DUBLIN CITY UNIVERSITY

Ollscoil Chathair Bhaile Átha Cliath

An Investigation of Novel Extraction Procedures used in the Preconcentration of Polycyclic Aromatic Hydrocarbons from Environmental Samples.

A thesis presented for the degree of Doctor of Philosophy

at

Dublin City University

by

Stephen Fitzpatrick B. Sc.

under the supervision of Dr. Mary Meaney

School of Chemical Sciences

September 1998

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of PhD is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

Signed: Stephen Fitzpatrick Date: 15/10/98

Stephen Fitzpatrick

Dedication:

Irene

Quotation:

"Success is Counted Sweetest

By those who ne'er Succeed

To comprehend a nectar

Requires sorest need"

Emily Dickinson 1878

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I wish to acknowledge a large number of people who have made this body of work possible.

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Table of Contents

Title Page..... i

Declaration..... ii

Dedication..... iii

Quotation..... iv

Acknowledgements..... v

Table of Contents..... vi

Abbreviations..... xv

Abstract..... xviii

Chapter 1.0 The Extraction of Polycyclic Aromatic Hydrocarbons from the Environment..... 1

1.1.Introduction..... 2

1.2.Sample types..... 2

1.2.1. Soils..... 2

1.2.1.1. Liquid Extraction..... 3

1.2.1.2.Supercritical Fluid Extraction..... 7

1.2.1.3.Other Extractive Techniques..... 9

1.2.2. Air Particulates..... 10

1.2.2.1. Soxhlet Extraction..... 11

1.2.2.2. Sonication..... 15

1.2.2.3. Supercritical Fluid Extraction..... 17

1.2.2.4. Other Extractive Techniques..... 20

1.2.3. Other Particulate Matter..... 22

1.2.4. Fly Ash..... 26

1.2.4.1. Sonication..... 26

1.2.4.2. Soxhlet Extraction..... 28

1.2.4.3. Other Extractive Techniques..... 29

1.2.5. Sediment..... 30

1.2.6. Miscellaneous Samples..... 32

1.2.7. Food Samples..... 37

1.2.8. Aqueous Samples..... 47

1.3. Conclusions..... 59

1.4. Bibliography..... 61

Chapter 2.0 An Examination of Offline Extraction procedures used for the enrichment of PAHs at Trace Concentrations from Aqueous Samples..... 70

2.1.Introduction..... 71

2.2. Chromatographic Method..... 71

2.2.1. Experimental..... 72

2.2.1.1. Apparatus..... 72

2.2.1.2. Materials..... 72

2.2.1.3. Optimum Conditions..... 72

2.2.2. Validation..... 73

2.2.2.1. Specificity..... 73

2.2.2.2. Linearity..... 74

2.2.2.3. Precision..... 75

2.2.2.4. Limit of Detection..... 76

2.2.2.5. Limit of Determination..... 76

2.3. Offline Extractive Procedures..... 77

2.3.1. Offline Liquid / Liquid Extraction..... 77

2.3.1.1. Experimental..... 78
2.3.1.1.1. Apparatus..... 78
2.3.1.1.2. Materials..... 78
2.3.1.1.3. Extraction Procedure..... 78
2.3.1.2. Results..... 79
2.3.1.3. Discussion..... 80
2.3.2. Offline Solid-phase Extraction..... 80
2.3.2.1. Experimental..... 81
2.3.2.1.1. Apparatus..... 81
2.3.2.1.2. Materials..... 81
2.3.2.2. Optimisation of Extractive Procedure..... 81
2.3.2.2.1. Sample Loading Flow Rate..... 82
2.3.2.2.2. Sample Elution Flow Rate..... 84
2.3.2.2.3. Volume of Solvent used..... 85
2.3.2.2.4. Reusing SPE Cartridges..... 86
2.3.2.2.5. Mobile Phase as the eluent for Offline Extraction..... 87
2.4. Conclusions..... 88
2.5. Bibliography..... 90

Chapter 3.0 Online Solid Phase Extraction as a Preconcentration Technique in the enrichment of PAHs from Aqueous Samples.. 91

3.1. Introduction..... 92
3.2. Experimental..... 94
3.2.1. Apparatus..... 94
3.2.2. Materials..... 97

3.2.3. Methods..... 97

3.2.3.1. Sample Preparation..... 97

3.2.3.2. Chromatographic Procedures..... 98

3.2.3.3. Sample Loading..... 98

3.3. Online Extraction procedure Results and Discussion..... 98

3.3.1. Precolumn Selection..... 98

3.3.2. Mass Loadability..... 101

3.3.2.1. Increasing Volume..... 102

3.3.2.1.1. 100ppb Five PAH mix..... 102

3.3.2.1.2. 10ppb Five PAH mix..... 105

3.3.2.1.3. 1ppb Five PAH mix..... 107

3.3.2.1.3.1. Low Flow Rate and Low Volume Study..... 108

3.3.2.1.3.2. High Flow Rate and High Volume Study..... 110

3.3.2.1.4. 100ppt Five PAH mix 112

3.3.2.2. Increasing Concentration..... 113

3.3.3. Limit of Detection Study 115

3.3.3.1. Flow Rate Investigation..... 115

3.3.3.2. Interferents from H_2O Blank Injections..... 116

3.3.3.3. Measurement of LOD for the System..... 117

3.3.3.4. Comparison of LOD with other Techniques..... 117

3.3.4. Reproducibility Study..... 118

3.4. Conclusions..... 119

3.5. Bibliography..... 122

Chapter 4.0 An Investigation of Matrix Solid Phase Dispersion (MSPD) as a Technique in the Simultaneous Extraction of Five PAHs from Spiked Milk Samples..... 124

4.1. Introduction..... 125

4.1.1. Process Description of MSPD..... 125

4.1.2. Animal Tissue Samples..... 125

4.1.3. Bovine Milk Samples..... 128

4.1.4. Fish Tissue Samples..... 130

4.1.5. Other Sample Matrices..... 131

4.2. Experimental..... 132

4.2.1. Chemicals..... 132

4.2.2. Instrumentation..... 132

4.2.3. Methods..... 132

4.2.3.1. Analytical Methods..... 132

4.2.3.1.1. Standard Preparation..... 133

4.2.3.1.2. HPLC Conditions..... 133

4.2.3.1.3. C_{18} Material Preparation..... 133

4.2.3.2. Sample Preparation..... 133

4.2.3.3. Calculation of Results..... 134

4.3. Results and Discussion..... 134

4.3.1. Optimisation of the amount of C_{18} packing used..... 135

4.3.1.1. 2ppm five PAH mixed spike..... 135

4.3.1.2. 500ppb five PAH mixed spike..... 136

4.3.2. Optimisation of Volume for the Milk Spike Sample..... 138

4.3.2.1. 100ppb five PAH mixed spike..... 138

4.3.2.2. 500ppb five PAH mixed spike..... 140

4.3.3. Optimisation of the Volume of ACN used to Elute the PAHs..... 141

4.3.4. An Investigation of the Reproducibility of the System..... 142

4.3.5. An Investigation of Different Packing Materials..... 143

4.3.6. An Investigation of the Addition of an Extra Solvent Wash..... 145

4.4. Conclusions..... 145

4.5. Bibliography..... 147

Chapter 5.0 An Evaluation of ATR/FTIR as a Technique for the Analysis of Polycyclic Aromatic Hydrocarbons in Aqueous Solutions..... 150

5.1. Introduction..... 151

5.1.1. The Theory of ATR..... 151

5.1.2. General Applications..... 152

5.1.3. Applications with Chlorinated Hydrocarbons..... 154

5.2. Experimental..... 159

5.2.1. Materials..... 159

5.2.1.1. Chemicals..... 159

5.2.1.2. Instrumentation..... 159

5.2.2. Methods..... 160

5.2.2.1.Polymer Coating..... 160

5.2.2.2.Generation of Spectra..... 160

5.3. Results and Discussion..... 161

5.3.1. Choice of Polycyclic Aromatic Hydrocarbon..... 161

5.3.2. Uncoated ATR/FTIR..... 162

5.3.3. Water Absorption..... 162

5.3.4. Types of Polymers in coated ATR/FTIR..... 163

5.3.4.1.Polyisobutylene..... 163

5.3.4.2.Teflon..... 165

5.3.5. Optimisation of Polyisobutylene Coating Procedures..... 166

5.3.5.1..Concentration of Coating Solution..... 166

5.3.5.1.1. 100ppm Fluoranthene..... 166

5.3.5.1.2. 10ppm Fluoranthene..... 167

5.3.5.2.Reusability..... 169

5.3.5.2.1. 10ppm Fluoranthene..... 170

5.3.5.2.2. 100ppm Fluoranthene..... 171

5.3.5.3.Physical coating procedure..... 172

5.3.5.3.1. Spread coating technique..... 172

5.3.5.3.2. Spin coating technique..... 178

5.3.6. Linearity Study..... 187

5.3.7. Limit of Detection..... 191

5.3.7.1.Static System..... 191

5.3.7.2.Dynamic System..... 192

5.4. Conclusions..... 193

5.5. Bibliography..... 195

Chapter 6.0 An Evaluation of Polymer Coated ATR/FTIR as a Technique for Analysing Four PAHs Simultaneously in Aqueous Solutions.....197

6.1. Introduction..... 198

6.2. Experimental..... 199

6.2.1. Chemicals..... 199

6.2.2. Materials..... 199

6.2.3. Instrumentation..... 199

6.2.4. Methods..... 200

6.2.4.1. Sample Loading..... 200

6.2.4.2. Spectrum Generation..... 200

6.2.4.3. Spiking Standards and Sample Preparation..... 201

6.3. Results and Discussion..... 201

6.3.1. The effects of H_2O/ACN Composition..... 201

6.3.2. Integration Techniques using 100 ppm Naphthalene..... 203

6.3.2.1 Manual Integration..... 205

6.3.2.2 Fixed Wavelength Integration..... 206

6.3.2.3 The First Derivative Technique..... 208

6.3.2.4 Kubelka Munk algorithm with manual integration..... 211

6.3.2.5 Kubelka Munk algorithm with fixed wavelength integration..... 214

6.3.2.6 Comparison of Correlation Data..... 215

6.3.3. The application of the quantitative technique to other PAHs..... 218

6.3.3.1 The enrichment profile for acenaphthene..... 218

6.3.3.2 The enrichment profile for fluorene..... 221

6.3.3.3 The enrichment profile for phenanthrene..... 223

6.3.4. Simultaneous analysis of the four PAHs..... 227

6.3.4.1. The optimum enrichment time.....227

6.3.4.2. Calculation of the Line Equations.....228

6.3.4.3. Difficulties with simultaneous analysis..... 228

6.3.4.4. Spiking Experiments.....231

6.3.4.4.1. High Concentration Experiments..... 231

6.3.4.4.2. Low Concentration Experiments..... 234

6.4. Conclusions.....237

6.5. Bibliography 240

Chapter 7.0 General Conclusions.....241

Appendices..... 245

Abbreviations:

1,2 DCB	1,2 Dichlorobenzene
1,2,4,TCB	1,2,4 Trichlorobenzene
ACN	Acetonitrile
ASW	Artificial Sea Water
ATD	Adsorption Thermal Desorption
ATR	Attenuated Total Reflectance
B[a]p	Benzo[a]Pyrene
CDx	Cyclodextrin
CF	Choroform
CHC	Chlorinated Hydrocarbons
CISS-RTL	Cyclodextrin Induced Solid Surface Room Temperature Luminescence
CV	Coefficient of Variation
DAD	Diode Array Detection
E/Pco	Ethylene/Propylene Copolymer
ED	Electrochemical Detection
EFOCS	Evanescent Fibre Optic Chemical Sensor
EWS	Evanescent waveguide
FID	Flame Ionisation Detector
FL	Fluorescence
FMW	Focussed Microwave Assisted Extraction
FTIR	Fourier Transform Infrared
GC	Gas Chromatography
GPC	Gel Permeation Chromatography
HPLC	High Performance Liquid Chromatography

K-D	Kuderna-Danish
LDPE	Low Density Polyethylene
LOD	Limit of Detection
MAE	Microwave Assisted Extraction
MASE	Microwave Assisted Solvent Elution
MCB	Monochlorobenzene
MIR	Mid Infrared
MPE	Mechanical Plunging Extraction
MS	Mass Spectroscopy
MSPD	Matrix Solid Phase Dispersion
MW	Molecular Weight
NaTC	Sodium Taurocholate
NBS	National Bureau of Standards
NIST	National Institute of Standards and Technology
OSPE	Online Solid Phase Extraction
PACs	Polycyclic Aromatic Compounds
PAHs	Polycyclic Aromatic Hydrocarbons
PBD	1,2 Polybutadiene
PDAD	Photo Diode Array Detector
PDMS	Polydimethylsiloxane
PIB	Polyisobutylene
PID	Photo Ionisation Detection
PLS	Partial Least Squares
ppb	Parts Per Billion
ppm	Parts per Million

ppt	Parts per Trillion
PUF	Polyurethane Foam
PVC	Polyvinyl Chloride
R114	1,2 Dichlorotetrafluoroethane
RPM	Revolutions Per Minute
RSD	Relative Standard Deviation
SDS	Soduim Dodecyl Sulfate
SFC	Supercritical Fluid Chromatography
SFE	Supercritical Fluid Extraction
SPME	Solid Phase Microextraction
TCE	Trichloroethylene
TDL	Tuneable Diode Laser
TeCE	Tetrachloroethylene
TLC	Thin Layer Chromatography
USEPA	United States Environmental Protection Agency
UV	Ultra Violet
ZnSe	Zinc Selidine

AN INVESTIGATION OF NOVEL EXTRACTION PROCEDURES USED IN THE PRECONCENTRATION OF POLYCYCLIC AROMATIC HYDROCARBONS FROM ENVIRONMENTAL SAMPLES.

Stephen Fitzpatrick, School of Chemical Sciences, DCU.

Abstract :

The accurate determination of PAHs at trace levels in environmental matrices is a complex problem and as a result, methods for the preconcentration and determination of PAHs are continuously under investigation. Techniques are required which are sufficiently sensitive and selective to enable the quantitation of PAHs at lower levels than ever before.

The importance of trace determination of PAHs in environmental samples is reviewed in chapter 1. Various sample matrices, extractive techniques and analytical methods were examined for the enrichment and determination of PAHs in the environment. The importance of accurate and complete extraction from various matrices is discussed. Particular mention was made to the universal problem of losses of low MW PAHs with the various techniques reviewed.

The extraction of five low MW PAHs compounds from aqueous samples is reported on in chapter 2 and chapter 3. Two standard techniques liquid/liquid extraction and solid phase extraction operated in offline mode were reviewed and applied to the first five PAHs recommended as priority pollutants by the USEPA.

A more novel extractive procedure was examined in chapter 3 for the same group of compounds. This was online solid phase extraction. In this case the compounds were extracted from the aqueous solution and transferred online to the analytical system by

the actuation of a switching valve. This system was then compared and contrasted with extractive systems previously described.

The concept of Multi Solid Phase Dispersion was reviewed in Chapter 4. It is a unique system which takes advantage of the physiochemical properties of C_{18} packing material. These properties allow the material interact with biological matrices in such away as to extract organic material preferentially. This extractive procedure was then applied to the extraction of PAHs from bovine milk samples.

The application of ATR/FTIR to the enrichment and detection of compounds is reviewed in chapter 5. A system was developed which allowed the detection of fluoranthene from aqueous samples.

The ability of the system to determine PAHs was assessed in chapter 6. Determination was achieved through the application of various mathematical algorithms and the effectiveness of the algorithms was compared. The system was then used to quantify a number of PAHs simultaneously.

Chapter 1.0

The Extraction of Polycyclic Aromatic Hydrocarbons from the Environment.

1.1. Introduction

The rapid growth in industry over the last century and particularly in the last several decades has resulted in more critical evaluations of environmental health hazards. Polycyclic aromatic hydrocarbons (PAHs) are the largest class of chemical carcinogens known today and as a result have become one of the most widely studied group of chemicals [1]. Polycyclic aromatic compounds (PACs) are ubiquitous in the environment and stem from both natural and anthropogenic sources, the latter by far being the major contributor [2]. PAHs are produced by the incomplete combustion of fossil fuels and other organic material from industrial processes and occur as particulates in the atmosphere [3].

The various extraction techniques used to remove these compounds from the environment depend on a number of factors, the nature of the sample investigated, the concentration of the compounds of interest, the presence of interfering compounds, the nature of the polycyclic aromatic compound being studied and in the case of particulates, the way they were collected. For the purpose of reviewing the various extraction techniques the papers have been divided on the basis of the nature of the sample studied.

1.2. Sample Types

1.2.1. Soil Samples.

Soil samples are of particular interest when evaluating the effects PAHs have on the environment, as the concentrations in the soil give a valuable assessment of the dangers to ground water by waste materials [1]. Soils can contain PAHs, their alkylated homologues and metabolites. They can also reflect the contamination levels

of PAHs in airborne particulates. The PAH content in soil samples is considered to be an indicator of the pollution level of PAHs in that particular environment [4].

The determination of PAHs in soil samples has been carried out using various modes of chromatography. High Performance Liquid Chromatography (HPLC) with Ultraviolet (UV) or Fluorescence (FL) detection has been widely used, other detection techniques have also been used such as Photo Diode Array (PDAD).

Gas Chromatography (GC) with Flame Ionisation Detection (FID) or Mass Spectrometry (MS) has also been used. Recently other novel techniques have been used such as Supercritical Fluid Chromatography (SFC). The most critical step in all the various techniques described is the extraction of the PAH from the sample matrix. This has been achieved a number of ways, liquid extraction, soxhlet and sonication, solid phase extraction and more recently supercritical fluid extraction (SFE) and micellular extractions have been investigated.

1.2.1.1 Liquid Extraction

The most common extraction procedures for liquid extraction of soils are soxhlet, ultrasonic and homogenised methods. Each of the techniques is evaluated based on the efficiency of the recovery, the processing time and the ease of use.

Auer et al [5] used liquid extraction with 2-methyoxyethanol and mechanical agitation to remove PAHs from soil samples. This solvent was used as it allows the omission of a drying step to remove H_2O prior to the extraction and thus reduces the possibility of losses due to evaporation of lower molecular weight (MW) PAHs. The PAHs investigated were naphthalene, phenanthrene, anthracene, fluoranthene and pyrene. Sample clean up was carried out on a C_{18} cartridge with elution with pentane and final

N_2 blow down. Analysis was carried by GC-FID.

The recoveries were between 80-90% except for naphthalene, which was 66%. Limits of Detection (LOD) were quoted at 15-35µg/Kg. Special attention was given to designing a spiking technique which simulated the natural incorporation of these compounds and allows 'real' recovery rates to be determined. They concluded that low recovery rates of naphthalene were associated with its relatively high vapour pressure which implied losses were due to evaporation.

Coover et al [6] compared a homogenised method with a soxhlet method for two types of soils. The homogenised method simply required suspending soil in dichloromethane in a tissumizer for 45 seconds. Soxhlet involved using $300cm^3$ dichloromethane for 16hrs with 60 solvent cycles. They concluded that soxhlet extraction gave significantly higher recoveries in general for 15 PAHs from acenaphthylene to indeno[123-cd]pyrene but the homogenised method is quicker and simpler.

Aamot et al [7] successfully applied ultrasonic extraction with dichloromethane to soil samples. Clean up was carried out using silica columns. They recorded recoveries of 66-90% for nine PAHs, naphthalene, biphenyl, acenaphthene, fluorene, phenanthrene, fluoranthrene, pyrene, chrysene, triphenylene and benzo[a]pyrene. It was concluded that 11-34% of PAHs were lost during the volume reduction step after the extraction and clean up procedure and that liquid/liquid partitioning proved ineffective at removing interferents.

Rose et al [8] reported using sonication for the extraction of soil samples with toluene, solvent replacement with propanol followed by clean-up on an aminopropyl/octyldecyl column and analysis by HPLC-PDAD. They reported good

recoveries for spiked samples 90-100% for anthracene to indeno[123-cd]pyrene.

Kicinski et al [9] used a combination of liquid and solid phase extraction to extract PAHs. The soil was first extracted by soxhlet or sonication in various solvents, evaporated and then reconstituted with propanol. This was then treated to solid phase extraction on C_{18}, Si or CN packing. Recoveries varied from 91-94% for the 13 National Bureau of Standards (NBS) PAHs, fluorene to indeno[123cd] pyrene. They also noted low recoveries for naphthalene, acenaphthylene and acenaphthene. They concluded that these low recoveries were related to evaporation and the highest recoveries were achieved with a combination of C_{18} and CN packings.

Spitzer et al [10] investigated different solid adsorbents for the separation of PAHs from contaminants prior to analysis. The sample was first extracted with toluene by soxhlet extraction. They proposed a one step clean up using adsorption chromatography on XAD-2 resin as being the most suitable. They make no reference to recoveries.

Czuczwa et al [11] used a combination of sonication and gel permeation chromatography for soil samples, it was noted that irreversible adsorption was not a problem in this method. Recoveries were between 89-96% for the six PAHs investigated, fluorene, pyrene, benzo[a]anthracene, benzo[a]pyrene, perylene and benzo[ghi]perylene.

Wenclawiak et al [12] compared SFE with a soxhlet method. They concluded that the SFE results were comparable to soxhlet. The soil sample was air dried and dynamically extracted with CO_2. They reported low recoveries for naphthalene, fluorene and phenanthrene, 30% approximately. They concluded that losses due to evaporation had occurred.

Tanaka et al [4] addressed a problem which is apparent in all fluorimetry methods used in the analysis of PAHs in soil. This is the presence of many PAH isomers and their substituted homologues which have similar fluorescence properties and which result in significant overlap. This problem was overcome by using HPLC linked via a switching valve to a synchronous fluorimeter. The PAHs, benzo[a]pyrene and benzo[a]anthracene were extracted from soil samples using acetonitrile (ACN) ultrasonically. They concluded that scanning fluorimetry of PAHs provided an improvement in selectivity through the reduction of spectral complexity while maintaining the sensitivity advantage of the scanning fluorescence method.

Gonalez-Villa et al [13] applied ultrasonication with clean up on alumina micro columns to soil/sludge samples and analysed them by GC-MS. They quoted recoveries at 78-100% for naphthalene, acenaphthene, fluorene, anthracene and pyrene. They concluded that the proposed method could be used for the rapid and accurate analysis of PACs present in very low concentrations, parts per billion (ppb), in complex matrices. They also successfully applied an ultrasonic dichloromethane extraction followed by clean up on alumina SepPak cartridges, then analysis by GC-MS to soils which had been contaminated by PAHs as a result of bio-mass combustion [14]. A variety of two- to four-ringed PAHs covering a wide range of concentrations were detected in the samples. Recoveries for the extraction procedure are quoted for internal standards at 90-95%.

An inter-laboratory study was reported for 9 laboratories which used the same samples but different extraction techniques for the 16 PAHs listed by the United States Environmental Protection Agency (USEPA) as priority pollutants [15]. Soxhlet, sonication and mechanical agitation were used. They concluded that large variations

in recoveries observed for the extractions were due to the different techniques and the different clean up procedures used.

Hechler et al [16] compared soxhlet using cyclohexane and sonication using THF extractive processes to extract PAHs from soil samples. Analysis was conducted using HPLC-PDAD . The concluded that the efficiencies were the same for the extractive processes.

1.2.1.2. Supercritical Fluid Extraction

The use of supercritical fluids for the extraction of organic pollutants from environment samples has received increasing attention because of the potential to dramatically reduce the time required for sample extraction as well as eliminating the need for large volumes of liquid solvents.

Hawthorne et al [17] used SFE to recover native PAHs from railroad bed soil and petroleum waste sludge. Recoveries were compared using different SFE solvents $CHClF_2$, N_2O, CO_2 and CO_2/methanol (MeOH). They concluded that $CHClF_2$ consistently yielded the highest extraction efficiencies. Extraction rates of individual PAHs from petroleum waste sludge were similar with $CHClF_2$ but decreased with increasing MW using CO_2 and N_2O. Recoveries were compared to those obtained by $MeCl_2$ sonication. They concluded that while the mechanistic reasons for the superiority of $CHClF_2$ are not clearly understood, $CHClF_2$ apparently more successfully removes matrix water from wet samples to expose adsorbed analytes and is more capable of competing with the sample matrix for the analyte.

Recently they achieved dramatic increases in extraction efficiencies from soil samples by increasing the temperature at which the SFE takes place [18]. It was shown that

SFE with CO_2 at 200°C gave recoveries between 98 and 200%. Recoveries were based on using certified standards and comparing results to certified concentrations. This implied that normal methods do not extract PAH quantitatively.

Hawthorne et al [19] compared the use of various supercritical fluids $CHClF_2$, N_2O and CO_2 with respect to the extraction of PAHs from a railway bed soil sample. They concluded that quantitative extraction by SFE requires specific interactions between the extracting solvent and not only the analyte but also the sample matrix. After SFE the samples were analysed by GC-FID. It was concluded that $CHClF_2$ yielded higher extraction efficiency for PAHs then normal CO_2 extraction. This was also the case for other solid samples such as waste sludge and sediment samples.

Reimer and Suarez [20] also compared soxhlet and SFE extractive procedures for the extraction of native PAHs from soil samples. They concluded that recoveries for the PAHs from soil samples were low and variable. This was attributed to apparent matrix effects and cyrogenic grinding of the sample may improve the performance of the SFE system.

SFE was combined with spectrofluorometry to give a screening technique for PAHs in soil samples. The technique was applied to six PAHs, benzo[f]fluoroanthene, benzo[a]pyrene, chyrsene, pyrene, benzo[a]anthracene and benzo[ghi]perylene[21]. Recoveries were quoted at between 89 to 103% with R.S.D. of 10%. The technique was deemed to be semi-quantitative as the analytes were not separated from each other, quantitation was based on the spectral data. The main advantage of the technique was that it gave real time information about the extractive process.

1.2.1.3. Other Extractive Techniques

Huettenhain and Windrich [22] used medium pressure liquid extraction to extract five PAHs acenaphthene, phenanthrene, pyrene, chyrsene and benzo[a]pyrene (B[a]p) from soil samples. They concluded that the results they got were at least as good as traditional soxhlet extraction. Recoveries ranged from 97% for acenaphthene to 101% for B[a]P.

Dean et al [23] compared three extraction methods soxhlet, microwave assisted extraction (MAE) and SFE for the extraction of 16 priority pollutant PAHs from soil samples. The three methods were compared under a number of headings, analysis time, ease of operation and cost. They concluded that soxhlet extraction is still very viable because of its cheap cost and simplicity, however its main disadvantage is the length time required for each extraction. MAE was the fastest and it also yielded the highest recoverable amounts when acetone was used as the solvent. They noted that volatile PAHs were not problematic for this extraction process. Percentage recoveries were not calculated for any of the extractions.

Budzinski et al [24] compared the relatively new technique of focussed microwave assisted extraction (FMW) with traditional soxhlet extraction for the extraction of PAHs from soil samples. They concluded that the recoveries were all above 90% for the 16 USEPA PAHs with repeatability and reproducibility that compared with the standard soxhlet method. The main advantages of FMW being its speed and the reduced amount of solvent required.

Kootstra et al [25] developed a solid phase extraction method for analysing the 16 USEPA PAHs. They claimed the method gave equal or better recoveries than

liquid/liquid extraction especially for the volatile compounds. Recoveries for naphthalene, acenaphthylene and acenaphthene were 80 – 90% which was higher than the liquid/liquid extraction method which they quoted at 70 - 76%.

A microemulsive extraction procedure [26] was developed which used H_2O/isoctane/Igepal CA-120 to extract PAHs from soil. Analysis was conducted using GC-FID. Recoveries were quoted in range 93 –112% for the PAHs from the microemulisve solutions. The actual recoveries from the soil samples were much lower ranging from 47 – 60 % for the PAHs investigated. The PAHs ranged from fluorene to Benzo[ghi]Perylene

The extraction of PAHs from soil samples is extremely complex as it depends on whether the analytes are on the surface of the soil or whether they are imbedded within the soil matrix.

It appears that certain types of SFE yield the highest recoveries of higher molecular weight PAHs from soil samples. The analysis of PAHs in soil samples presents difficult problems, as the physical nature of the soil and the type of contamination have a large influence on the efficiency of the sample preparation technique used. SFE appears to show advantages over the more traditional liquid extraction for soil samples as they are much faster and in many instances are more efficient. FMW appears also to show promising signs as a rapid reliable technique for the extraction of PAHs from soils.

1.2.2. Air Particulates.

PACs are generally considered not to exist as pure substances in the atmosphere but to be adsorbed on suspended particulate matter with an average diameter of less than

10μm[1]. It has been suggested that 70-90% of these compounds are associated with aerosol particulates in the respirable size range.

Air particulates have been subjected to a number of different extraction techniques that are discussed in detail here. The extraction procedures are subdivided on the basis of the types of extraction used, soxhlet, ultrasonication and SFE.

1.2.2.1. Soxhlet Extraction

Soxhlet extraction is the most widely used extraction technique for the extraction of PAHs from air particulates. It is the technique that is recommended by many official agencies and is therefore worth close scrutiny.

Keller et al [3] suggested that polyurethane foam (PUF) may be a good adsorbent for these compounds. They set up a sampling system with glass fibre traps in series with PUF traps. The PUF filters were soxhlet extracted overnight with pet-ether, glass filters were refluxed with $MeCl_2$, clean-up was carried out on an alumina column. Fractions were concentrated on Kuderna-Danish (K-D) apparatus and analysis was carried out using HPLC-Fl. PUF plugs were subjected to spiking experiments of four PAHs, phenanthrene, pyrene, benzo[a]pyrene, benzo[ghi]pyrene and recoveries were in the range of 90 ± 9 %, which is considered good.

May et al [27] applied two methods to the analysis of a standard reference material, SRM 1649, which represents urban air particulates. Reverse phase LC with programmable fluorescence detection and normal phase chromatography to isolate the aromatic compounds, followed by reverse phase LC with fluorescence was used. The sample extraction involved soxhlet extractions followed by liquid/liquid partition with cyclohexane and nitromethane. A slightly different approach was taken for normal

phase, the soxhlet was carried out using benzene / methanol. No percentage recoveries or LOD were mentioned.

Niles et al [28] describe a method for the determination of three to six ring PAHs in air and diesel particulate matter. The procedure included soxhlet extraction followed by fractionation using gel permeation chromatography and normal phase chromatography using an aminosilane stationary phase. The resulting fractions were examined by GC-FID. They concluded that the method took advantage of the ability of the aminosilane stationary phase to separate PAHs on the basis of the number of condensed rings thus minimising interferences. They did not quote recoveries or LODs.

Nunez et al [29] evaluated HPLC-Fl for the analysis of six PAHs fluorathrene, benzo[b]fluoranthene, benzo[b]pyrene, benzo[k]fluorathrene, ideno[123cd]pyrene and benzo[ghi]perylene. Air particulate samples were soxhlet extracted with n-hexane, the extract evaporated to dryness and the residue was dissolved in 25cm^3 n-hexane. LC analysis was carried out using a C_{18} spherisorb ODS column. They quoted recoveries at 80-95% and LODs at 7-10pg for water sample analysis but they make no mention of recovery from the particulate samples, it is presumed that similar levels would be attainable.

Bodzek et al [30] noted that cyclohexane used as an extractive solvent lead to lower recoveries then other solvents. Extraction was conducted by soxhlet extraction and analysis was done by MS.

Albaiges et al [31] describe an analytical procedure for the comprehensive determination of hydrocarbons in the atmosphere based on aerosol filtration and adsorption of vapour phase onto active carbon and PUF, fractionation was carried out

by gel permeation chromatography and HPLC. Analysis was carried out by GC-MS. Glass fibre filters were soxhlet extracted with $MeCl_2$/MeOH for 30hrs. Perdeuterated n-octyosane and anthracene were added as internal standard. Charcoal was extracted with carbon disulphide. The PUF plugs were soxhlet extracted with hexane. Extracts were fractionated using deactivated silica/alumina columns and analysed by GC. No mention of recoveries for PAHs were given.

Ligocki et al [32] compared two methods of collecting air samples, one using PUF and the other using Tenax. PUF plugs were soxhlet extracted using acetone/hexane, the extracts were concentrated on K-D apparatus. Concentrates were separated into acid, base and neutral compounds using a series of caustic and acid washes. Concentration was carried out prior to analysis by GC-MS. Tenax-GC cartridges required no sample pretreatment they were thermally desorbed and analysed by GC-MS. The recoveries ranged from 68-99% for PUF and 97-110% for Tenax. The PAHs ranged from acenaphthylene to perlyene. They concluded that PUF was more suitable than Tenax for collecting less volatile compounds. They concluded that for most compounds Adsorption Thermal Desorption (ATD)/Tenax GC is a versatile method that is well suited to sampling at low flow rates or high flow depending on the application.

Mumford et al [33] used XAD-2 resin along with glass filters to collect indoor air particulates. They were soxhlet extracted using $MeCl_2$ for 16hrs. Separate aliquots of the extract were concentrated and analysed for PAH content by MS. Most results were below the detection limit of 0.1ng for XAD-2 resin. The filter gave results of 50$\mu g/M^3$ for pyrene to coronene. No discussion of the recoveries quoted was given.

Karlesky et al [34] described a procedure whereby an air particulate sample was extracted by soxhlet and the PAHs were fractionated on the basis of aromatic ring number. This was achieved through the use of cartridges packed with amino polar bonded phases. They compared the Me_2SO /pentane extraction to extraction onto 'Bond Elut' amino bonded phases for clean-up after soxhlet extraction. They concluded that 'Bond Elut' is a viable alternative to Me_2SO clean up. The recoveries were quoted for PAHs from naphthalene to benzo[a]pyrene. In all cases the recoveries were higher for the 'Bond Elut' extraction however the percentages themselves are low 11-78 %. The authors also state that the percentage recoveries are low for the Me_2SO/pentane extractions used.

