	3 hrs 10 min	50 ± 5 ug	95
	"	800	4 hrs 20 min	10 ug	99
	1.0 g	"	4 hrs 20 min		
Aroclor 1016	500 ug	60	19 min	1.0 ±0.1	99

[a] Pesticides aldrin, dieldrin, DDT, DDE, and methoxychlor decomposed in 20 s of pulse treatment time.

[b] 10s pulse/180s dormant

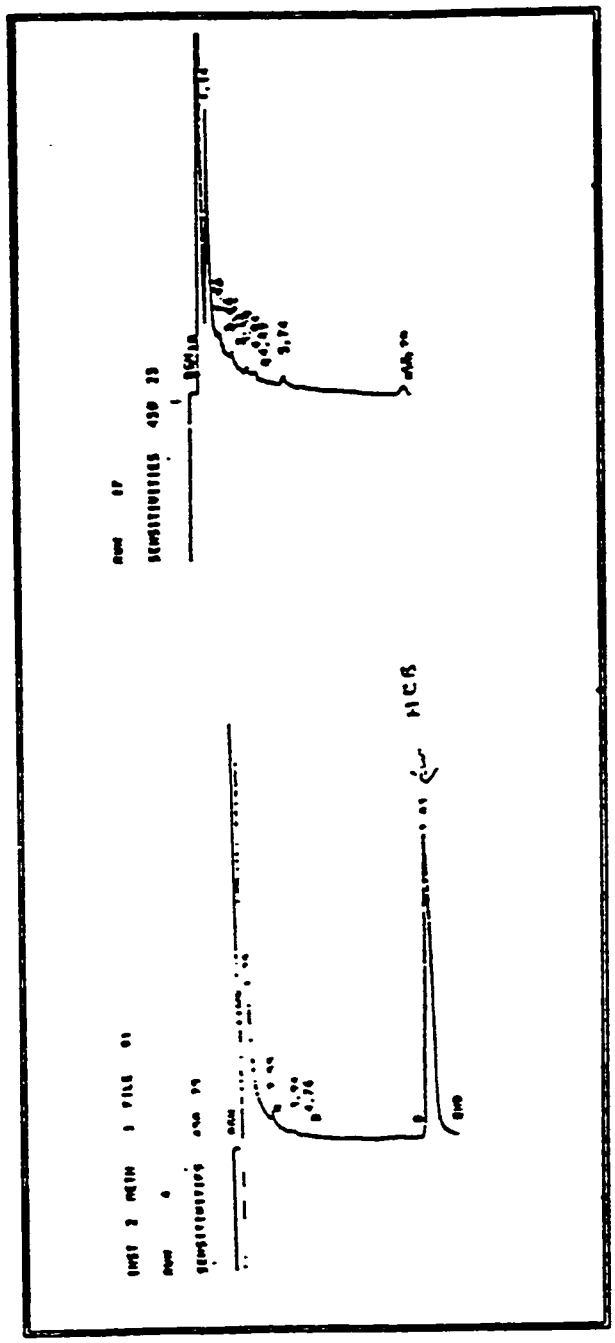


Figure 5.11 EC-GC chromatograms showing the hexachlorocyclohexane peak (A) and the response after 60s pulse microwave treatment (B)

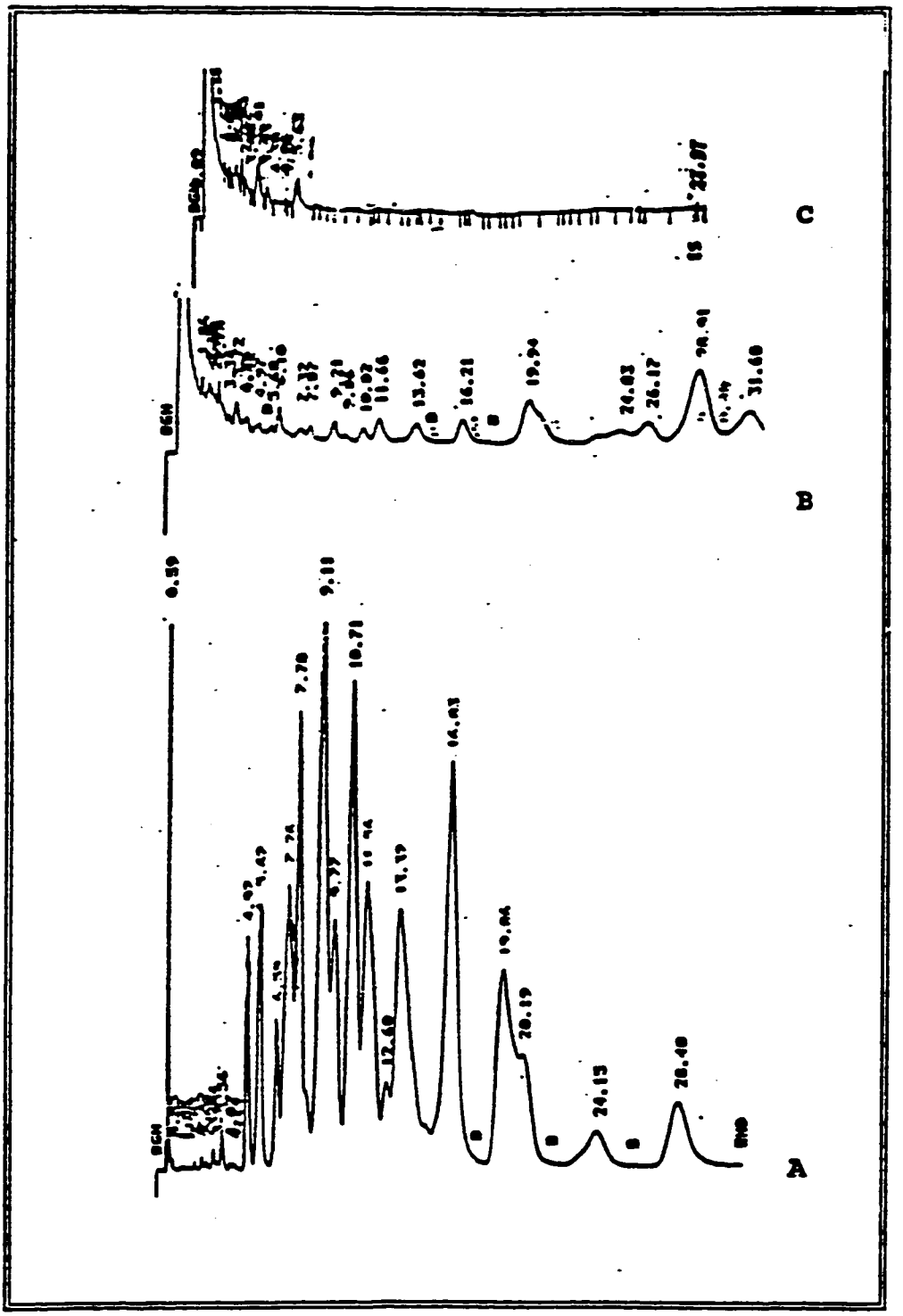


Figure 5.12 EC-GC chromatograms showing the decrease in concentration of Aroclor 1260 as a function of pulse microwave treatment time. Figure A = 10 ppm standard. B = 10,000 ppm after 240 s of pulse heating. C = 600s pf pulse heating.

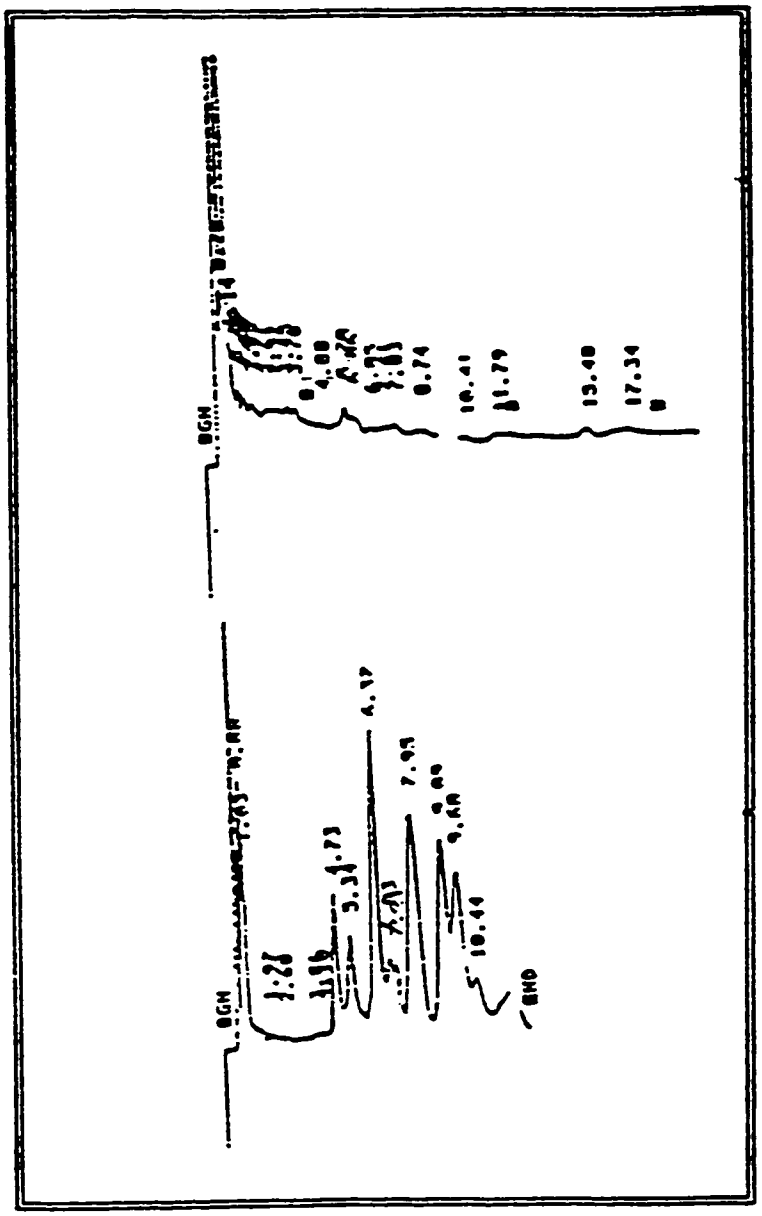


Figure 5.13 EC-GC chromatograms showing Aroclor 1016 standard and sample after 60s pulse microwave treatment time.

5.5 CONCLUSION

The pulse method to clean up biological samples for some refractory organic pollutants is deemed to bypass the traditional shortcomings of conventional procedure: several hours of sample preparation time, high cost of solvents and adsorbents, large variation in results, and interference of DDT and DDE with PCBs. This method can be used for quantitative analysis of highly stable PCBs and some pesticides at ppm level. In addition to quantitative recovery, longer pulse treatment time is effective in accomplishing a substantial and relatively rapid degradation of pollutants. It can be assumed that a number of toxic or non-toxic pollutants, besides these tested, can be decomposed by this method. This procedure requires no special preparation of reagents and a minimum of glassware.

5.5.1 Suggestions for Future Study:

Recovery rates for fortified samples at ppb level is useful to understand the extent of nujol protective function at nanogram levels. Unlike trace metal analytical methods, where several NIST reference materials are used for validation, all the data published to date have used exogenous fortification to study the analytical method. It is advisable to compare the results from EPA or Environment Canada approved method with the results from pulse wet ashing method for low level PCB in the presence of internal standard such as chrysene- d_{12} contaminated samples. The recovery of exogenous compound fortification should be same as the recovery of endogenous compound spike (environmental contamination).

Identification of products produced by treatment procedure (Section 5.4) is useful for better understanding of mechanism of degradation. Hexane extract of PCB

degradation product did not show any GC\MS response. It is possible that some of the products are polar and cannot be extracted into non polar hexane. Direct analysis of product by GC\MS is useful. Finally, quantitative analysis of HCl detected as one of the products of treatment procedure is vital.

CHAPTER 6

MECHANISM OF MICROWAVE REACTIONS UNDER REFLUX CONDITIONS [1]

[1] Accepted for presentation by 32nd Annual microwave symposium, Ottawa, Canada (July, 1997). I could not present due to tragic death of my brother,

6.1 INTRODUCTION

Microwave heating is used in a wide and growing range of chemical applications, from the speeding up of synthesis to the acceleration of sample preparation in analysis (Chapter 1). Recent reviews (63 - 69) and publications argue that the acceleration of reactions achieved with microwave heating is the result of an increase of boiling temperatures due to the rise of pressure and not by any superheating effects [2]. A small number of semi-quantitative observations was the basis for this conclusion (64,65). Gedye et al, (68) (Table 6.1) compared percent yield of n - propyl benzoate after heating equal amounts of reactants for four minutes in an oil bath at 160 ^{o}C and in the microwave oven in an open vessel. The yield of ester in these two reactions were identical and it was concluded that the faster rate of reaction in the microwave oven is due to the high pressure in the sealed teflon vessels, which allow the microwaves to superheat the reaction mixture.

Some suggested superheating effects based on indirect methods are the use of thermochromic liquid crystals or fibre optic methods. Mingos (69) used fibre optic technique to monitor the temperature in four spatially defined parts of a round bottomed flask during solvent heating. The round bottomed flask was connected to a reflux condenser, which passed through the floor of the microwave oven via a port.

---

[2] The author thanks Dr. O.S. Tee for recommending (ref # 63). The author presented the results given in Tables 6.5 and 6.6 to a weekly group meeting more than a year before the review was published. The results show that super heating occurs at reflux conditions and pulse microwave heating can be used to heat more effectively.

TABLE 6.1

Percent yield of n-propyl benzoate with conventional and microwave oven heating(68).

Conditions	Percent ester	Percent acid	Percent total recovery
Oil bath, 240s at 160 °C	29	64	94
Microwave, 240 s (1atm)	25	69	94

Whan (70) used thermochromic paint based on liquid technology that changed color at specific temperatures: red corresponded to temperatures between 99 °C and 101°C; green represented 103°C. to about 110 ° C. The flask was painted with thermochromic crystals and the boiling point of the distilled water was monitored in microwave oven and conventional heating. The reflux temperature of the water in microwave oven was about 105 °C.

This chapter reexamines the issue by studying detailed kinetic experiments using the Arrhenius parameters to compare kinetic results of conventional and microwave oven based reflux and pulse reactions.

Chemical reactions are generally very sensitive to temperature and, as a rough sort of guide, the rate will often double for a 10 °C rise in temperature. Arrhenius (70b) found the variation of a rate constant k with temperature could be expressed by the equation

$$\log_{10} k = \log_{10} A - E/2.303\ RT$$

or

$$k = Ae^{-E/RT}.$$

Thus, a plot of log k against 1/T (where T is the absolute temperature) is a straight line, where A and E are constant for a particular reaction. These can be evaluated from the intercept and slope, respectively. The pre-exponential factor A is known as the frequency factor and E as the activation energy.

Arrhenius parameters from fast reaction (Hydrolysis of trans$(Co(en)_2Cl_2)^+$) in water bath and slow reaction (Aromatic nitration of p-Nitrobenzoic acid) in oil bath were used to determine the effective reaction temperature in microwave oven based reflux reactions.

6.1.1 Hydrolysis of Trans$(Co(en)_2Cl_2)^+$:

The substituted reactions of Co (III) complexes in aqueous solutions have been extensively investigated (71-77). All the reactions were found to be first order in the complex concentration and zero order in the entering field. Analogous studies of these reactions with water in acid solutions show that the reaction proceeds in two steps. In the first step, the original anionic ligand is replaced by water molecule. This is the rate determining step. In the second step, water molecule is replaced by an entering anionic ligand. This entering anion is involved in the rate law only for the second step. As a result, the only reactions for which the rate constants have been obtained in acidic aqueous solutions are the acid hydrolysis reactions (Eq 6.1) and the anation reactions (Eq 6.2).

$$Co(NH_3)_5X^{2+} + H_2O \longrightarrow Co(NH_3)_5OH_2^{3+} + X^- \qquad (6.1)$$

$$Co(NH_3)_5OH_2^{3+} + X^- \longrightarrow Co(NH_3)_5X^{2+} + H_2O \qquad (6.2)$$

The reactions represented by Eq.(6.1) is called acid hydrolysis because at pH greater than 5, base hydrolysis reaction, illustrated by Eq. (6.3) takes place. Base hydrolysis is commonly second order: first order with respect to the complex and first order in hydroxide ion.

$$Co(NH_3)_5X^{2+} + OH^- \longrightarrow Co(NH_3)_5OH^{2+} + X^- \qquad (6.3)$$

The trans Co complex salts and their solutions are green, changing to pink upon hydrolysis. The half life of hydrolysis corresponds closely to a colourless stage, which

6.1.2 Nitration of p-Nitrobenzoic Acid

The electrophilic substitution reaction that has received by far the closest study is nitration of aromatic ring and it probably provides the most detailed mechanistic picture. Nitration reaction is most frequently carried out with a mixture of concentrated H_2SO_4/HNO_3, the so called nitrating mixture. Nitration is slow in the absence of sulphuric acid, which by itself has virtually no effect on benzene under the conditions normally employed. H_2SO_4 functions as a highly acid medium in which NO_2^+ (Nitronium ion) can be released from $HO\text{-}NO_2$ (78).

Nitronium ion produced by the HNO_3/H_2SO_4 nitrates p-nitrobenzoic acid to give 2,4 dinitrobenzoic acid. Carboxylic and nitro groups on the aromatic ring induce overall slower attack by the NO_2^+ ion due to the presence of deactivating nitro and carboxylic functional groups on the aromatic ring.

Arrhenius parameters from fast reaction (Hydrolysis of $trans(Co(en)_2Cl_2)^+$) in water bath and slow reaction (Aromatic nitration of p - nitrobenzoic acid) in oil bath were used to determine the effective reaction temperature in microwave oven based reflux reactions.

6.2 EXPERIMENTAL SECTION

6.2.1 Instruments:

Liquid chromatography: A Waters model 510 liquid chromatograph consisting of a dual pump and variable wavelength detector was used. The column was Waters C18, 10um, 50 cm H 3.9 mm reversed - phase .

6.2.2 Apparatus:

Modified Microwave oven: In the modification illustrated in Figure 6.1, the solution is contained within the round bottomed flask, which is connected via ground glass joints to an air condenser. The condenser passes through a hole drilled cavity wall of the conventional microwave oven and then connected to the water condenser located outside the microwave oven. This allows the solution to heat rapidly in the microwave oven and reflux safely at atmospheric pressure.

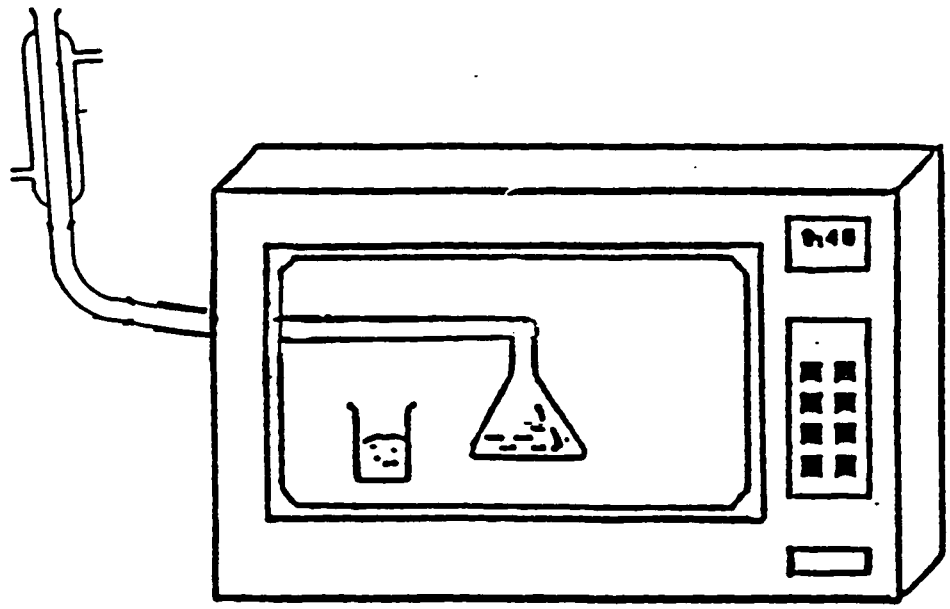


Figure 6.1: Schematic diagram of modified microwave oven

6.2.3 Reagents:

Water was double deionized nanograde. All acids were Fisher Scientific trace metal grade. p-Nitrobenzoic acid was purchased from Eastman organic chemicals. $CoCl_2$ and methanol were purchased from Mallinckrodt and ethylenediamine was purchased from Aldrich.

6.2.4 Procedure:

6.2.4.1 Preparation of Trans$(Co(en)_2Cl_2)Cl$:

The trans$(Co(en)_2Cl_2)Cl$ was prepared by the method given by Bailar (79). The precipitate was washed with alcohol, followed by ether, and dried at 110°C.

Table 6.2

Experimental conditions used for HPLC analysis

Compounds separated	Composition of mobile phase	Detector wave length	Flow Rate (ml/min)
Cl benzoic acid, p-nitrobenzoic acid and 2,4 dinitrobenzoic acids	Nanopure water (80:15:5) + 0.5 ml AcOH\L	254nm	1.0 ml

6.2.4.2 Hydrolysis of Trans$(Co(en)_2Cl_2)^+$:

A) Water bath:

49.0 ml of water/methanol (1:1) mixture in a three-necked flask equipped with reflux condenser was heated in a water bath. The flask was acclimatized to required temperature (49 °C, 55 °C, 60 °C, 80 °C), at which time the solvent mixture was spiked with freshly prepared 1.0 ml of 0.1 M aqueous trans $(Co(en)_2Cl_2)^+$(27.6 mg) stock solution. The mixture was monitored colorimetrically exploiting the complementarity of the colours of the reactant and the product to easily identify the half time. At half time, the solution changed from green to colourless.

B) Microwave oven (reflux):

49.0 ml of water/methanol (1:1) mixture in a three necked flask was heated in a modified microwave oven for 60s. The mixture was then spiked with freshly prepared 1.0 ml of 0.1 M aqueous trans $(Co(en)_2Cl_2)^+$ (27.6 mg) stock solution and continued to be heated. The mixture was monitored colorimetrically to determine half life.

6.2.4.3 Nitration of p - Nitrobenzoic Acid:

A) Oil bath:

1.0 ml of 0.05 M p-nitrobenzoic acid (8.4 mg) dissolved in acetone was injected into a three- necked flask. Acetone was evaporated under a slow stream of nitrogen and the flask was acclimatized to the required temperature (92 °C, 106 °C, or 118 °C). 20 mL of H_2SO_4/HNO_3 (1:1) acid mixture in a Erlenmeyer flask equipped with reflux condenser was acclimatized to the required temperature, at which time the acid mixture was added to the three necked flask. This reaction was followed for various durations of time for rate constant studies. For example, as demonstrated in Figure 6.3, at 92 °C, reactions were carried out for durations of 1 hr, 3 hrs, 4 hrs, and 5 hrs. For each different time duration, the experiment was repeated at least twice. Similar procedures were followed for 108 °C and 118 °C. The sample was cooled and the contents were

added to water and diluted to 200 ml. 50 ml of diluted solution's pH was adjusted to pH6 with NaOH and diluted to 100 ml in volumetric flask. 10 ul final solution was injected into HPLC equipped with C18 column. HPLC parameters are summarized in Table 6.2.

B) Microwave oven (reflux):

1.0 ml of 0.05M p-nitrobenzoic acid (8.4 mg) dissolved in acetone was injected into a three- necked flask. Acetone solvent was evaporated under a slow stream of nitrogen. 20.0 ml of H_2SO_4/HNO_3 (1:1) acid mixture was added to the flask. The mixture was refluxed in a modified microwave oven equipped with reflux condenser by continuous heating for various durations of time. For example, as demonstrated in Figure 6.14, the reflux reactions were carried out for 10 min, 15 min, 20 min, 30 min, 45 min and 1 hr. For each time duration, the experiment was repeated at least twice. The sample was cooled and the contents were added to water and diluted to 200 ml. 50 ml of diluted solution's pH was adjusted to pH 6 with NaOH and diluted to 100 ml in volumetric flask. 10 ul final solution was injected into HPLC equipped with C18 column.

6.3 RESULTS AND DISCUSSION

Kinetic studies of two different reactions at boiling points in two different media: the hydrolysis of trans $(Co(en)_2Cl_2)^+$ in a mixture of CH_3OH/H_2O (1:1) (Tables 6.3 and 6.4) and an aromatic nitration in mixed HNO_3/H_2SO_4 (Tables 6.5 and 6.6) are reported here. Both reactions were carried out in vessels open to constant room pressure.

Table 6.3 shows rate constant results of hydrolysis of trans$(Co(en)_2Cl_2)^+$ at 49 °C, 55 °C, 60 °C and 80 °C (average of two runs each). The temperature of water bath was maintained above 90 °C for 80° C experiment. The rate constant results calculated from $t_{1/2}$ values in column 2 are given in column 4 ($k = \ln 2/t_{1/2}$). The ln rate constant values versus 1/T are plotted to get Arrhenius plot as shown in Figure 6.2. Table 6.4 shows rate constant results in microwave oven under reflux conditions.

The nitration product was 2, 4 dinitrobenzoic acid. The nitration reaction was monitored by HPLC using chromosorb-LC column or C_{18}. The concentration values of p-nitrobenzoic acid at 92 °C, 108 °C and 118 °C for different runs in conventional oil bath are plotted as ln[PNBA] versus time for each run in Figures 6.3 - 6.12[3].

The average rate constant values obtained from the slopes of Figures 6.3 - 6.12 for each 92 °C ,108 °C and 118° C experiment are given in Table 6.5. The ln rate constant values vs 1/T are plotted to get Arrhenius plot as shown in Figure 6.13. The concentration values of PNBA for microwave reflux reactions are plotted as ln[PNBA] versus time for each run in Figures 6.14 - 6.15.

---

[3]The concentration values are given as inset in Figures 6.3 - 6.12

Table 6.3

Rate constant studies : Hydrolysis of trans$(Coen_2Cl_2)^+$ in water bath[a,b].

Temp (K)	$t_{1/2}$ (s)a	Coeff.V(%)	Rate constant (s^{-1})	1/T	ln k
322.16 ± 0.5	752.5 ± 10.6	1.4	0.09×10^{-2}	31.1×10^{-4}	-7.01
328.16 ± 0.5	495.0 ± 21.2	4.3	0.14×10^{-2}	30.0×10^{-4}	-6.57
333.16 ±0.5	203.5 ± 4.9	2.4	0.34×10^{-2}	30.0×10^{-4}	-5.68
353.16 ± 0.5	31.5 ± 0.7	2.2	2.20×10^{-2}	28.3×10^{-4}	-3.82

Average of 2 replicate runs

B.P of 50% methanol is 80 ^{0}C

Temp (k) $1/T \times 10^4$	ln K
31.06	-7.01
30.49	-6.57
30.03	-5.68
28.33	-3.82

Linear Regression Analysis

Intercept:	30.04773
Slope:	-1.1948
Std Err of Slope:	0.086
Correlation Coefficient:	0.9948

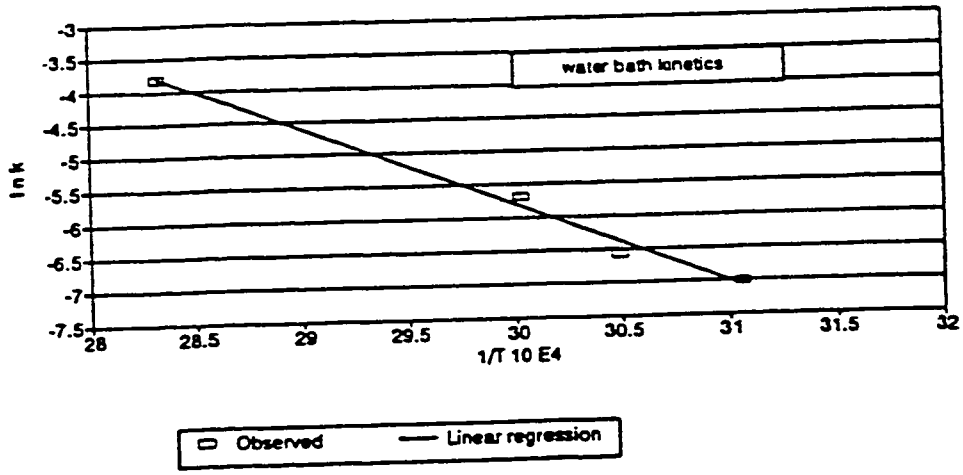


Figure 6.2
Arrhenius plot: Hydrolysis of trans$(Co(en)_2Cl_2)^+$ in water bath rate constant studies.

TABLE 6.4

Hydrolysis of trans$(Coen_2Cl_2)^+$ in microwave oven under reflux conditions [a]

Heating method	Duty cycle(%)	$t_{1/2}$	Rate constant (s^{-1})	ln K	Extrapolates temp at t_0	Extrapolates temp at $t_{1/2}$	Calculated temp (K) from Arrhenius plot
Reflux(MWO)	100	14.9	4.7×10^{-2}	-3.07	353K (80 °C)	353K (80 °C)	366.26

[a] Average of 4 replicate runs

Time (min)	Conc (mmoles)[1]	ln [PNBA]
0	0.050	-2.997
60	0.0445	-3.112
180	0.036	-3.324
240	0.029	-3.54
300	0.027	-3.612

[1] The concentration in mmoles was found in 200 ml of dilute acid mixture

Linear Regression Analysis

Intercept:	-2.98642
Slope:	-0.00212
Std Err of Slope:	0.000149
Correlation Coefficient:	0.9926

Rate constant = $0.35 \times 10^{-4} \pm 0.25 \times 10^{-5}\ s^{-1}$

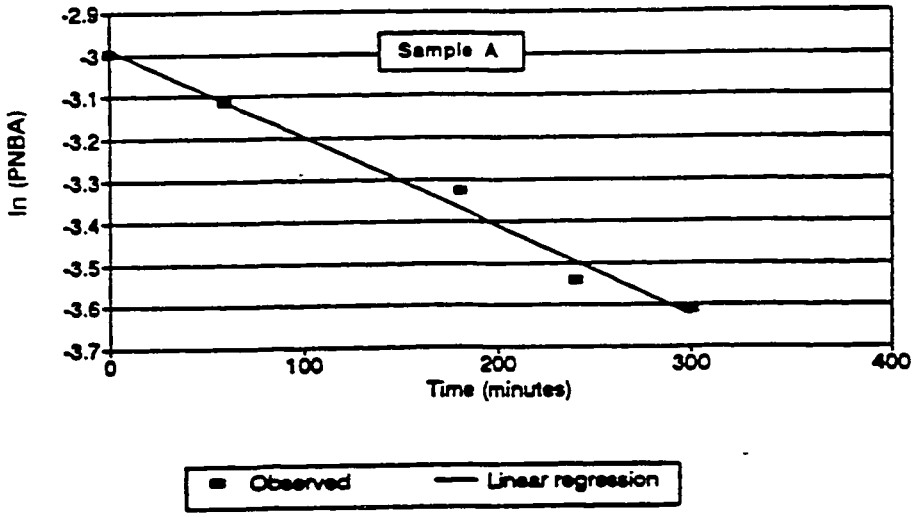


Figure 6.3
Oil bath rate constant studies:Nitration of p-Nitrobenzoic acid at 365K(92 °C) (Sample A)