Barroso et al [35] examined the use of fluorimetry in organised media to analyse PAHs, the media used was micellular Brij-35. Air particulate samples were ultrasonically extracted with cyclohexane and concentrated to 0.5cm^3. Clean up was carried out using silica gel. The sample was then concentrated, the residue was dissolved in methanol prior to analysis by fluorimetry. It was concluded that the detection limit for benzo[a]pyrene was 4pg/cm^3. LODs for naphthalene to dibenzoanthracene ranged from 20-200pg/cm^3. The method was successfully applied to three PAHs pyrene, benzo[ghi]pyrene and benzo[a]pyrene.

Pibarot et al [36] compared two types of extraction, soxhlet and steam distillation, for the extraction of the 16 PAHs, recommended as priority pollutants by USEPA, from active carbon used to purify air in closed atmospheres. They concluded that soxhlet extraction was the most efficient. However, recoveries and desorption yields were very low for higher molecular weight compounds. This implies that this method as given is not suitable for quantitative analysis. A detailed examination of spiking

efficiency was also conducted and spiking was deemed to be a satisfactory method for investigating recoveries with efficiencies quoted at 86.6-98.1% for the PAH spike. PAHs investigated ranged from naphthalene to benzo[ghi]perylene.

Hiel et al [37] compared a high volume dust sampler with a glass wool based filter-impinger as ways of sampling industrial workplace air. Filters from both systems were subjected to soxhlet extraction for 24 hours with cyclohexane followed by evaporation prior to analysis by GC-MS. Percentage recoveries were not calculated or discussed.

1.2.2.2. Sonication

Hawley-Fedder et al [38] noted that a major problem exists with one sampling technique for air particulates. This was the high volume samplers. The problem being that because a large range of particulate sizes was collected in the one filtration it makes it difficult to determine toxic or hazardous compounds associated with respirable particles. The dichotomous sampler was developed to overcome these problems. Hawley-Fedder et al evaluated such a filter system using teflon dichotomous with ultrasonication in a solvent [$MeCl_2$]. The extracts were condensed, using a semi-micro rotary evaporator to $0.30cm^3$ and further reduced to $0.10cm^3$ using N_2 stream. They were then analysed by GC-FID. Recoveries are quoted for fluorene, fluoranthrene, benzo[a]pyrene and a range of polycyclic compounds. The recoveries quoted were fluorene 71±46%, fluoranthrene 132±56% and benzo[a]pyrene 104±10%. The recoveries were for a 100ng/µl concentration and were corrected for losses which occurred during concentration. They concluded that ultrasonication is as good as soxhlet extraction and predicted LODs with GC-MS of $10pgM^{-3}$. No attempt was made to explain the large variations in the accuracy of results recorded.

Most ambient air sampling for PAHs is done by drawing large volumes of air through glass fibre filters. It has been suggested that for volatile PAHs complete collection may not take place under such conditions.

Maher et al [2] examined air particulates using $MeCl_2$ sonication followed by N_2 blow down with HPLC-UV/Fl. When necessary clean up was carried out on SepPak C_{18}. The 12 PAHs examined ranged from fluorene to indeno[123cd]pyrene. Recoveries were quoted to be in the range 80-100%. Spiking experiments were conducted to assess losses due to evaporation. They concluded that the lower MW compounds were most effected. LODs were 50-100 pg.

Deshpande et al [39] compared sonication and soxhlet for the extraction of PAHs from particulates in air samples. Extractions were carried out using cyclohexane for ten PAHs from naphthalene to benzo[a]pyrene, sonication was preferred to soxhlet. It was concluded that quantitative recoveries could be achieved in 30 minutes using sonication. Analysis was carried out by GC. Quantitative recoveries were estimated based on spiking experiments using fluoanthrene and pyrene.

Simoneit et al [40] gave a detailed description of the extraction of aerosols using $MeCl_2$ with ultrasonication. Fractionation was carried out by Thin Layer Chromatography (TLC), no mention was made of percentage recoveries or LODs.

Pool et al [29] used TLC coupled to fluorescence scanning densitometry to study PAHs. They evaluated a whole series of extraction techniques liquid/liquid, liquid/solid chromatography and gel permeation columns. For particulate samples, air and diesel, solvent extraction using a soxhlet apparatus or ultrasonications were found to be preferable, followed by silica gel column chromatography for clean up. Six

PAHs were used to evaluate the system anthracene, fluoathrene, benzo[a]anthracene, perylene, pyrene and coronene.

One advantage of using TLC is its ability to tolerate crude extracts that are unsuitable for analysis by column chromatography. This arises because the separation medium, the TLC plate, is used once and then discarded.

They noted that with prolonged sublimation considerable losses of more volatile compounds were seen. These losses can be controlled by using a two step sublimation.

For soxhlet extraction the extractor should be shielded from light to avoid photodecompostion. Using $MeCl_2$ for 8hrs was sufficient to recover greater than 90% of PAHs. Ultrasonication is suitable for rapid preparation of small scale extracts.

Spurious results can be obtained if losses due to spluttering and vaporization of volatile PAH are not avoided by using low temperature and slow evaporation rates.

The TLC system does not provide sufficient resolving power to separate all PAHs of environmental interest however under certain circumstances it is a valid alternative.

1.2.2.3. Supercritical Fluid Extraction

In recent years SFE has become an alternative to liquid solvent extraction for the recovery of organic air pollutants. Supercritical fluids have several potential advantages for the extraction and recovery of organics collected on PUF. Besides their solvating ability, SFs have higher solute diffusivities and lower viscosities than liquid solvents, which should result in improved mass transfer during extractions. The solvent strength of a supercritical fluid can be controlled by simply changing pressure and to lesser extent temperature. The addition of modifiers can also influence the

extraction characteristics [42].

Miller et al [43] used SFE utilising CO_2 to extract five PAHs from an urban particulate sample NBS SRM 1674. Internal standards were added before extraction so that the amount of PAH in the particulate matrix, not in the extract, would be measured. The results compared with certified values except for higher molecular weight PAHs. Recovery was 30% higher for benzo[ghi]perlyene. This suggests that SFE is more efficient than soxhlet extraction in removing the larger PAH species.

Langenfeld et al [44] extracted two certified standards for PAHs in air particulate matter and contaminated soil with SFE using pure CO_2 extraction. They concluded that high temperature CO_2 yielded recoveries that met or exceeded recoveries obtained in previous reports using alternative fluids such as $CHClF_2$, N_2O and CO_2 / 5% MeOH. Recovery studies were conducted on National Instititue of Standards and Technology (NIST) certified standards for a series of PAHs. They also concluded that extraction efficiency is very much dependent on the sample matrix which the PAH are extracted from.

They also recently examined the effect modifiers have on SFE on certified standards of air particulates containing PAHs [45]. The most common fluid for SFE has been CO_2 it was chosen because of its critical properties, low toxicity and chemical inertness. However poor recoveries associated with certain sample/matrix combinations of real environmental samples indicate that a suitable SF must not only be able to solvate analytes of interest but must possess properties that allow it to interact with the analyte, for the matrix to effectively partition the analyte into the bulk SF. $CHClF_2$ and N_2O have the ability to significantly improve extraction rates and

efficiencies of a wide variety of analyte/matrix combinations. These fluids would not be acceptable on a routine basis because of the ozone depletion abilities of $CHClF_2$ and the explosive nature of N_2O. Modified CO_2 supercritical fluid can have similar properties to $CHClF_2$ and N_2O.

Eight different modifiers with different chemical properties were examined for the extraction of native PAH analytes by SFE using CO_2 from certified standard reference material which contain PAHs in urban air particulate matter [SRM 1649], diesel soot was also examined. After the various extractions were carried out the extracts were then concentrated under a gentle stream of N_2 to a volume of $2cm^3$ approx. Analysis was carried out by GC-MS.

They noted surprisingly high recoveries, greater than 200%, based on the values reported by NIST using soxhlet extraction. A series of blank experiments were conducted which demonstrated that the high recoveries were real and not due to any artifacts or carryover. A detailed discussion of modifiers showed a complex picture for the extraction of PAHs from air particulates.

They concluded that certain modifiers such as aniline, acetonitrile (ACN), MeOH / Toluene and hexane significantly enhanced the extraction efficiency for low MW PAHs, increasing the concentration of these modifiers had little effect. These modifiers did not have the same effect on higher MW PAHs. CO_2 with 10% toluene showed little dependency on PAH MW and reasonable agreement with certified standard concentration was obtained for nearly all PAHs. For high MW PAHs dichloromethane and diethylamine gave the highest recoveries but only at a concentration of 10%.

PAHs extracted from diesel soot showed that SFE with CO_2 / 10% toluene gave

recoveries up to two times greater than CO_2 on its own. These results verify that SFE of a carbonaceous soot system might benefit more from an aromatic modifier such as toluene. It was concluded that modifiers should be selected on the basis of the matrix characteristics and the target analytes.

The efficiency of six adsorbants florisil, XAD, Tenax, C_{18}, Silica gel and PUF spiked with mixtures of PAHs were investigated [46]. One gram of adsorbent was spiked with 30ppm of acenaphthylene, benzo[a]anthracene, indeno[1,2,3-cd]pyrene and benzo[g,h,i]perylene. SFE was carried out using CO_2 and the extract was then evaporated and injected onto a GC column for analysis. They concluded that florisil foam was the best sampling adsorbent with recoveries of 85,90,89 and 74% respectively. This method was also applied to SRM 1694.

1.2.2.4. Other Extractive Techniques

Kopcznski et al [47] extracted atmospheric particles by vacuum sublimation, the PAHs were collected by cold trapping and analysed by GC-FID. The atmospheric sample used was the certified standard for dust particles SRM 1694. The sixteen priority pollutants recommended by the USEPA were analysed. They concluded that as the volatility decreases so does the desorption efficiency, less then 25% of the benzo[a]pyrene present was recovered and none of the benzo[ghi]pyrene was recovered.

Recently MAE has been applied to the extraction of PAHs from particulate matter [48]. The results of this extractive process were compared with conventional soxhlet extraction with mechanical plunging extraction (MPE) for 15 PAHs and seven deuterated internal standards on a PUF plug. They concluded that percentage

recoveries from MAE were higher than for MPE except for the higher MW PAHs. For these compounds recoveries were comparable. They noted that low MW compounds exhibited low recoveries for both systems and that this was possibly due to their volatility.

Janssen et al [49] reported on a new type of extraction material for sampling air particulates. They used polydimethylsiloxane (PDMS) particles as a 'solid liquid' for extracting analytes from gaseous samples. They applied this extractive method to a series of spiked PAH samples and analysed them by GC. They concluded that using PDMS in this manner was a viable alternative to solid phases like Tenax . They also concluded that using this material for volatile compounds was not a problem.

A combination of a glass fiber filter, PUF plug and XAD-2 resin were used to trap PAHs over a large volatility range from air particulates[50]. Deuterated PAH analogues were added to control and correct for any sample losses. The PAHs ranged from naphthalene to benzo[ghi]perylene. Sample extraction was carried out using dichloromethane. Recoveries exceeded 90 % with LODs recorded at $5pg/M^3$. The method allowed low levels to be detected because of the large sample volumes used.

The efficient extraction of PAHs from air particulates is a difficult task. Three main procedures are used in the sample preparation step, these are sonication, soxhlet and SFE. Soxhlet extraction is the standard method used, but it is time consuming, uses $MeCl_2$ in many cases and losses due to photodecomposition and evaporation are a problem. Sonication is an alternative however again dangerous solvents may be required. Supercritical fluids have been extensively used for the extraction of PAHs. It

is an extremely fast and effective technique. In some instances recoveries quoted for standard samples were better then the recommended technique which indicates that SFE is a more effective technique.

More recently MAE has been applied to the extraction of these compounds from pariculates and may show some promise as it combines the simplicity of liquid extraction with the speed of the more modern techniques. PDMS as an extractive material also shows promise as its characteristics are well understood from GC technology.

The main draw back of many of these extractive methods is the need for a solvent volume reduction or replacement prior to analysis either by HPLC or GC. It is this step which can introduce the possibility of losses of the volatile PAHs.

1.2.3. Other Particulate Matter.

Naikwadi et al [51] examined the particulate emissions from wood stoves. The samples were collected by high volume filtration using glass filters. A complex series of extractions were then carried out on the collected samples. First soxhlet extraction with toluene was carried out, followed by acid/base extraction to remove soluble inorganic impurities. Aliphatic compounds were then separated from aromatics using normal phase HPLC. HPLC fractions were then analysed by GC-MS. The recoveries for selected PAHs after acid/base treatment were above 85%. The recoveries of a standard mixture of 20 PAHs in acid/base extraction and HPLC separation were determined. No recovery data were quoted. It was concluded that the amount of PAH identified in the wood stove particles varied from 0.1 to 1.1μg/g.

Chuang et al [52] used XAD-4 and XAD-2 resins to collect both particulates and

organic matter from indoor air of both smoker's and non-smoker's homes. Prior to sampling adsorbent cartridges were spiked with perdeuterated PAHs. The cartridges were extracted with soxhlet using a range of solvent combinations. The main interest in this study was the development of an extraction technique for both PAHs and nicotine simultaneously. It was concluded that soxhlet extraction with $MeCl_2$ followed by ethyl acetate can remove both PAHs and nicotine from XAD-4 resin. Quantitative recoveries were quoted based on spiking experiments for nine PAHs and nicotine. The PAHs ranged from naphthalene to benzo[a]pyrene.

Tan et al [53] took part in an inter-laboratory investigation into the analysis of PAHs in diesel particulates. They carried out both ultrasonic and soxhlet extraction for comparison purposes. The sample preparation procedure was as follows a) soxhlet: 20mg sample with $MeCl_2$ for 18hrs b) 20mgs in toluene ultrasonication for 15 minutes. These extracts were then subjected to gel filtration chromatography with Sephadex LH-20 packing followed by passage through a silica gel column. The effluent was evaporated and analysed by GC-MS. Results were quoted for both sonication and soxhlet for 11 PAHs phenathrene, anthracene, fluoranthrene, pyrene, benzo[a]anthracene, benzo[a]pyrene, benzo[e]pyrene, indeno[123cd]pyrene, benzo[ghi]perylene, benzo[k]fluorathrene and dibenzanthracene. They concluded that in nine out of the eleven cases, the exceptions being anthracene and indeno[123cd]pyrene, ultrasonic extraction was the most effective. They also found that a combination of Sephadex gel filtration chromatography and silica gel adsorption chromatography is very effective in separating PAHs from this particular sample matrix.

Hawthorne et al [42] successfully applied SFE with CO_2 for the extraction of

particulates collected on PUF from diesel exhaust, cigarette smoke and roof tar volatiles. Recovery studies were also carried out using PUF plugs spiked with an acetone solution of PAHs. The SFE was also coupled directly to a GC-MS. Recoveries were quantified for naphthalene, phenanthrene, pyrene and chrysene to be 100%. Recovery for perylene was 97%. The most powerful advantage of SFE is the fact that class selective extractions can be achieved by simply changing the extraction pressure, and quantitative recovery of most organics can be achieved in 10-20 minutes compared with several hours normally required for soxhlet extraction.

Paschke et al [54] found that $CHClF_2$ gave the best recoveries for the SFE of particulates from diesel soot. They extracted the certified standard SRM 1650 and found that although CO_2 / 10% toluene gave lower results then $CHClF_2$ the recoveries were considered to be good. The following PAHs were examined phenathrene, fluoranthene, pyrene, benzo[a]anthracene, chyrsene, indeno[123cd]pyrene and benzoperylene.

Davis et al [55] applied a novel technique to the examination of diesel exhaust particulates. The analysis of PAC adsorbed on sooty particles is a difficult task as they are present at only trace levels in a highly complex hydrocarbon matrix. Micro-HPLC was used to obtain group type separation of the sample using normal phase chromatography with direct on-line transfer of the aromatic fraction to a GC-MS. No recoveries were quoted.

Classens et al [56] compared the recoveries of three extraction methods, ultrasonic ether, soxhlet toluene and a soxhlet extraction with liquid CO_2 for a typical soot

sample. The recoveries for the sixteen PAHs recommended by USEPA were examined. A detailed discussion of CO_2 extraction is given, it was concluded that a combination of SFE and ultrasonic extraction with ether or toluene gave the highest recoveries. It was also noted that significant losses in ultrasonic and soxhlet extractions due to evaporation may occur and therefore CO_2 extraction would be a superior method in that respect. Improvements were also made in the sample pretreatment step by using a diol column for the fractionation instead of a silica column that had been the previously recommended method.

Gotze et al [57] applied a new type of liquid extraction to diesel soot samples, soxtec-extraction. The procedure is as follows; Phase 1. Sample was extracted in boiling not condensed solvent as in soxhlet. Phase 2, The lifted extraction thimble was washed out nearly quantitatively by refluxed solvent. Phase 3. The cleaned solvent was collected in the upper part of the system for the next analysis while the sample was concentrated in the lower part for 4hrs instead of 16hrs for soxhlet extraction. Clean up was carried out on a SiO_2 column and fractionation was conducted on Al_2O_3 using benzene to elute the PAHs. Recoveries of above 95% were achieved based on standard injections. A LOD of between 5×10^{-10} to 3×10^{-8} grams was reached using HPLC-UV/Fl.

Hoener et al [58] extracted a range of PAHs from exhaust fumes from industrial boilers. Gases were trapped on glass fibers and PUF plugs. The analytes were extracted using SFE with CO_2/toluene and analysed by HPLC-UV/Fl. Recoveries were quoted in the 79-107% range. The PAHs ranged from naphthalene to coronene with the lower recoveries being recorded for the heavier PAHs.

1.2.4. FLY ASH

It has been reported that fly ash from municipal incinerators, coal-fired power plants and fluidized bed coal combusters contains respirable particulates, which have shown mutagenic activity. However little is known about the contribution of organic compounds to the mutagenicity of these solids. Therefore it is important to know the concentration of PAHs adsorbed on this solid matter and its desorption characteristics [59].

In the various steps of the PAH analysis irreproducible losses of PAHs complicate quantitation. Sublimation, chemical reactivity and irreversible adsorption of PAHs on a variety of substrates such as coal fly ash have been shown to play a predominant role. The extraction procedures for removing PAHs from fly ash has been subdivided based on the type of extraction carried out.

1.2.4.1. Sonication

Janssen et al [59] investigated the properties of PAHs on fly ash by using radio labelled B[a]P. They noted that one should keep in mind that the adsorption and desorption properties of 14C-spiked B[a]P could differ significantly from that of B[a]P already present on the surface of fly ash.

The PAHs were extracted from the fly ash by means of ultrasonication with a combined extractant/liquid scintillating solution, recoveries were very low 8-50 %. No detailed explanation is given as to why the quoted recoveries are so low, however they do note that thermal treatment reduces the recovery of benzo[a]pyrene significantly. Junk et al [60] commented that studies of the recoveries of PAHs from fly ash are limited and as noted above 14C labelled B[a]P spikes gave low recoveries and

inconsistent results. In this study pyridine proved to be the most effective solvent when compared to more standard solvents commonly used to extract PAHs from air particulates such as benzene, toluene or dichloromethane. They concluded that soxhlet extraction was more efficient for this particular sample matrix then sonication. Even at this recoveries for spiked experiments were low. 75-82% was the range recovered for PAHs from phenanthrene to coronene. Low MW PAHs gave better recoveries. To improve recoveries water with inorganic complexing agents was used to pretreat the fly ash but this had little effect. It was shown that pyridine is a solvent which can improve extraction efficiency.

Griest et al [61] using an ultrasonic technique found that the extraction of C_{14} labelled B[a]P with benzene from coal fly ash was incomplete and that the unextracted tracer remained on the fly ash. It was suggested that the association of PAH with fly ash surface might involve complexing of the aromatic compounds with metals on the fly ash surface. They also noted the dependence of recovery on the PAH molecular size. They improved recoveries for B[a]P from stack fly ash for ultrasonic extraction by increasing the power/mass ratio for the extraction of spiked radio labelled B[a]P at sub-ppm concentrations[62]. They concluded that the extraction behaviour is consistent with sorption models for the fly ash. One such model, being the sorbed C_{14}-B[a]P, is extracted from porous particles, where it is readily available or extracted from near the surface of these particles. However some may have penetrated deeply into the inner structure of the particle and much more time would be required to remove the inner B[a]P due to the diffusive-limited processes. The recoveries rose from 25% to 75% with this increase in power/mass ratio.

1.2.4.2. Soxhlet Extraction

One study [63] found that sequential soxhlet extraction with MeOH/benzene or benzene soxhlet of fly ash yielded the best results for the PAHs examined, biphenyl fluorene, fluoranthrene, pyrene and anthracene. Quantitation was carried out on GC/MS, no recovery figures were quoted.

Classens et al [64] examined a series of soxhlet and ultrasonic extraction techniques for PAHs from aerosol samples of stove smoke which is mainly carbonaceous. They concluded that ultrasonic extraction with dimethylsulfoxide at 100°C produced the best results especially for high MW compounds. The recoveries quoted are particularly poor for low MW compounds. They did note that with this approach it was uncertain whether spiked PAH material behaved the same as the original PAH aerosol deposit. Analysis by HPLC-UV/Fl was carried out after the sample was first prefractionated with HPLC. The PAHs analysed were the sixteen listed by the USEPA as priority pollutants.

Desilets et al [65] successfully applied Liquid Chromatography - Diode Array Spectroscopy (LC-DAS) to the analysis of PAHs in fly ash. This technique is especially well suited to the determination of PAHs were many isomers are difficult to resolve chromatographically. They are easily distinguished by their electronic spectra. The sample was extracted in a soxhlet extractor with $MeCl_2$ and cleaned up on silica gel. PAH recoveries were between 75-100% depending on the compound. Low molecular weight hydrocarbons tended to be more susceptible to the greatest losses. These losses were a result of sample handling, volatilisation and oxidation, according to the authors. One major disadvantage of LC-DAS is its very high detection limits compared with fixed wavelength detection.

1.2.4.3. Other Extraction Techniques

Sotyls et al [66] carried out a kinetic study of the extraction of B[a]P from doped fly ash using a continuous flow extractor and three different solvents $MeCl_2$, cyclohexane and benzene. They found that extraction efficiency depended on the ash composition and the solvent. The extraction mechanism was envisaged as a two-step process involving physical desorption coupled with diffusion out of extremely porous particles. They concluded that benzene was the most effective solvent. They also concluded that in the presence of large amounts of carbonaceous material or low PAH concentrations that there is the possibility of incomplete recovery of these compounds.

Mangani et al [67] presented a novel method for extracting PAHs from fly ash. It involves the packing of the ash into a tube and the passage of hot toluene (100°C) through the column at 0.5cm^3/min. Analysis was carried out by GC-MS/SIM. This technique was compared to soxhlet $MeCl_2$ and soxhlet toluene extractions. The results of the new technique are comparable with toluene soxhlet for most PAHs. Problems were noted with B[a]p for all methods. No explanation is offered but photodegradation may have been a problem with these methods. They concluded the main advantages of this method were its speed and ease of operation.

The extraction of PAHs from fly ash yielded poor recoveries in general. Radio labelled benzo[a]pyrene C_{14} was used in a number of spiking experiments to evaluate the various extraction procedures. One possible reason for the low recoveries may be the formation of complexes between the aromatic compounds and metals on the

surface of the ash. Recoveries are dependent on the nature of the ash, whether the PAHs are bound to the surface of the ash or whether they are combined within the matrix of the ash itself and the actual solvent used to extract the PAHs.

Photodegradation is also a possible reason for PAH loss particularly during soxhlet extractions. Low molecular weight PAHs show lower recoveries rates than the higher molecular weight compounds. Again here the reason for this is physical losses of these compounds during the extraction procedure.

1.2.5. Sediment Samples

The examination of marine sediment for PAHs is becoming more important because it is felt that the concentration will be an indicator of pollution in the marine environment as a whole.

Dunn et al [68] extracted PAHs from a number of samples including marine sediment using alkaline digestion followed by clean up on florisil and partitioning between isooctane and dimethyl sulfoxide. Finally fractionation was carried out on Sephadex LH-20 prior to analysis by HPLC UV/Fl. Radiolabelled B[a]P was used to monitor and correct for losses during sample purification and handling procedures. No recovery figures were quoted.

Ramos et al [69] reported a two step procedure that combines silica gel and Sephadex LH-20 chromatography using contaminant free solvent systems to isolate PAHs from extracts of sediment. It is an environmentally safer method than the standard benzene/methanol mixture using Sephadex LH-20. Recoveries for naphthalene, phenathrene, flouranthrene, chyrsene, benzopyrene were 93-100%. Also because of the relatively simple chromatograms isolated by this procedure it was possible to

quantitate the PAHs directly using GC.

Doerge et al [70] used LC with particle beam MS (PBMS) for the analysis of PAHs in marine sediment. Liquid extraction with $MeCl_2$ / EtOAc was used followed by fractionation by LC. They suggested that evaporative losses resulting from the nature of LC-PBMS was one reason for the low recoveries for low MW PAHs. They concluded that this method could be used in combination with USEPA 620 for the analysis of PAHs.

Sims et al [71] applied SFC with photoionisation detection (PID) to marine sediment samples which had been extracted by soxhlet extraction with $MeCl_2$ for twenty four hours followed by clean-up on silica gel and then fractionation on Sephadex LH-20. The main advantages of PID over other more standard types of detection are much less detector 'spiking', a smoother and flatter baseline and a molar response which is much less dependent on the nature of the individual analyte. A mixture of various PAHs were used to assess the chromatography. Detection limits are quoted as being a few hundred picograms. No recovery studies were conducted.

Hawthorne et al [72] applied a rapid extraction and analysis technique to a number of samples. The technique was SFE directly coupled to a GC. The main advantage is that losses are minimised since the extracted species are quantitatively transferred into the GC column where they are cryogenically focused prior to GC-FID or GC-MS. The technique was applied to PAHs in river sediment and treated wood. No recovery studies were conducted.

Yang et al [73] investigated temperature-modifier effects on SFE using CO_2 for the extraction of PAHs from marine sediment. They concluded that extractions with diethylamine at 200°C gave the highest recoveries.

Fernandez et al [74] compared SFE with CO_2 with and without modifiers to soxhlet extraction for the extraction of PAHs from marine sediment. They concluded that PAHs were only wholly extracted by CO_2/MeOH at 150°C. In general SFE extracted more than what was obtainable by soxhlet but soxhlet was a more reproducible technique. The PAHs ranged from phenanthrene to dibenzo[ah]anthracene.

The extraction of PAHs from marine sediment presents unique problems due to the physical characteristics of the sediment itself. Often a final fractionation procedure is introduced into the sample clean-up step to ensure a suitable mixture is available to be introduced into the analytical system. The need for this rigouous clean up procedure obviously introduces the possibility of physical losses of the PAHs to evaporation or irreversible adsoption onto a clean-up column. This would particularly be the case for the low molecular weight compounds.

1.2.6. Miscellaneous Samples.

Ronchetti et al [75] proposed a procedure for the determination of PAHs in lubricating oil and fuel oils. The procedure consisted of dissolving the sample in cyclohexane and extracting it with N-Methylpyrrokine-water-phosphoric acid. The PAH fraction was separated by TLC and analysed by GC-MS. There was no mention of recoveries for the PAHs examined. Blanco et al [76] extracted coal tar pitch and residue with toluene in an ultrasonic bath. Analysis was done by GC-MS, recoveries were not quoted. Lanças et al [77] compared two types of SFE, static and dynamic extraction for the extraction of PAHs from coal. The extract was fractionated by LC prior to GC-MS

analysis. From an examination of the total ion chromatograms it was concluded that dynamic extraction extracted more material than static SF. Recoveries were not used in this study.

Hertz et al [78] proposed the use of organised media in a fluorescent study of PAHs in coal liquids as it helps to reduce matrix effects and intermolecular interactions between solutes. This was achieved by isolating the analyte molecule in a uniform microenvironment within the sample. In this paper sodium taurocholate [NaTC] is compared with sodium dodecyl sulfate [SDS] with fluorescence analysis. They concluded that NaTC was superior to SDS as a micellar media as it gave a two-fold increase in sensitivity for fluoresence detection in ethanol compared to SDS. They concluded in general that both NaTC and SDS micellar media improved the ability of fluorescence analysis to discriminate between different coal liquids. Recoveries were not studied.

Bonifanti et al [79] investigated the collection of flue gases from a combuster on XAD-2 resin followed by soxhlet extraction with toluene. Analysis was carried out on GC-MS. They quoted the LOD to be between 0.2-0.1 μg/g of coal.

Blyshak et al [80] examined the use of cyclodextrin (CDx) modified solvents for the extraction of PAHs from complex matrices such as oil samples and air sample adsorbates. CDx as an aqueous modifier enhances the extraction of selected species into an aqueous layer while retaining other species in the organic layer. This is particularly useful when a complex mixture needs to be simplified. The PAHs examined were pyrene, benzo[e]pyrene, perylene, benzo[ghi]pyrene and coronene. Following the extraction of the analytes of interest present into the aqueous phase they may be studied without removal from the CDx or by back extraction into cyclohexane

and analysis by HPLC. Extraction efficiency varied from 34-98% depending on the PAH investigated. It was noted that efficiency increased with increasing MW.

Bello et al [81] characterised mixtures of PAHs using Cyclodextrin-Induced Solid-Surface Room-Temperature Luminescence [CISS-RTL] which was used to analyse extracts from coal liquids. They concluded that a range of PAHs from naphthalene to decacyclene could be identified in mixtures at nanogram levels. However they noted that complete extraction of the compounds into the α-cyclodextrin-sodium chloride mixture was not possible making this technique unsuitable for quantitative analysis.

Wright et al [82] mentions the use of SFE to extract PAHs from hazardous waste. A comparison of various SFE extractions for two samples was shown, a soil sample and a waste stream sample. They concluded that the use of modifiers such as methanol and ethanol with CO_2 resulted in a higher percentage extraction for the aqueous waste and did not have the same success for soil samples. They also mention the use of SF fractionation for resolving complex coal and petroleum derived PAC mixtures based on the number of aromatic rings. These fraction were obtained using supercritical CO_2 and columns packed with NH_2 modified silica particles. They concluded that this fractionation gave excellent selectivity based on ring numbers.

Rein et al [83] examined the factors which govern SFE of PAHs from octadecyl-bonded sorbents. They spiked C_{18} packing at a concentration of 200ppm for the PAH studied. They concluded that although the choice of SF had the greatest overall effect on the achievable recoveries, in the present study it was clear that other variables, including microextractor cell geometry, can have effects that are similar in magnitude and, therefore must be considered when optimising a SFE system. They concluded

that relative recoveries increased by up to a factor of two, for coronene, by employing short broad extraction vessels compared to using narrow vessels.

Jenkins et al [84] successfully applied SFE to the extraction of PAHs from an internal combustion engine valve deposit. Analysis was carried out by GC-MS. However certain limitations of GC-MS were noted, these included the problem of isomers being indistinguishable in MS and the nonvolatility of high MW PAHs for GC analysis. They propose the use of SFC/GC-FTIR-MS as a hyphenated technique, which would overcome these problems.

Miller et al [85] reported an extraction method which used static SFE. Following SFE the analytes were collected by rapidly depressurizing (3-30 secs) the CO_2 effluent through a 178μm i.d. stainless steel tube. It was noted that multiple static extractions were required for quantitative recoveries of PAHs from spiked sand samples. This may indicate the need for a modifier in the CO_2 system.

Burford et al [86] undertook an extensive study of extractions of PAHs from heterogeneous environmental samples. The PAHs examined ranged from naphthalene to benzofluoranthrene.

These PAHs were extracted from petroleum waste sludge, a standard representing urban air particulate matter, SRM 1694, and railroad bed soil. Both native PAH and spiked samples were investigated using SFE with CO_2 and CO_2/MeOH systems and compared with sonication in $MeCl_2$. Various spiking methods were also used. A number of interesting conclusions were reached from this work; regardless of the spiking method used, injection of the spike or suspension of the sample in the spiked solution, or the ageing time of the extraction rates, most of the recoveries for spiked d-PAHs were substantially higher than for the same native PAHs. Air particulate and

sludge samples show large differences in the extraction rates of the spiked d-PAHs and native PAHs. They concluded that this is consistent with the idea that the native PAHs are formed with the sample matrices and are therefore located in less accessible locations throughout the particles matrix. Railroad bed soil in contrast is contaminated after the soil has formed and it therefore seems likely that a high proportion of native PAH would be located at surface sites.

The largest difference for native and spiked extraction rates existed for the lowest MW PAH naphthalene whether it was SFE or sonication extraction. They surmised that since naphthalene persistence in the solid environment samples is low that any molecules that are present in well aged samples must be tightly associated with the sample matrix. This would be consistent with the slow extraction rates of the native naphthalene. They concluded therefore that spiking of heterogeneous sample matrices does not give an accurate estimation of extraction efficiencies from such samples.

Langenfeld et al [87] conducted a similar investigation into a range of environmental samples contaminated with native PAHs and spiked with d-PAHs. These samples included fly ash, railway bed soil and marine sediment. They concluded that SFE with CO_2 at 200°C improved the extraction efficiency of the process and that native compounds were extracted more slowly then the spiked ones suggesting that other processes were involved in the extraction of the native PAHs.

Low et al [88] conducted a study of the photochemical degradation of PAHs in solvent solutions. It was felt necessary as many articles cited this as a major source for reduced recoveries estimated by standard addition experiments. Losses have also been attributed to poor extraction efficiency and evaporation during solvent removal after

the extraction. They concluded the use of polar solvents such as ACN, $MeCl_2$, acetone or MeOH for solvent extraction is not recommended unless light can be rigorously excluded. Slower photodegradation occurs in non-polar solvents such as toluene or hexane, however, the choice of these solvents for an analytical method must be weighed against the extraction efficiency of the solvent. The PAHs investigated were acenaphthene, phenanthrene, fluoranthene, benzo[a]anthracene, benzo[k]fluoranthrene, benzo[a]pyrene and dibenzo[ah]anthracene. They concluded that B[a]p and B[a]a degraded rapidly.