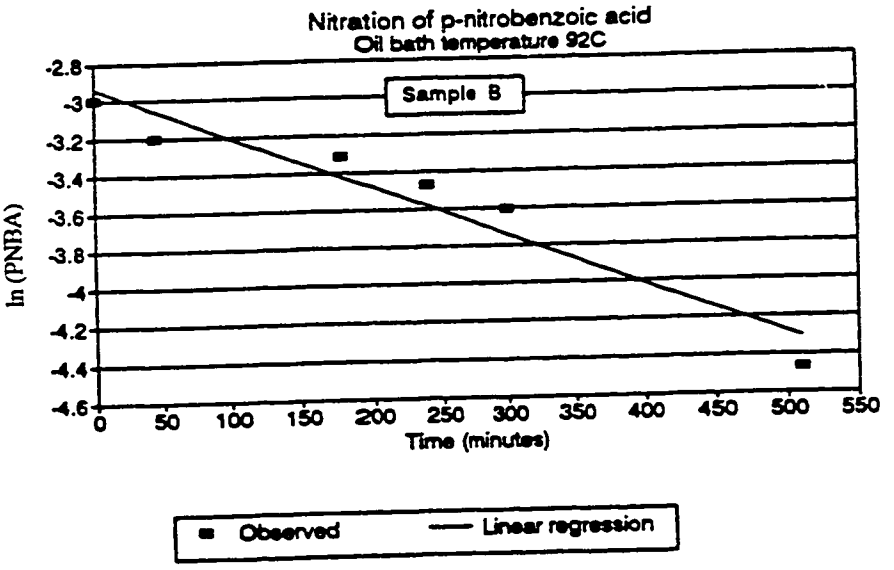
Time (min)	Conc (mmoles)[1]	ln [PNBA]
0	0.050	-2.996
45	0.0407	-3.202
180	0.0365	-3.31
240	0.0311	-3.471
300	0.0272	-3.605
510	0.0115	-4.465

[1] The concentration in mmoles was found in 200 ml of dilute acid mixture

Linear Regression Analysis

Intercept:	-2.93881
Slope:	-0.00268
Std Err of Slope:	-0.00368
Correlation Coefficient:	0.9643

Rate constant = $0.45 \times 10^{-4} \pm 0.61 \times 10^{-5} s^{-1}$



Rate constant $= 0.45 \times 10^{-4} \pm 0.61 \times 10^{-5}$

Figure 6.4
Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid at 365K (Sample B)

Time (min)	Conc (mmoles)[1]	ln [PNBA]
0	0.050	-2.996
20	0.032	-3.442
30	0.038	-3.27
45	0.031	-3.474
60	0.032	-3.442
90	0.015	-4.2

[1] The concentration in mmoles was found in 200 ml of dilute acid mixture

Linear Regression Analysis

Intercept: -3.00322

Slope: -0.01145

Std Err of Slope: 0.002651

Correlation Coefficient: 0.9074

Rate constant = $1.91 \times 10^{-4} \pm 0.44 \times 10^{-5} s^{-1}$

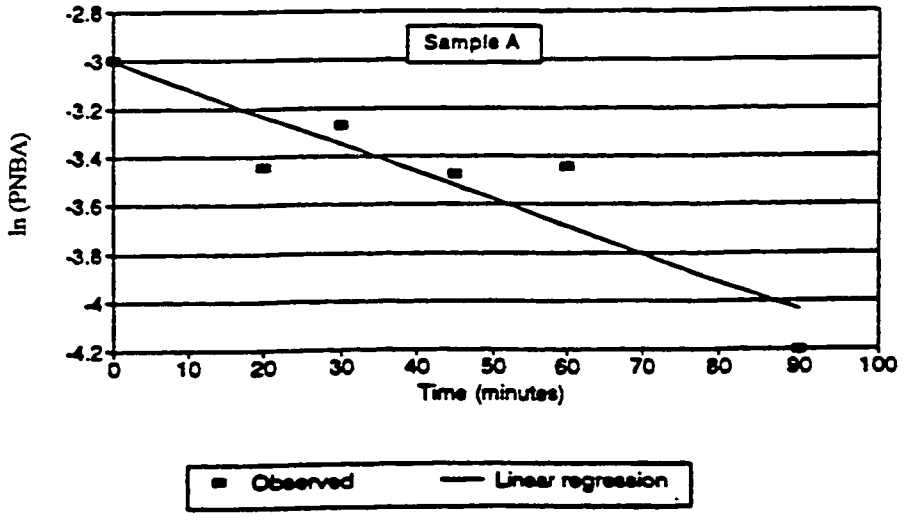


Figure 6.5
Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid at 381K(108 °C) (Sample A)

Time (min)	Conc (mmoles)[1]	ln [PNBA]
0	0.050	-2.996
30	0.039	-3.244
60	0.030	-3.507
90	0.017	-4.075

[1] The concentration in mmoles was found in 200 ml of dilute acid mixture

Linear Regression Analysis

Intercept: -2.9308

Slope: -0.01168

Std Err of Slope: 0.001797

Correlation Coefficient: 0.9765

Rate constant = $1.95 \times 10^{-4} \pm 0.3 \times 10^{-4} s^{-1}$

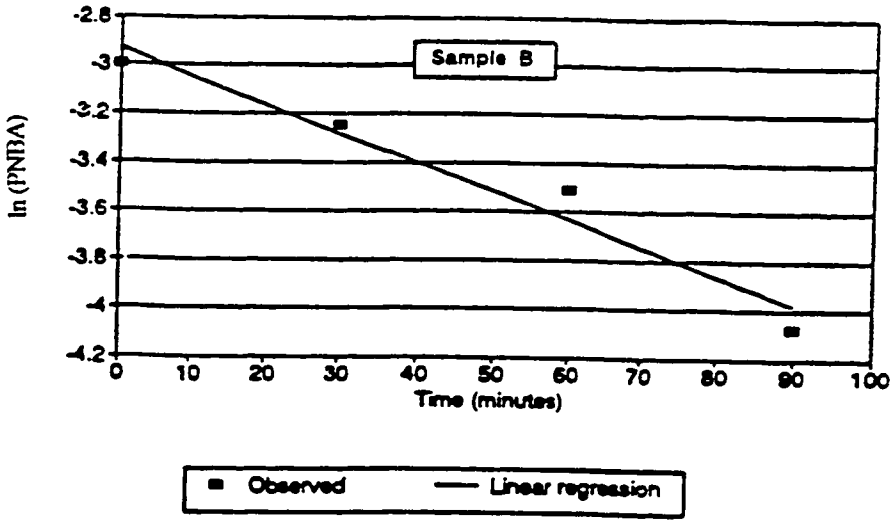


Figure 6.6
Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid at 381(108 ^{o} C) (Sample B)

Time (min)	Conc (mmoles)[1]	ln [PNBA]
0	0.050	-2.996
20	0.042	-3.17
30	0.036	-3.324
45	0.030	-3.507
60	0.025	-3.689
90	0.023	-3.772

[1] The concentration in mmoles was found in 200 ml of dilute acid mixture

Linear Regression Analysis

Intercept: -3.03241

Slope: -0.00924

Std Err of Slope: 0.001141

Correlation Coefficient: 0.9708

Rate constant = $1.5 \times 10^{-4} \pm 1.9 \times 10^{-5}\ s^{-1}$

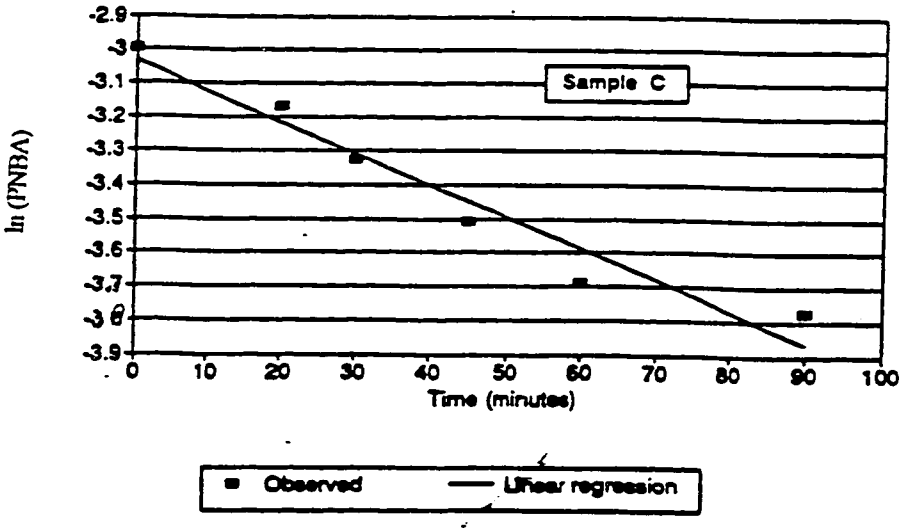


Figure 6.7
Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid at 381K (108°C) (Sample C)

Time (min)	Conc (mmoles)[1]	ln [PNBA]
0	0.050	-2.996
30	0.044	-3.124
60	0.037	-3.297
90	0.027	-3.612

[1] The concentration in mmoles was found in 200 ml of dilute acid mixture

Linear Regression Analysis

Intercept: -2.9541

Slope: -0.00674

Std Err of Slope: 0.001012

Correlation Coefficient: 0.9782

Rate constant = $1.12 \times 10^{-4} \pm 1.7 \times 10^{-5}\ s^{-1}$

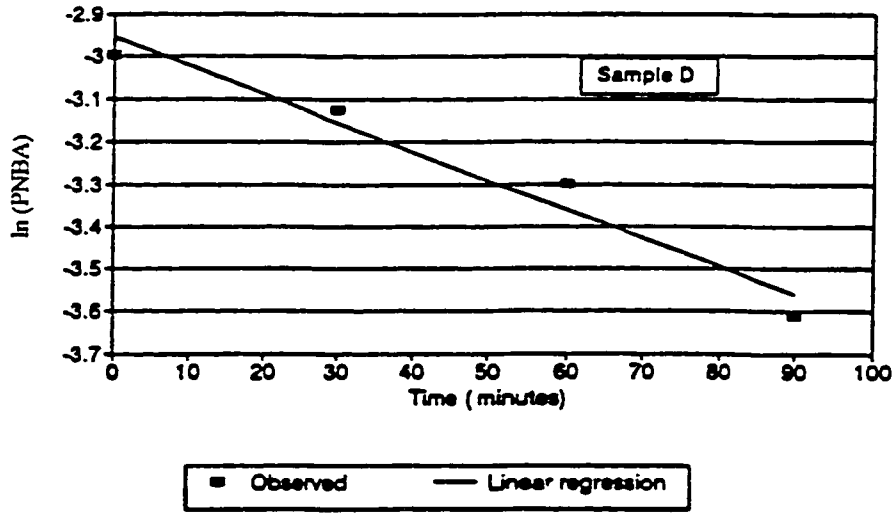


Figure 6.8
Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid at 381K (108 ^{o}C) (Sample D)

Time (min)	Conc (mmoles)[1]	ln [PNBA]
0	0.050	-2.996
10	0.044	-3.124
20	0.027	-3.612
30	0.020	-3.912
45	0.019	-3.963
60	0.011	-4.510
90	0.010	-4.605

[1] The concentration in mmoles was found in 200 ml of dilute acid mixture

Linear Regression Analysis

Intercept: -3.1259

Slope: -0.01898

Std Err of Slope: 0.002776

Correlation Coefficient: 0.95047

Rate constant = $3.2 \times 10^{-4} \pm 4.6 \times 10^{-5}\ s^{-1}$

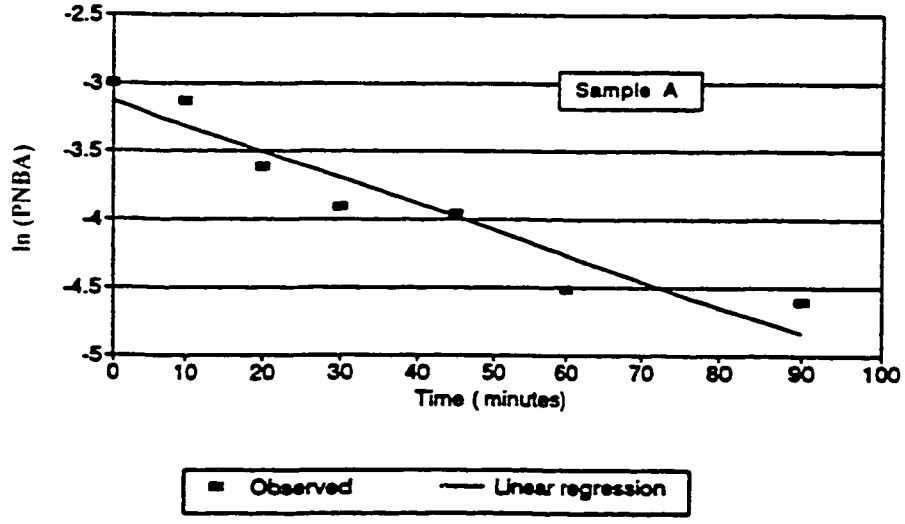


Figure 6.9
Oil bath rate Constant studies: Nitration of p-Nitrobenzoic acid at 391K (118 ^{o} C) (Sample A): Boiling point of acid mixture = 391(118 ^{o}C)
Oil bath temperature = 433K (160 ^{o} C)

Time (min)	Conc (mmoles)[1]	ln [PNBA]
0	0.050	-2.996
20	0.042	-3.170
30	0.033	-3.411
45	0.029	-3.540
60	0.025	-3.689
90	0.019	-3.963

[1] The concentration in mmoles was found in 200 ml of dilute acid mixture

Linear Regression Analysis

Intercept: -3.01442

Slope: -0.01095

Std Err of Slope: 0.000765

Correlation Coefficient: 0.9930

Rate constant = $1.83 \times 10^{-4} \pm 1.3 \times 10^{-5}\ s^{-1}$

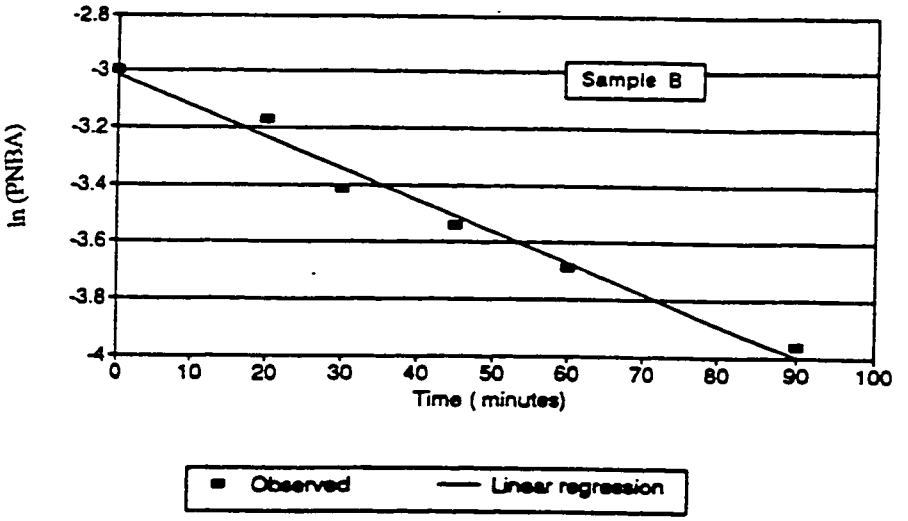


Figure 6.10
Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid at 391K (118 °C) (Sample B): Boiling point of acid mixture = 391K (118 °C) - Oil bath temperature = 433K (160 °C)

Time (min)	Conc (mmoles)[1]	ln [PNBA]
0	0.050	-2.996
20	0.039	-3.244
30	0.033	-3.411
45	0.030	-3.507
60	0.024	-3.730
90	0.015	-4.20

[1] The concentration in mmoles was found in 200 ml of dilute acid mixture

Linear Regression Analysis

Intercept: -2.98009

Slope: -0.01309

Std Err of Slope: 0.000654

Correlation Coefficient: 0.9951

Rate constant = $2.2 \times 10^{-4} \pm 1.1 \times 10^{-5}\ s^{-1}$

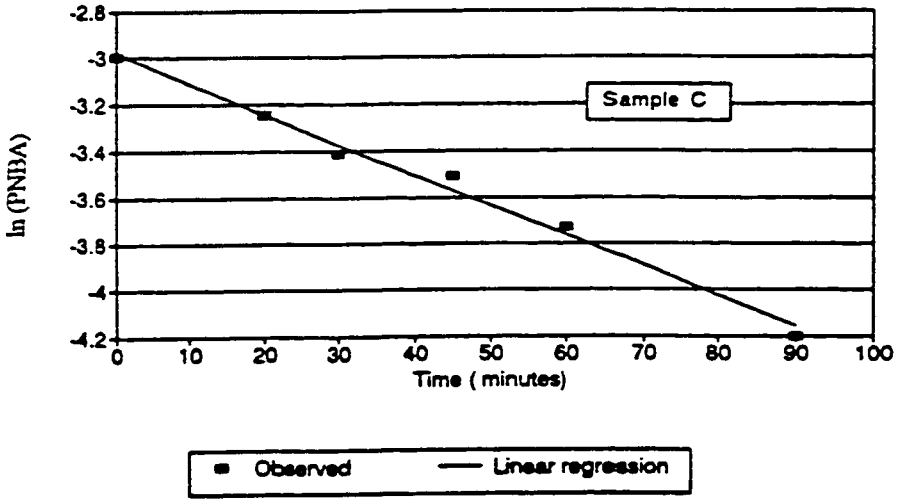


Figure 6.11
Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid at 391K (118 °C) (Sample C): Boiling point of acid mixture = 391K (118 °C) - Oil bath temperature = 433K (160 °C)

Time (min)	Conc (mmoles)[1]	ln [PNBA]
0	0.050	-2.996
10	0.046	-3.079
20	0.027	-3.612
30	0.020	-3.912
45	0.018	-4.017
60	0.017	-4.075
90	0.013	-4.343

[1] The concentration in mmoles was found in 200 ml of dilute acid mixture

Linear Regression Analysis

Intercept: -3.16892

Slope: -0.0151

Std Err of Slope: 0.002954

Correlation Coefficient: 0.9162

Rate constant = $2.5 \times 10^{-4} \pm 4.9 \times 10^{-5}$ s^{-1}

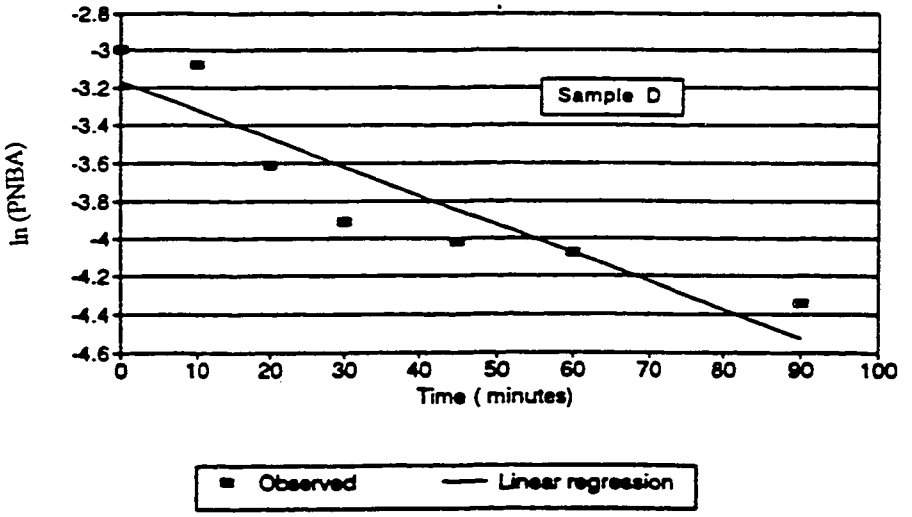


Figure 6.12
Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid at 391K (118 °C) (Sample D): Boiling point of acid mixture = 391K (118 °C) - Oil bath temperature = 433K (160°C)

Table 6.5

Oil bath rate constant studies: Nitration of p-nitro benzoic acid[a,b,c]

Acid mixture volume : 20 ml H_2SO_4/HNO_3 (1:1)

Heating method	Temperature (K)	Rate constant (s^{-1})
Conventional (Oil bath)	365 (92 °C)	$0.4 \times 10^{-4} \pm 0.6 \times 10^{-6}$
	381 (108 °C)	$1.60 \times 10^{-4} \pm 2.7 \times 10^{-5}$
	391 (118 °C)	$2.4 \times 10^{-4} \pm 2.3 \times 10^{-5}$

[a] Average of 2 replicates

[b] Average of 4 replicates

[c] Boiling point of HNO_3/H_2SO_4 (1:1) mixture is 391 K (118 °C) and oil bath temperature was maintained at 433 K (160 °C)

Temp (k) $1/T \times 10^4$	Ln k
27.4	-10.13
26.25	-8.74
25.58	-8.33

Linear Regression Analysis

Intercept:	17.681
Slope:	-1.013
Std Err of Slope:	0.1567
Correlation Coefficient:	0.9882

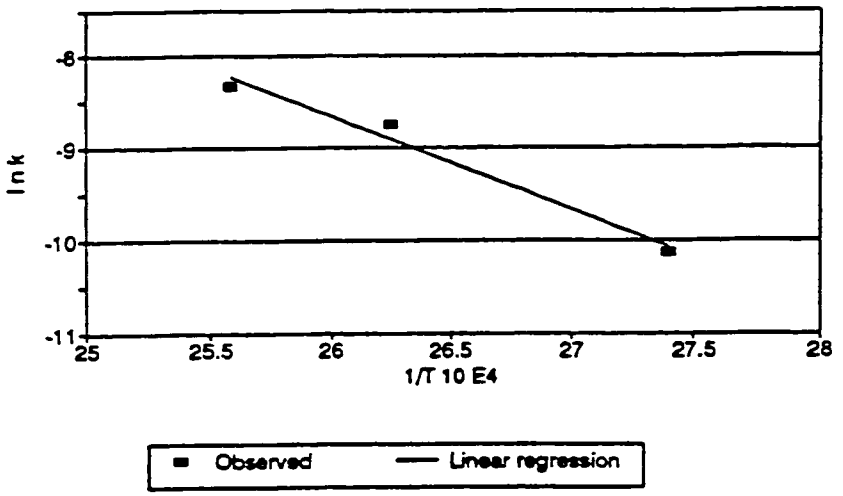


Figure 6.13
Arrhenius plot: Nitration of p-Nitrobenzoic acid in oil bath

Time (min)	Conc (mmoles)[1]	ln [PNBA]
0	0.050	-2.996
10	0.044	-3.124
15	0.037	-3.297
20	0.024	-3.730
30	0.025	-3.689
45	0.022	-3.817
60	0.014	-4.269

[1] The concentration in mmoles was found in 200 ml of dilute acid mixture

Linear Regression Analysis

Intercept: -3.04009

Slope: -0.02023

Std Err of Slope: 0.003018

Correlation Coefficient: 0.9486

Rate constant = $3.4 \times 10^{-4} \pm 5.1 \times 10^{-5}$ s^{-1}

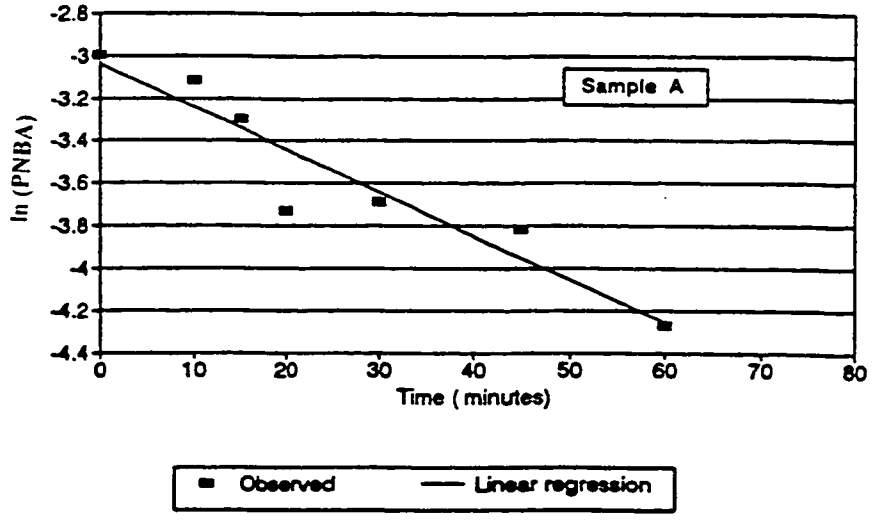


Figure 6.14
Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid reflux reaction in microwave oven. (Sample A): Boiling point of acid mix = 391K (118 °C)

Time (min)	Conc (mmoles)[1]	ln [PNBA]
0	0.050	-2.996
10	0.045	-3.101
20	0.032	-3.442
30	0.03	-3.507
45	0.021	-3.863
60	0.006	-5.116

[1] The concentration in mmoles was found in 200 ml of dilute acid mixture

Linear Regression Analysis

Intercept: -2.78456

Slope: -0.03223

Std Err of Slope: 0.006349

Correlation Coefficient: 0.9303

Rate constant = $5.4 \times 10^{-4} \pm 1.1 \times 10^{-5}$ s^{-1}

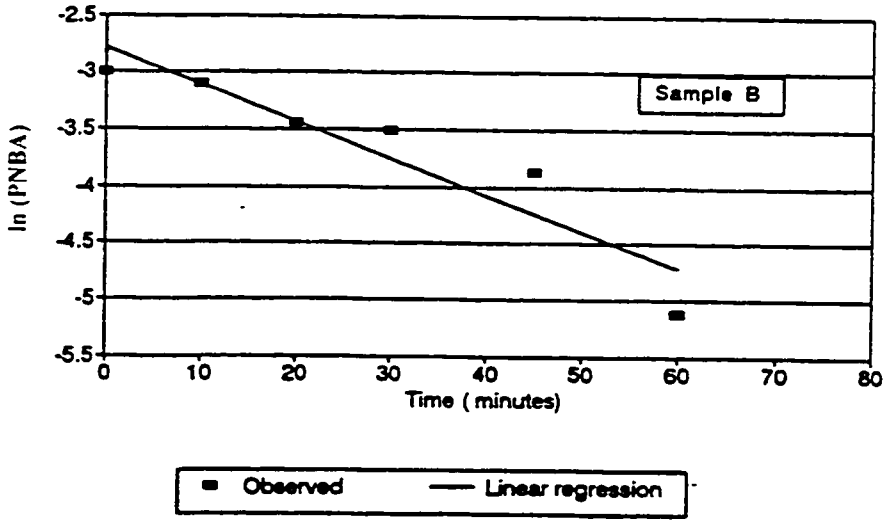


Figure 6.15
Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid reflux reaction in microwave oven. (Sample B): Boiling point of acid mix = 391K(118 ^{0}C)

TABLE 6.6

Microwave oven rate constant studies : Nitration of p-nitrobenzoic acid

Heating method	**Temperature (^{0}C)**	**Duty cycle(%)**	**Rate constant (s^{-1})**	**ln k**	**Extrapolated temp(K)**	**Effec temp. (K)**
Microwave oven	Reflux	100	$4.40 \times 10^{-4} \pm 3.1 \times 10^{-5}$	-7.729	391 (118 ^{0}C)	398 (125 ^{0}C)

The hydrolysis reaction was monitored colorimetrically exploiting the complementary of the colours of the reactant and the product to readily identify the half time, a method introduced by Basolo et al (72). Hydrolysis of trans$(Co(en)_2Cl_2)^+$ complex in aqueous solution is too fast to study in microwave oven reflux (and pulse) conditions. To facilitate study in microwave reflux and pulse conditions, trans$(Co(en)_2Cl_2)^+$ complex in several compositions of MeOH/H_2O were hydrolysed. The infinity UV spectrum at 512 agreed with the spectrum of solvo product. The reaction was carried out at higher concentration in water with little acid then diluted with methanol to give the same solvent composition. This is defined as authentic aquo product. The aquo product was compared with reaction run after 7 half lives. Although 60%, 65% and 70% methanol solution slowed the hydrolysis rate substantially, infinity spectrum did not agree with solvo product. It was found that 50% MeOH solution was optimum for rate constant studies.

The microwave pulse sequence was repeated for 60s prior to sample injection to bring the system to a quasi-steady state. This time was just sufficient to bring the solvent to reflux. The temperature dependence of the reactions was determined using the conventional baths. Conventional hydrolysis rate constants (Table 6.3) of trans$(Co(en)_2Cl_2)^+$ at 49 °C , 55 °C, 60 °C and 80 °C (average of two runs) were used to get Arrhenius parameters (Figure 6.2). The Arrhenius equation (linear fitting with $R>0.99$ was used to calculate the effective reaction temperature (93 °C) for the microwave's run under reflux conditions (Table 6.4) from the observed effective "rate constants". The effective reaction temperature calculated was higher than the maximum temperature measured conventionally.

It is apparent from rate constant results from hydrolysis reaction that superheating occurs in a microwave oven under reflux. In the case of slower reaction, linearity for each run in Figures 6.3 - 6.12 proves that the reaction is first order. The average conventional rate constants at 92 °C,108 °C and 118 °C are given in Table 6.5. The rate constant values are plotted as lnk versus 1/T. The effective temperature of 125 °C for microwave oven based nitration rate constant under reflux conditions was calculated from Arrhenius plot (6.13). This temperature is 7 °C higher than the solvent mixtures boiling point of118 °C. In this study, the conventional heating bath was set at a temperature well above the reflux temperature of the solvent mixture so that a rapid heat transfer analogous to the microwave oven would occur for both cases. Nevertheless, the rate constant under reflux with microwave heating is larger than observed.

It can only be concluded that superheating effects can arise from the mechanism of microwave heating and that these can be important even when there is no opportunity for the confining pressure to increase so that boiling points rise.

6.4 CONCLUSION:

Detail kinetic experiments under reflux conditions of two very different reactions in different conditions: a) Hydrolysis of trans$(Co(en)_2Cl_2)^+$ in a mixture of CH_2OH/H_2O and b) Aromatic nitration of p - nitrobenzoic acid H_2SO_4/HNO_3, showed that super heating occurs in microwave oven. Subsequently, other workers confirmed the presence of superheating effects based on indirect electronic probe methods

APPENDIX A

Kinetic Studies by Pulse Sequence Heating

The information given in appendix is complex. The author acknowledges that it may require more simplicity and precision. However, the work shown in this section is incomplete and need not be considered. Nevertheless, the information herein is significant, indeed requiring further study, and thereby worthy of consideration in its own right and may point to new area of research.

Arrhenius parameters (Hydrolysis of trans$(Co(en)_2Cl_2)^+$) in water bath and slow reaction (Aromatec nitration of p-Nitrobenzoic acid) in oil bath (Chapter 6) were used to determine the effective reaction temperature in microwave oven based pulse reactions for different duty cycles (pulse heating followed by cooling). The maximum and minimum temperatures were determined by extrapolation techniques as described in section A.2.