1.2.7. Foods.

PAHs have been the subject of numerous studies related to foods because of their possible effects on human health. This is because of the carcinogenicity of a number of members of this compound class. The PAH contamination arises from several sources including the processing of foods e.g. smoking, direct drying, cooking, natural sources and environmental contamination of air, water or soil. It has been pointed out that vegetables, not smoked foods or grilled meat may be the greatest source of PAHs for humans. It has also been estimated that food intake may in fact surpass tobacco smoking as a major contributor to PAH exposure [89].

Lawrence and Weber [89] determined eighteen PAHs in a variety of foods available in Canada. These include smoked and unsmoked seafood products, meat spreads and fried and char-broiled meats. All samples were carried through a saponification step followed by liquid/liquid partition with H_2O/MeOH and cyclohexane. Clean up was carried out on a florisil column, and then a second liquid/liquid partition with Me_2SO/hexane. HPLC-Fl was used routinely to analyse the extract with high or

suspicious results confirmed by GC-MS. The PAHs studied ranged from fluoranthene to dibenzo[ai]pyrene. The HPLC detection limit was defined as twice noise and estimated to range from 2 to 27 pg/injection. Detection limits by GC-MS were in sub microgram per kilogram range, which is comparable to that of HPLC-Fl. Recoveries were estimated using spiked samples of fresh fish. The recoveries were quoted at three concentrations. Recoveries varied from 28 to 119% depending on the PAH. No obvious trend was noted for these recoveries. In fact no reason is given as to why recoveries varied so much. The florisil clean up is mentioned as being a major source of losses of PAHs. It must also be noted that recoveries were based on spiked samples and no attempt was made to investigate if native occurring PAHs would react differently to the extraction regime. They concluded that elevated levels of carcinogenic PAHs resulted from char-broiling of meats, environmental contamination of mollusks and lobsters and to a lesser degree the smoking of foods.

Joe et al [90] presented a rapid sensitive and reliable LC method for the determination of twelve PAHs in various smoked foods. The PAHs were extracted with 1,1,2 trichloro-1,2,2 trifluoroethane [freon 113] from the KOH digests of the samples. The extracts were then purified by column chromatography through a deactivated silica gel/alumina column and liquid/liquid partitioning between Me_2SO and cyclohexane before separation of PAHs by LC with UV and FL detection. Recovery studies were carried out using a 2.5ppb spike for twelve PAHs. Recoveries ranged from 81-98% with a coefficient of variation of 4.4% for freon 113 extractions. The LOD was defined as twice noise which gave LODs of 0.02ppb - 0.39ppb for UV and 0.03ppb - 0.44ppb for fluorescence detection depending on PAH examined. Recovery studies

were also conducted on several smoked foods, which had been spiked with the twelve PAHs. Recoveries ranged from 65-107% depending on the PAH and the particular food involved. All recoveries were corrected for control values. No explanation was given for the considerable losses noted for the blank extract experiments. They concluded that trace levels of PAHs were present in all smoked foods analysed but generally at levels < 1ppb.

Takatsuki et al [91] presented a method for the determination of PAHs in fish and shellfish. The procedure involved alcoholic KOH digestion, extraction with n-hexane, silica gel chromatography and LC-FL analysis. They found that B[a]P, a commonly used representative of PAHs, was decomposed easily by the analytical procedure. Recovery experiments were carried out on spiked fish samples. They concluded that B[a]P was decomposed by the coexistence of alkaline conditions, light, oxygen, by peroxide in aged ethyl ether and by oxygen when adsorbed on silica gel. To overcome these problems the following recommendations were made: protect from light during all analytical steps, add Na_2S to the alkaline digestion mixture as an antioxidant; remove peroxides from ethyl ether before using it; quick column chromatography on silica gel; prevent air from coming into contact with adsorbent. Recoveries were quoted for seven PAHs, the PAHs ranged from benzo[a]pyrene to benzo[ghi]perylene. Recoveries were quoted as 92.8-106.0%, based on spiked fish experiments. Results were also quoted for concentrations of PAHs in mussels, oysters, corb-shells and shortnecked clams. However no attempt was made to calculate recoveries of natural PAHs. The experiments assumed quantitative extractions from fish based on the spike experiments.

Lawerance et al [92] describe the development of a method for the analysis of eight

target PAHs using HPLC-Fl and GC-MS/SIM. The methodology was applied to forty-eight selected food products. Ten to one hundred fold improvements in determination limits, compared to earlier work, were achieved through the use of modified extraction, clean up and the use of a HPLC column with improved selectivity, Vydac 201TP [5µm 4.6mm x 25cm] with a C18 ultrasphere ODS 5µm precolumn.
A detailed description was given of the extraction and clean up techniques used for the various food groups analysed. However the reasons why one clean up method is used for one food group, e.g. fish/meat used silica gel with fractionation on Sephadex LH 20, and not used for another food group, cereal/diary products used a florisil column, were not discussed. Limits of detection for HPLC-Fl are quoted at 5-264pg depending on the PAH examined. An extensive recovery study was also carried out which involved both method and control blank experiments. Ocean porch was chosen to represent meat/fish and bran cereal, and coffee whitener represented dairy produce. For fish the average recoveries were greater than 62% at three different spike concentrations. They noted low recoveries for dibenzo[ah]anthracene and dibenzo[ai]pyrene at 25ug/kg spike. No apparent reason could be put forward as to why recoveries of the latter PAH increased as the spiking level decreased. They noted that bran exerts a type of matrix effect on the recovery of B[a]p. They concluded that seafood contained significantly higher levels of PAHs then the cereal groups.

Rainio et al [93] presented data on the PAH content of blue mussel and fish from the Finnish archipelago sea. Samples were treated to soxhlet extraction prior to saponification and liquid/liquid partitioning with Me_2SO prior to clean up, followed by extraction with cyclohexane and analysis by GC-MS. Recovery experiments were

carried out using spiked samples. The PAHs spiked were naphthalene, phenanthrene, anthracene, fluoranthene, pyrene and triphenylene. The recoveries varied from 49% to 97%. The average recovery was 78%. No explanation is given as to why certain recoveries were very low. The concentration of individual PAH components varied from less than 0.5 to 109 µg/kg for mussels and from 0.5 to 27 µg/kg for the four PAHs in the fish samples.

Musial et al [94] presented a simple and rapid procedure for the analysis of PAHs in shellfish. The main difference between this and other methods is that this method is targeted at more highly contaminated marine shellfish. A smaller sample size is required, 1-8g of tissue. Saponification with ethanolic KOH followed by partitioning into 2,2,4-trimethylpentane and gel permeation chromatography on biobeads was the sample preparation method used. When individual PAH measurements were required, Gel Permeation Chromatography (GPC) fractions were subjected to LC-Fl. The advantage of this method was, the reduced amounts of expensive reagents required , there was no need for multiple liquid/liquid partitions or for lengthy LC procedures. Eleven PAHs were investigated using this procedure, they ranged from fluoranthrene to indeno[123cd]pyrene. Recovery studies were carried out using µg and ng spiked samples. Analysis was done in triplicate. Initial erratic recoveries [40-85%] were suspected to be a result of volatilisation losses. This was overcome through extreme care during rotatory evaporation steps and the addition of a keeper solution [1µl 0.2% paraffin oil in methanol / chloroform] to the solution prior to evaporation also special care was taken during the final dilution to 100µl prior to injection on LC. Recoveries quoted varied from 87.7% - 108.9% at µg spike and 75.3% - 96.2% at ng levels. No

explanation is given for the difference in percentage recoveries except that ηg spike was left overnight which may have lead to oxidation.

Grimmer and Jacob [95] reported on a collaborative study conducted using extraction with trichlorotrifluorethane, and clean up on silica and sephadex LH 20 column prior to GC analysis. However no recovery experiments for the extraction procedure were carried out. They concluded that large coefficients of variation existed and that the determination of PAH by the above method was not satisfactory.

Alonge et al [96] presented an unusual method for the analysis of PAHs based on the officially recommended method. This work involved mincing frozen meat samples, dissolving them in boiling water. The sample was then mixed with $25cm^3$ butan-2-one, $25cm^3$ cyclohexane and 50g relite. The solvents were evaporated off and the PAHs were eluted with propylene carbonate. The solution was then extracted with benzene, cleaned up on florisil and analysed by TLC. Spiked experiments with B[a]P were carried out on meat samples, 90-100% recoveries were reported. There was no description of the spiking method used.

Birkholz et al [97] presented a procedure for the analysis of fish samples. The analytical procedure included soxhlet extraction with $MeCl_2$ followed by GPC on Bio-Beads SX-3, cleaned up on florisil and elution with hexane. Analysis was both by GC-FID and GC-MS. The average recovery for naphthalene was quoted as 87% for fortified tissues at 0.24 and 0.024 µg/g.

Bazylak and Maslowska [98] investigated the presence of PAHs in mineral oils. These oils were proposed for use in crop protection products. They analysed for seventeen PAHs using gradient HPLC. The extraction procedure involved liquid/liquid with

DMF/H_2O followed by cyclohexane, size exclusion with silica gel followed by clean-up on Sephadex LH 20. They concluded that the use of adsorption HPLC on silica columns enables only group separation of PAHs extracted from investigated oils. This chromatography can only be usefully employed during the clean up step of PAHs extracts. No recovery data were presented or discussed. They concluded that refined mineral oils contain different quantities of carcinogenic PAHs and the use of these oils in agriculture enhances the risk of exposure to this class of compounds.

Speer et al [99] conducted a comprehensive study of PAHs in vegetable oils and mussels. Oysters and bream from the river Elbe were also analysed. Clean-up was carried out on silica gel and GPC Biobeads SX-3 prior to GC-MS. Sixteen PAHs were analysed. There was no discussion of the efficiency of the method.

Geahachan et al [100] presented a paper which both reviewed standard liquid/liquid extraction techniques for mineral oils and also presented a new method which offers many improvements. They found these methods gave similar but poor recoveries, as low as 12% for B[a]p. The major improvements made were changing the solvent ratios and using a two step liquid partition on Sephadex LH-20. Analysis was by LC-FL. Twenty-nine PAHs were analysed. Recoveries ranged from 39% for B[a]p to 103% for methyl-3-cholanthrene using a total spiked concentration of 7ppm. LODs were quoted in the range of 0.2ppt for benzofluoranthene and 200ppt for acenaphthene. Recoveries were corrected with an internal standard to 92.1%-111.4%, no details are given as to how these corrections were achieved.

Coates et al [101] developed a method for determining PAHs in plant tissues. They

compared four extraction procedures sonication, liquid/solid batch extraction, continuous liquid/solid extraction and soxhlet extraction. A sonication extraction method was found to be reliable, convenient and most effective. After sonication PAHs were partitioned into pentane. The pentane extract was fractionated on a micro silica acid column and was then analysed by GC-FID. Recovery studies were conducted for the concentration steps, the clean-up steps and the overall method with different extraction techniques. Recovery studies were evaluated by spiking extracts with NBS SRM 1674 standard, which contains the 16 PAHs recommended as priority pollutants by USEPA. Recoveries for the concentration and clean-up stages were quantitative in most cases. However the overall method showed recoveries as low as 47% for naphthalene. No explanation was given for these low recoveries. They concluded that the differential accumulation by the plants of selected PAHs was probably related to their different physical characteristics.

Stive and Diserens [102] present an alternative extraction technique for the extraction of PAHs from spiked cocoa butter and corn oil using adsorption on synthetic calcium silicate [calflo E]. This method allows large sample portions, 30g to be used. The formation of emulsions during sample preparation is usually a problem with large sample sizes. This is not the case with this technique. The technique was also applied to difficult substrates like lecithin and essential oils. Recoveries were compared with standard saponification methods. They concluded that the calflo method worked well for PAHs, however, the results quoted showed that in all cases investigated saponification showed higher recoveries.

Meier and Aubart [103] examined a number of dried mushrooms using a

saponification technique with silica gel clean-up and analysis by GC-MS. PAHs ranged from biphenyl to benzo[ghi]perylene. LOD for benzopyrenes was 20pg. The mean recovery based on spiked experiments was 82±7%. No discussion as to how this was carried out was given.

Ignesti et al [104] determined the levels of five PAHs in olives to see if they act as indicators for pollution. The olives were saponified and partitioned with DMF/H_2O and isooctane, clean-up was on a silica column and the samples were analysed by HPLC. Recoveries of 36.9±12% were quoted but the basis as to how this was achieved is not mentioned. The PAHs examined were benzo[a]pyrene, fluoranthrene, benzo[ghi]perylene, benzoanthracene and pyrene.

Ligon et al [105] presented an unusual method for the extraction of PAHs from chicken fat. They used azeotropic distillation with glycerol to extract the PAHs, analysis was carried out by GC-MS. A chicken sample was spiked with PAH followed by GC analysis. Azeotropic distillation offers a number of advantages over soxhlet extraction. It is often quicker and more effective than soxhlet extraction for many sample types. In addition, many interfering substances typically encountered in environmental analyses such as large triglycerides do not form azeotropes and therefore are excluded from the analysis. It also produced enriched samples for GC-MS analysis.

The effect of SFE flow rate was investigated in relation to extraction efficiency for the extraction of PAHs from potato chips and lemon peel [106]. They concluded that the extraction rate from potato chips, which is controlled by the solubilisation/elution process showed direct correlation with CO_2 flow rate and inverse correlation to sample size. This correlation was not present for extractions which are based, in their

opinion, on the kinetics of the desorption step which is the case for the lemon peel sample.

Lodovici et al [107] conducted an investigation into the presence of PAH in the Italian diet. Extraction was conducted by refluxing the sample in MeOH / H_2O with KOH for 1.5 hours followed by extraction into iso-octane and evaporation. Analysis was conducted by HPLC. No recoveries for the method were mentioned.

H. Kaupp and M. Sklorz [108] used a soxhlet extraction method with toluene to extract PAHs from maize leaves. This was followed by clean up using biobeads SX8 and silica gel. Analysis was carried out using GC-MS. Recoveries for all the PAHs studied were greater than 93% except for perylene and indeno[123 cd]pyrene which were 51 and 55 % respectively. There was no reason given for these low recoveries.

Chen et al [109] investigated the use of soxhlet extraction with clean-up using SepPak florisil cartridges. Analysis was conducted by HPLC UV/Fl. The 16 priority PAHs were extracted from grilled duck and frozen meat. They did not assess the technique's ability to recovery samples quantitatively.

Cejpek et al [110] used a simplified extractive and clean up procedure for extracting 12 of the USEPA priority pollutants from meat products. The samples were ground and homogenised with $CHCl_3$, the organic extract was filtered and applied to a biobeads S-X3 GPC column for clean prior to evaporation and analysis by HPLC. LODs were quoted at between 0.02 and 1 mirco gram/kg. The examined PAHs ranged from phenanthrene to indeno[123cd]pyrene. The recoveries quoted ranged from 53% for phenanthrene and 112% for benzo[k]fluoranthene.

Due to the varied nature of food-stuffs, the extraction and analysis of the PAHs are often specific for the individual type of food. In general similar problems with sample preparation which were noted for other sample matrices were noted here. These included losses due to evaporation, photodecomposition and physical losses.

1.2.8. Aqueous Samples.

The examination of aqueous environments for the presence of PAHs has become increasingly important in recent years. Although PAHs are not very soluble in water due to their non-polar nature they may be present in suspensions and are therefore a health hazard.

Symons et al [111] reported on a method of extracting PAHs, which were recommended by USEPA as priority pollutants, from an aqueous environment. These PAHs ranged from naphthalene to dibenz[ah]anthracene. The PAHs were extracted from water samples by enrichment onto disposable Sep-Pak C_{18} cartridges followed by direct injection onto HPLC-UV/FL. They found that because of the limited solubility of PAHs in water, representative spiking prior to the enrichment step proved difficult. Recovery experiments were done by adding 500cm^3 of a PAH mixture in ACN to 500cm^3 of H_2O and passing through Sep-Pak. They noticed that the recoveries got increasingly worse as the number of rings present in the molecule increased. Adsorption onto the container surface was considered to be a main cause of bad recoveries for the larger PAHs. The addition of 20% MeOH to the sample greatly improved the recoveries. They also concluded that the recoveries for the lighter PAHs were higher for a liquid/liquid extraction procedure than for the SPE, the reason for this was not given. LODs were quoted to range from 0.005-10μg depending on the

analyte and the method of detection.

Rostad et al [112] applied off-line solid phase extraction using a series of bonded phases to the extraction of PAHs from hazardous waste water. Spiked water samples were used to investigate recoveries for different concentrations of PAHs and different bonded phases. They concluded that for most compounds the recoveries were not effected by concentrations. The notable exceptions were fluorene, phenanthrene and anthracene at 200µg/L which gave low recoveries. Phenyl bonded phases seemed to give slightly higher recoveries for spiked samples. Analysis was carried out by GC-MS. One advantage of using SPE is the ability to collect the analyte directly onto the sorbent. This implied that possible alterations between the time of sampling and analysis would be eliminated.

Desideri et al [113] determined PAHs in 1000cm^3 of sea water by first extracting with n-hexane/CCl_4 followed by acid/base extraction, clean-up on SiO_2 and Al_2O_3 columns. The PAHs were eluted with n-pentane/CCl_4. Analysis was carried out by GC-FID. The efficiency of extraction was evaluated by spiking the sea water. Recoveries for PAHs varied between 47-84% for naphthalene to benzo[ghi]perylene. No explanation was given as to why the recoveries for the overall method were low. The solvents efficiencies were evaluated separately and recoveries at this step were concluded to be good. This implies losses arose from the concentration or column chromatography steps. LODs are quoted as being between 25-45 ng/L.

Van Noort et al [114] noted that results of the extraction of PAHs from canal water and rain water depended on whether the PAHs were concentrated directly on SepPak C_{18} or concentrated after filtration with further analysis of the particulate matter. For the canal water higher total PAH concentrations were obtained when suspended

material was also analysed. Recoveries were calculated for spiked deionized water containing 15% 2-propanol. Recoveries ranged from 74% for fluoranthrene and 59% for indeno[123]cd pyrene with a gradual reduction as the molecular weight increased. No explanation for these relatively low recoveries was given. It was noted that spiked PAHs may react differently from PAHs which are native to the sample matrix.

Ghaolu et al [115] compared the extraction of PAHs from spiked water samples on various solid adsorbents. These adsorbents included a number of C_{18} , C_8, Thermotrap TA and phenyl bonded packings. Analysis was carried out by GC. It was concluded that the same C_{18} from different suppliers was found to have different extraction capabilities. Although Thermotrap TA was shown to be best adsorbent for fluorene, anthracene, phenanthrene and pyrene, phenyl reversed phase sorbent proved to be better for chyrsene and benzo[a]pyrene. It is therefore difficult to find a single adsorbent that would extract all of the components of interest. No explanation was given as to why one bonded phase was better than another. One problem with the sample preparation used was the need for solvent replacement prior to analysis. The procedure was also compared to liquid/liquid extraction. No attempt was made to optimise the conditions for the SFE extraction used.

Geisert et al [116] presented an alternative solid sorbent matrix for the extraction of B[a]P and fluoranthene from the aqueous environment. The alternative adsorbent was called blue pearl [bp]. It is a specific mutagen adsorbent of solid polymethacrylamide carrier beads (Eupergit® - Diol) with covalently linked trisulfocopper-phthalocyanine (TCP residues),. BP has an affinity for fused planar ring structures which makes it very selective. Compounds are adsorbed from an aqueous environment by passage

through a packed column. They are eluted with $20cm^3$ $MeOH/NH_4OH$. The solvent was then replaced with DMSO and then the sample was assayed by TLC. The recovery was quoted at 90% for the PAHs investigated. No details about these efficiency experiments were given.

Junk and Richards [117] reported on the application of SPE using octadecyl bonded columns for the extraction of PAHs from the aqueous environment. The unique feature of this research was the combination of small water volumes, fast flow rates, small columns and small eluate volumes that obviate solvent removal prior to GC analysis. All the other advantageous features of SPE were retained. The PAHs were examined at a $10\mu g/cm^3$ concentration and recoveries were compared for elution with ethyl acetate and benzene. They found that when a normal procedure of drying for several minutes before elution with ethyl acetate was used it resulted in low recoveries for low MW compounds. This was concluded to be the result of evaporation. They concluded that the best results were achieved using a partially dried column and benzene as the eluant. They stated that the reason why ethyl acetate gave lower recoveries may be related to its solubility, 10% in water. This may result in small fractions of these compounds being solubilized in the leading phase containing 1:10 ethyl acetate: water during extraction. Recoveries for several high MW compounds were calculated to range from 88-96%. These were achieved using the predried ethyl acetate method. The SPE procedure was also applied to a field sample and compared with a conventional extraction with $MeCl_2$. It was concluded that there was excellent agreement between the two techniques in terms of quantities recovered. This agreement would suggest that the SPE method is the better method to use because it is a more convenient procedure.

The Dept. of the Environment (UK) discussed [118] two analytical procedures, HPLC and TLC, for the analysis of six PAHs, fluoranthene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[ghi]perylene, benzo[l]flouranthene and indeno[123]pyrene. The six PAHs are used as indicators of PAHs occurring in nature. The procedure involved extraction with cyclohexane followed by concentration first with a rotary evaporator and then with N_2 blow down. The residue was then taken up in 200µL of MeOH. They noted that losses of PAHs may occur during this concentration procedure. They were then analysed by HPLC-FL and TLC. No details were given as to how recovery experiments were carried out, efficiencies were quoted in the range 70-90%.

Valenburg et al [119] examined liquid extraction procedures for the removal of PAHs from aqueous samples. They investigated the order in which the water was acidified or basefied, whether a continuous extraction or a separatory funnel procedure was used and whether a micro-snyder apparatus or nitrogen blow down was used for concentrating the sample. They concluded that a continuous extraction method with acidification followed by basification with fresh $MeCl_2$ for each extraction gave the highest recoveries. This was particularly the case for low MW compounds. They also found that the only difference with nitrogen blow down was that it seemed to produce more reproducible results.

Yunker et al [120] compared the adsorption of spiked aqueous samples on chromosorb T and XAD-2 resin. They concluded that chromosorb T columns gave good recoveries for PAHs of three or more rings at a concentration of 0.4ng/l/component, and XAD-2 resin gave good recoveries for PAHs of four and more rings at a concentration of 0.06ng/l/component. Both sorbents gave poor recoveries

for low MW compounds.

Mazzeo et al [121] described a rapid and simple procedure whereby PAHs are analysed by LC-electrochemical detection (ED). This involved the derivatisation of PAH to quinones using Ce(IV) oxidation and EC detection by cyclic voltammetry. The method was validated for spiked tap water. Recoveries are not discussed. They concluded the detection limits were poor when compared with HPLC-FL. The advantage of the described method was its selectivity as it allows one or two PAHs present in a complex mixture to be determined, this makes the method ideal for specific applications.

Dix et al [122] proposed a method which combined steam distillation and SPE for the removal of PAHs from aqueous samples. The distillation is used because of problems that were encountered when samples containing solids cause blockages in the SPE system. In this work condensate is drawn through a small resin containing tube by vacuum and the trapped organics are eluted from the resin with ethyl acetate. Good recoveries were noted for low MW compounds, 93.1-83.55% for PAHs ranging from naphthalene to pyrene in spiked samples.

Ong et al [123] described a rapid and simple method for the analysis of PAHs in aqueous environments. A micro scale on-line SFE-SFC system using CO_2 was employed. The unusual feature of this system was the addition of an extractor. The extractor is a cartridge-like chamber filled with packing material, it facilitates extraction from liquid samples and also acts as a clean-up precolumn. The method was compared with a standard liquid/liquid extraction for the extraction of four PAHs

fluoranthene, benzo[b]fluoranthene, benzo[a]pyrene and benzoperylene. These are known to act as pollution indicators in drinking water. They concluded that the extraction efficiencies obtained using on-line SFE-SFC system were generally higher (91-88%) than the liquid/liquid extraction method (58-68%). The additional clean-up step required in the latter method seems to be the main cause of the lower extraction efficiency. No detailed description is given of the spiking experiments that these recoveries are based on.

Jani et al [124] employed an extraction procedure involving liquid/liquid extraction with $MeCl_2$, evaporation, reconstitution in cyclohexane, extraction with nitromethane followed by cleanup by TLC and finally analysis by HPLC. The sixteen PAHs recommended by the USEPA as priority pollutants were investigated. No investigation of the extraction efficiency for the described method was carried out. They concluded that the levels of exposure to these PAHs from drinking water in India were higher than that experienced in Europe. In particular the level of exposure to benzo[a]pyrene was two and ten times greater than the maximum allowable concentration in the USA or USSR.

García et al [125] developed a HPLC-FL gradient technique for the separation of the sixteen PAHs listed as priority pollutants by the USEPA. The separation was applied to water and solid samples. The efficiency of the extraction procedure was evaluated for six PAHs. Coal washings were extracted with $MeCl_2$, concentrated and injected onto the HPLC column. After the solid samples (sediments) were ultrasonically extracted, cleanup was carried out on a SiO_2/Al_2O_3 column prior to analysis. They concluded that the recoveries observed for the six individual PAHs from a spiked coal washing sample were better than 85%. The PAHs examined were benzo[a]anthracene,

chrysene, benzo[b]fluoranthene, benzo[a]pyrene, dibenzo[ah]anthracene and indeno[123 cd] pyrene. In the case of suspended solids recovery rates were in the range 80-88% for the six PAHs. The LOD ranged from 0.008ng/cm^3-0.125ng/cm^3 depending on analyte.

Klarska et al [126] described the solid phase extraction of PAHs particularly B[a]p from aqueous solutions using XAD-2 resin. The effectiveness was evaluated for thirteen PAHs ranging from naphthalene to benzo[ghi]perylene. Recoveries of 41-64% were quoted which are very poor. No explanation was offered for these low recoveries.

Tang et al [127] investigated the use of liquid/solid extraction on C_{18} cartridge/disk followed by supercritical fluid elution as an alternative method for the extraction of PAH from spiked water samples. SFE conditions were opitimised based on recovery experiments. They concluded that disk rather than cartridge forms of C_{18} packing gave the highest efficiency. They also concluded that high pressure increases extraction efficiency for most of the PAHs from the disks. Increasing the volume of CO_2 used resulted in the highest extraction efficiencies with the optimum being a volume of seven times that of the extraction cell volume. Changing temperature had the least effect on the recoveries of PAHs in this study.

Bokelen and Niessner [128] applied different non-ionic surfactants for the micellar extraction and enrichment of PAHs from aqueous media. Detection was achieved by a spectroscopic method using synchronous fluorescence. Detection limits of 6.8mg/L and 2.6mg/L for benzofluoranthene and benzo[a]pyrene were recorded. Recoveries of up to 100% were achieved for these compounds, however, no details are given as to how this was assessed. They stated that the great advantage of this technique was that

there was no longer a need for large volumes of inflammable and toxic organic solvents.

Pinto et al [129] used micellar extraction with a nonionic surfactant, Triton X-114, to concentrate PAHs from waste aqueous and solid samples. Analysis was carried out by HPLC-FL. The advantage of using micellar extraction with X-114 is its ability to prevent sorption of the analytes onto sample containers such as glass and polyethylene. They concluded that micellar solutions of triton X-114 are as effective as traditional organic solvents for the extraction of PAH from solid matrices. This lead to a procedure in which organic solvents are eliminated in the pretreatment and preconcentration steps used in preparing the sample.

An insitu filtration / extraction system was developed for the recovery of trace organics in solution and on particles from deep ocean water [130]. Six PAHs were recovered using a SPE system that used XAD-2 resin as the extractive material. No recoveries were quoted for the individual PAHs examined.

R. el Harrak et al [131] compared two SPE based systems where a 1000cm^3 sample of 85% H_2O / 15 % IPA was passed through an Empore disc (05mm X 47mm) containing 500mgs of C_{18} bonded silica or styrene-divinylbenzene copolymer. PAHs were extracted with CH_2Cl_2 / EtOAc / ACN (5:3:2). They were concentrated and reconstituted with ACN and analysed by HPLC-Fl. LODs were quoted at 0.2 - 3.7ng / L with recoveries of between 70 - 99%. They concluded that the copolymer membrane gave better recoveries. They also noted an increase in recoveries when IPA was replaced with Brij-35.

Solid phase microextraction (SPME) is a relatively new technique and was applied to

the extraction of 6 PAHs from aqueous coal water [132]. SPME uses a fiber coated with PDMS to extract organic material from environmental samples. These analytes where then thermally desorbed prior to analysis by GC. The technique was compared with a liquid / liquid extraction that used deuterated internal standard. No percentage recoveries were calculated. They concluded that SPME minimizes disturbance of the natural matrix, requires only a small volume of sample and has a fast response time. J. Slobodik et al [133] developed an automated SPE -LCMS system for the extraction of the 16 priority pollutant PAHs. Spiked water samples were mixed with Brij-35 and extracted onto Boos silica (diol modified silica containing a copper phthalocyanine trisulfonic acid moiety). The PAHs were then eluted onto a HPLC column that gave separation of the compounds with a gradient assay. Detection was achieved using MS. They did not calculate % recoveries. They noted however that the method was not suitable for low MW compounds. The LODs were quoted at 0.003 -4.0 μg/L.

Kayali Sayadi et al [134] described a rapid online SPE process for the determination of PAHs in drinking water. In this case 1.5L of a water sample was subjected to SPE on SepPaK vac tc-18 cartridges eluted with ethyl acetate, evaporated, dissolved in MeOH and analysed by HPLC-Fl. Recoveries were between 60% and 96% for the 12 PAHs examined in the concentration range 2.33 –48.7 ng/L. No explanation was given for the low recoveries. The PAHs ranged from naphthalene to benzo[ghi]perylene.

Janda et al [135] investigated the various parameters involved in SFC and examined their effects on the extraction of acenaphthene, fluoranthene, pyrene and chrysene using CO_2. The results indicated that the presence of salt in the sample did not affect

the extraction, increasing the temperature from 50 to 95 °C increased recoveries and a higher flow rates produced high recoveries.

SPE with microwave assisted solvent elution (MASE) was developed for preconcentrating PAHs from aqueous samples [136]. Samples were first drawn through C_{18} extraction discs at 68kPa and dried. The loaded discs were then transferred to PTFE-lined vials with $20cm^3$ of acetone and exposed to microwave radiation. Recoveries were quoted for anthracene and benzo[a]pyrene at 70-86%. No explanation was given as to why recoveries improved with the presence of NaCl and decreased by the presence of humic acid.

Fernandez et al [137] compared SPE using SepPak and C_{18} cartridges to the standard method 610 for the extraction of 11 of the priority pollutant PAHs from H_2O. Analysis was carried using HPLC-Fl. They noted that recoveries were slightly higher for liquid / liquid extraction but were generally above 80% for SPE.

Kiss et al [138] applied a SPE method with HPLC UV for the extraction and analysis of 8 PAHs (3 - 6 ring EPA priority pollutants). A procedure was optimised that required evaporation and reconstitution prior to analysis. Recoveries were quoted at > 90% no attempt was made to account for any possible losses.

Manoli and Samara [139] compared three extraction methods for the extraction of the 16 priority pollutants. These were, the EPA Method 610, liquid / liquid extraction using cyclohexane and SPE combined with sonication. They concluded that the best

results were achieved with liquid / liquid extraction using cyclohexane. They also noted that losses due to evaporation for the volatile compounds were unavoidable due to the fact that the samples had to be reconstituted in ACN.

Chen and Pawliszyn [140] demonstrated the ability of a SPME to extract four PAHs, fluoranthene, pyrene, benzo[a]anthracene and benzo[a]pyrene at 100ppb spiked in H_2O and introduced into HPLC system. No recoveries were quoted.

Lintelmann et al [141] described a SPE method were by once the water sample had been extracted onto the column (copper phthalocyanine modified silica) the column was then included into a HPLC system.The analytes were then flushed in backflush mode onto the analytical system. No % recoveries were recorded.

Six PAHs benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene indeno[123 cd] pyrene and benzo[ghi]perylene were extracted into hexane from an aqueous sample [142]. The extracts were then scanned at 200 - 500nm with a constant 120nm between the excitation and emission monochromators. Percentage recoveries were recorded at 77%.

1.3. Conclusion

Many traditional and novel techniques have been applied to the problem of extracting PAHs from various environmental samples. A number of parameters must be considered while assessing the success of these techniques.

When low MW PAHs were investigated many authors noted problems with recoveries of these compounds due to the fact that they are volatile and are therefore susceptible to loss by evaporation. The use of solvent distillation and N_2 blow down techniques to remove organic solvents or water during sample extraction and clean up can be a major draw back when preparing samples which contain low molecular weight compounds.

The type of spiking experiments used to assess an extraction technique can also influence the conclusions made. It must be noted that under certain circumstances if the spiking technique does not mimic the presence of PAHs in the natural environment, the conclusions drawn about the ability of the technique to extract PAHs from real samples must be questioned.

This is the case for solid or particulate samples when the PAHs are included during the formation of the substance. Therefore spiking samples where PAHs are placed on the surface of these samples will not mirror the presence of these compounds in natural samples. In these samples the PAHs are incorporated in less accessible parts of the sample, it may require more vigorous extraction than a spiking experiment for the PAHs to be removed quantitatively. The use of radiolabelled spikes has also been questioned in terms of its accuracy for many of the reasons outlined above and also it has been asked whether the radiolabelled PAH itself many respond differently than the naturally occurring PAH.

In this present work it is hoped that the removal of the need to use solvent replacement in sample preparations through the use of novel extraction techniques will overcome many of the problems encountered previously.

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Chapter 2.0

An Examination of Offline Extraction Procedures used for the Enrichment of PAHs at Trace Concentrations from Aqueous Samples.

2.1 Introduction

Trace enrichment of priority pollutants is critical if present day analytical techniques that are relatively inexpensive i.e. HPLC UV/Fl are to be used.