A.1 EXPERIMENTAL SECTION

A.1.1 Hydrolysis of Trans$(Co(en)_2Cl_2)^+$:

49.0 ml of water/methanol mixture in a three necked flask was heated by a given pulse sequence for a total time of 60s (includes pulse + dormant time). For example, for 50% duty cycle, the solvent mixture was heated for 5s followed by 5s of dormant time. This sequence was repeated six times. The solvent mixture was spiked with freshly prepared 1.0 ml of 0.1 M aqueous trans $(Co(en)_2Cl_2)^+$(27.6 mg) stock solution. and immediately the solution was subjected to 5s heating/5s dormant time sequence. The mixture was monitored colorimetrically to determine half life. Similar procedure was followed for 60%, 70%, 80%and 90% for 10s and 20s duty cycles.

A.1.2 Nitration of p-Nitrobenzoic acid in Microwave oven (pulse heating):

1.0 ml of 0.05M p-nitrobenzoic acid (8.4 mg) dissolved in acetone was injected into a three- necked flask. Acetone solvent was evaporated under a slow stream of nitrogen. 20 ml of H_2SO_4/HNO_3 (1:1)(v/v) acid mixture was added to the flask. The mixture was heated in a modified microwave oven equipped with reflux condenser by pulse heating for various durations of time. For example, as demonstrated in Figure A.14, the 5s pulse sequence reactions were carried out for 25 minutes, 100minutes and 120minutes. For each time duration , the experiment was repeated at least twice. The sample was cooled and the contents were added to water and diluted to 200 ml. 50ml of diluted solution's pH was adjusted to pH6 with NaOH and diluted to 100 ml in volumetric flask. 10 ul final solution was injected into HPLC equipped with C18 column.

A.2 RESULTS AND DISCUSSION

Tables A.1 and A.2 show rate constant results of hydrolysis of trans$(Co(en)_2Cl_2)^+$ for 10s and 20s pulse heating sequence respectively. Duty cycle (%) gives the percent time the microwave oven was on for different pulse heating durations. Figure A.1 - A11 show extrapolated temperature for 50% duty cycles for t_o and $t_{1/2}$. Similar procedure was followed for other duty cycles. The Arrhenius parameters calculated from conventional water bath method (Table 6.3) are used to determine effective kinetic temperature. Figures A.12 and A.13 show the data of Tables A.1 and A.2 plotted as in rate constant values versus 1/T to get Pseudo Arrhenius plot for 10s and 20s pulse sequence experiments respectively.

The concentration values of PNBA for microwave pulse sequence reactions for different runs are plotted as ln[PNBA] versus time for each run in Figures A.14 - A.19[1]. The average rate constant values (Table A.3) calculated from the slopes of Figures A.14 - A.19 for each 5s, 7s and 10s pulse sequence experiments are plotted as lnk versus 1/T to get Pseudo Arrhenius plot[1].

[1]Concentration data are given as inset in Figures C.14 - C.19

The microwave pulse sequence was repeated for 60s prior to sample injection to bring the system to a quasi-steady state. This time was just sufficient to bring the solvent to reflux[2] when the microwave duty cycle was 100%. The maximum temperatures were measured by inserting a thermometer at the end of a heating pulse after reaching the $t_{1/2}$. No further pulse was applied and the temperature readings were recorded as a function of time. The cooling curve was then extrapolated to time zero to obtain the temperature just at the end of a heating cycle. The minimum temperature given was the extrapolated temperature at the point of sample injection for each duty cycle. For example, the following pulse sequence was used for 90% duty cycle:

A) Temperature extrapolation to t_0:

1) 9s pulse/1s dormant 2) 9s pulse /1s dormant 3) 9s pulse/1s dormant 4) 9s pulse/1s dormant 5)9s pulse/1s dormant 6) 9s pulse/ 1s dormant . Started timer at the end of dormant cycle to extrapolate the temperature to t_0.

B) Determination of $t_{1/2}$:

1) 9s pulse/1s dormant 2) 9s pulse /1s dormant 3) 9s pulse/1s dormant 4) 9s pulse/1s dormant 5) 9s pulse/1s dormant 6) 9s pulse/ 1s dormant. Quasi-steady state. Injected Co complex at the end of dormant cycle and the 9s pulse/1s dormant cycle continued to determine $t_{1/2}$. 7) 9s pulse/ 1s dormant 8) 9s pulse/ 1s dormant (Co complex changed to colorless at this point). $t_{1/2}$ for 90% duty cycle is 20s

---

[2] 50% methanol solvent reached boiling point in 60s.

C) Temperature extrapolation to $t_{1/2}$:

1) 9s pulse/1s dormant 2) 9s pulse /1s dormant 3) 9s pulse/1s dormant 4) 9s pulse/1s dormant 5) 9s pulse/1s dormant 6) 9s pulse/1s dormant. Quasi steady state. Pulse heating continued. 7) 9s pulse/1s dormant 2)9s pulse/1s dormant (Total heating time after quasi state - 20s). Started timer at the end of 9s pulse and the temperature was extrapolated. This temperature is the hottest point in the duty cycle.

The reported maximum and minimum conventional temperatures for different duty cycles are consistently below the effective kinetic temperatures. The temperature dependence of the reactions was determined using the conventional baths, and the Arrhenius equation (linear fitting with $R>0.99$ was used to calculate the effective reaction temperature for each of the microwave runs from the observed effective "rate constants". Each effective hydrolysis reaction temperature calculated was higher than the maximum temperature measured conventionally at the hottest point in the duty cycle

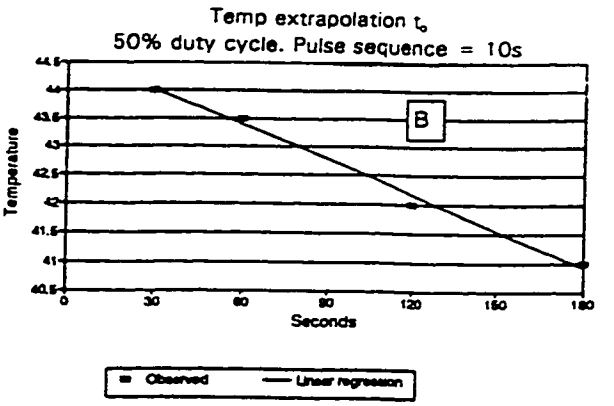
Although each nitration pulse heating sequence run could be fitted well to first order kinetics, the parameters are not true rate constants since the temperature is not constant. Each effective reaction temperature calculated was higher than the maximum temperature measured conventionally at the hottest point in the duty cycle.

It can only be concluded that transient superheating effects can arise from the mechanism of microwave pulse heating and that these can be important even when there is no opportunity for the confining pressure to increase so that boiling points rise. It is particularly interesting in the context of maximization of energy efficiency of reaction conditions to consider fully the controlled use of pulse heating.

A.3. CONCLUSION:

The pulse microwave may be used to exploit repeated superheating phenomena and accelerate rates to values above average temperatures that can be measured by an extrapolation technique. This level of overheating will ultimately offer the opportunity to optimize the energy efficiency of microwave heated synthesis. Pulse experiments point to an area of research. How can you optimize the use of energy to accelerate chemical reactions? Transient "overheating" may be a highly effective strategy, since the reaction rates have an exponential dependence on temperature so that a short time at a high temperature may well be very efficient in the use of energy.

Time (s)	Temp °C
30	44.0
60	43.5
120	42.0
180	41.0



Linear Regression Analysis

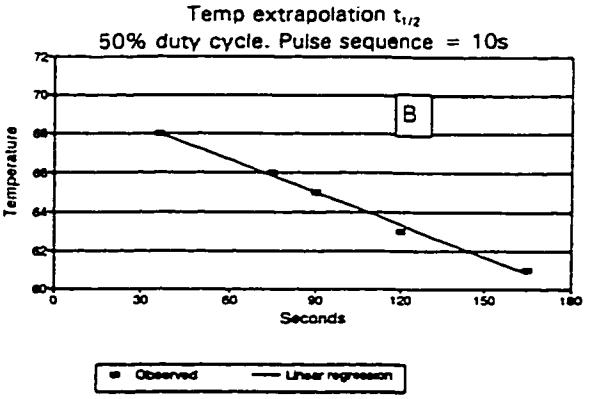
Correlation coefficient: 0.9963

Intercept (extrapolated temp) = 44.6°C

Slope: -0.02062

Figure A.1 (a)
Solvent temperature extrapolation to t_o (A) at 50% duty cycle for pulse heating sequence of 10s

Time (s)	Temp °C
36	68.0
75	66.0
90	65.0
120	63.0
165	61.0



Linear Regression Analysis

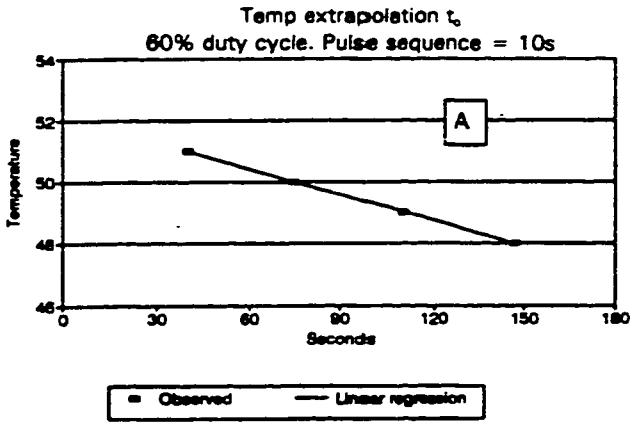
Correlation coefficient: 0.9971

Intercept (extrapolated temp) = 70°C

Slope : -0.05556

Figure A.1 (b)
Solvent temperature extrapolation to $t_{1/2}$ (B) at 50% duty cycle for pulse heating sequence of 10s

Time (s)	Temp °C
40	51.0
75	50.0
110	49.0
147	48.0



Linear Regression Analysis

Correlation coefficient: 0.9999

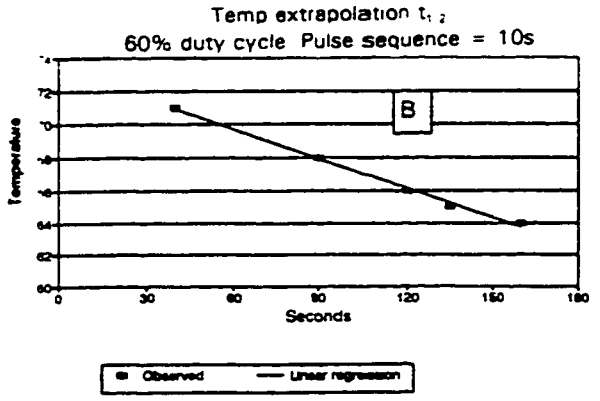
Intercept (extrapolated temp) = 52°C

Slope: -0.02808

Figure A. 2 (a)

Solvent temperature extrapolation to t_o (A) at 60% pulse heating sequence of 10s

Time (s)	Temp °C
40	71.0
90	68.0
120	66.0
135	65.0
160	64.0



Linear Regression Analysis

Correlation coefficient: : 0.9975

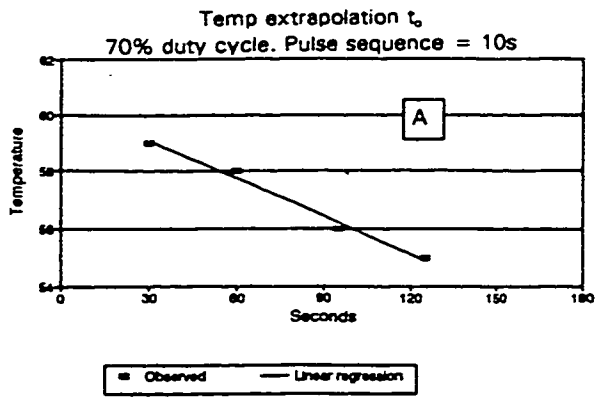
Intercept (extrapolated temp) = 73.3°C

Slope: -0.05998

Figure A. 2 (b)

Solvent temperature extrapolation to $t_{1/2}$ (B) at 60% duty cycle for pulse heating sequence of 10s

Time (s)	Temp °C
30	59.0
60	58.0
95	56.0
125	55.0



Linear Regression Analysis

Correlation coefficient: 0.9939

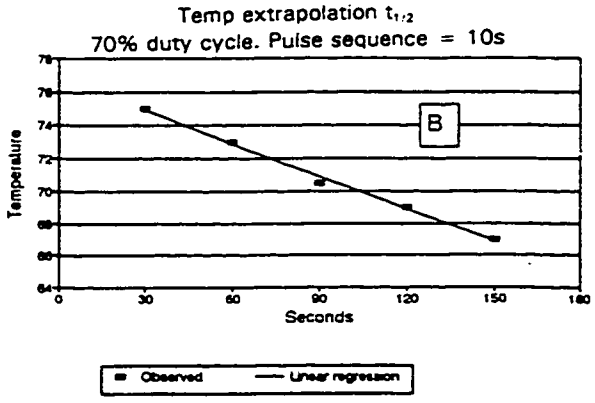
Intercept (extrapolated temp) = 60.4 °C

Slope: -0.0439

Figure A. 3 (a)

Solvent temperature extrapolation to t_o (A) at 70% duty cycle for pulse heating sequence of 10s

Time (s)	Temp °C
30	75.0
60	73.0
90	70.5
120	69.0
150	67.0



Linear Regression Analysis

Correlation coefficient: 0.9975

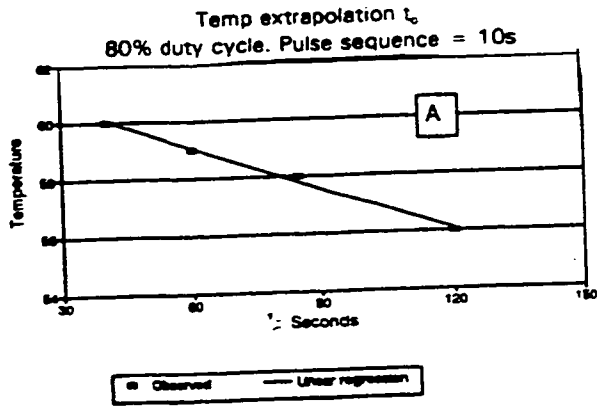
Intercept (extrapolated temp) = 76.9°C

Slope: -0.06667

Figure A. 3 (b)

Solvent temperature extrapolation to $t_{1/2}$ (B) at 70% duty cycle for pulse heating sequence of 10s

Time (s)	Temp °C
40	60.0
60	59.0
85	58.0
120	56.0

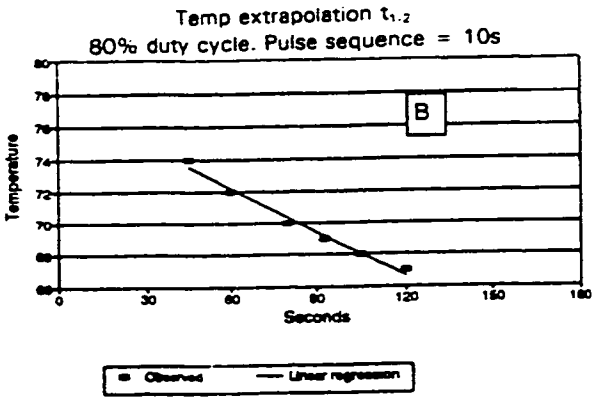


Linear Regression Analysis

Correlation coefficient:	0.9974
Intercept (extrapolated temp) =	62°C.
Slope:	-0.04939

Figure A. 4 (a)
Solvent temperature extrapolation to t_o (A) at 80% duty cycle for pulse heating sequence of 10s