PAHs are a particular class of carcinogens, which are of great interest in sample enrichment because of their presence in our environment, and because of their hazard to health at even minute concentrations.

Offline extractive techniques for the removal and enrichment of PAHs from the aqueous environment have been discussed in detail in chapter 1 (see section 1.2.8.).

The aim of this investigation was to assess two offline extraction procedures used for the enrichment of PAHs from aqueous samples. A HPLC method was developed and validated for the five PAHs investigated. These were naphthalene, acenaphthylene, acenaphthene, fluorene and phenanthrene. The ability of liquid/liquid and a solid/liquid extraction were assessed in relation to the removal of these five PAHs from aqueous samples.

2.2. Chromatographic Method.

For the purpose of investigating the various extraction techniques, it was decided to develop a HPLC separation for the first five compounds listed by the USEPA as priority pollutants. The five PAHs were chosen as they have the lowest molecular weights of the sixteen priority pollutants listed, their low boiling points and vapour pressure would make them most sensitive to extraction techniques. Previous studies of these compounds have indicated that more traditional extractive techniques have shown poor reproducibility and

sensitivity towards these compounds. The compounds investigated were naphthalene, acenaphthylene, acenaphthene, fluorene and phenanthrene.

2.2.1.Experimental

2.2.1.1. Apparatus.

The instrument arrangement consisted of a Rheodyne 7125 fixed 20µl loop injection system, one Waters Model 501 solvent delivery pump, one Waters Model 486 UV absorbance tuneable detector. The analytical column was a Spherisorb 10 ODS 1 5µm (25cm X 4.6mm). Data was collected on a Waters 746 integrator.

2.2.1.2. Materials

All chemicals used were of analytical reagent grade. Labscan supplied Acetonitrile. H_2O was purified using a Milli-Q water purification system supplied by Millipore. Chem Services Alltech supplied the Polycyclic Aromatic Hydrocarbons. The purities of the certified standards were as follows: naphthalene 98.5%, acenaphthylene 97.0%, acenaphthene 99.0%, fluorene 99.0% and phenanthrene 99.0%. All solvents were filtered and sonicated before use. Stock solutions of the Polycyclic Aromatic Hydrocarbons ($1.0mg/cm^3$) were prepared in ACN and stored at 2°C in the dark. From these solution a 1.0mg/L stock standard containing the five PAHs mentioned above was prepared. A fresh 1mg/L stock standard was prepared every six weeks. This solution was also stored at 2°C. All glassware was detergent cleaned, rinsed with tap water and then with distilled water prior to use.

2.2.1.3. Optimum Conditions.

The optimum chromatographic parameters were achieved by the judicious choice of mobile phase composition and pump flow rate. The wavelength of detection was chosen because these compounds show maximum absorbance in this region. The optimum conditions for the separation of these compounds were defined as;

Wavelength	=	254nm
Mobile Phase	=	50% Acetonitrile / 50% H_2O.
Flow	=	$0.8cm^3/min$
Chart Speed	=	0.5mm/min

2.2.2 Validation

The HPLC method was validated under the following parameters: specificity, linearity, precision and limit of detection.

2.2.2.1 Specificity

Specificity for the assay was defined as the ability of the assay to separate the PAHs present. The conditions outlined in 2.2.1.3. were deemed to be satisfactory for this purpose. The separation was developed using injections of a 10ppm mixed standard containing all five compounds mentioned (see table 2.1). The identity of each compound was confirmed by injecting individual standards.

Retention Time (mins)	Compound
17.80	Naphthalene
21.39	Acenaphthylene
26.96	Acenaphthene
28.77	Fluorene
35.47	Phenanthrene

Table 2.1

A list of retention times (mins) for the five PAHs investigated using the optimum conditions for their separation on a Spherisorb 10 ODS 1 5μm (25cm X 4.6mm). These conditions were mobile phase 50/50 H_2O/ACN and flow rate = 0.8cm^3/min (see appendix 1)

2.2.2.2. Linearity

For the purpose of quantifying the amount of analyte present in the aqueous samples it was necessary to show that each analyte had a linear response in the concentration range which we investigated i.e. sub-ppm. A calibration curve was constructed by injecting a series of standards containing specific concentrations of analyte .The concentration range was from 900 - 100ppb, five points were used for the standard curve, each standard was injected twice and an average area recorded. Linear regression was carried out for each analyte and the correlation coefficient was calculated. All five compounds were deemed to have a linear response in this concentration range as their correlation coefficients were all above 0.994 (see table 2.2). The working calibration curve was verified by the injection of a 500ppb mixed standard prior to each experiment or when a fresh reservoir of mobile phase was prepared.

Compound	Correlation Coefficient
Naphthalene	0.999
Acenaphthylene	0.997
Acenaphthene	0.994
Fluorene	0.999
Phenanthrene	0.998

Table 2.2

Correlation coefficients calculated for the five PAHs investigated demonstrating a linear response for each compound. The linear response was calculated for each of the compounds based on the injection of five standards in the 100 to 900ppb concentration range (see appendix 2).

2.2.2.3. Precision

The precision of the manual injection system was assessed to ensure precise results were obtainable. Four injections of a standard containing the five compounds at a concentration of 500ppb were carried out using the conditions outlined in 2.2.1.3. The coefficient of variation (%CV) was calculated (see table 2.3).

Compound	% Coefficient of Variation
Naphthalene	4.03 %
Acenaphthylene	4.05 %
Acenaphthene	5.09 %
Fluorene	0.77 %
Phenanthrene	4.63 %

Table 2.3

Table of %CV calculated of each of the five compounds of interest demonstrating the compounds could be injected precisely at a concentration of 500ppb using the optimum conditions for their separation.

The precision data was deemed acceptable for all five compounds with only acenaphthene giving a result slightly above the 5.0 % level. This is possibly due to the fact that this peak elutes close to fluorene and at certain concentrations fluorene interferes with the integration of this peak.

2.2.2.4. Limit of Detection

The limit of detection was defined as twice the level of noise. The noise level was measured at Attenuation 1 to be 3mm.

L.O.D. = 3mm x 2 = 6mm

6mm = 1500 - 1700 area units.

A series of standard injections were carried out at various concentrations, 10ppb-70ppb, and were possible a plot of concentration versus area was constructed. For naphthalene, fluorene and phenanthrene it was possible to calculate the L.O.D. based on these plots. For acenaphthylene and acenaphthene L.O.D. was based on a visual examination of the chromatograms as the plots of concentration versus area were not linear due to the poor peak shape noted for these compounds at such low concentrations (see table 2.4).

Compound	Limit of Detection (ppb)
Naphthalene	16.4
Acenaphthylene	51.0
Acenaphthene	34.8
Fluorene	5.4
Phenanthrene	3.5

Table 2.4

Table showing the limits of detection for the five compounds of interested using the defined chromatographic technique. LODs were calculated for naphthalene, fluorene and phenanthrene using a plot of area versus concentration. LODs were estimated for acenaphthylene and acenaphthene due their poor peak size at low concentrations (see appendix 3).

2.2.2.5. The Limit of Determination

The limit of determination was defined as three times the level of noise.

Limit of Determination = 3mm X 3 = 9mm

9mm = 2250 - 2500 Area Units

Similar problems to the ones discussed for the limit of detection were encountered here; the non-linear response for both acenaphthylene and acenaphthene meant that the limits of determination were estimated based on the results calculated in section 2.2.2.4. (see table 2.5).

Compounds	Limit of Determination (ppb)
Naphthalene	27.35
Acenaphthylene	76.50
Acenaphthene	52.20
Fluorene	6.78
Phenanthrene	3.99

Table 2.5

Table of limit of determination for the five compounds of interest using the defined chromatographic technique. The limits of determination were calculated for naphthalene, fluorene and phenanthrene using plots of area versus concentration and estimated for acenaphthylene and acenaphthene due to their poor peak size at low concentrations.

2.3. Offline Extraction Procedures.

2.3.1. Offline liquid/liquid extraction

Liquid/Liquid extraction is the traditional method used to extract analytes from a liquid matrix. It is this procedure which is recommended by the United States Environmental Protection Agency (USEPA) Method 610 for the extraction of PAHs from aqueous samples [1]. The method outlined below is a method based on USEPA 610 in which the extraction procedure was followed as closely as possible. However as specific pieces of glassware were not available certain modifications to the method were made. These modifications relate specifically to the solvent replacement. A rotory evaporator was used to concentrate the dichloromethane to dryness and the sample was then reconstituted in acetonitrile prior to injection. This replaces a step in the method that involved the use of microdistillation equipment to carry out the replacement of dichloromethane with

acetonitrile. The use of the rotory evaporator and particularly the need to reconstitute the sample in acetonitrile makes this extraction technique much more sensitive to losses due to evaporation. This in turn may have lead to difficulties in getting accurate recoveries.

2.3.1.1 Experimental

2.3.1.1.1. Apparatus

The HPLC system is as described in 2.2.1.1.

The conditions used are outlined in 2.2.1.3.

The Extraction Apparatus was made up of the following pieces of glassware.

1 x 1000cm^3 Separating Funnel

1 x 250cm^3 Round Bottom Flask

1 x Gravitational Filtration Unit

1 x Buchi Rotavapor R-114 with a B-480 Waterbath

2.3.1.1.2. Materials

Dichloromethane HPLC grade was supplied by Labscan.

BDH LTD supplied sodium sulphate analar grade.

All other chemicals are as described previously (see 2.2.1.2.).

2.3.1.1.3. Extraction Procedure

A spiked H_2O sample was prepared by diluting 1cm^3 of a 500ppb-mixed standard to 500cm^3 with water in a 500cm^3 volumetric flask. The 500cm^3 sample was transferred to a 1000cm^3 separating funnel. 60cm^3 of dichloromethane were added and the mixture was shaken for 2 minutes with periodic venting. It was then allowed to settle for 10 minutes.

The dichloromethane was separated into a beaker. This procedure was repeated two further times.

10 grams of Na_2SO_4 were added to the 180cm^3 of dichloromethane, this was then stirred for 5 minutes. The Na_2SO_4 was filtered off using No. 1 Whatman filter paper. The Na_2SO_4 was washed with three 15cm^3 aliquots of dichloromethane.

The dichloromethane was transferred, followed by a 10cm^3 wash, to a 250cm^3 round bottom flask. This was evaporated to dryness on a Rotovap with H_2O vacuum system with the waterbath at ambient temperature. 1cm^3 of acetonitrile was then added to reconstitute the sample. This was then transferred to a plastic vial and sealed until the sample was analysed by HPLC. Two samples and one H_2O blank were prepared using the above procedure and percentage recoveries calculated by comparison of peak areas with peak areas for a 500ppb mixed standard.

2.3.1.2 Results.

Compound/ % Recovery	Sample 1	Sample 2
Naphthalene	88.8 %	55.8 %
Acenaphthylene	95.0 %	38.0 %
Acenaphthene	172.7 %	75.2 %
Fluorene	372.6 %	67.0 %
Phenanthrene	126.6 %	63.7 %

Table 2.6

Table of percentage recoveries for the liquid / liquid extraction procedure based on USEPA method 610. Recoveries were calculated by the comparison of sample peak area for each compound and the average peak area for a 500ppb mixed standard.

2.3.1.3 Discussion

The recoveries were calculated for each compound by comparison with average peak areas for four injections of a 500ppb mixed standard because the predicted final concentration in the $1cm^3$ aliquot of acetonitrile is 500ppb.

The main advantages of this method are that no complex pieces of equipment are required and the extraction is relatively simple to perform. A large amount of sample can be used which makes it a sensitive technique. It was possible to analyse a spiked sample with 500ppb of five PAHs using this technique.

The main problem with the method is it is not reproducible. There are large variations in recoveries between the two samples. The problem with the extraction technique is the fact that these compounds may be susceptible to evaporation during the distillation. Another problem is the way the sample is reconstituted in acetonitrile; the slightest variation in the amount of solvent used will have a great effect on the accuracy of the recovery recorded.

2.3.2 Offline Solid Phase Extraction.

An offline solid/liquid extraction procedure was developed for the extraction of the five PAHs listed in section 2.2.1.2 from aqueous samples.

2.3.2.1. Experimental

2.3.2.1.1 Apparatus

The HPLC equipment is as outlined in 2.2.1.1. The chromatographic conditions are outlined in 2.2.1.3. The offline extractions were carried out on 'Sep Pak C_{18} Light' cartridges supplied by Waters. Each cartridge contained 130mgs of mono-functional C_{18} packing.

2.3.2.1.2. Materials

The materials are as described previously in section 2.2.1.2.

2.3.2.2. Optimisation of the Extractive Procedure

The conditions for the extraction of the analytes from aqueous samples were optimised using the following basic procedure:

$10cm^3$ of 100ppb mixed standard were loaded onto the 'Sep Pak' cartridge. The analytes were then eluted using two aliquots of $2cm^3$ of acetonitrile. These aliquots were then analysed by HPLC.

A number of factors were investigated in relation to this procedure.

a) The sample loading flow rate.

b) The sample elution flow rate.

c) The volume of solvent used to elute the analytes.

d) A comparison of reusing a cartridge as opposed to using fresh cartridges for each extraction.

e) The effect of using mobile phase as the eluting solvent instead of acetonitrile.

2.3.2.2.1. The Sample Loading Flow Rate

Due to the nature of the extraction procedure i.e. manual extraction onto solid phase cartridge, it was felt that the flow rate at which the sample was loaded onto the precolumn would have an effect on the amount of analyte that would be retained on this C_{18} packing. The flow rate would influence the washing effect of the sample passing through the cartridge and thus effect the efficiency of the extraction.

A number of $2cm^3$ acetonitrile wash aliquots were collected from the cartridges. The flow rate, at which the original aqueous sample had been added, was varied. The volume of sample added was held constant at $10cm^3$ of 100ppb mixed standard. The volume and flow rate of acetonitrile used to elute the analytes from the cartridge were also held constant, $2cm^3$ of acetonitrile at a flow of $0.4cm^3$/min was used. The sample loading flow rate was varied from $10cm^3$/min to $0.7cm^3$/min (see table 2.7 and fig 2.1).

Rate (cm^3/min)	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
10.0	78.45%	81.40%	118.35%	75.03%	63.86%
2.0	69.53%	79.84%	108.75%	67.50%	57.20%
1.0	60.97%	65.56%	91.68%	58.91%	50.26%
0.7	62.90%	70.90%	94.48%	57.00%	46.26%

Table 2.7

Table of percentage recoveries for the five PAHs recovered from $10cm^3$ of 100ppb spiked aqueous sample after it was loaded at a number of flow rates onto a SPE cartridge and eluted using $2cm^3$ of ACN at a flow rate of $0.4cm^3$/min.

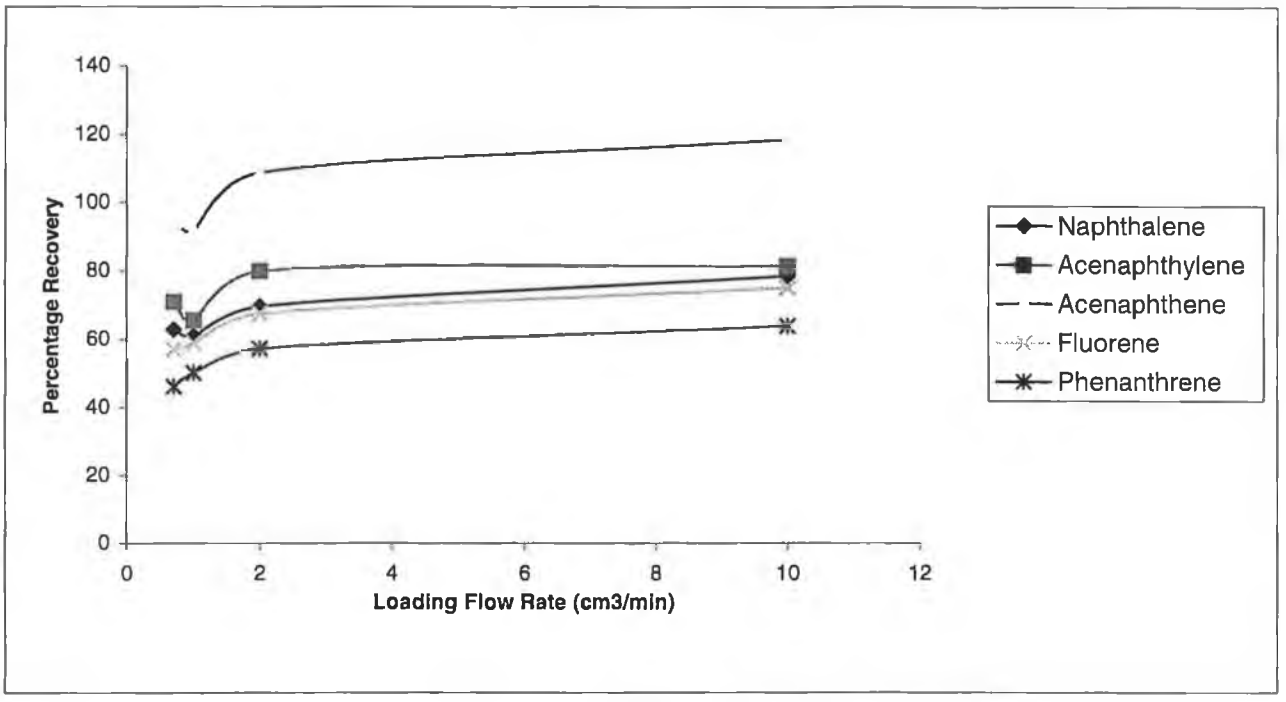

Fig 2.1
Plot of percentage recoveries versus loading flow rate (cm^3/min) for the five PAHs recovered from 10cm^3 of 100ppb spiked aqueous sample after it was loaded at a number of flow rates onto a SPE cartridge and eluted using 2cm^3 of ACN at a flow rate of 0.4cm^3/min.

Each compound reacts differently to this solid phase extraction procedure; this is reflected in the varying recovery rates observed. The high recoveries recorded for acenaphthene are related to poor integration of this peak. 10cm^3/min was chosen as the flow rate for loading the PAHs onto extraction column. Although it appears that a further increase in flow rate would give higher recoveries, the slope of the graph and difficulties encountered trying to maintain such a high flow rate offline meant that the gains possibly achievable by increasing flow rate any further would be minimal. It must be noted that because these extractions were carried out offline, problems were encountered while trying to control the flow rate by hand during both loading and elution of the precolumn. The optimum loading flow rate was concluded to be 10cm^3/min.

2.3.2.2.2. Sample Elution Flow Rate

The flow rate at which the compounds were eluted from the cartridge was also thought to have a significant effect on the efficiency of the extraction system.

A $10cm^3$ aliquot of 100ppb standard mix was loaded onto the cartridge at a flow rate of $10cm^3$/min. This was then eluted from the cartridge using $2cm^3$ of acetonitrile at various different flow rates. These aliquots were collected and analysed separately (see table 2.8 and fig 2.2).

Flow Rate(cm^3/min)	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
4	81.23%	73.31%	N/A	77.99%	91.09%
1.5	81.29%	90.86%	178.20%	95.65%	83.73%
0.4	79.77%	88.10%	181.40%	86.77%	85.21%
0.2	82.90%	92.06%	191.70%	95.97%	78.48%

Table 2.8

Table of percentage recoveries for the five PAHs recovered from $10cm^3$ of 100ppb spiked aqueous sample after it was loaded at a flow rate of $10cm^3$/min onto a SPE cartridge and eluted using $2cm^3$ of ACN at a range of flow rates.

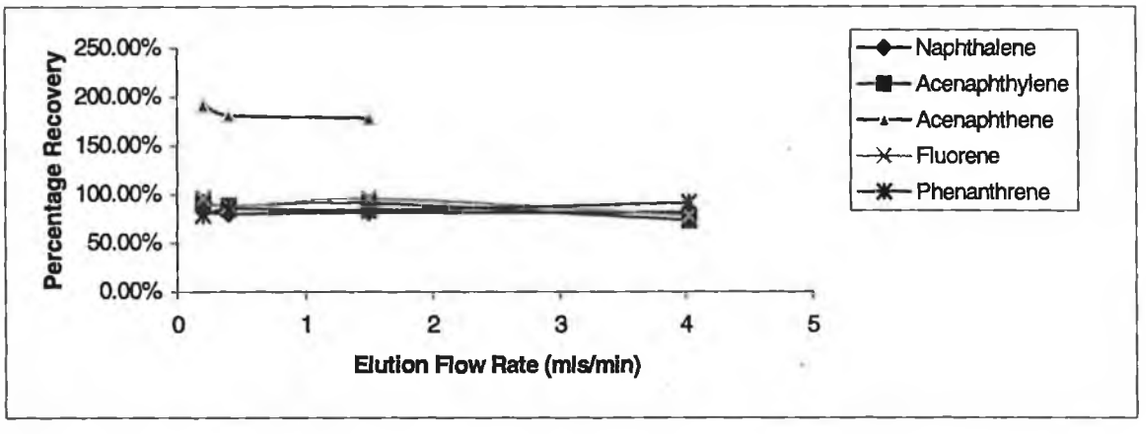

Fig 2.2

Plot of percentage recoveries for the five PAHs recovered from $10cm^3$ of 100ppb spiked aqueous sample after it was loaded at a flow rate of $10cm^3$/min onto a SPE cartridge and eluted using $2cm^3$ of ACN at a range of flow rates.

The difficulties outlined in 2.3.2.2.1. with regard to control of flow rate were also noted here. An examination of the plot of recovery versus flow rate reflects these difficulties, as

it is difficult to assign a definite trend to the recoveries quoted. Recoveries for acenaphthene were again unrealistically high due to poor integration. It was observed that inconsistencies in flow rate have a significant effect on recovery rates. The difficulty in controlling flow rate is a major source of errors in this procedure. It was not possible to define an optimum elution flow rate for this system.

2.3.2.2.3. Volume of Solvent Used.

It was thought that the amount of solvent used to elute the analytes would also have an effect on the efficiency of the extraction process.

A series of samples were prepared using the following extraction procedure.

10cm^3 of 100ppb mixed standard were loaded at 10 cm^3/min. The compounds of interest were eluted using acetonitrile at flow rate of 0.4 cm^3/min. The volume of acetonitrile used was varied between 1cm^3 and 3.0cm^3 (see table 2.9 and fig 2.3).

Volume of Elutent (cm^3) / % Recovery	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
1	85.18%	64.74%	86.27%	76.05%	72.89%
2	81.22%	88.10%	181.20%	86.77%	85.21%
3	84.50%	65.53%	93.32%	80.13%	77.15%

Table 2.9

Table of percentage recoveries for the five PAHs recovered from 10cm^3 of 100ppb spiked aqueous sample after it was loaded at a flow rate of 10cm^3/min onto a SPE cartridge and eluted using various volumes of ACN at 0.4cm^3/min.

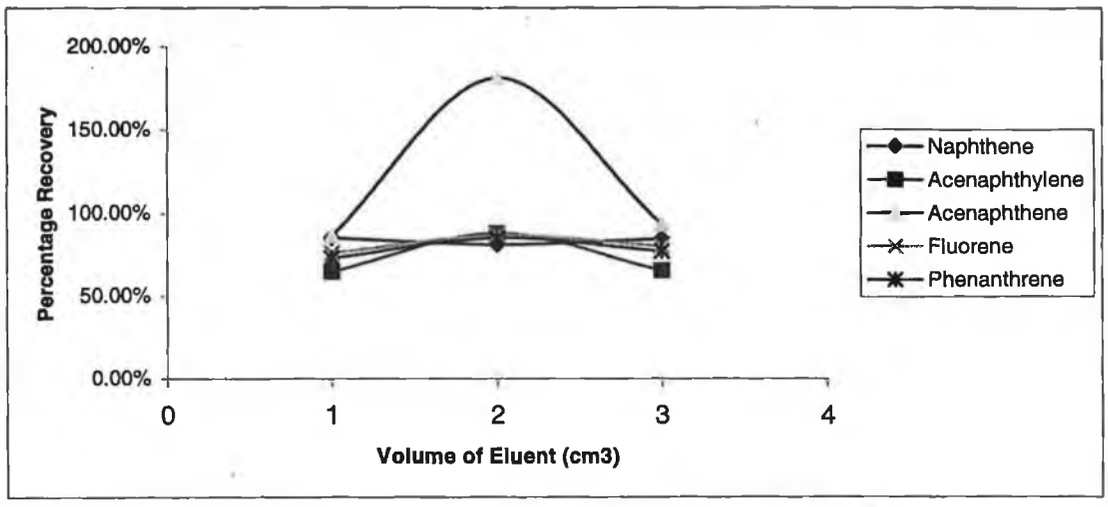

Fig 2.3

Plot of percentage recoveries for the five PAHs recovered from 10cm^3 of 100ppb spiked aqueous sample after it was loaded at a flow rate of 10cm^3/min onto a SPE cartridge and eluted using 2cm^3 of ACN at a range of flow rates.

Again here acenaphthene gave unrealistic recoveries. The optimum volume of ACN concluded to be 2cm^3. The problem of accurate flow control as well as volume measurement contributed to the variation in recoveries in this section.

2.3.2.2.4. Reusing one cartridge Vs Using fresh cartridges.

Since the manufacturer suggested that, in general, a fresh cartridge should be used for each extraction it was deemed necessary to investigate if any significant difference in efficiency existed between using the same cartridge repeatedly and using fresh cartridges. Five samples were prepared using the same cartridge and five were prepared using fresh cartridges (see tables 2.10 and 2.11).

Sample No.	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
1	27.96%	78.44%	77.60%	65.24%	68.32%
2	51.56%	64.21%	56.97%	65.08%	70.94%
3	59.36%	84.66%	67.23%	65.58%	74.10%
4	47.86%	69.35%	49.65%	63.07%	72.12%
5	50.24%	62.82%	51.15%	62.28%	68.34%
% C.V.	**24.66%**	**13.08%**	**19.47%**	**2.30%**	**3.52%**

Table 2.10

Table of percentage recoveries and percentage coefficient of variation for five extractions conducted on the same 'SepPak' cartridge. The extractions were carried out by first loading 10cm^3 of a 100ppb spiked aqueous sample at a flow rate of 10cm^3/min onto a SPE cartridge and eluted using 2cm^3 of ACN at a 0.4cm^3/min flow rate.

Sample No.	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
1	46.41%	72.44%	52.61%	62.87%	72.30%
2	55.87%	62.65%	45.05%	65.48%	74.04%
3	55.48%	80.05%	68.51%	67.29%	76.73%
4	55.67%	75.92%	52.96%	65.24%	74.91%
5	54.29%	70.46%	55.85%	66.25%	75.63%
% C.V.	**7.54%**	**9.01%**	**15.54%**	**2.50%**	**2.24%**

Table 2.11

Table of percentage recoveries and percentage coefficient of variation for five extractions conducted on the different 'SepPak' cartridge. The extractions were carried out by first loading 10cm^3 of a 100ppb spiked aqueous sample at a flow rate of 10cm^3/min onto a SPE cartridge and eluted using 2cm^3 of ACN at a 0.4cm^3/min flow rate.

It was concluded that there was no significant difference between reusing the cartridges and using fresh cartridges for fluorene and phenanthrene. However the performance for reusing the single cartridge for naphthalene and acenaphthylene was significantly poorer, in terms of reproducibility, than for using fresh cartridges.

2.3.2.2.5. Mobile Phase as the eluent for Offline Extraction

Since ultimately online solid phase extraction was to be investigated, it was decided to investigate if the use of the mobile phase for the analytical column as the eluent for the offline extraction would have a significant effect on efficiency of the procedure.

The extraction procedure used is as outlined in 2.3.2.2.4. except that 50% H_20 / 50% ACN was used instead of 100% ACN to elute the compounds from the SepPak cartridges. This procedure was carried out in duplicate and the average percentage recoveries recorded. These results were then compared with the average percentage recoveries for the fresh cartridge experiments (see table 2.12).

Eluent Composition / % Recovery	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
100% ACN	53.54%	72.30%	55.00%	65.43%	74.72%
50/50 ACN/H2O	48.39%	46.12%	29.80%	29.49%	17.93%

Table 2.12

Comparison of the percentage recoveries for the five PAHs recovered from $10cm^3$ of 100ppb spiked aqueous sample after it was loaded at a flow rate of $10cm^3$/min onto a SPE cartridge and eluted using $2cm^3$ of solvent, ACN or $2cm^3$ 50/50 ACN/H_2O at a flow rate $0.4cm^3$/min.

The use of mobile phase did not have any effect on the efficiency of the extraction of naphthalene. However for the other compounds it was less effective than the 100% ACN. The degree to which it was less effective was proportional to the molecular weight of the compound. The higher the molecular weight the less effective the mobile phase was at extracting the compound from the solid phase.

2.4. Conclusions

A chromatographic separation was developed and partially validated for five PAHs that are listed as priority pollutants by the USEPA. The five PAHs were naphthalene, acenaphthylene, acenaphthene, fluorene and phenanthrene. These PAHs were chosen because of their relatively low M.W.

The optimum separation conditions for these compounds were defined as:

HPLC column 25cm X 4.6mm Spherisorb ODS 10 5µm with a mobile phase of 50/50 ACN/H_2O and a flow rate of 0.8cm^3/min. Detection was conducted at 254nm.

Linearity was shown over the 100 to 900ppb concentration range for the five compounds and the manual injection system was shown to have adequate precision. The limit of detection range was from 3.5ppb to 51 ppb and the limit of determination ranged from 4ppb to 76.5 ppb for the five compounds.

An investigation of various extraction techniques for the enrichment of PAHs from aqueous solutions was carried out. A liquid/liquid extraction technique was initially investigated.

The procedure was based on a procedure recommended by the USEPA method 610. The main advantages of this method are that no complex pieces of equipment are required and the extraction is relatively simple to perform. A large amount of sample can be used which makes it a sensitive technique. It was possible to analyse a spiked sample with 500ppb of five PAHs using this technique.

One major problem with this technique was the need to evaporate dichloromethane to near dryness and reconstitute in acetonitrile. This lead to significant differences in recoveries recorded.

An investigation of extraction onto solid phase was carried out in offline mode. The offline system was optimised for both sample loading, flow rate and elution flow rate. It could be seen that each compound reacted differently to the solid phase extraction procedure.

An optimum of 10cm^3/min was chosen as the loading flow rate for solid phase extraction of the PAHs. An examination of the plot showed that no significant gain would be

achieved by increasing the flow rate any further. It was difficult to assign particular trends to the elution flow rate and volume in terms of the effect on PAH recoveries. It was observed that inconsistencies in flow control had significant effects on recovery rates. The inaccurate control of both loading and elution flow rates proved to be a major draw back with this offline technique as it lead to inconsistencies in washing effects in both loading and elution modes. It was concluded that the reuse of a solid phase extraction cartridge was not as reproducible as using a fresh cartridge for each extraction. An investigation of the use of mobile phase was carried out. This showed that for higher M.W. PAHs mobile phase was less effective at extracting compounds then acetonitrile. The offline solid phase extractive technique was seen to overcome some of the major drawbacks of the liquid/liquid extraction technique with the elimination of dichloromenthane and the distillation step from the procedure. However for the technique to be of real significance the flow rates must be controlled better.

2.5 Bibliography

1. J.E. Longbottom and J.J. Lichtenberg, USEPA Technical Report, EPA 600/4-82-057 PBB 3-201798, (1982) 92 – 101.

Chapter 3.0

Online Solid Phase Extraction as a Preconcentration Technique in the Enrichment of PAHs from Aqueous Samples.

3.1. Introduction

The need for sensitive and selective detection techniques to analyse organic compounds at trace concentrations is well recognised. Sample handling procedures have been developed which are more sophisticated than conventional extraction techniques. A promising approach is to enrich compounds onto a sorbent material and then transfer the analytes online to the system, which chromatographically separates them and allows them then to be detected.

Preconcentration using online solid-phase extraction (OSPE) has been applied to numerous types of analytes from metals in aqueous solutions [1] to drug metabolites in plasma [11]. In all cases it has shown itself to be a selective and sensitive technique of extraction.

Pesticides from river water have been enriched and determined using OSPE [2]. The precolumn was packed with PLRP-S and a sample of $100cm^3$ was loaded onto it, detection was achieved using mass spectroscopy (MS). The limits of detection (LOD) were quoted to be in the 0.1 – 200ppt range. Spiked samples containing pesticides were enriched using OSPE with a C_{18} precolumn and separated on an analytical HPLC column [3]. The calibration range was from 1 – 40 µg/L, no LODs were discussed.

Phenols have been successfully preconcentrated from aqueous solutions by using a precolumn containing a mixture of activated Carbon and XAD-4 [4]. Analysis was conducted by HPLC. Recoveries and relative standard deviations (RSD's) were considered to be good with LODs of approximately 0.1ppb for phenol and chlorophenols.

Phenolic compounds were determined in surface water using OSPE [5]. In this case two precolumns were used to clean up and preconcentrate the analytes. One precolumn

contained PLRP-S and the other contained ENVI-Chrom. Fourteen phenols were determined with LODs ranging from 0.1 - 1μg/L.

OSPE has also been used to extract phenols from wine [6]. In this case LiChrolut EN cartridges were used as the precolumn and a PC controlled the operation.

Hogendoorn et al [7] used two analytical columns of Hypersil ODS in a column switching mode to extract a metabolite of the ethylene (bisdithiocarbamate) fungicides from ground water samples. The LOD was measured at 1ppb.

Herbicides have been enriched from water samples using OSPE. A Supeclean Envi-18 packing was used as the precolumn packing [8]. Analysis was conducted by HPLC with UV detection. The LODs were quoted at 0.1ppb range.

Aldehydes have been determined in drinking water using OSPE [9]. In this case the aldehydes were first derivatised then enriched onto an RP-18 Newguard precolumn prior to analysis by HPLC with UV detection. The LODs were quoted at 1ppb. They noted for formaldehyde the LOD was lower then that achieved by the recommended EPA method.

OSPE has been used with an ion exchange based precolumn to determine low molecular mass organic acids in fogwater [10].

OSPE has also been successfully applied to the extraction of drug substances and their metabolites from biological fluids prior to analysis by HPLC [11]. Solutions of proteins have been enriched using beta-cyclodextrin sulfate-immobilized as the precolumn [12]. The method was applied to the extraction of lysozyme from a chicken egg white.

OSPE of aqueous samples has also been applied prior to analysis by GC. Difficulties are inherent with such a technique because of the need for a gaseous liquid interface. One

approach is to use thermal desorption techniques [13,16]. The other is to use standard OSPE precolumns [14,15,17] and to elute the analytes using a solvent like ethylacetate to facilitate direct injection onto the GC column. The LODs for phenols using standard OSPE with XAD-2 resin as the precolumn were 0.3-2-μg/L for the phenols examined [17].

The aim of this project was to develop an OSPE procedure that would enable the enrichment and analysis of low MW PAHs from aqueous samples. This was achieved by the optimisation of certain boundary conditions. These conditions included the selection of a suitable sorbent material for the precolumn, analyte loadability onto the precolumn, sample volume, loading flow rate and the limits of detection of the system.

The system reproducibility was also assessed and compared with the offline extractive procedures for liquid/liquid and solid/liquid extraction outlined in chapter 2.

3.2. Experimental

3.2.1. Apparatus

The instrument configuration incorporated a six port two-way switching valve, Rheodyne 7000, two 'Waters' 501 solvent delivery pumps and one 'Waters' 486 UV absorbance tuneable detector. The analytical column was a Spherisorb 10 ODS 1 5μm (25cm X 4.6mm). All data was collected on a Waters 746 integrator with the exception of the limit of detection study where the response of the detector was recorded on a Mega Series integrator. A 20μl injection port, Rheodyne 7125, was mounted before the analytical column to facilitate direct injection of samples.

Eluent flow was controlled by manual actuation of the switching valve. Column switching was operated in the backflush mode. Whereby when the switching valve is in position 1 the components of interest are passed to the precolumn where they are retained. The effluent from this column then goes to waste thereby bypassing the analytical column preventing its contamination with excipients. At the same time the mobile phase for the analytical column flows via the switching valve to the analytical column thus allowing the analytical column to equilibrate with the eluent specified for the separation (see fig 3.1). After the six port valve was switched to position 2 the compounds which were retained on the precolumn are eluted in a narrow band as they are eluted in the opposite direction to the one in which they were loaded, using the analytical mobile phase. They then pass to the analytical column where they are separated by the interaction of analytes, stationary phase and the mobile phase (see fig 3.2).

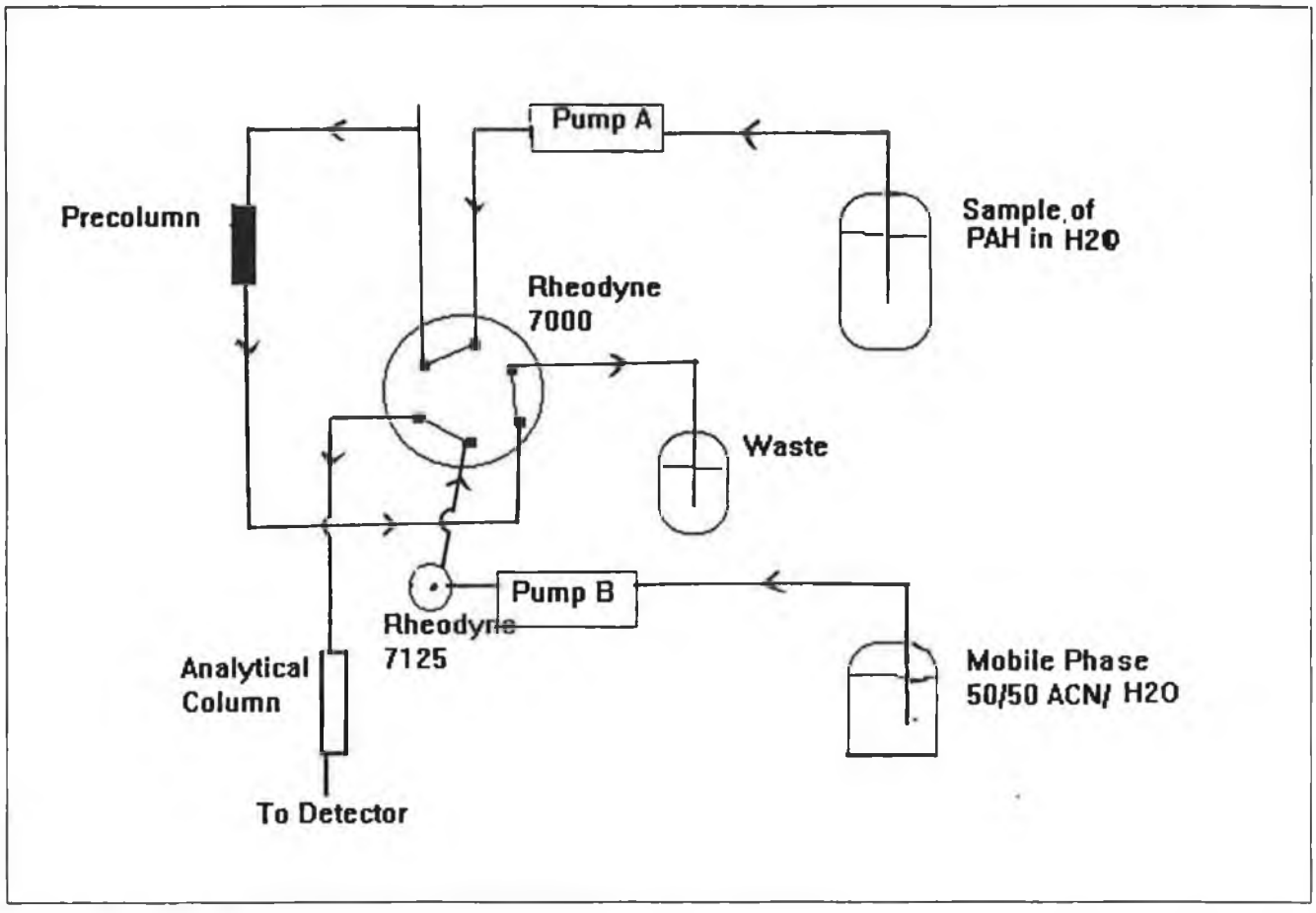

Fig 3.1

Schematic diagram of an Online Solid Phase Extraction system used for the preconcentration of low MW PAHs from aqueous samples.

Note: The switching valve, Rheodyne 7000, is shown in position 1. This allows the preconcentration of the analytes onto the precolumn while simultaneously allowing the mobile phase for their analysis equilibrate on the analytical column.

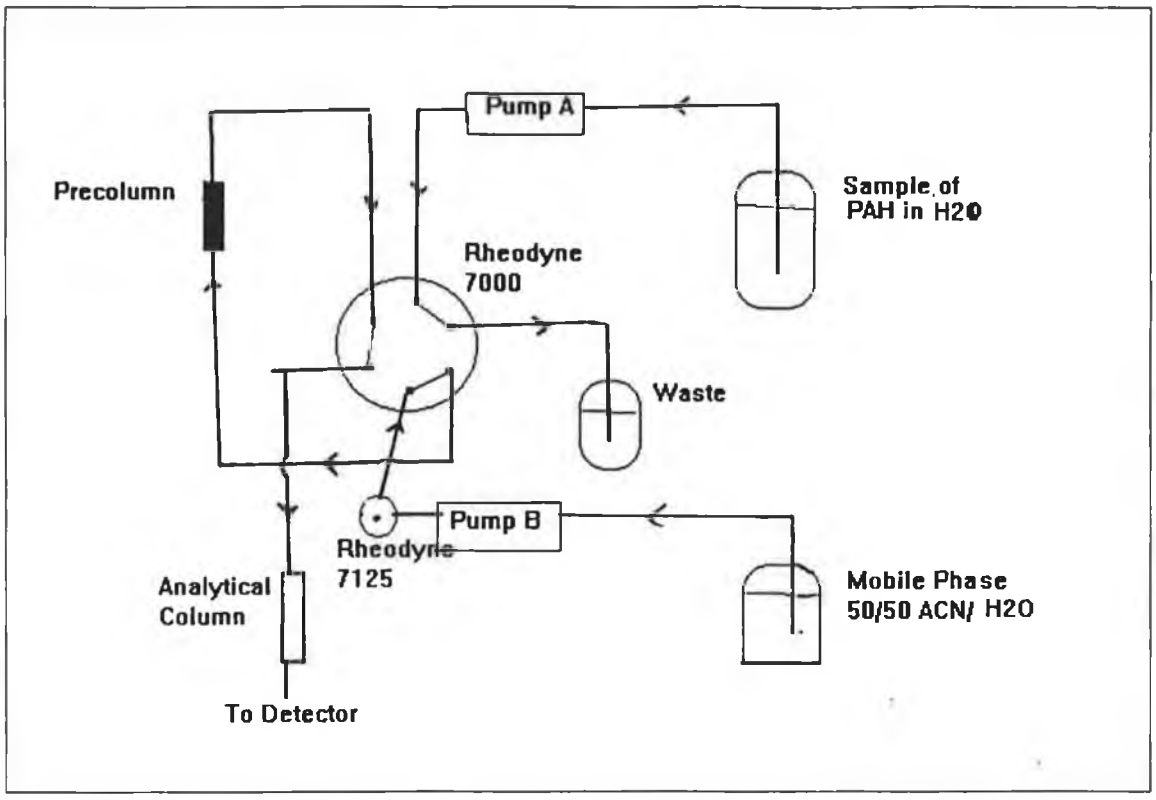

Fig 3.2

Schematic diagram of an Online Solid Phase Extraction system used for the preconcentration of low MW PAHs from aqueous samples.

Note: The switching valve, Rheodyne 7000, is shown in position 2. The analytes are eluted in backflush mode. This enables the 50/50 ACN/H2O mobile phase to elute the analytes from the precolumn to the head of the analytical column in a narrow band. The analytical separation then takes place.

3.2.2. Materials

Materials are as stated in 2.2.1.2. with one exception, the C_{18} material used for the precolumn was supplied by Alltech.

3.2.3. Methods

3.2.3.1 Sample Preparation

Spiked Polycyclic Aromatic Hydrocarbons, samples were prepared by serially diluting the 1mg/L standard mentioned in 2.2.1.2. with Milli-Q H_2O. These samples varied in concentration from 100ppb to 100ppt.

3.2.3.2. Chromatographic Procedures

The optimum conditions for separation of the PAHs are discussed in detail in 2.2.1.3. The conditions for the separation of the five PAHs are summarised here: Wavelength 254nm, Mobile Phase 50/50 ACN/ H_2O, Flow rate A 0.5cm^3/min, Flow rate B 0.8cm^3/min, Attenuation 4, Chart Speed 0.5mm/min, Peak Threshold 30.

3.2.3.3. Sample Loading

Samples were prepared as stated in 3.2.3.1. These samples were then loaded onto the precolumn at a specific flow rate and a defined volume. The Rheodyne 7000 was then switched to position 2, thus allowing the sample to be analysed as mentioned in 3.2.1. In the following series of experiments it is the concentration of sample, loading flow rate, sample volume and the precolumn packing which have a significant bearing on the efficiency of the online extraction system.

3.3. Online Extraction Procedure Results and Discussion

3.3.1. Precolumn Selection

The aim of this online study was to improve the sensitivity and selectivity for the extraction and analysis of the five PAHs listed in 2.2.1.2. The chromatographic procedure described earlier gives the desired separation for these compounds. It was therefore necessary to choose a precolumn packing suitable for the extraction of these PAHs from an aqueous environment.

The precolumn must display high retentive characteristics towards the PAHs in aqueous solutions during the preconcentration step. The precolumn should also have an ability to

retain large concentrations of these compounds. During the desorption stage retention on the precolumn should be minimal to minimise extra column band boardening effects. When the PAHs are introduced to the preconcentration system in an aqueous solution they will bind strongly to a C_{18} reversed phase sorbent material whose hydrophobic nature will attract these organic compounds. Elution from the precolumn would then be possible when an eluent of a much higher elutropic strength, e.g. mobile phase, is introduced to the system. The fact that this eluent flow is opposite to the flow which the compounds were loaded is also of significance. This ensures that the preconcentrated sample passes to the head of the analytical column in a very narrow band, which ensures component separation is efficient. Commercially available reversed phase C_{18} material with a particle size of 30-50 um are well suited for use in sample enrichment.

An investigation of naphthalene at a concentration of 150ppb in aqueous solution confirmed that the C_{18} packing for the precolumn would be suitable for the study of PAHs using column switching. The peak shape was excellent and the response in terms of peak area was large. This investigation involved the loading of 147ppb naphthalene standard at various volumes and a plot of the loading volume versus peak area was constructed.

A standard of 147ppb naphthalene was loaded at 0.5cm^3/min until the specified volume was reached. The sample loading volume ranged from 2.5 to 20.0cm^3. Once the requisite volume was reached the sample was injected by the actuation of the Rheodyne 7000 valve and the peak areas recorded (see Table 3.1 and fig 3.3.).

Volume Loaded on Precolumn (cm^3)	Peak Area for 150ppb Naphthalene
$2.50cm^3$	1784
$5.00cm^3$	97248
$7.50cm^3$	166681
$10.00cm^3$	210064
$20.00cm^3$	271277

Table 3.1

Table showing the volume of 150ppb naphthalene standard loaded onto the precolumn versus the peak area recorded for naphthalene. The peak area was recorded after naphthalene had been preconcentrated onto the C_{18} material from the aqueous standard solution at a flow rate $0.5cm^3$/min and then eluted using 50/50 ACN/H_2O at a flow rate of $0.8cm^3$/min.

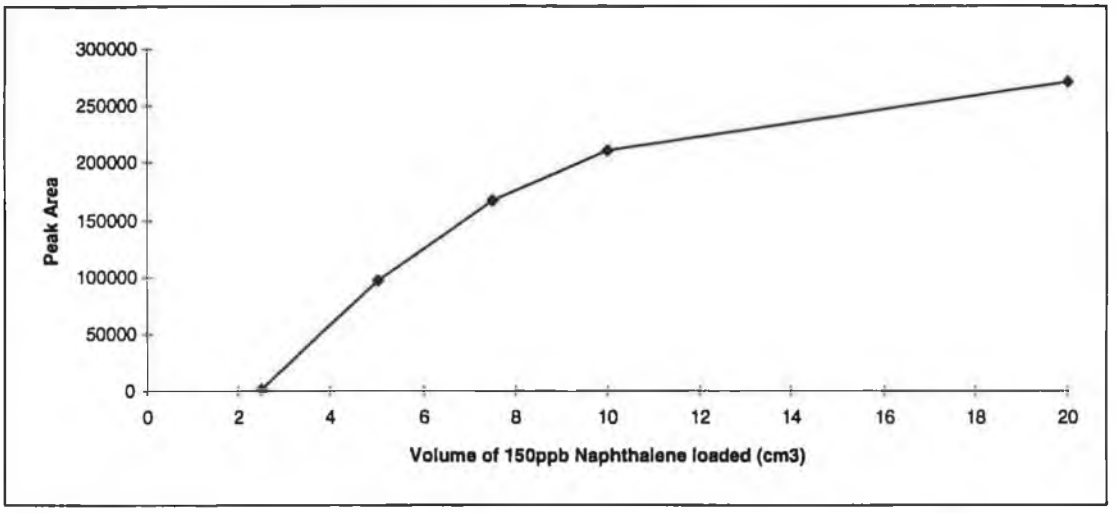

Fig 3.3

Plot showing the volume of 150ppb naphthalene standard loaded onto the precolumn versus the peak area recorded for naphthalene. The peak area was recorded after naphthalene had been preconcentrated onto the C_{18} material from the aqueous standard solution at a flow rate $0.5cm^3$/min and then eluted using 50/50 ACN/H_2O at a flow rate of $0.8cm^3$/min.

It was concluded from this plot of area versus volume that C_{18} material shows significant concentrating properties and was therefore an excellent material for the preconcentration of low MW PAHs from aqueous solutions.

3.3.2. Mass Loadability.

The problem of mass loadability was addressed with respect to the maximum sample concentration that can be loaded onto the precolumn. This was investigated from two perspectives.

The first by increasing the volume of a single concentration which was loaded onto the precolumn and the second by increasing the concentration of a sample with a fixed volume.

Due to the nature of the system different concentrations of PAHs at different volumes will have different loadability characteristics. At different concentrations the compounds will be subjected to various washing effects both when the samples are loaded onto the precolumn and when they are eluted from the precolumn prior to analysis. For this reason a series of loading volume versus response, peak area, studies for a series of concentrations were carried out. The concentrations were:

1) 100ppb Five PAH mixed standard.

2) 1ppb Five PAH mixed standard - Low volume / Low flow

- High volume / High flow

3) 10ppb Five PAH mixed standard.

4) 100ppt Five PAH mixed standard.

The second investigation of mass loadability examined loadability in terms of increasing concentration. The concentration was increased from 10-100ppm at a fixed volume. A plot of concentration versus response was constructed. This is discussed in detail in section 3.3.3.

3.3.2.1. Increasing Volume

By varying the sample loading time while keeping the loading flow rate constant it was possible to determine the most suitable wash volume. The wash volume was defined as the volume of aqueous sample containing PAH spike, which it is possible to wash onto the precolumn without causing elution of the retained analytes due to the washing effect of the solvent. Increasing aliquot volumes were individually loaded onto the preconcentration column; the retained PAHs were then backflushed onto the analytical column and analysed at 254nm.

When the precolumn capacity was exceeded only a fraction of the loaded PAHs were retained on the precolumn indicating that the unretained portion had been washed off the precolumn during this loading procedure i.e. breakthrough at a certain loading volume of analyte had occurred.

Breakthrough volume studies were carried out for the five PAH analyte mix at a number of concentrations. It was necessary to study the breakthrough characteristics of different concentrations because of the different wash effects that would be experienced by the analytes at different concentrations. These studies also made it possible to define various concentration ranges and flow rates that the system could tolerate.

3.3.2.1.1. 100pbb Five PAH mix.

A series of samples were investigated with a concentration of approximately 100ppb for the five PAHs. These samples were loaded at a fixed flow rate of 0.5cm^3/min. The volume of sample loaded was increased from 0 to 30cm^3. The samples were then

analysed by manual actuation of the Rheodyne 7000. A plot of sample volume loaded versus peak area was constructed for each of the compounds present and the breakthrough volume was established (see Table 3.2 and Fig 3.4).

Volume (cm^3) / Area	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
0.0	0.0	0.0	0.0	0.0	0.0
5.0	45733	26798	26291	244680	429358
10.0	96411	116705	130177	1162936	1682908
15.0	145832	194946	253674	3415914	9917972
20.0	166580	204035	269317	3771295	11543889
25.0	173033	207377	281258	4041723	13261705
30.0	150712	207891	283915	4154715	14233789

Table 3.2

Table showing the volume of 100ppb five PAH mixed standard loaded onto the precolumn versus the peak area recorded for each individual PAH. The peak areas were recorded after the PAHs had been preconcentrated onto the C_{18} material from the aqueous standard solution at a flow rate 0.5cm^3/min and then eluted using 50/50 ACN/H_2O at a flow rate of 0.8cm^3/min.

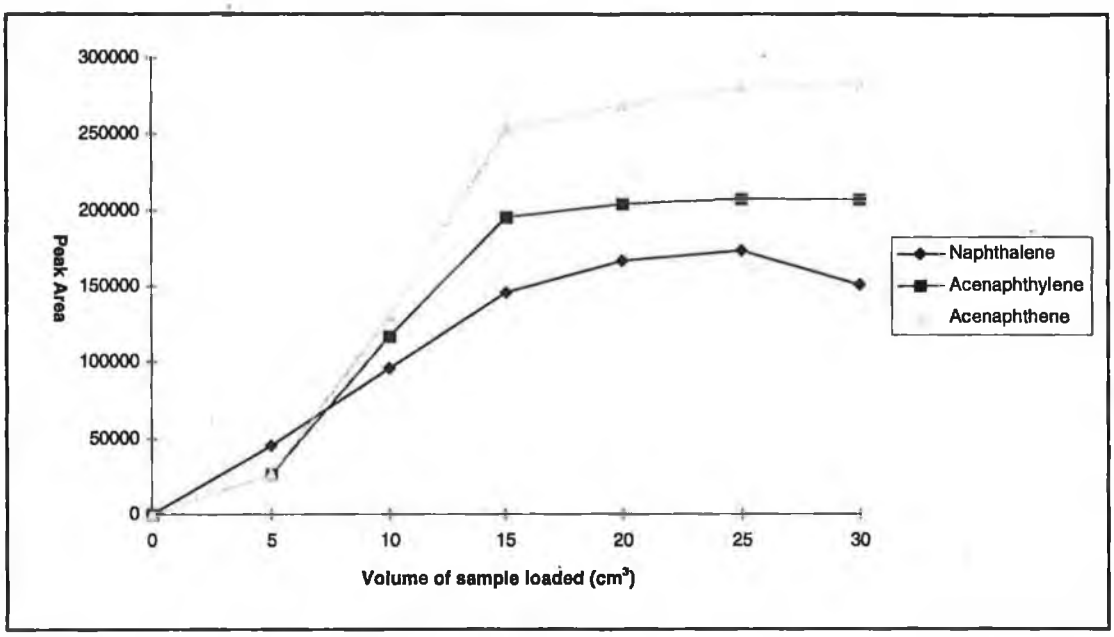

Fig 3.4a

Plot showing the volume of 100ppb five PAH mixed standard loaded onto the precolumn versus the peak area recorded for the first three PAHs. The peak areas were recorded after the PAHs had been preconcentrated onto the C_{18} material from the aqueous standard solution at a flow rate 0.5cm^3/min and then eluted using 50/50 ACN/H_2O at a flow rate of 0.8cm^3/min.

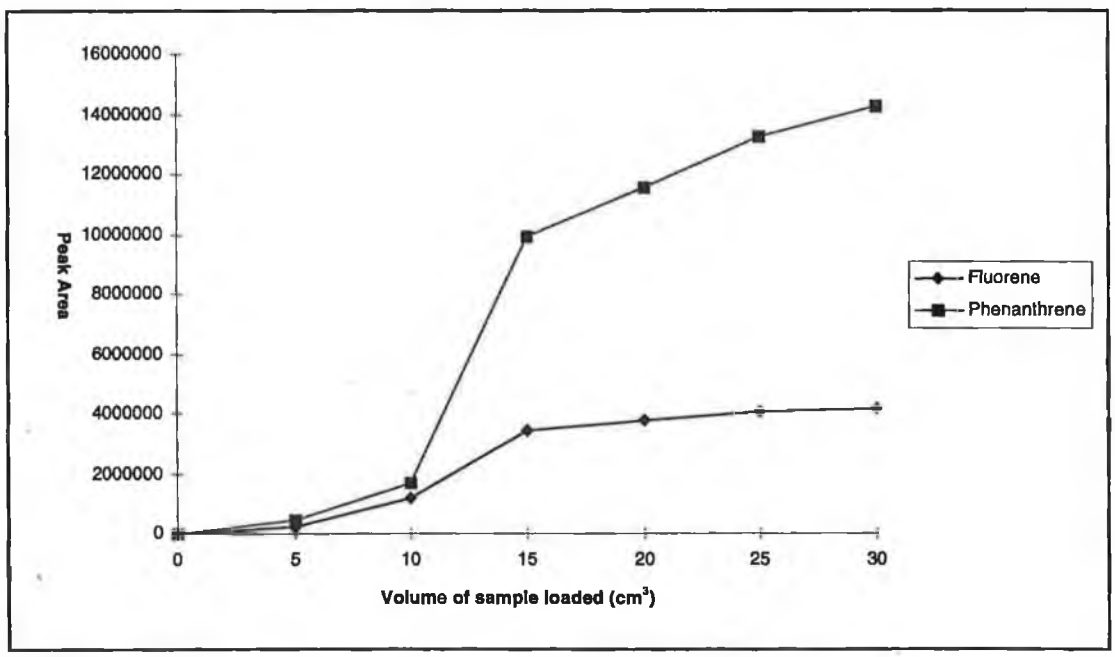

Fig 3.4b

Plot showing the volume of 100ppb five PAH mixed standard loaded onto the precolumn versus the peak area recorded for the last two PAHs. The peak areas were recorded after the PAHs had been preconcentrated onto the C_{18} material from the aqueous standard solution at a flow rate 0.5cm^3/min and then eluted using 50/50 ACN/H_2O at a flow rate of 0.8cm^3/min.

From an examination of the plot of volume versus peak area for the five PAHs at a concentration of 100ppb (see fig 3.4a and 3.4b) it can be seen that each of the PAHs investigated responds differently to the system. This is as would be expected as with increasing molecular weight the compounds would have different hydrophobic characteristics which results in a slightly different interaction with the C_{18} precolumn packing.

As the molecular weight increases the levelling-off effect on the graph seen when breakthrough begins to occur becomes less severe. It can be concluded that a volume of $15cm^3$ is the volume at which breakthrough occurs for the five PAHs examined at 100ppb when they were loaded onto the C_{18} precolumn at a flow rate of $0.5cm^3$/min and eluted with mobile phase $0.8cm^3$/min.

An inflection was noted at the beginning of the graph for these compounds. It was more noticeable for the higher molecular weight compounds. One explanation may be the active sites of the precolumn are being irreversibly coated with the PAHs initially and thus was causing a certain amount of the analytes to be retained on the column. One possible way of eliminating this problem would be to pretreat the column with non-polar organic solvent or with the PAHs themselves.

3.3.2.1.2 10ppb Five PAH mix

The procedure outlined previously for investigating maximum loadability was repeated for a 10ppb sample. The volume of sample loaded was increased from 0 to $30cm^3$ and

the peak area recorded. A plot of peak area versus volume of 10ppb mixed sample loaded was constructed (see table 3.3 and fig 3.5).

Volume (cm^3) /Area	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
0.0	0.0	0.0	0.0	0.0	0.0
5.0	24784	14295	11970	59014	61151
10.0	94915	63285	50264	157634	63292
15.0	144051	155549	167488	921072	847288
20.0	152681	185503	243390	1855598	2864300
30.0	144436	179638	263205	2405851	4671603

Table 3.3.

Table showing the volume of 10ppb five PAH mixed standard loaded onto the precolumn versus the peak area recorded for each of the PAHs examined. The peak areas were recorded after the PAHs had been preconcentrated onto the C_{18} material from the aqueous standard solution at a flow rate 0.5cm^3/min and then eluted using 50/50 ACN/H_2O at a flow rate of 0.8cm^3/min.

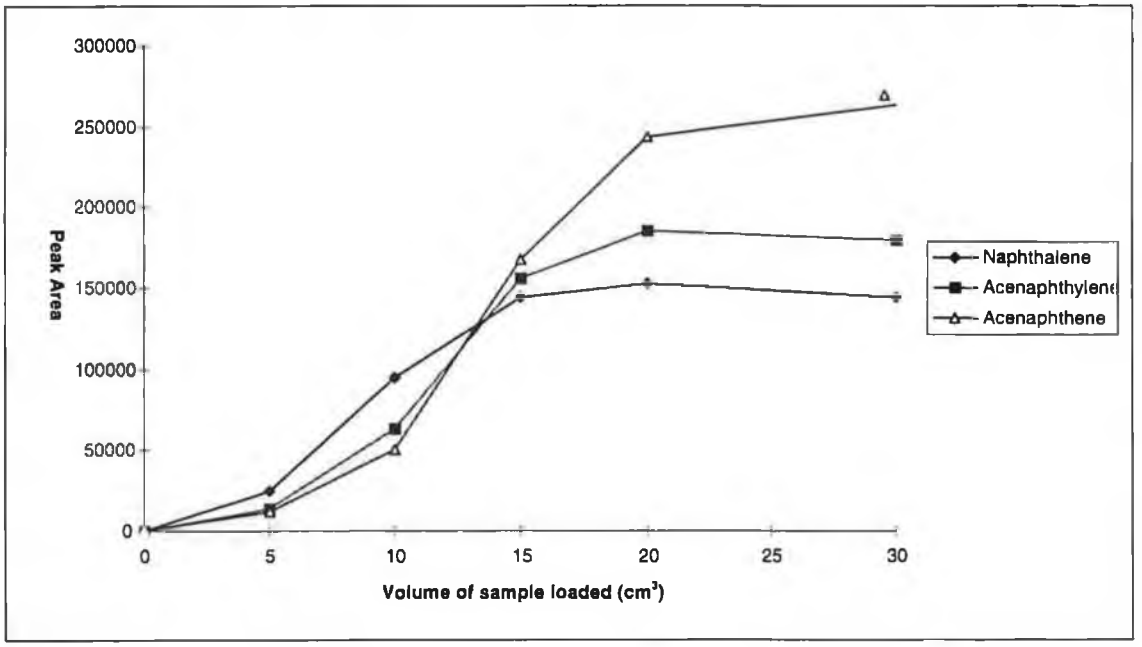

Fig 3.5a

Plot showing the volume of 10ppb five PAH mixed standard loaded onto the precolumn versus the peak area recorded for the first three of the PAHs examined. The peak areas were recorded after the PAHs had been preconcentrated onto the C_{18} material from the aqueous standard solution at a flow rate 0.5cm^3/min and then eluted using 50/50 ACN/H_2O at a flow rate of 0.8cm^3/min.

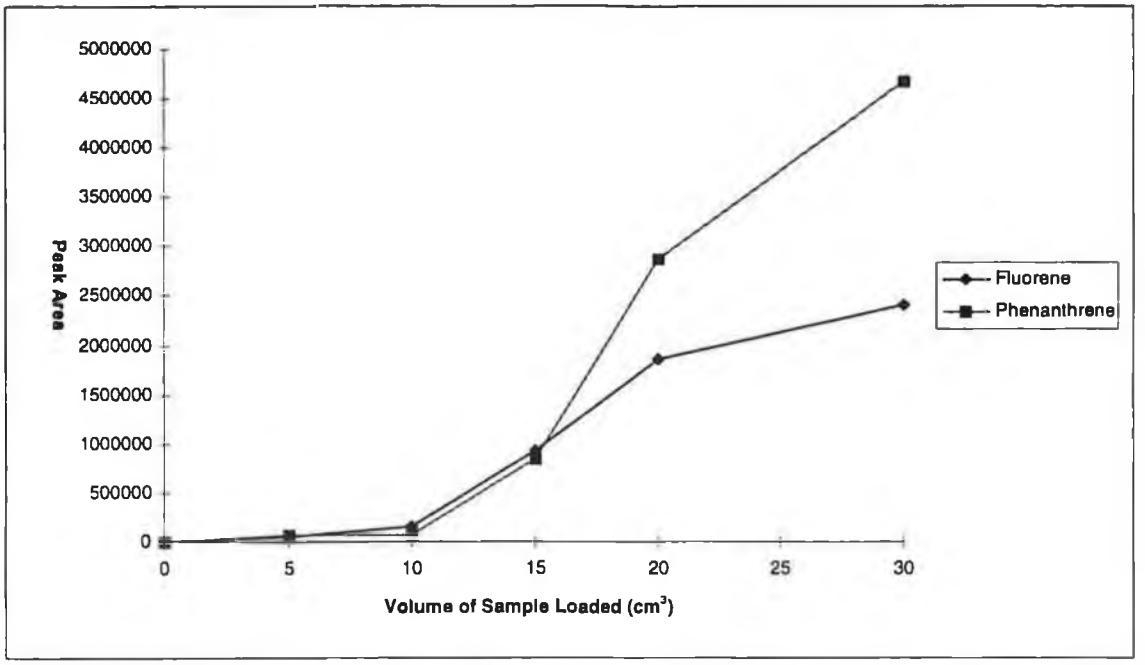

Fig 3.5b

Plot showing the volume of 10ppb five PAH mixed standard loaded onto the precolumn versus the peak area recorded for the last two of the PAHs examined. The peak areas were recorded after the PAHs had been preconcentrated onto the C_{18} material from the aqueous standard solution at a flow rate 0.5cm^3/min and then eluted using 50/50 ACN/H_2O at a flow rate of 0.8cm^3/min.

It can be seen from an examination of the results (see fig 3.5a and 3.5b) that the conclusions from these experiments are similar in general terms to the conclusions for the 100ppb study. The only difference being that the inflection at the lower volumes is more pronounced then for the 100ppb standard.

The analytical column was reversed prior to this experiment and a large increase in sensitivity in terms of peak area was noted.

3.3.2.1.3. 1ppb Five PAH mix

Two separate investigations were carried out at this concentration.

3.3.2.1.3.1 Low Flow Rate and Low Volume Study.

The first investigation was carried out under the same conditions noted above, with what has been termed low flow (0.5 cm^3/min) and low volume (0-30cm^3). These were the conditions which were used to investigate breakthrough volumes for the higher concentrations. However at this concentration (1ppb) the preconcentration effect of the column switching proved ineffective as the peak areas recorded were so close to the limit of detection that accurate integration of naphthalene, acenaphthylene and acenaphthene proved impossible (see table 3.4 and fig 3.6). Breakthrough volumes were estimated for fluorene and phenanthrene. The breakthrough volume for both compounds was 20cm^3.

Volume (mls) /Area	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
0.0	0.0	0.0	0.0	0.0	0.0
5.0	4530	1791	N/D	10585	13600
7.5	1147	2060	1834	19838	32545
10.0	806	2052	4833	27680	67066
20.0	1646	1863	5540	38033	132592
30.0	2894	2832	3142	42490	132311

Table 3.4

Table showing the volume of 1ppb five PAH mixed standard loaded onto the precolumn versus the peak area recorded for each of the PAHs examined. The peak areas were recorded after the PAHs had been preconcentrated onto the C_{18} material from the aqueous standard solution at a flow rate 0.5cm^3/min and then eluted using 50/50 ACN/H_2O at a flow rate of 0.8cm^3/min.