Time (s)	Temp °C
45	74.0
60	72.0
80	70.0
93	69.0
105	68.0
120	67.0

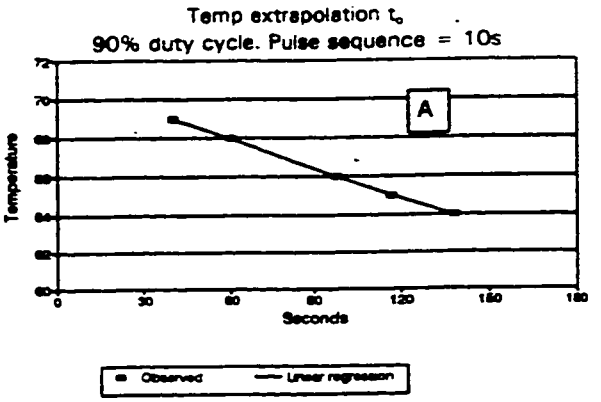


Linear Regression Analysis

Correlation coefficient:	0.9929
Intercept (extrapolated temp) =	77.7°C
Slope:	-0.09235

Figure A. 4 (b)
Solvent temperature extrapolation to $t_{1/2}$ (B) at 80% duty cycle for pulse heating sequence of 10s

Time (s)	Temp °C
40	69.0
60	68.0
98	66.0
117	65.0
138	64.0

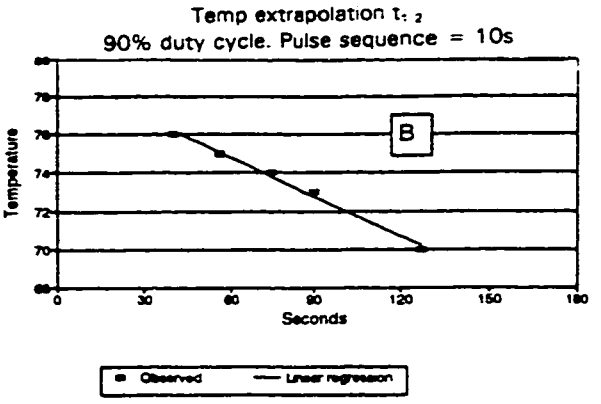


Linear Regression Analysis

Correlation coefficient: 0.9998
Intercept (extrapolated temp) = 71.1°C
Slope: -0.05145

Figure A. 5 (a)
Solvent temperature extrapolation to t_o (A) at 90% duty cycle for pulse heating sequence of 10s

Time (s)	Temp °C
40	76.0
56	75.0
75	74.0
90	73.0
127	70.0

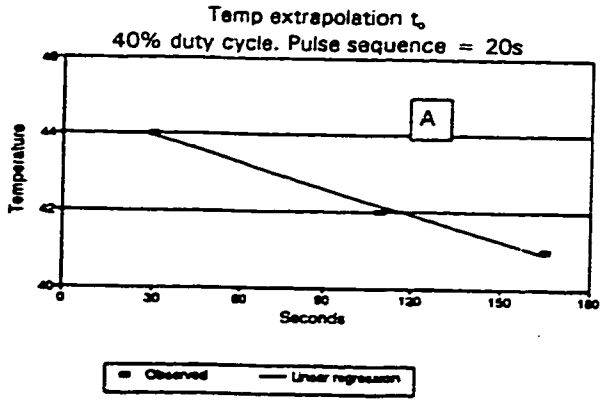


Linear Regression Analysis

Correlation coefficient: 0.9953
Intercept (extrapolated temp) = 78.9°C
Slope: -0.06846

Figure A. 5 (b)
Solvent temperature extrapolation to $t_{1/2}$ (B) for 90% duty cycle for pulse heating sequence of 10s

Time (s)	Temp °C
30	44.0
110	42.0
165	41.0



Linear Regression Analysis

Correlation coefficient: 0.9965

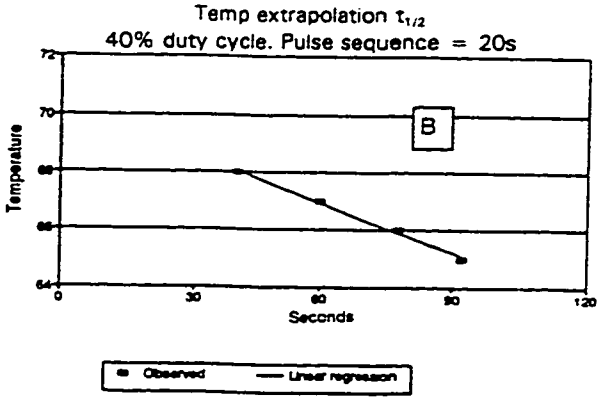
Intercept (extrapolated temp) = 44.6°C

Slope: -0.02242

Figure A. 6(a)

Solvent temperature extrapolation to t_o (A) at 40 % duty cycle for pulse heating sequence of 20s

Time (s)	Temp °C
40	68.0
60	67.0
78	66.0
92	65.0



Linear Regression Analysis

Correlation coefficient: 0.9970

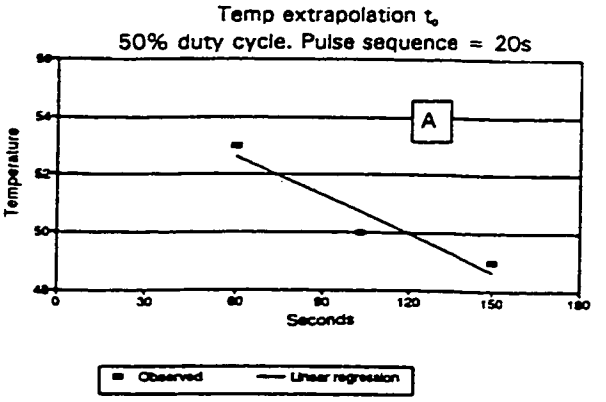
Intercept (extrapolated temp) = 70.4°C

Slope: -0.05712

Figure A. 6 (b)

Solvent temperature extrapolation to $t_{1/2}$ (B) at 40% duty cycle for pulse heating sequence of 20s

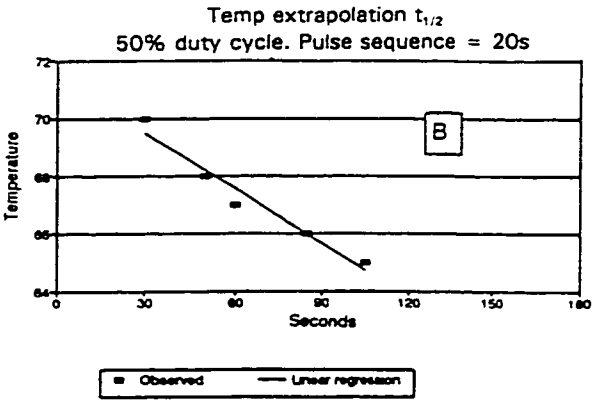
Time (s)	Temp °C
60	53.0
103	50.0
150	49.0



Linear Regression Analysis
Correlation coefficient: 0.9533
Intercept (extrapolated temp) = 55.3°C
Slope: -0.04409

Figure A. 7(a)
Solvent temperature extrapolation to t_o (A) at 50% duty cycle for pulse heating sequence of 20s

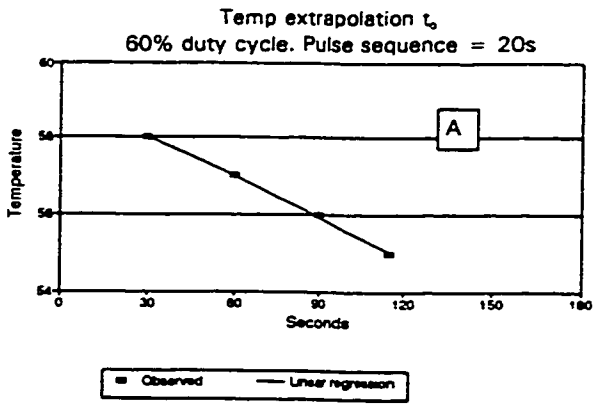
Time (s)	Temp °C
30	70.0
50	68.0
60	67.0
85	66.0
105	65.0



Linear Regression Analysis
Correlation coefficient: 0.9752
Intercept (extrapolated temp) = 71.4°C
Slope: -0.06369

Figure A. 7 (b)
Solvent temperature extrapolation to $t_{1/2}$ (B) at 50% duty cycle for pulse heating sequence of 20s

Time (s)	Temp °C
30	58.0
60	57.0
90	56.0
115	55.0



Linear Regression Analysis

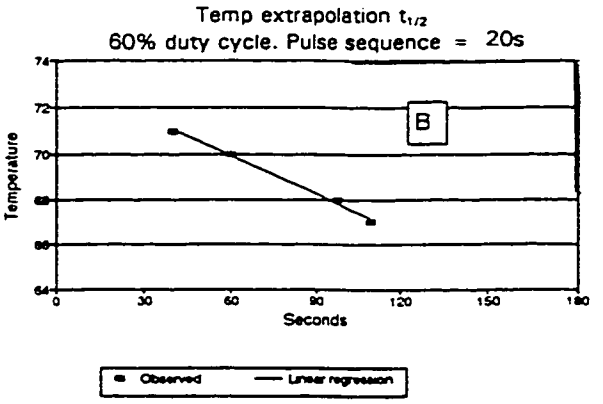
Correlation coefficient: 0.999

Intercept (extrapolated temp) = 59.1°C

Slope: -0.03502

Figure A. 8 (a)
Solvent temperature extrapolation to t_o (A) at 60% duty cycle for pulse heating sequence of 20s

Time (s)	Temp °C
40	71.0
60	70.0
98	68.0
110	67.0



Linear Regression Analysis

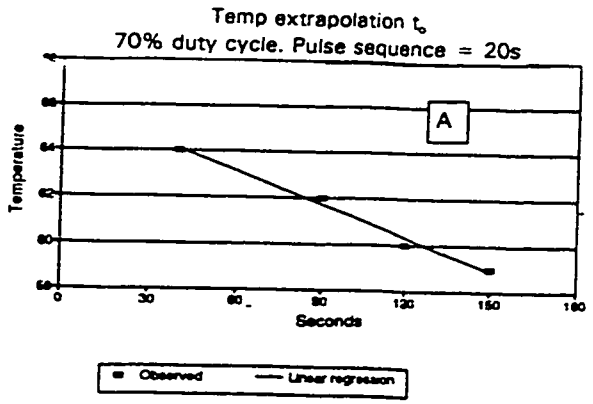
Correlation coefficient: 0.9969

Intercept (extrapolated temp) = 73.3°C

Slope: -0.05583

Figure A. 8 (b)
Solvent temperature extrapolation to $t_{1/2}$ (B) at 60 % duty cylce for pulse heating sequence of 20s

Time (s)	Temp °C
40	64.0
90	62.0
120	60.0
150	59.0

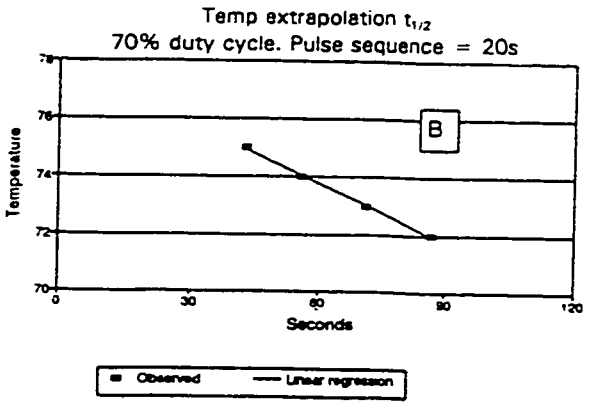


Linear Regression Analysis

Correlation coefficient: 0.9920
Intercept (extrapolated temp) = 65.9°C
Slope: -0.04697

Figure A. 9 (a)
Solvent temperature extrapolation to t_o (A) at 70% duty cycle for pulse heating sequence of 20s

Time (s)	Temp °C
43	75.0
56	74.0
71	73.0
87	72.0

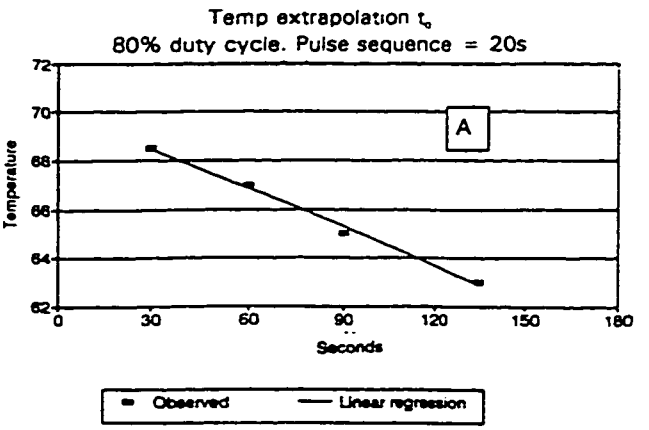


Linear Regression Analysis

Correlation coefficient: 0.9989
Intercept (extrapolated temp) = 77.9°C
Slope: -0.06788

Figure A. 9 (b)
Solvent temperature extrapolation to $t_{1/2}$ (B) at 70% duty cycle for pulse heating sequence of 20s

Time (s)	Temp
30	68.5
60	67.0
90	65.0
135	63.0



Linear Regression Analysis

Correlation coefficient 0.9969

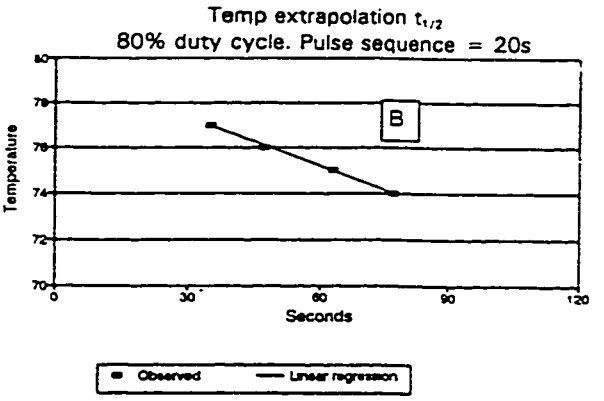
Intercept (extrapolated temp) = 70.1°C = 343.26 K

Slope: -0.05327

Figure A. 10 (a)

Solvent temperature extrapolation to t_o (A) at 80% duty cycle for pulse heating sequence of 20s

Time (s)	Temp °C
35	77.0
47	76.0
63	75.0
77	74.0



Linear Regression Analysis

Correlation coefficient: 0.9986

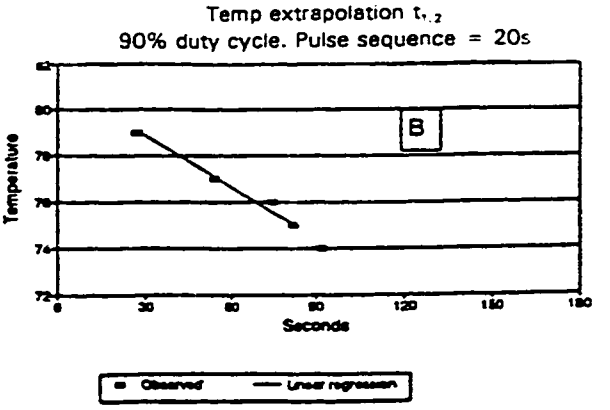
Intercept (extrapolated temp) = 79.4°C = 352.56 K

Slope: -0.07023

Figure A. 10 (b)

Solvent temperature extrapolation to $t_{1/2}$ (B) at 80% duty cycle for pulse heating sequence of 20s

Time (s)	Temp °C
30	76.0
55	74.0
90	71.5
115	70.0
150	68.0

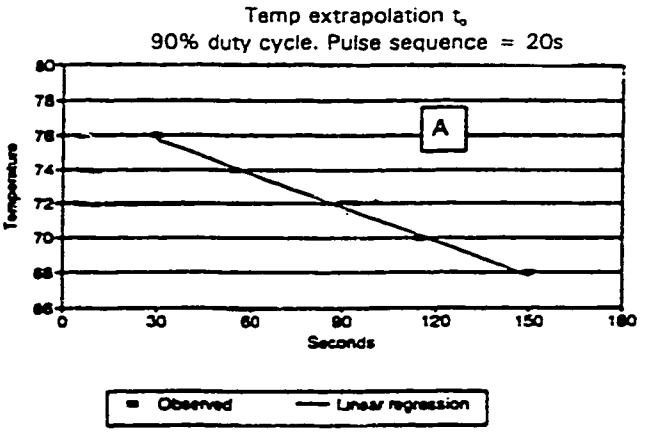


Linear Regression Analysis

Correlation coefficient:	0.9975
Intercept (extrapolated temp) =	77.8°C
Slope:	-0.06656

Figure A. 11(a)
Solvent temperature extrapolation to t_o (A) at 90% duty cycle for pulse heating sequence of 20s

Time (s)	Temp °C
27	79.0
54	77.0
75	76.0
82	75.0
92	74.0



Linear Regression Analysis

Correlation coefficient:	0.9895
Intercept (extrapolated temp) =	81.1°C
Slope:	-0.07356

Figure A. 11 (b)
Solvent temperature extrapolation to $t_{1/2}$ (B) at 90% duty cycle for pulse heating sequence of 20s

Table A.1

Rate constant studies : Hydrolysis of trans($Coen_2Cl_2$)$^+$ in microwave oven[a].

Total pulse heating cycle = 10s

Heating method	Duty cycle(%)	$t_{1/2}$	Rate constant (s^{-1})	ln k	Extrapolated temp(K) at t_0	Extrapolated temp(K) at $t_{1/2}$.	Calculated temp(K) from Arrhenius plot
9s pulse/1s dormant	90	20.0	3.5×10^{-2}	-3.36	344.	352	362
8s pulse/2s dormant	80	23.0	3.0×10^{-2}	-3.51	335	350	360
7s pulse/3s dormant	70	52.0	1.3×10^{-2}	-4.34	333	350	350
6s pulse/4s dormant	60	76.0	0.9×10^{-2}	-4.71	325	346	350
5s pulse/5s dormant	50	117	0.6×10^{-2}	-5.12	317	343	341

TABLE A.2

Rate constant studies : Hydrolysis of trans$(Coen_2Cl_2)^+$ in microwave oven[a].

Total pulse cycle = 20s

Heating method	Duty cycle(%)	$t_{1/2}$	Rate constant (s^{-1})	ln k	Extrapolated temp (K) at t^0	Extrapolated temp (K) at $t_{1/2}$	Calculated temp (K) from Arrhenius plot
18s pulse/ 2s dormant	90	16.0	4.3×10^{-2}	-3.15	350	354	360
16s pulse/ 4s dormant	80	28.8	2.8×10^{-2}	-3.58	343	353	355
14s pulse/ s dormant	70	30.4	2.3×10^{-2}	-3.77	339	351	353
12s pulse/ 8s dormant	60	42.0	1.6×10^{-2}	-4.14	332	346	350
10s pulse/ 10s dormant	50	63.5	1.1×10^{-2}	-4.51	328	344	345

Temp (K) $1/T \times 10^4$	ln k
28.33	-3.06
28.42	-3.35
28.49	-3.51
28.58	-4.34
28.93	-4.71
29.16	-5.12

Linear Regression Analysis

Intercept:	65.6491
Slope:	-2.431
Std Err of Slope:	0.3903
Correlation Coefficient:	0.95

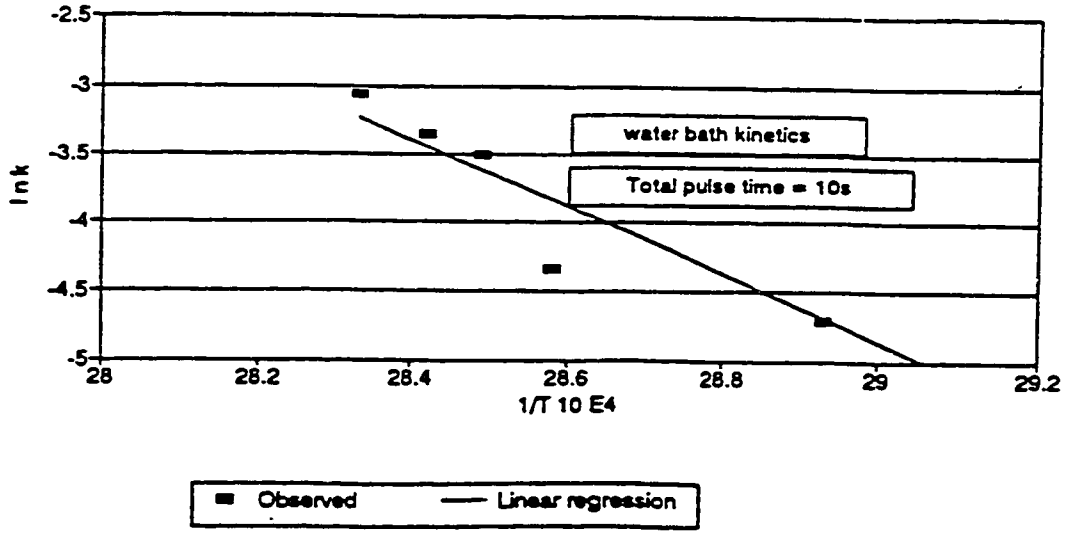


Figure A.12
Pseudo Arrhenius plot (10s):Hydrolysis of trans$(Co(en)_2Cl_2)^+$ in Microwave oven

Temp (K) $1\backslash T \times 10^4$	ln k
28.33	-3.06
28.24	-3.15
28.5	-3.58
28.47	-3.77
28.88	-4.14
29.04	-4.51
29.12	-4.96

Linear Regression Analysis

Intercept:	51.0321
Slope:	-1.9164
Std Err of Slope:	0.2186
Correlation Coefficient:	0.969

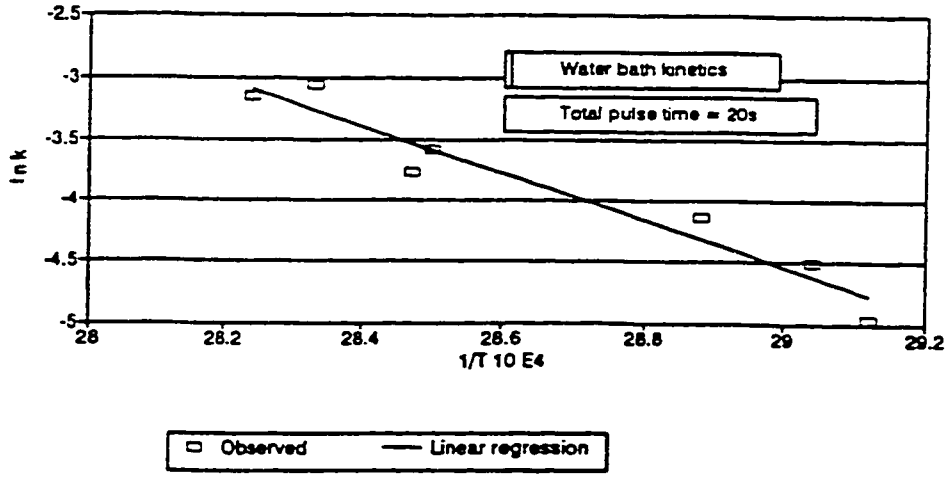


Figure A.13
Pseudo Arrhenius plot (20s):Hydrolysis of trans$(Co(en)_2Cl_2)^+$ in Microwave oven

Time (min)	Conc (mmoles)[1]	ln [PNBA]
0	0.050	-2.9960
25	0.0499	-2.9977
100	0.0479	-3.0386
120	0.0420	-3.170

[1] The concentration in mmoles was found in 200 ml of dilute acid mixture.

Linear Regression Analysis

Intercept: -2.97836

Slope: -0.00118

Std Err of Slope: 0.000559

Correlation Coefficient: 0.8306

Rate constant = $1.97 \times 10^{-5} \pm 0.93 \times 10^{-5}\ s^{-1}$

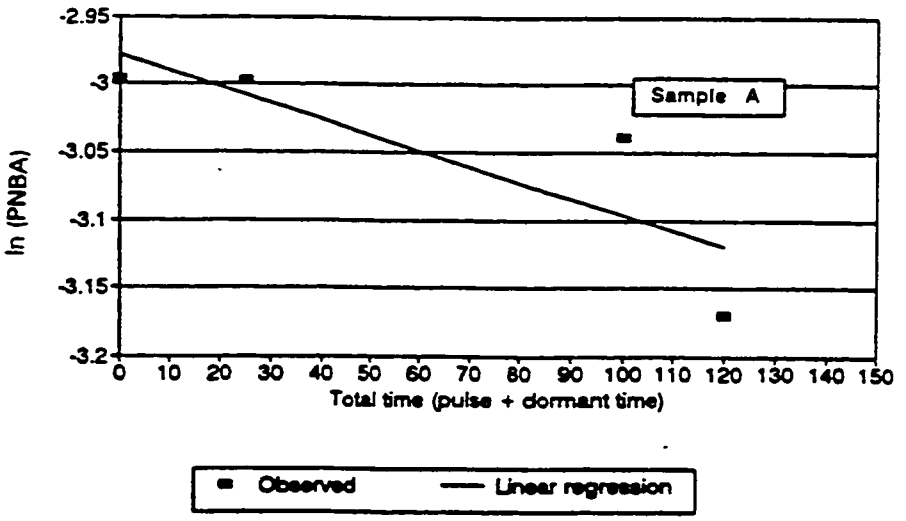


Figure A.14
Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid (5s pulse) (Sample A)

Time (min)	Conc (mmoles)[1]	ln [PNBA]
0	0.050	-2.9960
36	0.04866	-3.0229
75	0.04788	-3.0391
106	0.04745	-3.0481

[1] The concentration in mmoles was found in 200 ml of dilute acid mixture.

Linear Regression Analysis

Intercept: -3.0019

Slope: -0.00049

Std Err of Slope: 7.07E-05

Correlation Coefficient: 0.9795

Rate constant = $0.82 \times 10^{-5} \pm 0.12 \times 10^{-5}$ s^{-1}

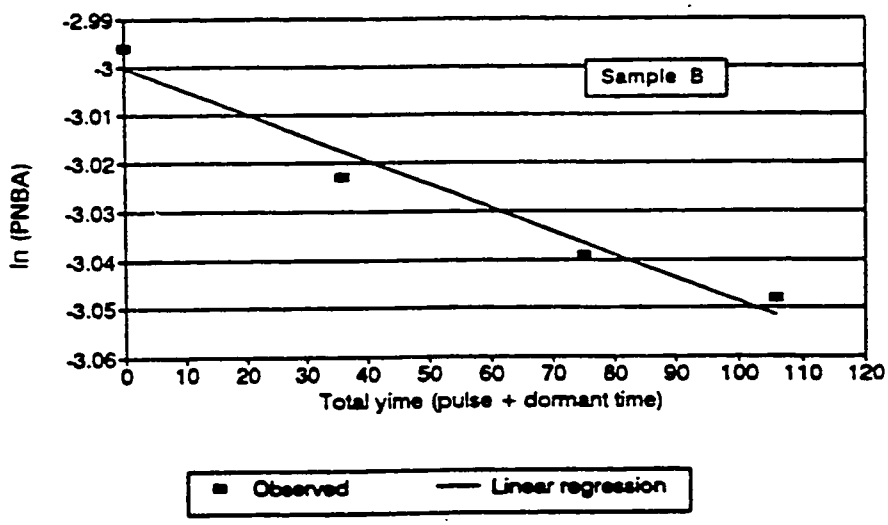


Figure A.15
Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid (5s pulse) (Sample B)

Time (min)	Conc (mmoles)[1]	ln [PNBA]
0	0.050	-2.996
45	0.0505	-2.986
105	0.0488	-3.020
150	0.0471	-3.055
195	0.0473	-3.051

[1] The concentration in mmoles was found in 200 ml of dilute acid mixture.

Linear Regression Analysis

Intercept:	-2.98545
Slope:	-0.00037
Std Err of Slope:	9.23E-05
Correlation Coefficient:	0.9160

Rate constant = $0.62 \times 10^{-5} \pm 0.15 \times 10^{-5}\ s^{-1}$

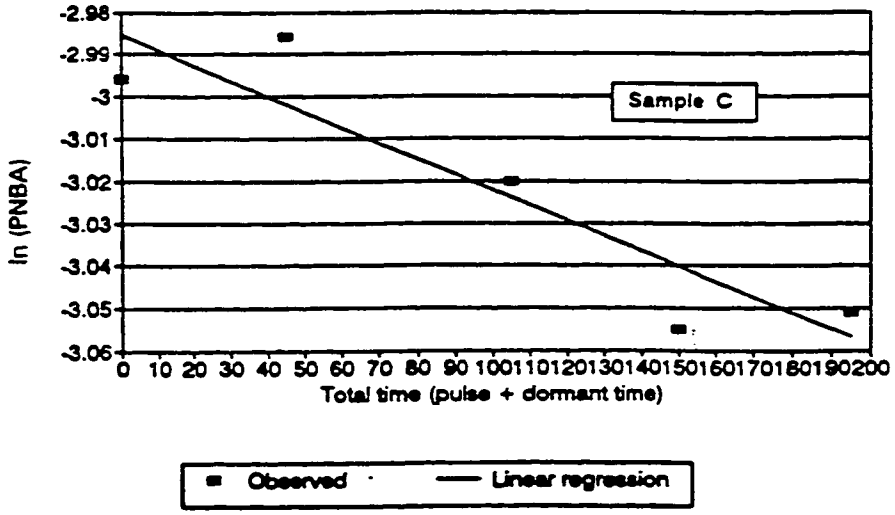


Figure A.16
Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid (5s pulse) (Sample C)

Time (min)	Conc (mmoles)[1]	ln [PNBA]
0	0.050	-2.996
50	0.0489	-3.0180
75	0.0442	-3.1190
105	0.0426	-3.1560
135	0.0387	-3.2520

[1] The concentration in mmoles was found in 200 ml of dilute acid mixture.

Linear Regression Analysis

Intercept:	-2.96715
Slope:	-0.00194
Std Err of Slope:	0.00034
Correlation Coefficient:	0.9563

Rate constant = $3.2 \times 10^{-5} \pm 0.57 \times 10^{-5}$ s^{-1}

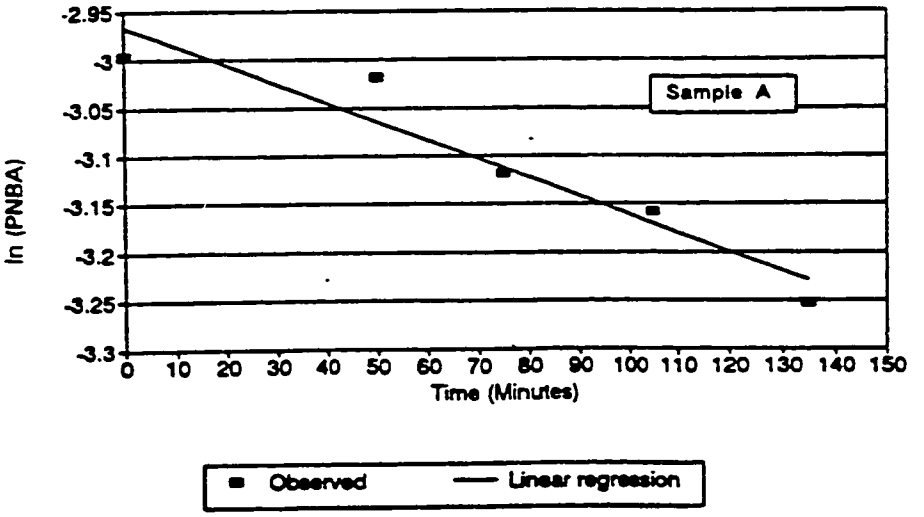


Figure A.17
Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid (7s pulse) (Sample A)

Time (min)	Conc (mmoles)[1]	ln [PNBA]
0	0.050	-2.9960
40	0.0455	-3.090
60	0.0424	-3.1610
80	0.0375	-3.2834
100	0.0317	-3.4514

[1] The concentration in mmoles was found in 200 ml of dilute acid mixture.