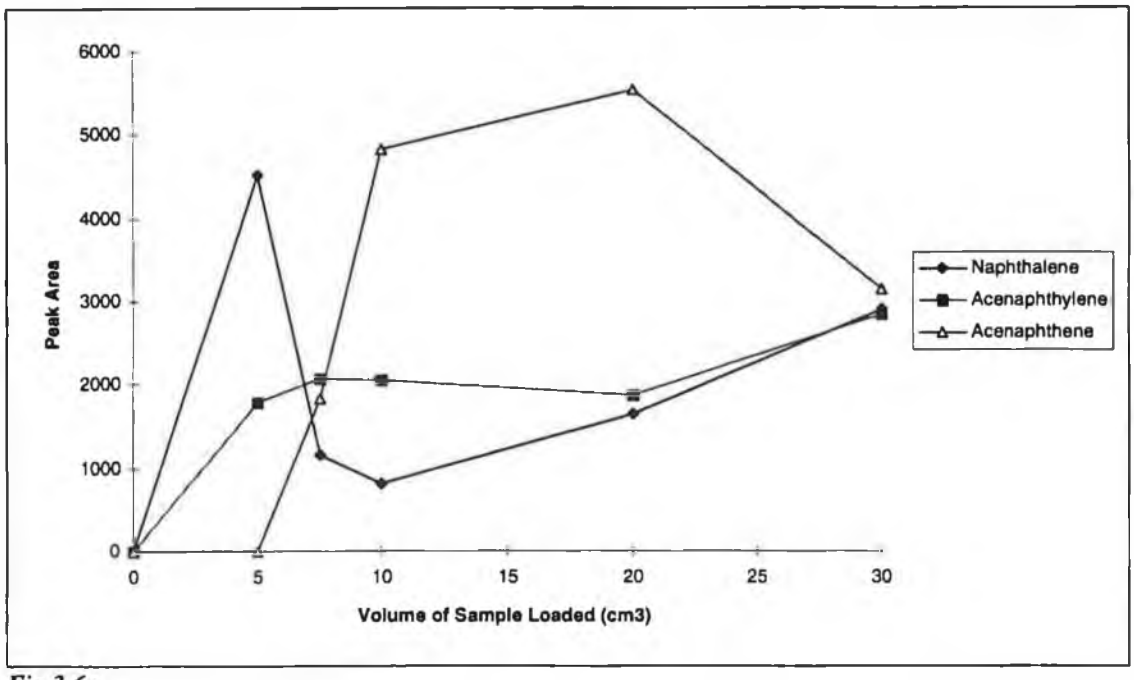

Fig 3.6a

Plot showing the volume of 1ppb five PAH mixed standard loaded onto the precolumn versus the peak area recorded for the first three of the PAHs examined. The peak areas were recorded after the PAHs had been preconcentrated onto the C_{18} material from the aqueous standard solution at a flow rate 0.5cm^3/min and then eluted using 50/50 ACN/H_2O at a flow rate of 0.8cm^3/min.

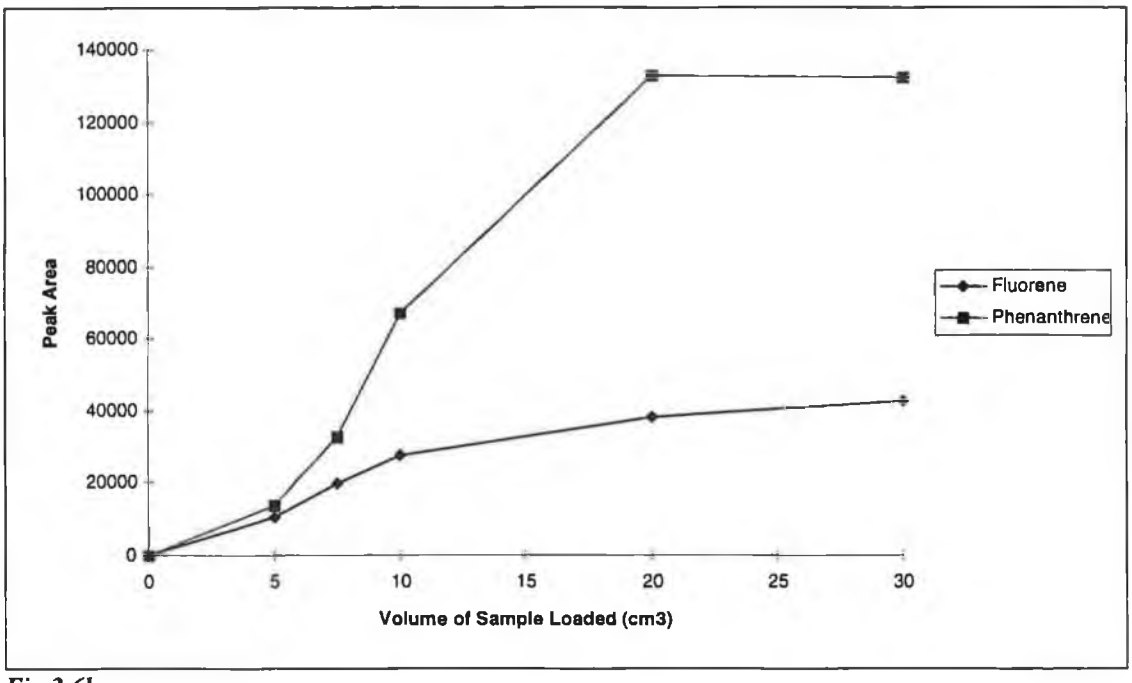

Fig 3.6b

Plot showing the volume of 1ppb five PAH mixed standard loaded onto the precolumn versus the peak area recorded for the last two of the PAHs examined. The peak areas were recorded after the PAHs had been preconcentrated onto the C_{18} material from the aqueous standard solution at a flow rate 0.5cm^3/min and then eluted using 50/50 ACN/H_2O at a flow rate of 0.8cm^3/min.

3.3.2.1.3.2. High Flow Rate and High Volume Study

The second study was carried once the conditions for the system had been optimised for much lower concentration studies this was achieved during the limit of detection study (see section 3.3.3).

A flow rate for sample loading of $5cm^3$ /min was chosen. Due to this high flow rate the washing off effects are noticeable during the sample loading step (see table 3.5 and fig 3.7). This is particularly the case for the lower molecular weight compounds. However the gain in sensitivity is large enough to justify the use of this high flow rate. The maximum loadability for fluorene and phenanthrene at 1ppb loaded at a flow rate of $5cm^3$/min was concluded to be $150cm^3$. For the five compounds in a single solution a loading volume of $100cm^3$ was considered the most appropriate, as above this breakthrough was occurring.

Volume (cm^3)	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
50.0	14965	15206	21063	222311	585529
100.0	16872	19526	24746	279518	944261
150.0	13226	18948	41682	359265	1346707

Table 3.5.

Table showing the volume of 1ppb five PAH mixed standard loaded onto the precolumn versus the peak area recorded for individual PAHs examined. The peak areas were recorded after the PAHs had been preconcentrated onto the C_{18} material from the aqueous standard solution at a flow rate $5.0cm^3$/min and then eluted using 50/50 ACN/H_2O at a flow rate of $0.8cm^3$/min.

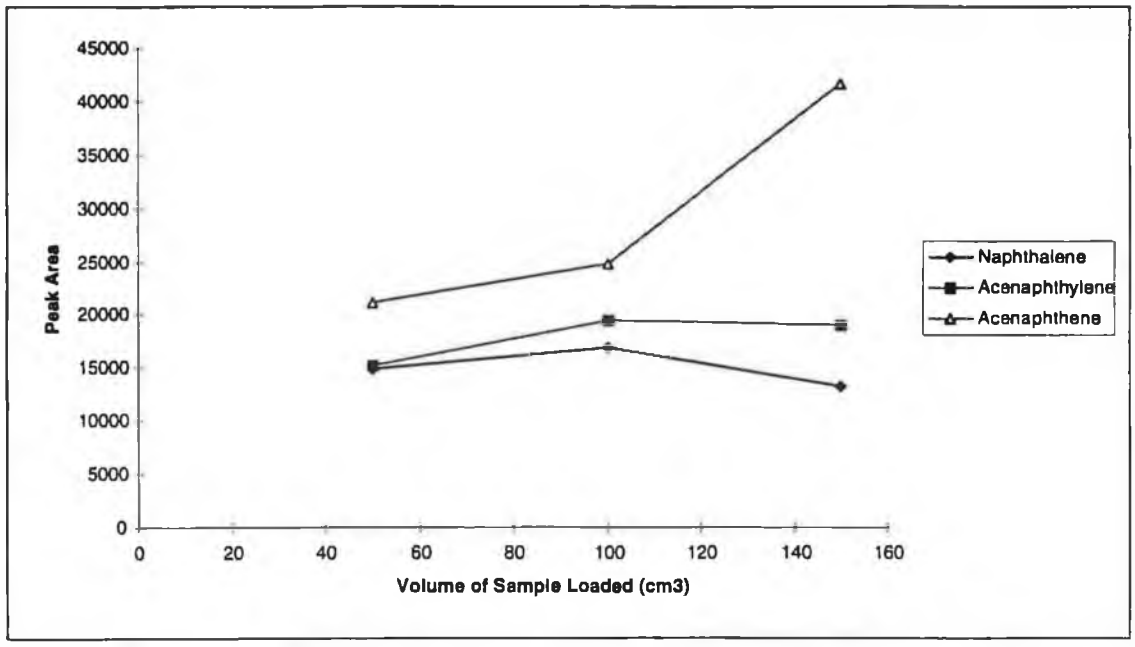

Fig 3.8a

Plot showing the volume of 1ppb five PAH mixed standard loaded onto the precolumn versus the peak area recorded for the first three of the PAHs examined. The peak areas were recorded after the PAHs had been preconcentrated onto the C_{18} material from the aqueous standard solution at a flow rate 5.0cm^3/min and then eluted using 50/50 ACN/H_2O at a flow rate of 0.8cm^3/min.

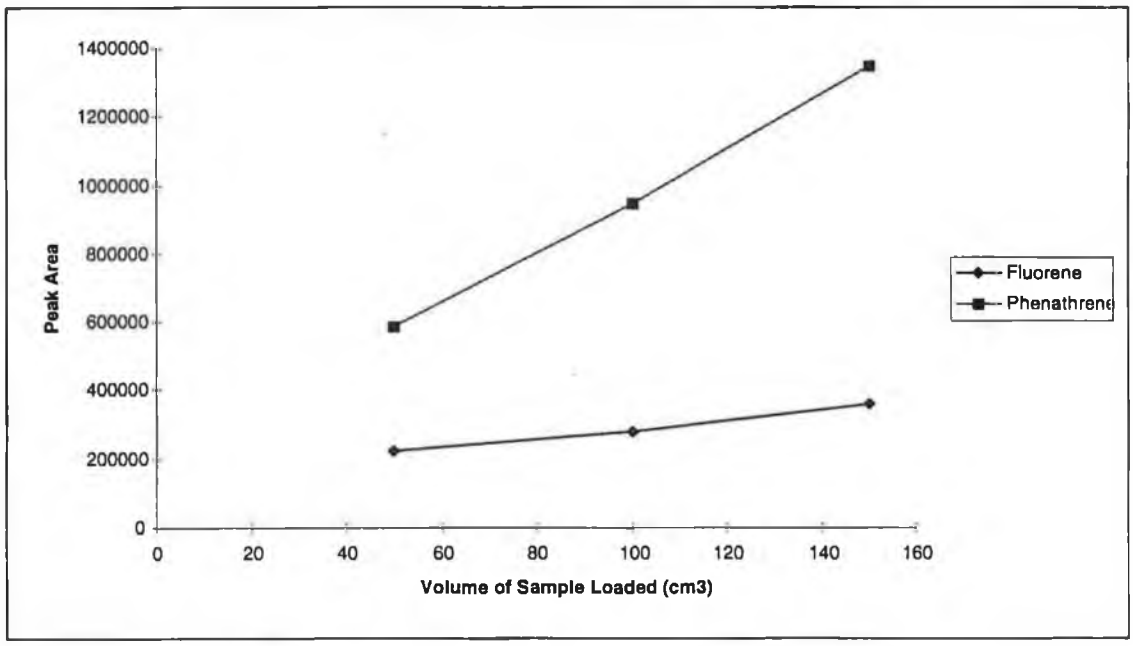

Fig 3.8b

Plot showing the volume of 1ppb five PAH mixed standard loaded onto the precolumn versus the peak area recorded for the last two of the PAHs examined. The peak areas were recorded after the PAHs had been preconcentrated onto the C_{18} material from the aqueous standard solution at a flow rate 5.0cm^3/min and then eluted using 50/50 ACN/H_2O at a flow rate of 0.8cm^3/min.

3.3.2.1.4. 100ppt Five PAH mix.

Due to the gain in sensitivity experienced for a 1ppb mixed sample at high loading volumes it was decided to try a lower concentration. A 100ppt solution was enriched at a flow rate of 5cm^3/min and the areas for various volumes recorded (see table 3.8).

Volume (cm^3) / Area	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
150	3089	2193	9824	66372	298385
500	N/D	8431	11390	63544	295960

Table 3.8

Table showing the volume of 100ppt five PAH mixed standard loaded onto the precolumn versus the peak area recorded for the individual PAHs examined. The peak areas were recorded after the PAHs had been preconcentrated onto the C_{18} material from the aqueous standard solution at a flow rate 5.0cm^3/min and then eluted using 50/50 ACN/H_2O at a flow rate of 0.8cm^3/min.

It can be concluded from table 3.8 that an increase in sensitivity was possible for acenaphthylene and acenaphthene. However due to problems with interferents at the increased loaded volume of 500cm^3 no results could be quoted for naphthalene. Also no increase in sensitivity was noted for fluorene and phenanthrene. This implies that an appropriate loading volume for these compounds was at 150cm^3 and any further loaded material was not retained on the precolumn.

Breakthrough volumes for the five PAHs investigated are very much dependent on the individual compound concentration, the volume of sample loaded and the flow rate at which the compounds are loaded onto the C_{18} precolumn.

The breakthrough volumes were defined as follows:

15cm^3 for the 100ppb and 10ppb five PAH mixed standards loaded at a flow rate of 0.5cm^3/min.

100cm^3 for 1ppb five PAH mixed standard loaded at a flow rate of 5.0cm^3/min.

150cm^3 for 100ppt five PAH mixed standard loaded at a flow rate of 5.0cm^3/min.

3.3.2. Increasing Concentration

It is also possible to investigate the efficiency of the preconcentration step by keeping the volume constant during the sample loading and increasing the concentration of the PAH in the sample being extracted.

It should be possible to reach a maximum concentration of analyte that can be loaded onto the precolumn for a given volume. Above this concentration no active sites would be available on the precolumn to absorb any more analyte. The excess analyte would then be removed in the effluent from the precolumn.

The five PAHs in an aqueous solution were loaded at various concentrations at a flow rate of 0.5cm^3/min and the peak areas recorded (see table 3.9 and fig 3.10). In the study carried out, which was in the range 10-100ppm, no maximum was attained. Since the working range of the system would never go above the concentration of 100ppm in reality, as PAHs are pollutants which are only present at trace concentration levels in real life aqueous samples, it was deemed unnecessary to investigate further as to what the exact maximum concentration reachable was.

Concentration (ppm) / Area	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
10	17618	15702	21301	178458	223846
20	40707	35018	48303	417510	744442
30	63141	46736	59522	573617	1110082
40	91453	90493	100331	1003757	1988586
50	109252	104006	125432	1275360	2889809
100	225905	212958	236351	2408535	5402690

Table 3.9

Table of peak areas for a series of standards with different concentrations of the five PAHs ranging from 10 to 100ppm loaded onto the C_{18} precolumn at a flow rate of 0.5 ml/min for 20mls. These analytes where then eluted onto the analytical column using 50/50 ACN/H_2O at a flow rate of 0.8cm^3/min.

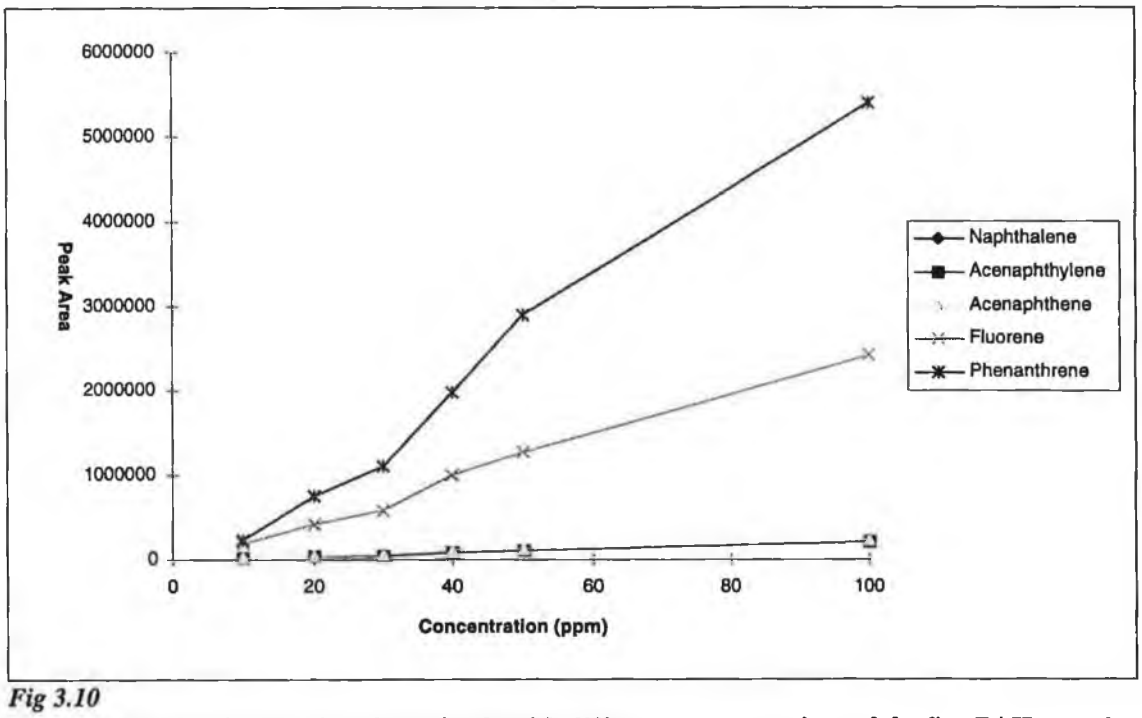

Fig 3.10

Plot of peak areas for a series of standards with different concentrations of the five PAHs ranging from 10 to 100ppm loaded onto the C_{18} precolumn at a flow rate of 0.5 ml/min for 20mls. These analytes where then eluted onto the analytical column using 50/50 ACN/H_2O at a flow rate of 0.8cm^3/min.

3.3.3 Limit of Detection Study

It was obvious from the loadability studies conducted that the system set up in column switching mode was much more sensitive then the direct injection technique. It was therefore necessary to see how much more sensitive the system was.

From the loadability experiments it was concluded that the maximum loadability by increasing volume in the 1ppb-100ppt concentration range was 150cm^3, for the five PAHs, which agrees with the results reached for 10ppb experiment which a maximum of 15-20cm^3.

3.3.3.1. Flow Rate Investigation

Due to the excessive time required to load a volume of 150cm^3 at a flow rate of 0.5mls/min. It was necessary to carry out an investigation into the effects of increasing the flow rate. This was achieved by loading 150cm^3 of 1ppb 5 PAH mix onto the precolumn at a range of flow rates,0.5cm^3/min to 5.0cm^3/min (see table 3.10 and fig 3.11).

Analysis Time	Flow Rate/Area	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
5.0 hrs	0.5mls/min	16791	22259	38635	353550	1552489
2.5 hrs	1.0mls/min	15262	18478	33182	332866	1497687
0.5 hrs	5.0mls/min	13226	18948	41682	359265	1346707

Table 3.10

Table of peak areas for the loading of 1ppb five PAH mixed standard at various flow rates ranging from 0.5cm^3/min. to 5.0cm^3/min. and a fixed load volume of 150cm^3. The analytes were then eluted onto the analytical column using 50/50 ACN/H_2O at a flow rate of 0.8cm^3/min.

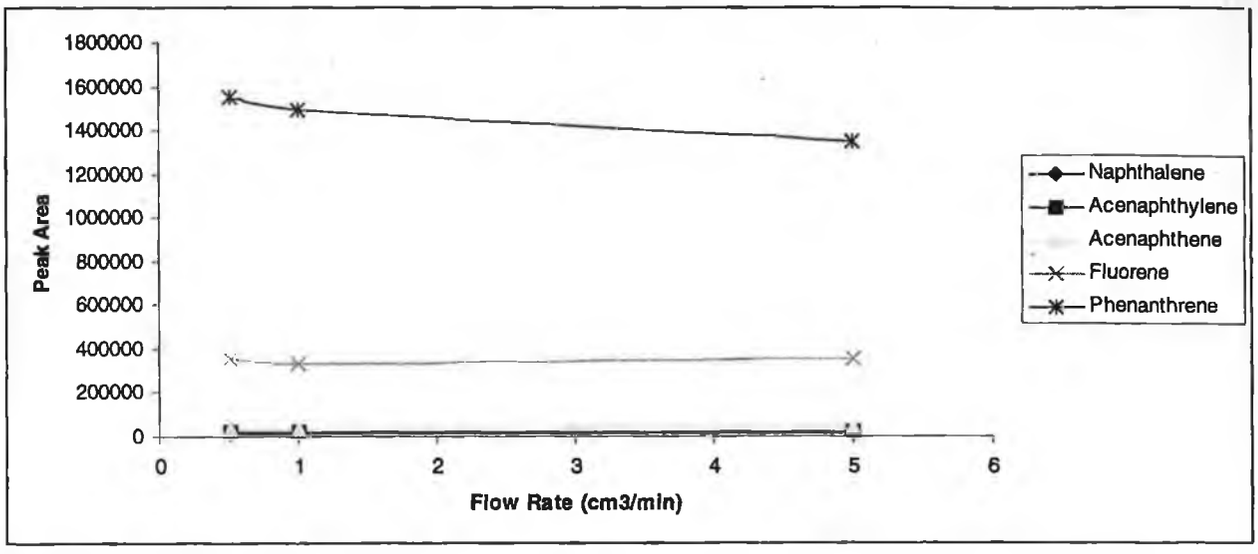

Fig 3.11

Plot of peak areas versus flow rate for the loading of 1ppb five PAH mixed standard at various flow rates ranging from 0.5cm^3/min. to 5.0cm^3/min. and a fixed load volume of 150cm^3. The analytes were then eluted onto the analytical column using 50/50 ACN/H_2O at a flow rate of 0.8cm^3/min.

From this study it was concluded that a 5cm^3/min. flow rate was acceptable although there is a slight decrease in peak areas for the PAHs there is a significant gain in analysis time 30mins as opposed to 5hrs. Therefore a volume of 150cm^3 at a loading flow rate of 5cm^3/min was chosen for the L.O.D. study.

3.3.2.2. Interferents from H_2O Blank Injections

A number of interferents were noted due to the fact that large sample volumes were being used to estimate the LOD for the system.

A series of H_2O blanks were studied and a number of interferents were noticed which interfered with the PAHs to be investigated. Four blank runs were investigated and the average peak areas recorded (see table 3.11).

Compound / Peak Area	PAH Retention Time	Interferent Retention Time	Interferent Area
Naphthalene	15.70	15.12	4474*
Acenaphthylene	19.40	20.58	2922*
Acenaphthene	24.40	24.22	1719
Fluorene	25.60	26.06	200
Phenanthrene	31.60	31.87	6171

Table 3.11

Table of interferents retention times and average peak areas noted from a series of four H_2O blank injections carried on the online solid phase extraction system. These blanks were achieved by loading 150cm^3 of H_2O onto the precolumn at flow rate of 5cm^3/min an eluted onto the analytical column using 50/50 ACN/H_2O at a flow rate of 0.8cm^3/min

***Peaks do not intefer with analyte concentrations below 1ppb at a loading volume of 150cm^3 (see appendices 4 and 5).**

These interferents cause a slight decrease in the sensitivity of the system. I defined a PAH as being detectable when its area is twice that of any interfering peak present.

3.3.3.3. Measurement of L.O.D. for the System

A series of standards was prepared in the 0.1ppt -200ppt range. These samples were loaded at a fixed volume of 150cm^3 and at a flow rate of 5cm^3/min. No analyte gave a detectable peak for the 0.1ppt, 1.0ppt, 2.0ppt injections. The table 3.12 below details the limits detectable for the various PAHs analysed.

Compound/ LOD	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
Concentration	187ppt	170ppt	166ppt	11.2ppt	10.0ppt

Table 3.12

Table of LODs for the five low MW PAHs studied using the Online Solid Phase Extraction system. LODs were estimated by loading 150cm^3 of five PAH mixed standards onto the precolumn at a flow rate of 5cm^3/min . The standard concentrations ranged from 0.1pp to 200ppt.

3.3.3.4. Comparison of LOD with other Techniques

Table 3.13 shows a comparison of the limit of dete\ction for USEPA610 [18], Direct Injection and Column Switching.

Compound/LOD.(ug/L)	USEPA 610	Direct Injection	Column Switching
Naphthalene	1.80	16.4	0.187
Acenaphthylene	2.30	51.0	0.170
Acenaphthene	1.80	34.0	0.116
Fluorene	0.21	5.0	0.011
Phenanthrene	0.64**	2.0	0.010

Table 3.13
Comparison of LODs for various techniques used to investigate PAHs in the aqueous environment
**** Measured by fluorescence**

Column Switching is at least ten times more sensitive than the Method 610 published by USEPA. The sensitivity of the online system could be further improved by the use of a lower flow rate if analysis time can be sacrificed. The use of fluorescence detection could also improve the sensitivity of the method, as it is a more selective method of detection for phenanthrene in particular.

3.3.4. Reproducibility Study

As it is envisaged that the technique will be applied to real aqueous samples. It was necessary to investigate if the whole system, preconcentration, manual actuation of the switching valve and separation on the analytical column resulted in a reproducible procedure.

Four 'injections' were carried out of a 10ppb Five PAH mix at a flow rate of 0.5cm^3/min for 10 mins and statistical analysis was carried out. The percentage Relative Standard Deviation (%RSD) was calculated (see table 3.14).

Injection No	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
1	28280	23811	29577	280970	626007
2	28067	24289	32239	310318	783294
3	27310	24629	32920	310774	750716
4	29263	25813	27497	277236	711743
Average	28230	24364	30558	294824	717940
std. dev	697	739	2164	15778	588112
%RSD	**2.40**	**3.00**	**7.08**	**5.35**	**8.19**

Table 3.14

Table of peak areas and %RSDs for each of the five PAHs investigated. Four injections of a 10ppb mixed standard were carried by loading the standard for 10 minutes at a flow rate of 0.5cm^3/min followed by elution with 50/50 ACN/H_2O at a flow rate of 0.8cm^3/min.

All percent RSDs were below 10% for the five compounds investigated. It was concluded that the system was very reproducible when the washing effects of loading and extracting the analytes from the precolumn are considered.

3.4. Conclusions

An online solid phase extraction procedure was developed. The trace enrichment by column switching online extraction proved to be an effective technique for the extraction of PAHs from the aqueous environment. The judicious choices of precolumn packing, loading flow rate and sample volume as well as the use of an appropriate eluent solvent all enhanced the ability of a column switching system to extract the five low MW PAHs at trace concentrations.

The problem of mass loadability was addressed in terms of increasing volume at various concentrations. Breakthrough volume studies were conducted at concentrations ranging from 100ppb to 100ppt.

The breakthrough volumes were defined as follows:

15cm^3 for the 100ppb and 10ppb five PAH mixed standards loaded at a flow rate of 0.5cm^3/min.

100cm^3 for 1ppb five PAH mixed standard loaded at a flow rate of 5.0cm^3/min.

150cm^3 for 100ppt five PAH mixed standard loaded at a flow rate of 5.0cm^3/min.

Mass loadability was also investigated in terms of increasing concentration. A study was conducted in the range 10-100ppm for the five PAHs mentioned. It was concluded that no maximum was attained. It was concluded that since in reality PAHs exist at trace concentrations in the environment was deemed unnecessary to investigate increasing concentrations further.

The limit of detection was determined for the online system. It was concluded that the LODs for the five PAHs investigated on the online system were at least ten times lower then the LODs quoted by the USEPA Method 610 and one hundred times lower then the LOD calculated for direct injection on the HPLC using a 20µl injection loop.

It was concluded that the online system was reproducible with all percent RSDs calculated to be less then ten percent for four 'injections' on the system.

Method / Parameters	**Liquid/Liquid Extraction**	**Offline Liquid/Solid Extraction**	**Online Liquid/Solid Extraction**
Reproducibility (%CV)	23.0 - 69.5%	24.0 - 2.3%	2.4% - 8.19%
L.O.D.	16.0 - 2.0ppb	3.2 - 0.4ppb	0.18 - 0.01ppb
Problems	Evaporation	Inconsistent flow rates	None

Table 3.15

Comparison of the three extractive techniques investigated in chapters 2 and 3 in terms of reproducibility, LOD and various problems encountered. The three systems were used to extract the lowest MW PAHs at trace concentrations from aqueous samples.

The table 3.15 gives a brief over view of the extraction techniques investigated. This shows that the online method developed here has major advantages over the offline techniques in terms of reproducibility and limit of detection. The problems encountered due to evaporation are completely eliminated through the use of column switching. This is significant as many of the techniques discussed in chapter 1 site this as a major problem. The column switching system allows consistent flow rates for both loading and injecting samples, which was a major draw back of the offline solid extraction system. The LODs quoted for these compounds using the online technique are excellent and would be further advanced if PDAD or fluorescence detectors were coupled to the system.

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Chapter 4.0

An Investigation of Matrix Solid Phase Dispersion (MSPD) as A Technique in the Simultaneous Extraction of Five PAHs From Spiked Milk Samples.

4.1. Introduction

Matrix solid-phase dispersion (MSPD) was developed a decade ago, by researchers at Louisiana State University School of Veterinary Medicine, to simplify the isolation of drug and chemical residues from biological samples. It has been applied to many biological matrices for the extraction of organic analytes such as antibiotics and pesticides.

It was discovered that C_{18} silica based bonded phases can solubilize samples rich in lipids, cause the lysis of cells and interact with organic matter present within the cell [1]. It is this characteristic which is exploited in MSPD extractions [1 - 3].

MSPD has been applied to a number of biological matrices. These include milk, animal tissues, fish tissue, fruit and vegetables as well as Infant formula.

4.1.1. MSPD Process Description

The general process requires that the biological matrix, normally 0.5 grams, be mixed with C_{18} packing material. The analyte molecules are bound to the surface of the C_{18} material. The fat from the biological sample is not bound and therefore can be easily removed. The resulting mixture is packed into a column. This column may then be washed with a solvent to remove excipients. The compounds are then eluted with $8cm^3$ of eluent e.g. ACN. This solution may then be treated further prior to analysis.

4.1.2. Animal Tissue Samples

MSPD is a microscale method when compared to the classical approach of isolating drugs from tissues. Normally 10 – 50grams of tissue would be homogenised in a blender

in the presence of a large volume (100 – 500cm^3) of an extracting solvent [4]. MSPD combined with SPE is an extremely rapid and sensitive method for the determination of a number of compound types, organophosphates, benzimidazole, anthelmintics and β-lactam antibiotics from animal tissue [1]. Recoveries ranged from 93.64 % for cruformate eluted with benzene to 59.75% for ampicillin eluted with methanol. This technique was then applied to a large number of drug compounds from a number of biological samples which demonstrated the generic nature of the technique and its ability to work with a number of sample matrices [3].

Long et al [5] investigated the use of MSPD in conjunction with LC to analyse five benzimidazole antihelmintics in fortified beef liver. The analytes were eluted with ACN followed by evaporation and H_3PO_4 treatment prior to injection. The LOD was quoted at 0.1 ug/g. Schenck at al [6] used a complex derviatisation process after ivermectin was eluted by MSPD from beef liver tissue recoveries were quoted at 72 - 77% with a percentage coefficient of variation (%CV) of 7.3 to 12.9% which is considered poor. Rosen et al [7] required a clean up procedure using a Bond-Elut C_{18} SPE column and evaporation prior to analysis when they used MSPD to extract acetylgestagens from bovine kidney fat. The recoveries they quoted were 59% +/- 5%, which are low. The main losses were attributed to irreversible bonding of the analyte during the MSPD process. Further losses were also noted at the evaporation step where difficulties were encountered trying to reconstitute the analyte prior to analysis. However they noted that the recoveries compare with the standard extraction technique.

MSPD extraction was also applied to the extraction of pesticides from beef fat [8]. The procedure outlined above was used with one exception. The C_{18} / sample mixed paste

was transferred to a column which already contained florisil. This material is normally used in clean up procedures prior to analysis. In this case the material was packed into the same column as the MSPD material to effect this clean up procedure. When this was eluted with ACN it produced an extract with minimal interferents. Recoveries were quoted at 82 +/- 3.4% to 102 +/- 5.0 % for the nine pesticides investigated. They concluded that this methodology was acceptable for extraction and determination of these residues in beef fat.

MSPD was also applied to other animal tissues like chicken [9,10] and pork [11-15].

MSPD was used to extract nicarbazin [9] from chicken tissue. Recoveries were quoted at between 80.1% and 96.9 % with CV's from 1.2% to 8.4%. The results achieved compared favourably with a conventional technique. MSPD was also applied to the extraction of furazoline from chicken tissue [10]. A complex pre-treatment prior to analysis involving evaporation and centrifugation was required. The recoveries quoted were excellent at 99.8+/- 4.4%.