Linear Regression Analysis

Intercept: -2.94965

Slope: -0.0044

Std Err of Slope: 0.000762

Correlation Coefficient: 0.9579

Rate constant = $0.73 \times 10^{-5} \pm 0.13 \times 10^{-5}$ s^{-1}

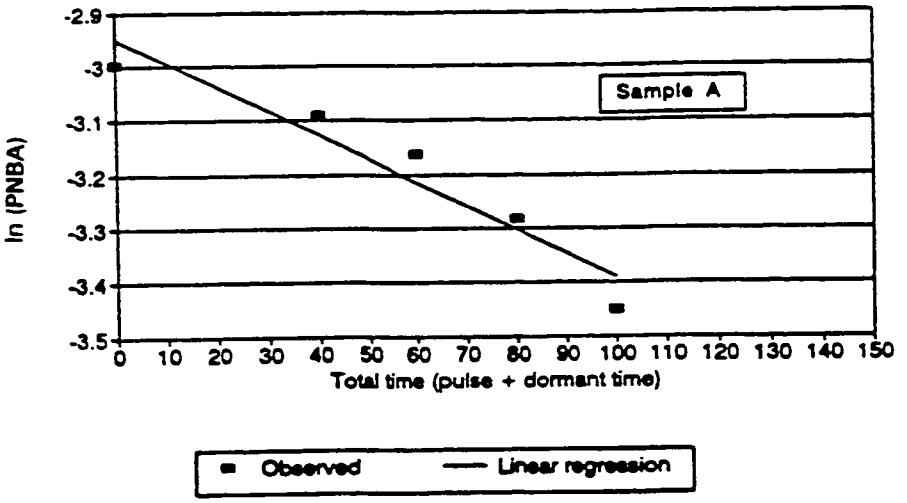


Figure A.18
Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid (10s pulse) (Sample A)

Time (min)	Conc (mmoles)[1]	ln [PNBA]
0	0.050	-2.9960
60	0.0438	-3.1281
80	0.0399	-3.2214
100	0.0343	-3.3726

[1] The concentration in mmoles was found in 200 ml of dilute acid mixture.

Linear Regression Analysis

Intercept:	-2.96971
Slope:	-0.0035
Std Err of Slope:	0.000792
Correlation Coefficient:	0.9523

Rate constant = $5.8 \times 10^{-5} \pm 1.3 \times 10^{-5}$ s^{-1}

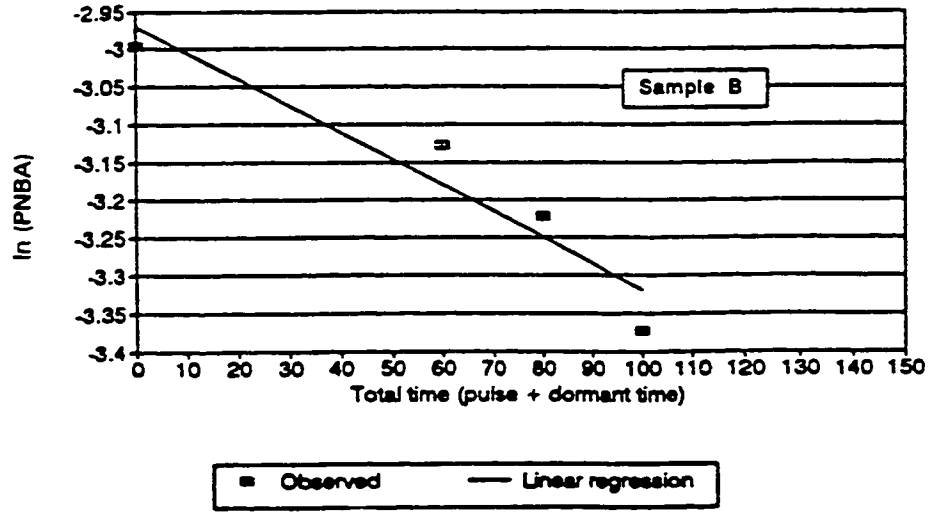


Figure A.19
Oil bath rate constant studies: Nitration of p-Nitrobenzoic acid (10s pulse) (Sample B)

TABLE A.3

Microwave oven rate constant studies : Nitration of p-nitro benzoic acid

Pulse heating cycle	Duty cycle(%)	Rate constant (s^{-1})	Ink	Extrapolated temp(K)[a] .	Effec temp.
10s pulse	5.3	$6.60 \times 10^{-5} \pm 1.30 \times 10^{-6}$	-9.626	357(348)	371
7s pulse	3.7	$3.20 \times 10^{-5} \pm 5.70 \times 10^{-6}$	-10.35	345 (335)	362
5s pulse	2.7	$1.36 \times 10^{-5} \pm 1.3 \times 10^{-5}$	-11.205	328(321)	350

Temp (K) 1/T 10 E4	ln k
30.49	-11.21
28.99	-10.35
27.95	-9.63
25.58	-7.73

Linear Regression Analysis

Intercept:	10.4780
Slope:	-0.7152
Std Err of Slope:	0.0417
Correlation Coefficient:	0.996

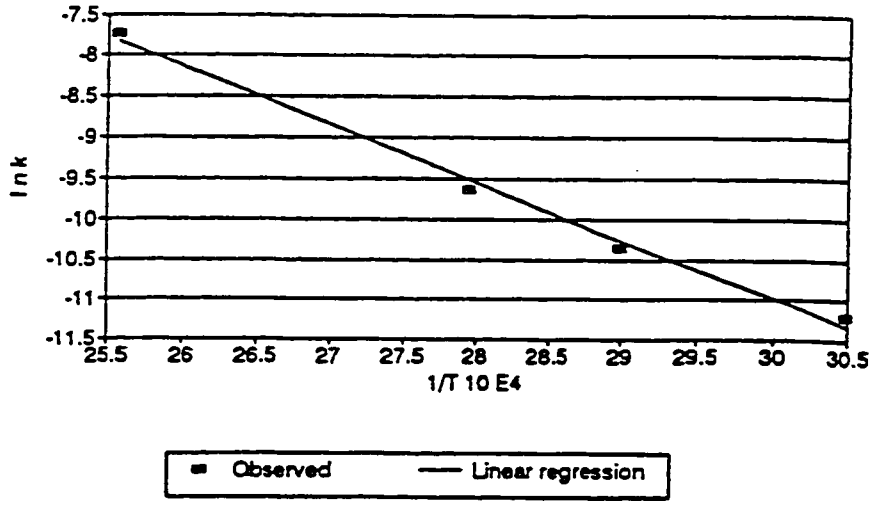


Figure A.20
Pseudo Arrhenius plot: Nitration of p-Nitrobenzoic acid

REFERENCES

REFERENCES

1. Lead poisoning: Strategies for prevention, Baltimore: Maryland Department of Health and Mental Hygiene (1984).
2. Deflora, B.M., Serra, D., Mutant Res., 238, 99 (1990).
3. Rab, S., J. PMA., 1990, 40 (1990).
4. Gorsuch, T.T., "The Destruction of Organic matter", Pergamon Press: New York,(1990).
5. Anderson, J., At. Abs.Newsl., 14, 129 (1972).
6. Official Method of Analysis, Publisher: Association of Official Analytical Chemists (1985).
7. Analytical Methods Committee, Analyst, 92, 403 (1967).
8. Egaas, E., and Julsham, K., At. Abs. Newsl. 17, 35 (1975).
9. Official Method of Analysis. Publisher: Association of Official Analytical Chemists (1987).
10. Blanchar, R.W., Rehmand, G., Caldwell, A.C., Soil Sci. Soc. Am. Proc., 29, 71 (1965).
11. Butters, B., and Chenery, E.M., Analyst, 84, 239 (1959).
12. ASTM Publication, 564.
13. Perkin Elmer A.A.S. Instruction Manual (1977).
14. Abu-Samra, A., Morris, J.S., Koirtyohann, S. R., Anal. Chem., 47, 1475 (1975).
15. Gedye, R., Smith, F., Westaway, K., Ali, H., Baldisera, L., Laberge, L., Tetrahedron, Lett., 27 (3), 279 (1986).
16. Mahan, K.I., Foderaro, T.A., Garza, T.L., Martinez, R. M., Maroney, G. A.,

17. Kingston, H. M., Jassie, L. N., Fassett, J. D., Presented at the 190th National Meeting of the American Chemical Society, Chicago, IL., Paper No. 10., September (1985).

18. Veillon, C., Patterson, K. Y., Kingston, H. M. , Presented at the 28th Rocky Mountain Conference, Denver, CO., Paper No. 8., August (1986).

19. Veillon, C., Patterson, K. Y., Kingston, H. M., Presented at the 13th FACSS, St. Louis, MO., Paper No. 689, September (1985).

20. Kingston, H.M. and Jassie, L.B., "Introduction to Microwave Sample Preparation" ACS Professional Reference Book, Washington, D.C., 1st Edition (1988).

21. Encyclopedia of Analytical Sciences, Academic Press (1995).

22. Vandecasteele, C., and Block, C.B., "Modern Methods for Trace Element Determination" John Wiley and Sons Ltd. (1993).

23. Sloterdijk H., "Toxic chemicals in the St-Lawrence", Can-Am Conference, Montreal, Quebec, June (1984).

24. Environment Canada Technical Report (EPS 5/HA/1), April (1985).

25. Environmental Contaminants Committee of Environment Canada and Health and Welfare, "Background to the Regulation of PCBs in Canada", Report to the Task Force on PCBs, Ottawa, Technical Report 76-11 (1976).

26. Miller, S., Environ. Sci. Technol., 17, 11a (1983).

27. Josepson, J., Environ. Sci. Technol., 17, 124a (1983).

28. Durfee, R., Proc. Natl. Conf. PCB, 103 (1975).

29. Environmental Contaminants Committee of Environment Canada and Health and Welfare Task Force, Report 76 - 1 of the Task Force on PCBs, Ottawa, Technical Report 76-1 (1976).

30. Duffy, J.R. and Wong, N. J., Agr. Food Chem., 15, 457 (1967).

31. Saha, J.G., Craig, C.H., Janzen, W.K., J. Agr. Food Chem., 16 (4), 617 (1968).

32. Perring, F.H., Mellandy, K., "Ecological Effects of Pesticides", Academic Press, Inc., 1st Edition, New York, N.Y. (1977).

33. Porter, M.L., Young, S.J.V., and Burke, J.A., J. Assoc. Off. Anal. Chem., 53, 1300 (1970).

34. Mulhern, B.M., Cromartie, E., Reichel, W.L., and Belisle, A.A., J. Assoc. Off. Agric. Chem., 54, 548 (1971).

35. Collins, G.B., Holmes, D.C., Jackson, F.J., J. Chromatography, 71, 443 (1972).

36. Holden, A.V., and Marsden, K., J. Chromatography, 44, 481 (1969).

37. Encyclopedia of Physical Sciences and Tech., 15, Academic Press (1992).

38. Not used

39. Webb, R.G., McCall, A.C., J. Chromatogr. Sci., 11, 366 (1973).

40. Holden. A.V., PCB Conference 11, Stockholm, Swedish Environmental Protection Board, Publications : 4E, 22 - 33.

41. Jensen, S., Johnels, A.G., Olssen, M., Otterlind, G., Nature, 224, 247 (1969).

42. Paus, P.E., At. Abs. Newsl., 11, 129 (1972).

43. Leonard, E.N., At. Abs. Newsl., 10, 84 (1971).

44. Adrian, W.J., Analyst, 98, 213 (1973).

45. Same as ref #14

46. Barret, P., Davidowski, L.J., Penaro, K.W., and Copeland, T.R., Anal. Chem., 50, 1021 (1978).

47. Nadkarni, R.A., Anal. Chem., 56, 2233 (1984).

48. Matthes, S.A, Farrel, F.A., and Mackie, A.J., Tech. Prog. Rep. - U.S., Bur. Mines. (1983).

49. Perkin -Elmer Instruction Manual for Model 503 (1973).

50. Dokiya, Y., Yamamoto. Y and Fuwa, K., Spectroscopy Letters, 8, 759 (1975).

51. Allen, S., Anal. Biochemistry, 346-353 (1984)

52. National Bureau of Standards, Standard Reference Material, 1573 (1976).

53. Chao, S.S., and Picket, E.E., Anal.Chem., 52, 335-339 (1980).

54. American Society for Testing Materials (ASTM), Annual Book of ASTM Standards (1988).

55. Snyder, D., Bulletin of Environmental Contamination and Toxicology, 6, 385 (1971).

56. Stalling, D.L., Tindle, R.C., Johnson, J.L., J. Assoc. Off. Anal. Chem., 55 (1972).

57. Veierov, D., Aharonson, N., J. Assoc. Off. Anal. Chem., 63, 202 (1980).

58. Murphy, P.G., J. Assoc. Off. Anal. Chem., 55, 1360 (1972).

59. Stevens, A., Budde, A.L., Bellar, T.A., Anal. Chem., 57, 2452 (1985).

60. Environ., Sci., Technol., 25, 812 (1991).

61. Sedlak, D.L., Dean, K.E., Armstrong, D.E., Andren, A.W., Environ., Sci., Technol., 25, 1935 (1991).

62 Choudary, G., "Humic Substances", Science Publishers, London (1994).

63. Mingos, M.P., Baghurst, D.R., Chem. Soc. Rev., 20, 1 (1991).

64. Gedye, R.N., Smith, F., Westaway, K.C., Can. J. Chem., 66, 17 (1988).

65. Mingos, M.P., Chemistry and Industry., 18, 596 (1994).

66. Laurent, R., J. Org. Chem., 57, 7009 (1992).

67. Armstrong, B.F and Neas, E.D., Separation Sci. and Tech., 25, 2007 (1990).

68. Gedye, R.N., Rank, W., Westaway, K.C., Can. J. Chem., 69, 706 (1991).

69. Mingos, M.P and Baghurst, D.R., J. Chem. Soc. Chem. Commun., 9, 674 (1992).

70. Bond, G., Moyes, R.B., Pollington, S.D., Whan, D.A., Chemistry and Industry, 18, 686 (1991).

70b. Sykes, A.G., "Kinetics of inorganic reactions", Pergamon Press, 1st Edition, London (1966)

71. Langford, C.H and Gray, H. E., " Ligand substitution processes" , Benjamin, W.A, Inc., New York, N.Y. (1965).

72. Basolo, F., Stone, B.D., Pearson, R.G., J. Am. Chem. Soc., 76, 3 079 (1954).

73. Pearson, R.G., Moore, Y.W., Inorg. Chem., 3, 1334 (1964).

74. Adamson, A.W., Wilkins, R.G., J. Am. Chem. Soc., 76, 3379 (1954).

75. Ettle, G.W., Johnson, C.H., J. Chem. Soc., 1490 (1940).

76. Langford, C.H., Johnson, M.P., J. Am. Chem. Soc., 86, 229 (1964).

77. Basolo, F., Pearson, R.G., " Mechanisms of Inorganic Reactions", John Wiley & Sons Inc., New York, N.Y., 2nd Edition (1967).

78 Sykes, P., "A guide book to mechanisms in Organic Chemistry" Longman., 6th Edition, New York, N.Y (1986).

79 Bailor, J.C., Inorganic synthesis, 2, 222 (1946).