A range of drug compounds were extracted from pork tissues using MSPD. Seven sulphonamides were extracted using the method defined previously [1] with the one exception that dichloromethane was used to elute the MSPD column [11]. Recoveries were quoted at between 70.4% and 95.8% with %CV between 7% and 13%. They concluded that the method was simple and free from interferents. Five benzimidazole antihelmintics were extracted using 18 % ODS silica as the matrix solid [12]. Elution was achieved with ACN and clean up was conducted on alumina. The percentage recoveries ranged from 73% to 105 % with CV of between 6.4% to 7.9 %. Furazolidone was extracted using MSPD however this time the column was eluted with ethyl acetate

followed by complex evaporation and centrifugation prior to analysis [13]. Recoveries were quoted at 89.5% +/- 9.9%. It was therefore concluded that the method worked well for this extraction.

MSPD was compared with a standard solvent extraction technique for sulphonamides in pork tissue [14]. They concluded that MSPD compared well with recoveries quoted at > 80%. From this work an 'on site' analytical system was developed for sulphonamides [15]. The method involved the addition of petroleum spirit to the MSPD eluent and clean up on a microcolumn prior to analysis by TLC.

4.1.3. Bovine Milk Samples

MSPD has also been used to extract a range of drug compounds [16 -24] and pesticides [25] from bovine milk samples. The procedure used is similar to the general method outlined above. In most cases 0.5cm^3 sample was mixed with 2.0grams C_{18}, washed with hexane before elution with 8cm^3 of CH_2Cl_2 or ethylacetate.

Seven benzimidazole antihelmintics were extracted by MSPD and analysed by HPLC. In this case recoveries were quoted at between 70+/-9% and 107+/-2%. The concentration ranged studied was 62.5 to 2000ng/cm^3. Chlorofuron was blended with ODS silica, washed with hexane and eluted with CH_2Cl_2 [17]. Recoveries were quoted at 91.6+/-10 % for the same concentration range. Eight sulphonamides were also extracted using MSPD [18]. Recoveries were quoted as between 73.1+/- 7.4% and 93.7+/-2.7%. The limits of detection were quoted at 313.0 ng/cm^3 to 62.5 ng/cm^3. Both chloramphenicol [19] and furazolidone [20] were extracted from milk samples using a modification of the method previously described [1]. Chloramphenicol was recovered at between 60.8% and

79.0 % in the 62.5 to 2000ng/cm^3 concentration range. Furazolidone was recovered at a lower concentration range 7.8 to 500ng/cm^3. Percentage recoveries were quoted at between 68.2% and 87.9%.

MSPD was used to extract oxytetracycline; tetracycline and chlorotetracycline from milk in this case however 0.05g Na_2EDTA and 0.05grams oxalic acid were also mixed with the C_{18} packing and 0.5cm^3 spiked milk sample. The cited drug EDTA complex was eluted with ethyl acetate and ACN. The concentration range investigated was 100 to 3200 ng/cm^3. Recoveries were quoted at between 63.5 and 93.3%.

Clorsulon was extracted from milk using MSPD [22]. In this case however the analyte was eluted directly onto a florisil SPE cartridge and then eluted from this using ether. Recoveries were between 88.8% and 96.6% for the concentration range 50 to 200ppm. Barker and Long [23] described a general procedure that was used for the extraction and analysis of several drugs used in the dairy industry. The only modification from previous methods [16-23] was the addition of a disposable pipette tip on the end of the MSPD column that increased the residence time for the eluting solvents on the column. Ivermectin was recovered from spiked milk samples at between 1ppb and 8ppb. Which is deemed extremely sensitive [24]. Recovery rates of 81.5% to 99.5% were recorded. This was achieved using MSPD along with a series of SPE and a derivatisation step.

MSPD has also been used as a technique in the screening of pesticides from milk [25]. This was achieved by MSPD extraction using C_{18} silica followed by florisil SPE with Na_2SO_4 also present. Recoveries were quoted at between 76.0% and 97.8% for organochlorides and between 75.0% and 104.5% for organophosphorus pesticides. The

LODs were between 2 and 20ppb for the organochlorides and between 10 and 50ppb for the organophosphorus pesticides, which is considered to be very good.

4.1.4. Fish Tissue Samples

Fish muscle tissue has been extracted using MSPD for analysis of drug residues [26-28] and for pesticides [29].

Oxytetracycline was extracted using C_{18} along with Na_2EDTA and oxalic acid as mentioned previously [26]. No recoveries were quoted. Sulphadimethoxine was also extracted from fish muscle tissue [27] using MSPD. The method used was very similar to the method used for animal tissue [11]. No recoveries were quoted. Walker and Barker [28] used a modification of MSPD for the extraction of sulfadimethoxine and 4-N-acetylsulfadimethoxine from fish tissue and plasma. The plasma / C_{18} mixture was mixed by using vortex mixing. The sample size was also very small 100µl plasma mixed with 400mgs of C18. Recoveries were quoted at between 79 and 67 % for muscle and plasma tissues respectively, which is very good considering the sample size used.

Pesticides were also extracted from fish samples using MSPD [29]. A florisil clean up procedure was used and recoveries were quoted at between 82 +/- 4.8% and 97 +/- 3.6%. The interassay variability ranged between 5.0 +/- 2.7% and 16.9 +/- 6.5%. The clean extracts were a result of the combination column used. They concluded that the blending of the muscle tissue with the C_{18} disperses the tissue and allows an efficient extraction of the pesticide into the ACN elution solvent. Water and a small amount of residual lipids present eluting from this C_{18} / sample mix are removed as the eluent passes through the

florisil co-column. They concluded that the method was suitable for the determination and screening of pesticides in fish muscle tissue.

PAHs have been extracted from spiked catfish muscle tissue [2] using the technique described previously. 2grams of tissue were blended with C_{18} material and eluted with $8cm^3$ of ACN this was then evaporated and reconstituted prior to analysis by HPLC-UV.

The extractive process outlined has also been used to extract PAHs from other fish samples. Fourteen of the priority pollutant PAHs were extracted over the concentration range 100 – 2000ng/g with recoveries ranging from 72 – 112 %. The low recoveries were possibly due to evaporation prior to analysis by HPLC [2].

4.1.5. Other Sample Matrices

MSPD has also been applied to the extraction of seven sulphonamides from infant formula [30]. The method employed has been detailed previously [11]. Recoveries were measured to range from 75.9 to 112.0%. It has also been used to screen fruits and vegetables for pesticides [31]. Recoveries ranged from 41 to 108 % which is a broad range of percentage recoveries.

The aim of the present study was to develop an extractive procedure to extract the five lowest MW PAHs from bovine milk samples utilising MSPD. This was achieved by investigating and optimising a number of boundary conditions. These included the amount of sorbent material used, the volume of sample, the volume of eluent and the nature of the sorbent material.

4.2. Experimental

4.2.1. Chemicals.

All chemicals used were of analytical reagent grade. Labscan supplied (all HPLC grade) acetonitrile (ACN), methanol and dichloromethane (CH_2Cl_2).

Water was purified using a Milli-Q water purification system.

The PAHs, naphthalene (98.5%), acenaphthylene (97.0%), acenaphthene (99.0%), fluorene (99.0%) and phenanthrene (99.0%) were all supplied by Chem. Services Alltech. Chem. Services Alltech also supplied the C_{18}, silica and phenyl packing materials used in this investigation.

Becton Dickson supplied the $5cm^3$ syringes that were used as the extractive columns. All syringes were washed with soapy water, rinsed with Milli-Q water and left dry prior to use.

4.2.2. Instrumentation

The chromatographic instrumentation defined in chapter 2.2.1.1. was used for these experiments.

4.2.3. Methods

4.2.3.1. Analytical Methods

These methods relate specifically to the defining of the analytical technique used to analyse the samples generated from the MSPD extraction technique.

4.2.3.1.1. Standard Preparation

Stock solutions of the PAHs (1.0 mg/cm^3) were prepared in ACN and stored at 2°C in the dark. From these solutions a 1.0mg/L stock standard containing the five PAHs was prepared. A fresh 1mg/L stock standard was prepared every six weeks.

4.2.3.1.2. The HPLC Conditions.

These conditions were defined in chapter 2.1.3.1.

4.2.3.1.3. C_{18} Material Preparation.

Prior to using the C_{18} packing material it was thought necessary to condition it first to remove any solvents or chemicals used in its preparation. This was achieved by washing it with three volumes of dichloromethane followed by three volumes of ACN and allowing it to dry prior to using it.

4.2.3.2. Sample Preparation.

Milk (Avonmore Fresh Milk) samples were purchased from a local retail outlet.
PAH spiked samples were prepared by diluting the stock standards with the milk sample. A known weight of the C_{18} packing was then taken and mixed with a fixed volume of the spiked milk sample. This mixing was achieved by placing the packing and the sample onto a mortar and mixing it very gently with a pestle until a homogeneous paste was formed. Blank milk samples were prepared in the same fashion except that 0.5cm^3 ACN was spiked into the milk sample.

The resultant C_{18} / milk paste was placed into a $5cm^3$ syringe which had been plugged with a frit. The frit was made of cellulose, an inert porous material. The packing material was compressed with a syringe plunger from whom the rubber had been removed. The column was then eluted with $2cm^3$ of HPLC grade ACN. Flow through the column was gravity controlled. The eluate was filtered using 0.45μm filter prior to injection onto the HPLC system. If samples were not injected directly they were sealed and stored in the dark.

4.2.3.3. Calculation of Results.

A standard curve was constructed for each PAH investigated as detailed in chapter 2.2.2. This standard curve was then used to calculate the concentration of the individual PAH in a given sample. From this concentration the % recovery of the individual PAH was then calculated for the specific extractive procedure and its efficiency assessed.

4.3. Results and Discussion.

The extractive procedure outlined in 4.2.3.2. was optimised by examining a number of the parameters involved the process. The amount of packing used, the volume of the milk sample, the volume of eluent used and finally the composition of the material itself.

4.3.1. Optimisation of the amount of C_{18} packing used.

C_{18} was chosen as the packing material for the initial investigation as it had already been demonstrated in Chapter 3 that this material was suitable for extracting these five compounds very effectively from an aqueous environment.

The optimum weight of the C_{18} material was investigated at two separate concentrations 2ppm and 500ppb for the five PAHs investigated.

4.3.1.1. 2ppm Five PAH Mixed Spike.

A 2ppm milk spike was prepared for the five PAHs by diluting the 1mg/cm^3 stock solution. 100µl of this spiked solution was then mixed with a series of weights of the conditioned C_{18}. The compounds were then eluted by the addition of 2cm^3 ACN and gravity flow. Each sample was injected twice and the average peak area recorded. A plot of C_{18} weight versus % recovery was constructed (see table 4.1 and figures 4.1).

C18 weight /% Recovery	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
0.100	39.42	38.60		24.90	54.02
0.200	108.50	131.09		81.85	109.52
0.300	131.20	148.82		93.98	120.27
0.400	137.77	150.99		92.04	112.84
0.500	119.05	143.86		92.32	123.57

Table 4.1.

Table of % Recovery for the five PAHs extracted from a spiked milk sample at a concentration of 2ppm. Extraction was achieved by mixing 100µl of the spiked milk sample with various weights of C_{18} (0.10gram to 0.50grams) and eluting the PAHs with 2cm^3 ACN.

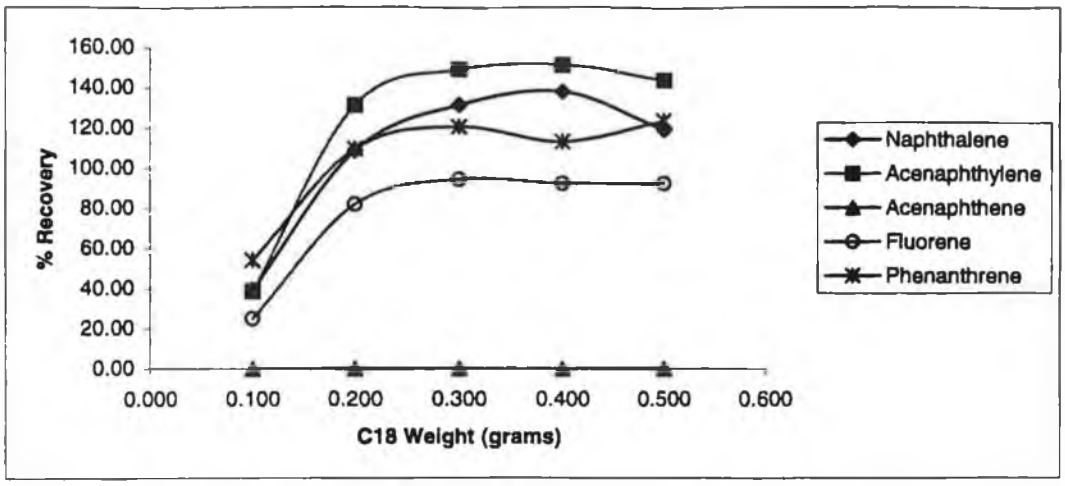

Fig 4.1

Plot of % Recovery versus weight of sorbent material used for the five PAHs extracted from a spiked milk sample at a concentration of 2ppm. Extraction was achieved by mixing 100µl of the spiked milk sample with various weights of C_{18} (0.10gram to 0.50grams) and eluting the PAHs with 2cm^3 ACN. This procedure was then repeated and a plot of C_{18} versus % Recovery constructed.

The first point to note is the fact that no results were recorded for acenaphthene. This was due to the fact that the initial concentration in the spiked milk sample was 2ppm. 100µl of this solution was then extracted with 2cm^3 of ACN giving a final concentration in the injected solution of 100ppb. This concentration was at or below the limit of determination for acenaphthene (see appendices 6 and 7).

It was concluded that a minimum of 0.30grams of sorbent material was required to extract the five compounds quantitatively. Below this not enough material was present to extract the compounds from the spiked milk samples.

4.3.1.2. 500ppb Five PAH Milk Spike.

The procedure carried out in 4.3.1.1. was repeated here except the initial milk spike concentration was reduced to 500ppb . It was obvious that using 100µl-spike volume and eluting with 2cm^3 ACN would not be practical in this case as it would give an injection concentration below the limit of determination for the HPLC assay. It was therefore

decided to increase the sample volume to 500µl. A range of C_{18} weights was investigated. A table and plot for the various weights used were constructed (table 4.2 and fig 4.2).

C18 weight / % Recovery	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
0.300	64.13	35.20	2.38	57.10	56.19
0.400	110.68	47.44	32.06	54.23	51.56
0.500	115.80	52.59	0.00	65.45	64.14
0.600	114.21	38.67	0.00	38.50	39.13
0.700	43.46	35.40	0.00	35.16	33.53
1.000	26.06	3.49	0.00	9.885	10.31

Table 4.2
Table of % Recovery for the five PAHs extracted from a spiked milk sample at a concentration of 500ppb. Extraction was achieved by mixing 500µl of the spiked milk sample with various weights of C_{18} (0.10gram to 1.00grams) and eluting the PAHs with 2cm^3 ACN.

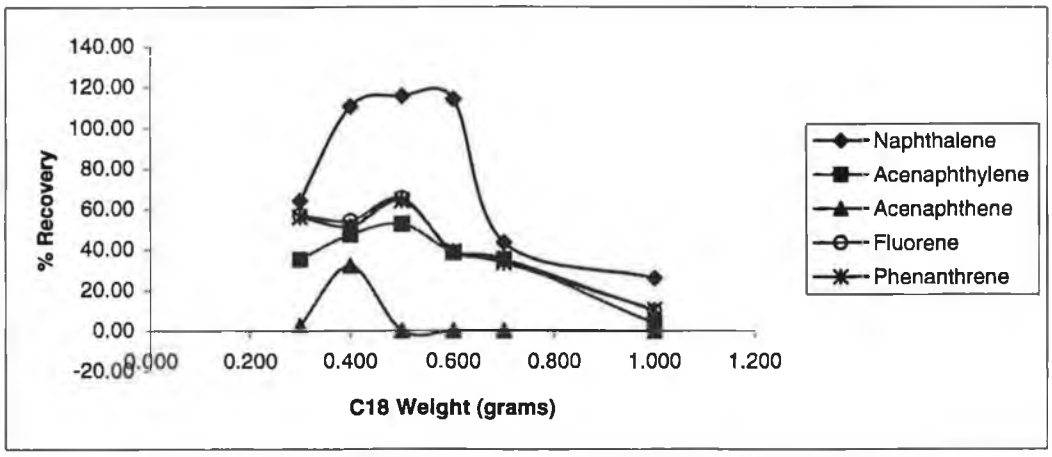

Fig 4.2
Plot of % Recovery versus weight of sorbent material used for the five PAHs extracted from a spiked milk sample at a concentration of 500ppb. Extraction was achieved by mixing 500µl of the spiked milk sample with various weights of C_{18} (0.10gram to 1.00grams) and eluting the PAHs with 2cm^3 ACN. This procedure was then repeated and a plot of C_{18} versus % Recovery constructed.

The general trend noted in the previous experiments was also noted here with a maximum recovery reached at approximately 0.5grams of C_{18} packing. Large variations were noted for the % recoveries recorded for the 500ppb-mixed spike. In this particular case again interferences caused problems for acenaphthene. The drop off in percentage recoveries is

very clear above 0.6 grams with very low recoveries recorded for the 1.0gram C_{18} packing experiment.

This indicates that below a certain weight of material (0.50grams) not all the PAHs are extracted by the C_{18} material and that above 0.6grams not all the compounds are removed from the C_{18} material by the $2cm^3$ of ACN.

It was concluded the optimum amount of sorbent material required for the MSPD extraction of these compounds is very much influenced by the concentration of the compounds.

It was decided to choose 0.50grams as the optimum C_{18} packing weight to use in the rest of the studies carried out because it appears to be the most effective over a range of milk spike concentrations.

4.3.2. Optimisation of the volume of the milk spike sample.

The procedure detailed in 4.3.1.1. was carried out with the following exceptions, the weight of C_{18} material was fixed at 0.500grams, the volume of milk sample loaded was varied and elution volume fixed at $1cm^3$ ACN. This optimisation procedure was conducted at two concentrations 100ppb and 500ppb.

4.3.2.1. 100ppb Five PAH Milk Spiked Sample.

A 100ppb five PAH milk spike was prepared and loaded onto the 0.500 grams of the C_{18} at a series of volumes and eluted with $1cm^3$of ACN (see table 4.3 and fig 4.3)

Milk volume (cm^3) / % Recovery	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
0.25	121.40	101.45		59.46	98.09
0.50	73.02	36.16		46.29	76.53
1.00	42.81	34.02		30.25	39.84
2.00	21.14	15.93	75.31	26.19	14.52
3.00	9.61	11.84	26.01	9.91	15.00
4.00	8.72	7.55	23.06	7.26	9.79
5.00	5.91	15.08		7.54	8.92

Table 4.3

Table of % Recovery for the five PAHs extracted from a spiked milk sample at a concentration of 100ppb. Extraction was achieved by mixing various amounts of the spiked milk sample with 0.50grams C_{18} and eluting the PAHs with $1cm^3$ ACN.

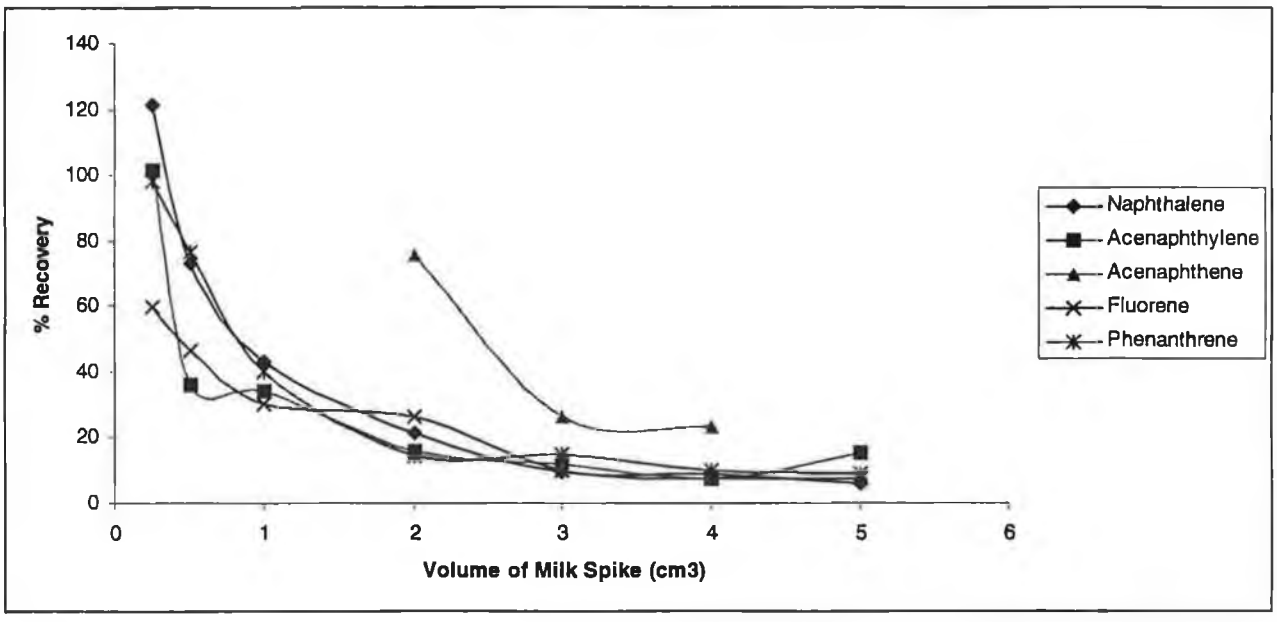

Fig 4.3

Plot of % Recovery versus volume of spiked milk used for the five PAHs extracted at a concentration of 100ppb. Extraction was achieved by mixing various amounts of the spiked milk sample with 0.50grams C_{18} and eluting the PAHs with $1cm^3$ ACN.

From an examination of these plots it can be seen that the most effective extraction procedure was the one where the least amount of sample was loaded onto the system. The reason for the reduction in % recoveries as larger sample volumes are used may be due to

a washing effect, which removed the analyte, while the sample was loading onto the C_{18} material. No results were recorded for acenaphthene at the earlier spike volumes because the actual peak size for these peaks was to small and therefore below the limit of detection.

4.3.2.2. 500ppb Five PAH Milk Spiked Sample.

The procedure outlined in 4.3.2.1. was repeated here with one exception the actual milk spike concentration used was 500ppb (see table 4.4 and fig 4.4).

Milk volume (mls) / % Recovery	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
0.25	118.48	78.30		101.80	92.43
0.50	68.24	51.19		77.35	91.36
1.00	30.43	34.86		36.69	31.32
1.50	27.75	36.10	22.81	32.24	23.90
2.00	35.84	29.59	25.46	34.42	30.94

Table 4.4

Table of % Recovery for the five PAHs extracted from a spiked milk sample at a concentration of 500ppb. Extraction was achieved by mixing various amounts of the spiked milk sample with 0.50grams C_{18} and eluting the PAHs with 1cm^3 ACN.

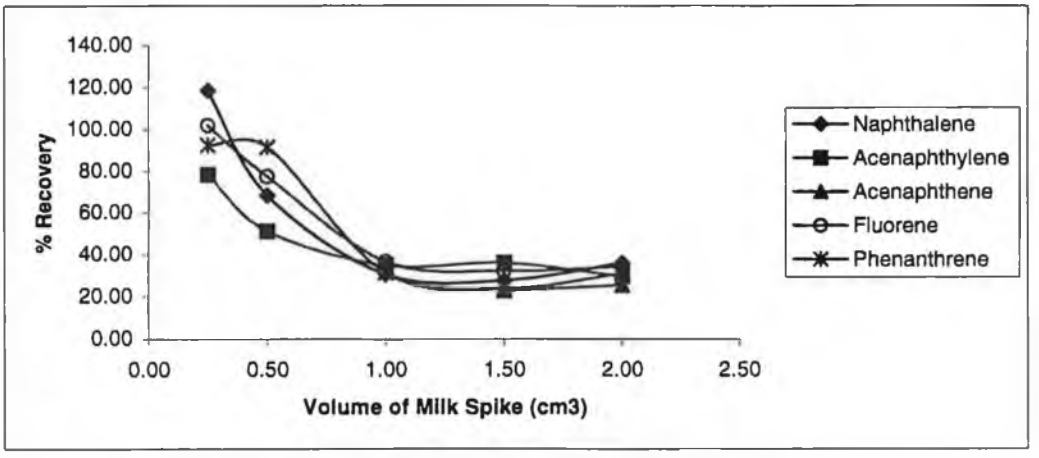

Fig 4.4

Plot of % Recovery versus volume of spiked milk used for the five PAHs extracted at a concentration of 100ppb. Extraction was achieved by mixing various amounts of the spiked milk sample with 0.50grams C_{18} and eluting the PAHs with 1cm^3 ACN.

Similar trends noted for 4.3.2.1. were also seen here with the minimum amount of milk spike sample giving the highest recovery results. Even though an eluent volume of $1cm^3$ was used to ensure a good chromatographic peak size no peak areas were recorded for the lower milk volumes for acenaphthene. This was due to interferents (see appendices 8 and 9).

It was concluded that the optimum sample volume to use for the extractive procedure was the minimum as it ensures good mixing with the C_{18} material and reduces any possible washing effects during the sample loading procedure. The most practical volume to use for the spiked milk samples in the 100 – 500ppb concentration range was deemed to be $0.5cm^3$.

4.3.3. Optimisation of the Volume of ACN used to elute the PAHs

The procedure outlined in 4.3.1.1. was repeated here except in this case the volume of ACN used to elute the compounds was varied. $0.50cm^3$ of 500ppb milk spike was added to 0.500grams of C_{18} and these analytes were then eluted with varying amounts of ACN (see fig 4.5 and table 4.5).

Volume ACN (mls) / % Recovery	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
1.00	53.07	22.25	5.30	43.33	44.13
2.00	98.86	72.97	33.74	96.58	85.26
3.00	0.00	76.75	0.00	114.54	102.72

Table 4.5
Table of % Recovery for the five PAHs extracted from a spiked milk sample at a concentration of 500ppb. Extraction was achieved by loading $0.50cm^3$ of the 500ppb spiked milk sample onto 0.50grams C_{18} and eluting the PAHs with various amounts of ACN (1.0 to $3.0cm^3$).

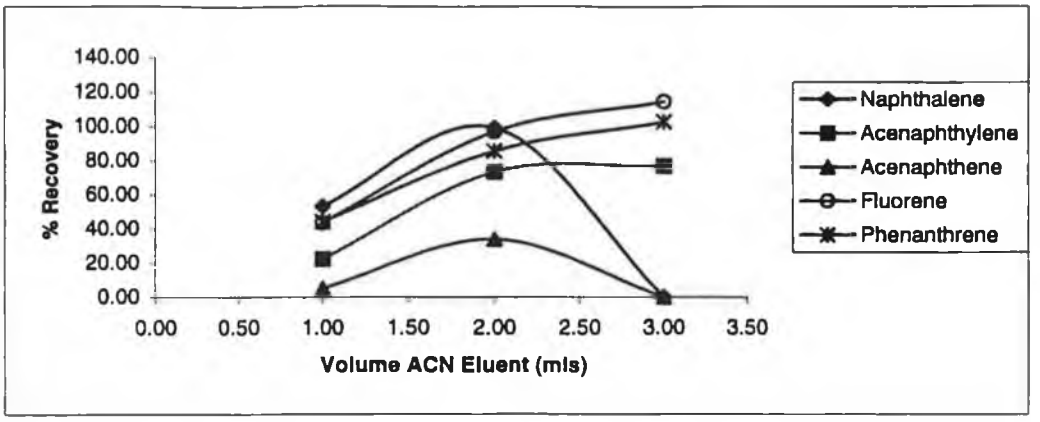

Fig 4.5

Plot of % Recovery versus volume of ACN used to elute the PAHs from the C_{18} material. Extraction was achieved by loading 0.50cm^3 of a 500ppb spiked milk sample onto 0.50grams C_{18} and eluting the PAHs with various amounts of ACN (1.0 to 3.0cm^3).

It was concluded that each compound reacted differently to the amount of ACN used to elute them. A minimum of 2cm^3 was required to elute all the higher MW PAHs because below this volume a certain amount of the material did not elute. Above 2cm^3 the dilution effect for the compounds with lower responses was too great leading to a loss in sensitivity.

4.3.4. An Investigation of Reproducibility.

It was decided to investigate the reproducibility of the optimised technique as some variation in % recovery had been noted during the optimisation procedure and for the technique to be of practical significance it is necessary to demonstrate that the procedure can be carried out in a repetitive manner.

A series of extractions were prepared using the technique described in 4.3.1.1. in this case a 0.5cm^3 aliquot of 500ppb mixed milk spike was added to 0.500grams and eluted with 2cm^3 of ACN and the % recovery calculated. This procedure was repeated six times and the % CV recorded (see table 4.6).

Sample No./ % Recovery	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
1	85.55	89.56	123.64	107.58	98.80
2	140.00	82.97	100.53	113.27	88.33
3	78.65	106.01	116.08	106.12	83.10
4	87.29	151.16	106.04	107.35	87.99
5	121.21	62.88	146.73	110.34	86.16
6	83.76	81.18		107.08	88.55
% CV	**25.20**	**31.96**	**15.24**	**2.47**	**5.97**

Table 4.6

Table of % Recovery data collected for a reproducibility study. Recoveries were calculated for a 0.5cm^{3}of a 500ppb five PAH milk spike sample loaded onto 0.500grams of C_{18} packing and eluted with 2cm^3 of ACN. This was repeated six times and the %CV calculated.

From an examination of this data it can be seen that the % recoveries vary considerably for certain PAHs and in some instances are well above 100%. For naphthalene and acenaphthylene the recorded % CV is poor at over 25 % in both cases.

The % CV for the heavier PAHs is good with fluorene and phenanthrene giving results of less than 6.0%, which is considered to be very good for this extractive procedure. The actual % recovery recorded for these two compounds of on average 108.6 and 88.8% are also deemed acceptable.

The reduced variations at higher MW may also be related to washing effects during the actual loading of the compounds with the lighter compounds not binding as well or as quickly to the C_{18} as the heavier compounds and thus resulting in variations in recoveries recorded.

4.3.5. Investigation of Different Packing Materials.

Since there is a wide range of materials available for packing into chromatographic columns it was decide to investigate some of these materials as possible materials to be

used in MSPD. The difference between these packings is based on the functional groups attached to a neutral base substance (see table 4.7).

Name	Particle Size (µm)	Supplier
C18	70 - 30	Alltech
Cyano	70 - 30	Alltech
Silica 60	70 - 30	Fisons
Phenyl	10	Shandon

Table 4.7
List of types of material used as the sorbent for the MSPD extractions along with the particle size and the supplier name.

Each packing material was conditioned as outlined in section 4.2.3.1.3. prior to use. The sample preparation was followed as outlined in 4.3.4. with the exception that 0.500 grams of each of the materials listed above was used. The results for the C_{18} material was based on the average results recorded in 4.3.4 (see table 4.8).

Name of Material / % Recovery	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
C18	99.41	95.63	118.60	108.62	88.82
Cyano	79.83	76.50	126.85	90.99	80.62
Silica 60	92.50	76.38	177.28	4.00	87.70
Phenyl	67.32	48.71	89.73	49.07	46.14

Table 4.8
% Recoveries recorded for 500ppb mixed milk spike loaded, at a volume of 0.5mls, onto 0.500grams of different packing material and eluted with 2cm^3 ACN.

It was concluded that the properties of the extracting material have an effect on the ability of the extractive system to recover the analytes of interest. C_{18} in most cases gave the most efficient extraction. The one exception being acenaphthene with silica 60 giving a recorded recovery of 177%. It must be noted for this compound that the actual peak size is small and may have lead to significant variations in results.

4.3.6. An Investigation of the Addition of an Extra Solvent Wash.

An extra solvent wash of the packed C_{18} / Milk spike paste was introduced to see if it removed any interferences noted in the blank milk runs or if it had any effect on the extractive efficiency. This was achieved by the introduction of an extra $1cm^3$ wash prior to the ACN elution of the packed column (see Table 4.9).

Name of Solvent / % Recovery	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene
None	99.41	95.63	118.60	108.62	88.82
H2O	44.98	58.90	167.47	69.30	66.10
Hexane	60.44	52.15	92.28	556.40	67.09

Table 4.9

Table comparing the % recoveries for the addition of an extra solvent wash to the extraction system and the normal extraction procedure. In the normal case % recoveries were calculated for a 500ppb mixed milk spike loaded, at a volume of $0.5cm^3$, onto 0.500grams of C_{18} and eluted with $2cm^3$ ACN. The extra solvent wash was achieved by washing with $1cm^3$ of the specified solvent prior to the $2cm^3$ ACN wash.

From an examination of this table it is possible to conclude that the addition of an extra wash either H_2O or hexane did not have a positive effect on the ability of the system to recover the analytes of interest. Hexane seems also to intefere with the fluorene peak significantly. The clean up procedures therefore had detrimental effects on the ability of the system to recovery the analytes.

4.4. Conclusions

MSPD was demonstrated to be a viable alternative to traditional extractive techniques used to extract PAHs from biological aqueous environments. The technique was used to extract five PAHs namely naphthalene, acenaphthylene, acenaphthene, fluorene and phenanthrene from spiked milk samples. The extractive technique was optimised based on a number of parameters.

The initial investigations were carried out using C_{18} as the solid phase material. This material was chosen because it had previously been demonstrated to have a high affinity for the extraction of these compounds from aqueous environments.

Various boundary conditions for the MSPD extractive system were investigated and the following optimum parameters achieved:

The amount of sorbent material was deemed to be 0.50grams.

The volume of spike milk sample was deemed to be 0.50cm^3.

The volume of ACN eluent was deemed to be 2.0cm^3

The reproducibility of the method was also examined. It was deemed to be very reproducibile for the higher MW PAHs i.e. fluorene (2.47%CV) and phenanthrene (5.97%CV). This was not the case for the other three compounds examined with %CVs at over 15%.

The nature of the sorbent material was also examined and C_{18} was concluded to give the best recoveries.

The addition of an extra clean up step involving another solvent was also investigated and in this particular case no advantage was gained from the introduction of this step.

The MSPD method used in this case eliminates many of the problems associated with classical isolation techniques. It uses small sample sizes, has a minimum number of steps and no chemical manipulations e.g. distillation and requires the minimum amount of solvent. The savings in terms of time and solvent requirements make this procedure an attractive alternative to classical isolation methods.

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Chapter 5.0

An Evaluation of ATR/FTIR as a Technique for the Analysis of Polycyclic Aromatic Hydrocarbons in Aqueous Solutions.

5.1 Introduction

Due to the increasing demands placed by US agencies and European directives concerning the quality of drinking water, the treatment of waste water and the protection of water resources the need for rapid sensitive techniques for analysing pollutants is becoming increasingly important. Online chemical sensors are becoming increasingly important in the area of detecting pollutants as they give real time data and do not require complex sample pre-treatment.

Attenuated total reflection fourier transform infrared spectroscopy (ATR/FTIR) is a well established surface spectroscopic technique and recently has been applied to various samples with the view of developing it as a chemical sensor.

5.1.1. The Theory of ATR

The principle of ATR/FTIR is that when light passes through a waveguide internal reflection occurs and this reflected light interacts with the external surface. When total internal reflection occurs at this external surface some radiation penetrates a short distance outside the waveguide. When this occurs in a vacuum this evanescent wave decays exponentially outside the waveguide over a distance of about one wavelength. In a vacuum this is not accompanied by any energy absorption. On the other hand if instead of a vacuum the waveguide is immersed in a liquid or an other substance, the evanescent wave may be partially or totally absorbed and the transmission through the waveguide is reduced. This will occur at those wavelengths that correspond to the absorption spectrum of the sample. The transmission loss spectrum is the basis of ATR spectroscopy, which is also called evanescent wave spectroscopy [1].

It can be proven that in a total internal reflection process there is interference between the incident and the reflected waves which gives rise to standing waves along the Z axis,

perpendicular to the interface in medium 2. Medium 2 had a refractive index $n_{2,}$. The electric field E decays exponentially with distance Z (fig 5.1).

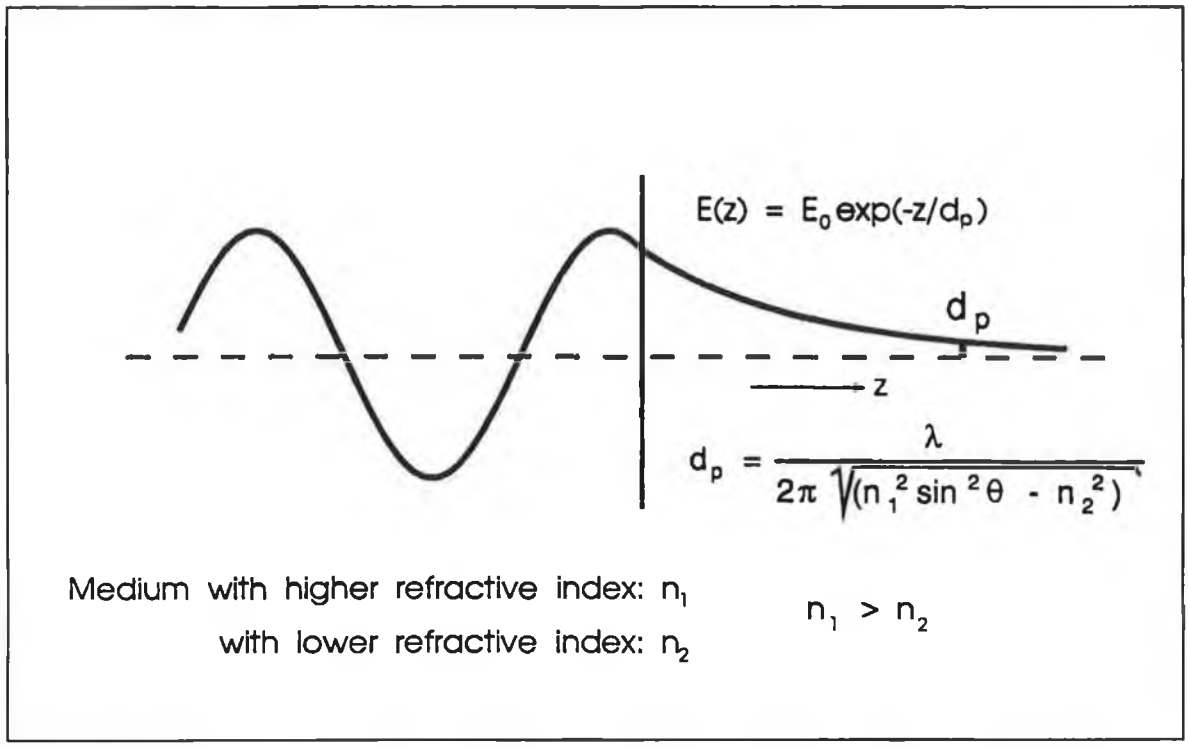

Fig 5.1

Propagation of the evanescent wave. Medium with higher refractive index: n_1. Medium with lower refractive index n_2. Where dp is the depth of penetration (the distance when E decays by time e^{-1}). Therefore the penetration depth is large for angles of incident near θ_c and for fractionally small difference between n_1 and n_2. Typically dp is of the order of the light wavelength in medium 2 [12].

5.1.2. General Applications

Crystal ATR has been used to rapidly quantitate total carboxylate [COO^{-1}] concentration in aqueous solution [2]. This was achieved by integrating the area of the asymmetric stretching peak, which is considered to be nearly independent of compound structure. They noted that the precision of the application was limited by the need to use solvent subtraction and because of instrument noise. The limits of detection were not quoted but due to the fact that the crystal was used without an enrichment coating and the samples were aqueous based the limits would be expected to be poor.

In ATR/FTIR the penetration depth of the evanescent wave is small and therefore high concentrations of analyte are required for detection. This is particularly the case for

aqueous solutions as water can be an inteferent. Various techniques have been employed to increase the sensitivity of ATR/FTIR. Optical fibers have been used as evanescent waveguides in place of the more traditional crystal based systems. Fiber based ATR is a technique with substantial potential. This is due to the fact that it has a large number of reflections per unit length; it's ideally suited for remote sensing and new developments in the manufacturing of IR transparent fibers.

A 10cm piece of chalogenic fiber which had the cladding removed was used as an ATR waveguide [3]. They used this bare fiber to sense for a number of individual gases hexane, trichlorotrifluoroethane, methane and acetone as well as various mixtures. The detected concentration range was 1 - 10 v/v% with reproducibility of 2 -3 %.

Fiber ATR has been used to detect gases using a commercially available teflon - clad fluoride glass optical fiber as the ATR/FTIR waveguide [4]. Propane gas was detected at 3.36μm and it is mentioned that other hydrocarbons could be detected using this technique. There is no mention of the LOD for the method.

Due to development of silver halide optical fibers by Katzir the optical window between 1000 and 700 cm^{-1} became accessible for optical mid-IR sensors [5,6]. Improvements in sensitivity were noted when these fiber based systems were used as the number of reflections through the waveguide increased dramatically [6,7].

Another way of improving the sensitivity of ATR/FTIR systems is to concentrate the analyte into the region where the evanescent wave penetrates.

Thin polymer layers have been shown to readily absorb organic molecules upon exposure [8]. Recently research has shown that coating an ATR crystal or an infrared optical fiber, which is essentially an ATR waveguide, with a polymeric phase can improve the detection limit for an analyte if that analyte has an affinity for the polymeric phase [1,6,12,17,18,19].

Much research has been carried out in the area of polymer coated ATR waveguides.

The use of ATR for studying thin films is a well established technique. It has been used to examine polymer reactions [9] and biomolecules. Meuse and Tomellini [8] demonstrated that thinly coating an ATR crystal with a suitable polymer, in this case PVC, improved the ability to detect test analytes in aqueous solution. They noted that the concentration of methanol in the aqueous solution had effects on the rate of this diffusion. The analytes investigated were nitrobenzene and ethylbenzoate. No LODs were quoted for the technique outlined.

Ertan-Lamontagne et al [6] investigated the use of plasticizers as a way of improving diffusion rates into a polymer coated waveguide. In this case the optical waveguide was a ZnSe crystal. They concluded that for the analytes nitrobenzene and benzonitrile the addition of chloroparaffin to PVC resulted in an increase in magnitude of the absorbance observed for the two analytes investigated.

5.1.3 Applications with Chlorinated Hydrocarbons (CHCs)

The development of sensors for chlorinated hydrocarbons in aqueous solutions using polymer coated evanescent waveguide spectroscopy (EWS) has been examined extensively.

The polymers most commonly used for the enrichment of these compounds are polyisobutylene (PIB) and low density polyethylene (LDPE). The analytes are enriched within the area of the evanescent field, where they are detected by their absorption peak in the range 1000 - 700 cm^{-1}. When selecting a polymer film it must have the following properties, there should be weak IR bands associated with the polymer in the spectral region of interest; the substances analysed must be held reversibly in the film; the enrichment time should be short; and the polymer must adhere well to the substrate.

The first application of a Mid-IR fiber optic sensor for CHCs was as a result of the development of polycrystalline silver halide fibers, which in this case were coated with low density polyethylene [5,10,11]. This set-up enabled the simultaneous in situ detection

of chlorobenzene and trichloroethylene at 1 to 50 ppm levels in aqueous solution. They claimed good agreement with independent results using headspace GC.

The principle of coating silver halide fibers with polymers was used to analyse different CHCs in water simultaneously [7]. The fiber was coated with a solution of 3 w/w % LDPE in decalin. Decalin has a boiling point that is approximately 80 °C above the boiling point of toluene. Therefore it does not evaporate quickly during subsequent air drying leaving behind a more stable and homogeneous LDPE coating than previously achieved. The compounds examined were trichloroethylene (TCE), tetrachloroethylene (TeCE), monochlorobenzene (MCB), 1,2 dichorobenzene (1,2 DCB), 1,2,4, trichlorobenzene (1,2,4,TCB) and chloroform. LODs ranged from 50 to 5ppm.

Mizaikoff et al [12] presented a review of their research in the area of mid-infrared (MIR) fiber optical chemical sensing systems. They describe two types of devices they developed thick film > 10um and thin film < 10um.

The thick film device were films which consisted of either LDPE or PIB at a film thickness of between 10μm and 20μm. Five chlorinated hydrocarbons were investigated in water, MCB, 1,2 DCB, TCE, 1,2,4 TCB and TeCE, simultaneously at the 50ppm concentration range. The replacement of the FTIR spectrometer with a tuneable diode laser (TDL) system reduced the detection limit significantly to 100ppb as well as the measurement time to three minutes. This sensor has also been used to investigate gaseous samples of 1,2-dichlorotetrafluoroethane (R114). The limit of detection was measured at 0.1 % v/v.

The second type of sensor reviewed was the thin layer sensor. This involved the immobilisation of an enzyme on chalcogenide fibers. The enzyme was used to facilitate the conversion of glucose to gluconic acid. The monitoring of the concentration of reaction products in the surrounding aqueous solutions by EWS presumes an enzyme layer thinner than the penetration depth of the IR radiation but within the maximum reactivity of the catalytically active surface to provide a fast sensor response. They

concluded that the enzyme activity was lower than expected based on experiments carried out on glass surfaces.

A ZnSe crystal was used as an ATR element that was coated with polymers to investigate diffusion and enrichment behaviour for three different CHCs, MCB, chloroform (CF) and TeCE into various polymers [13]. They concluded that PIB gave the best results of the polymers investigated. 1,2-polybutadiene (PBD) gave the poorest response and was very irreproducible. The reason given for this was a strong IR band at 910 cm^{-1} for the polymer that caused interference problems. They also noted that the amount of water that diffuses into the polymer coating greatly affects the reproducibility of the measurements. Aliphatic polymers LDPE, PIB and poly (4-methyl-1-pentane) show less water enrichment then PBD.

Gobel et al [14] investigated the use of silver halide fiber coated with polymers LDPE, PIB and ethylene / propylene copolymer (E/P co). They noted that the chosen polymer must not have strong absorption bands in the spectral region below 1000 cm^{-1} as they would intefere with CHC detection. This limits the application polymers to the small group listed above. Ten environmentally relevant CHCs were detected at concentrations of 1 to 50ppm. They noted that the high solubility of CHCs in aqueous solutions diminishes the partition coefficients of CHCs between water and polymer membrane thus reducing possible enrichment. They also investigated the temperature dependence of the sensor response. They concluded that no real trend could be observed in the temperature range they investigated between 0 and 22 °C.

They also applied this sensor to artificial seawater (ASW) [15]. The sensor was used with and without a protective polymer coating. They investigated the influence of high salt concentrations on the sensor response. They concluded that in contrast to the bare fiber the coated fibers remained stable in contact with the chloride solution. This enabled the development of a fiber evanescent waveguide spectroscopy (FEWS) sensor to quantitate CHCs in seawater. TeCE was the analyte investigated in this study.

Gobel et al further tried to improve on the sensitivity of the FEWS system for quantitating CHCs in water [16]. They did this by using a TDL as the IR source and a mercury cadmium tungsten detector.

They investigated ways of increasing the evanescent wave absorption. Since

$$A \approx N.\ a \qquad N = (l.\mathrm{Tan}\theta) / d$$

where A is the responsiveness of a FEWS sensor, N is the number of internal reflections, a is the absorption per internal reflection, l is the length of fiber, d is the diameter of the fiber and θ is the angle of incident.

From this they concluded that there are three ways of increasing the evanescent wave absorption.

1. Increase the fiber length l, this was not viable due to attenuated losses of light
2. Decreasing the fiber diameter d, this is achieved by flattening the fiber diameter and was applied in this case.
3. Increase the angle of incidence, since maximum absorbance was found to occur at incident angles of 55 - 60°, an angle of incidence of 60° was used for this work.

They concluded an LOD of 50ug/L for TeCE which is ten times better than had previously been seen.

Tapered chalogenic fibers were also used to detect low levels of benzene, $CHCl_3$ and nitrobenzene in aqueous solutions in this case the polymer coating used was PVC [17]. The limits of detection were quoted as 0.02, 0.11 and 0.006% (v/v) for benzene, $CHCl_3$ and nitrobenzene respectively. They concluded that the coating of the fiber increased the sensitivity of the ATR element, as the above compounds were undetectable using the uncoated fibers.

Regan et al [18] used teflon coated silver halide fiber to analyse TCE in aqueous solution. The useful calibration range had a correlation coefficient of 0.9990 and relative standard deviation of 0.0002 in the range between 5 - 50ppm. A limit of detection of 1ppm was achieved for TCE determined using a teflon coated optical fiber. A dip coating technique

was used to coat the fiber and the dipping rate was investigated. It was concluded that faster dipping speed lead to thicker polymer coats.

An ATR sensor was used to investigate TCE and TeCE in muddy water samples. The sensor used silver halide as the waveguide and it was coated with LDPE [19]. They looked at the interference effects of benzene and toluene on the quantitative technique and concluded that these interferences had little effect on the result.

A novel system for detecting TCE and toluene was developed [20], which used diffusion through a polydimethysiloxane film and an evanescent fiber optic chemical sensor (EFOCS). In this case the EFOCS was 2 meters of coiled silica bore fiber and the near IR spectra were collected. They concluded that the system could be used to monitor organic analyte concentrations in aqueous solutions. Linear chemometric algorithms such as partial least square (PLS) were used to model the CHCs in mixtures between 20 -300 ppm.

ATR/FTIR has also been used to analyse in situ changes of enzymes under different immobilisation procedures [21]. The feasibility of using MIR transparent waveguides, and specifically chalogenic fibers, as a spectroscopic probe to analyse surface layer formation was demonstrated.

Silicone rubber was used as both an evanescent waveguide and a selective membrane into which specific analytes could diffuse [22]. The sensor was used to detect volatile organics, TCE, 1,1,dichloroethylene and benzene. The time for 90% maximum absorbance to be reached (t_{90}), was 30 minutes for an aqueous solution and 3 minutes for a gaseous headspace sample. The LOD for TCE was measured at 1.1 ppm in aqueous solution. They also investigated temperature effects on the enrichment process. They concluded that temperature effects were related to the degree of polymer swelling which occurred. They also noted that because variations seen in absorbance were wavelength

dependent that the changes in absorbance were related to not only temperature but also changes in refractive index and the reflectivity at the two polymer quartz interfaces.

In this present study an analytical method was developed and validated which utilised a polymer coated ATR element to analyse for PAHs in an aqueous environment. Flouranthene was chosen to represent this group of compounds. The method was developed by optimising the various parameters involved in coating and using the ATR crystal.

5.2. Experimental

5.2.1. Materials

5.2.1.1. Chemicals

Teflon® (4,5 Difluoro 2,2 Bistrifluoromethyl 1,3 Dioxole polymer with tetrafluoroethylene) was supplied by Dupont. Polyisobutylene was purchased from Aldrich. Labscan supplied Acetonitrile. Fluka Chemika supplied Decahydronaphthalene (decalin). Aldrich supplied the Polycyclic Aromatic Hydrocarbons (PAHs). A stock solution of fluoranthene was prepared in ACN at 0.1mgs/cm^3 and stored at ambient in the dark.

5.2.1.2. Instrumentation.

The Infra Red Spectrophotometer used for these experiments was a PE2000 FTIR supplied by Perkin Elmer. Data was collected using Grams Analyst 2000, which is a windows based FTIR package supplied by Perkin Elmer. This software was run on an Elonex 466 PC. All spectra were obtained at nominal resolution of 2 cm^{-1}. The paragon

1000, the attenuated accessory used for this study was supplied by Perkin Elmer. The zinc selinide (ZnSe) crystal was mounted into a sample trough and this was then mounted on a paragon 1000 on the baseplate of PE2000 FTIR. The angle of incidence was 45°. The manufacturer assumed a refractive index of 2.403 at $1000cm^{-1}$ for the ZnSe element and concluded that there were twelve reflections of light along the crystal at this angle of incidence.

5.2.2 Methods

5.2.2.1. Polymer Coating

Polymer coatings were applied to the crystal using two separate techniques. The initial technique was a spreading technique. $2cm^3$ of solution were spread as evenly as possible with a pasture pipette and it was then left to dry overnight. The actual thickness of the coated film was not measured.

5.2.2.2. Generation of Spectra

Once the solvent had evaporated the polymer was equilibrated with a blank solution which in all cases was $2cm^3$ of 90/10 H_2O/ACN solution for an optimum amount of time which was evaluated to be 50 minutes. This optimum time was achieved by loading the coated crystal with the blank solution and taking IR spectra, every 5 minutes, until no change was seen between the spectrum and the previous one. The solution was then removed and a solution containing the analyte was then added to the sample trough. Spectra were obtained for a period of time usually under 30 minutes. Absorbance spectra

were produced by the subtraction of a background spectrum that was a spectrum of the crystal, the polymer and a blank solution after 50 minutes, from the sample spectra. The absorbance peak of the analyte was then integrated at between 770 cm^{-1} and 790 cm^{-1} and the area recorded. A plot of area v time was then constructed and the effect of the extraction into the polymer layer was assessed.

5.3 Results and Discussion

5.3.1. Choice of Polycyclic Aromatic Hydrocarbon (PAH)

The ability of coated ATR/FTIR to analyse PAHs in an aqueous environment was assessed by first looking at one PAH. Fluoranthene was the PAH chosen after an examination of IR spectra for the sixteen PAHs listed in EPA method 610 [23]. Fluoranthene was chosen because it gave strong absorbance at 772cm^{-1} and it is considered to be relatively non toxic compared to other members of this group of compounds (see fig 5.2).

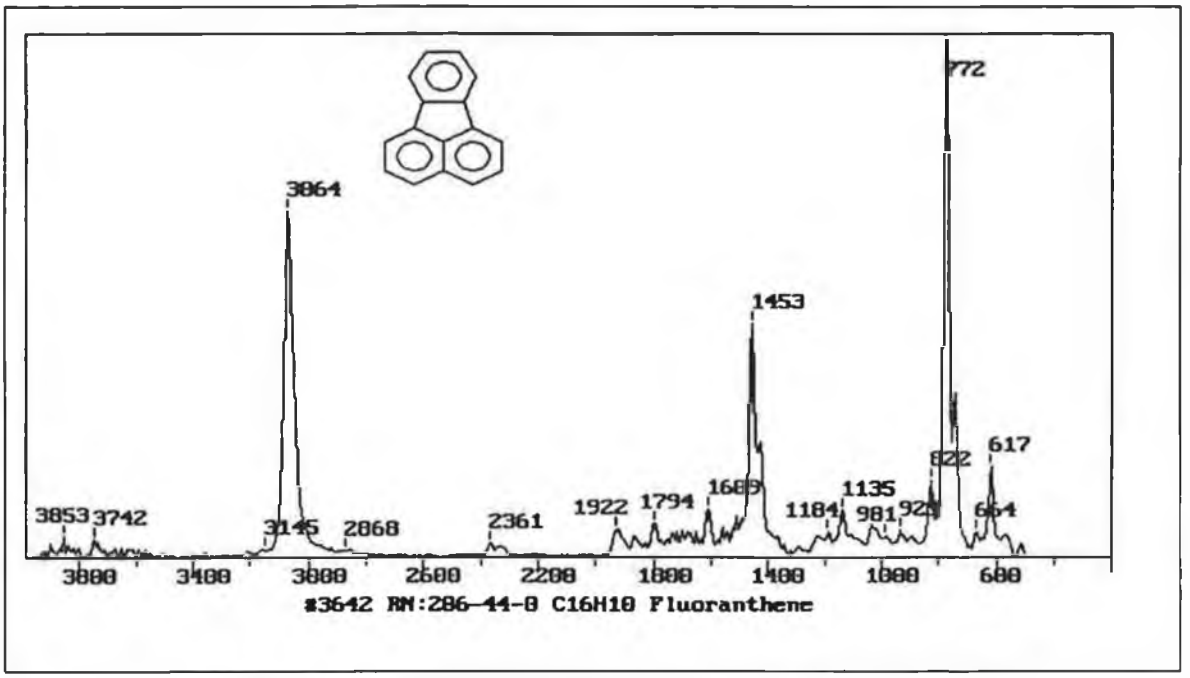

Fig 5.2

This diagram depicts the infrared spectrum and molecular structure of fluoranthene. The strong absorbance peak at 772cm^{-1} was chosen to measure the response for fluoranthene.

5.3.2. Uncoated ATR/FTIR

A 100ppm solution of fluoranthene was prepared in 90/10 H_2O/ACN, as it is insoluble in 100% H_2O. This solution was placed onto the ZnSe crystal and ATR/FTIR spectra were recorded. No detectable response was noted for this 100ppm solution of fluoranthene. It was therefore concluded that traditional ATR/FTIR was not an appropriate technique for the analysis of fluoranthene at this concentration.

5.3.3. Water Absorption

Initially it had been thought necessary to use thick coatings of polymers (>10μm) to exclude disruptive water bands. However it was found that by using thin layers (2 - 5μm)

and saturating the polymeric material with water prior to analysis, that satisfactory absorbances and response times for the analyte were achieved. Thicker layers prevent water ingress, however absorption of the analyte into the polymeric region is significantly slower [18].

90/10 Water/ACN spectra were taken until no significant difference was noted over a 5 minute time period in the water band region at approximately 3600cm^{-1}. This was then taken to be the background absorption and was repeated prior to each loading of the crystal.

5.3.4. Types of Polymers in Coating ATR/FTIR

Previous research has shown that various types of polymers had been investigated to enhance the response of pesticides using coated ATR/FTIR [18]. It was decided in this case to investigate two polymers, polyisobutylene (PIB) and teflon.

5.3.4.1. Polyisobutylene

Medium M.W. PIB (density = 0.920) was initially chosen to coat the ATR crystal. All PIB coating solutions were prepared in decalin. Decalin was chosen because it has a high boiling point and therefore it evaporates slowly leaving behind a very stable homogeneous PIB coating on the surface of the crystal [7].

An 8% PIB in decalin solution was coated onto the ZnSe crystal using the spread technique, as described previously and left to dry overnight. The 100ppm solution of fluoranthene was placed on this coating and IR spectra measured and recorded. Initial

results indicated that the coating of this crystal (fig 5.3) enhanced the response of fluoranthene.

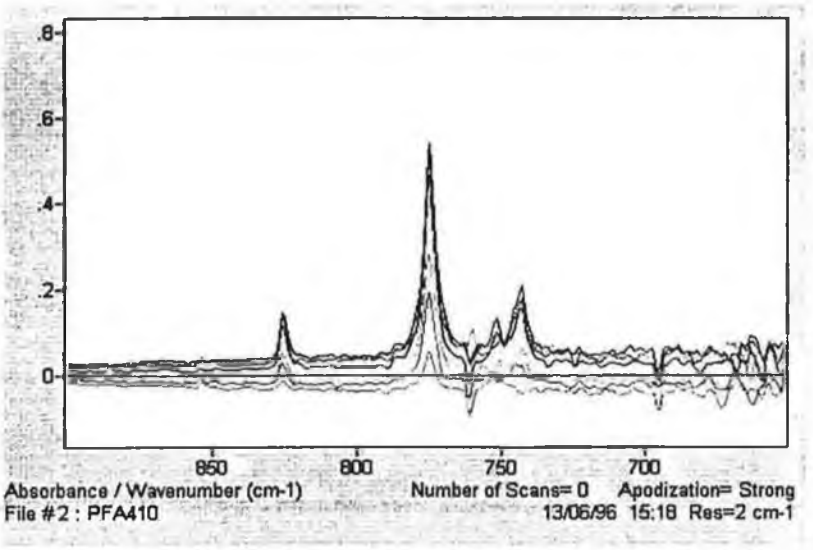

Fig 5.3

Overlay of a series of spectra, which demonstrate the enrichment of fluoranthene at a concentration of 100ppm into a polymer film on an ATR waveguide. The polymer coating was achieved by spreading 8% (w/w) PIB onto the surface of a ZnSe crystal and allowing it to dry over night. Spectra were recorded at various times over 90 minutes and are overlaid here.

The peak areas were recorded over a period of time for the enrichment of 100ppm fluoranthene on the 8% PIB coated ZnSe crystal (see table 5.1 and fig 5.4).

Time (minutes)	Area at 772cm^{-1}
5.00	0.8732
15.00	2.2165
30.00	2.5772
50.00	2.6264
60.00	2.6310
90.00	2.5426

Table 5.1.

Table of peak areas for the peak at 772cm^{-1} recorded when a 100ppm fluoranthene solution was enriched onto an 8% PIB polymer coating on a ZnSe crystal over a period of 90 minutes. The peak was integrated between 770 – 790cm^{-1} using the Grams Analyst software and the areas recorded.

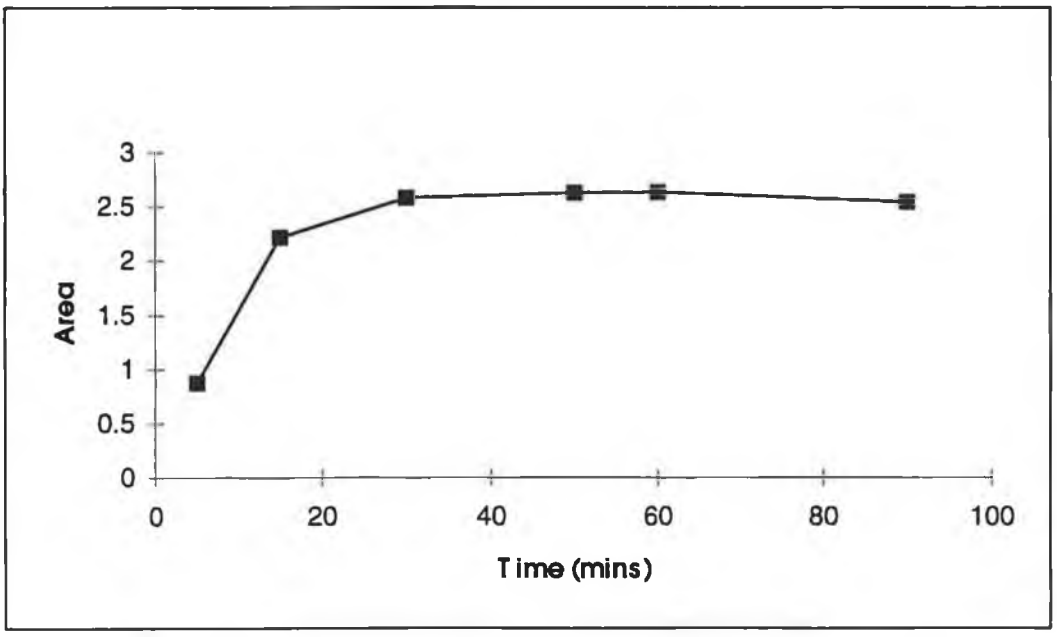

Fig 5.4

Graph of peak areas versus time (minutes) for the peak at 772cm^{-1} recorded when a 100ppm fluoranthene solution was enriched onto an 8% PIB polymer coating on a ZnSe crystal over a period of 90 minutes. The peak was integrated between 770 – 790cm^{-1} using the Grams Analyst software and the areas recorded.

5.3.4.2. Teflon

Previous work [18] had indicated that Teflon was also possible as a coating material and it was therefore decided to investigate it. The same procedure used in section 5.3.4.1, for PIB, was used for teflon coating. In this case however water proved to be a major interferent making it impossible to identify any response due to fluoranthene. The polymer coating itself showed signs of lifting from the crystal. As a result of this lifting a curing procedure was introduced in the hope it would bind the teflon better. The curing procedure was as follows, 50°C for 5 minutes, 120° C for 5 minutes and finally 165°C for

5 minutes. This proved to be unsuccessful, as water was a major interferent, which prevented the response of fluoranthene from being assessed. It was therefore decided to optimise the procedures involved coating PIB on the ZnSe crystal.

5.3.5. Optimisation of Polyisobutylene Coating Procedures

5.3.5.1. Concentration of Coating Solution

A series of polyisobutylene solutions were prepared in decalin, 1w/v%, 2w/v%, 5w/v% and 8w/v%. An attempt was made to make up a higher concentration, 10w/v % however it was virtually impossible to get the material into a homogeneous solution.

5.3.5.1.1. 100ppm Fluoranthene

The solutions were coated onto the ZnSe crystal using the technique described previously in section 5.2.2.1. and left overnight to allow the decalin evaporate. The coatings were conditioned using 90/10 H_2O/ACN mix for 50 minutes and a background spectrum was taken. The sample trough was filled with $2cm^3$ of 100ppm fluoranthene and spectra recorded over time (see table 5.2 and fig 5.7).

Time (minutes)/ % (w/v) PIB	1 %	2 %	5 %	8 %
5.00	0.1454	0.1138	0.4577	0.0714
10.00	0.2571	0.7332	1.0241	0.2601
15.00	0.3322	1.0744	1.6095	0.3286
20.00	0.5650	1.4666	1.9747	0.4097
25.00	0.2583	2.3958	2.3070	0.5464
30.00		2.0817	2.3972	0.6899
35.00		2.2447	2.4159	0.7349
40.00		1.7023	2.4733	0.8095
45.00			2.6258	0.9535
50.00			2.6837	0.9570
55.00			2.6483	0.9940

Table 5.2.

Table of peak areas integrated between 770 - 790 cm^{-1} when a 100ppm fluoranthene solution was enriched onto various PIB coating concentrations ranging from 1w/v % to 8w/v % over a period of 55 minutes.

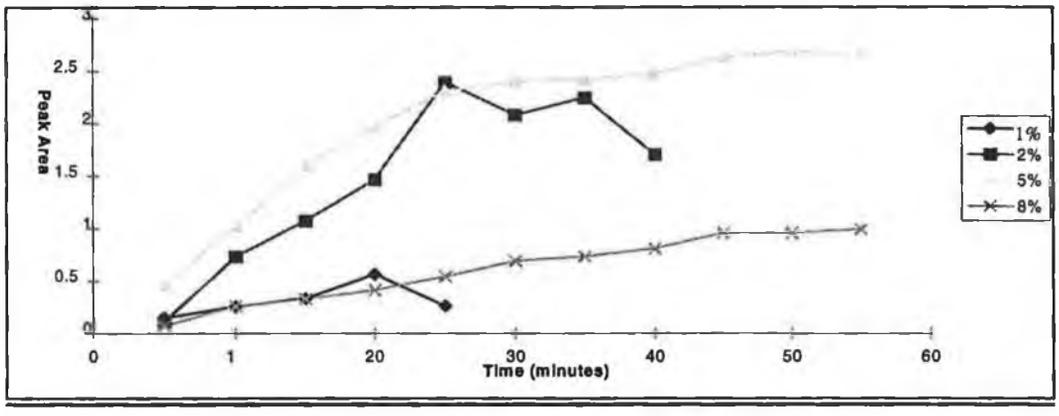

Fig 5.7

Graph of peak areas integrated between 770 - 790 cm^{-1} versus time (minutes) when a 100ppm fluoranthene solution was enriched onto various PIB coating concentrations ranging from 1w/v % to 8w/v % over a period of 55 minutes.

5.3.5.1.2. 10ppm Fluoranthene

This procedure was repeated using 10ppm fluoranthene as the analyte extracted by the PIB coating (see table 5.3 and fig 5.8).

Time (minutes)/ % PIB	5w/v %	8w/v %
1.00	0.0465	0.0008
5.00	0.1086	0.0010
10.00	0.1711	0.0002
15.00	0.1743	0.0011
20.00	0.2074	0.0007
30.00	0.2111	0.0042
40.00	0.2138	0.0086
50.00	Not Available	0.0133
60.00	0.2483	0.0162
70.00	0.2521	0.0201
90.00	Not Available	0.0259
120.00	0.2827	Not Available

Table 5.3

Table of peak areas integrated between 770 - 790 cm^{-1} when a 10ppm fluoranthene solution was enriched onto 5w/v % and 8w/v % PIB coating concentrations over a period of 120 minutes.

Note: It was not possible to record any results for the 1% and 2% coating due to water interferences.

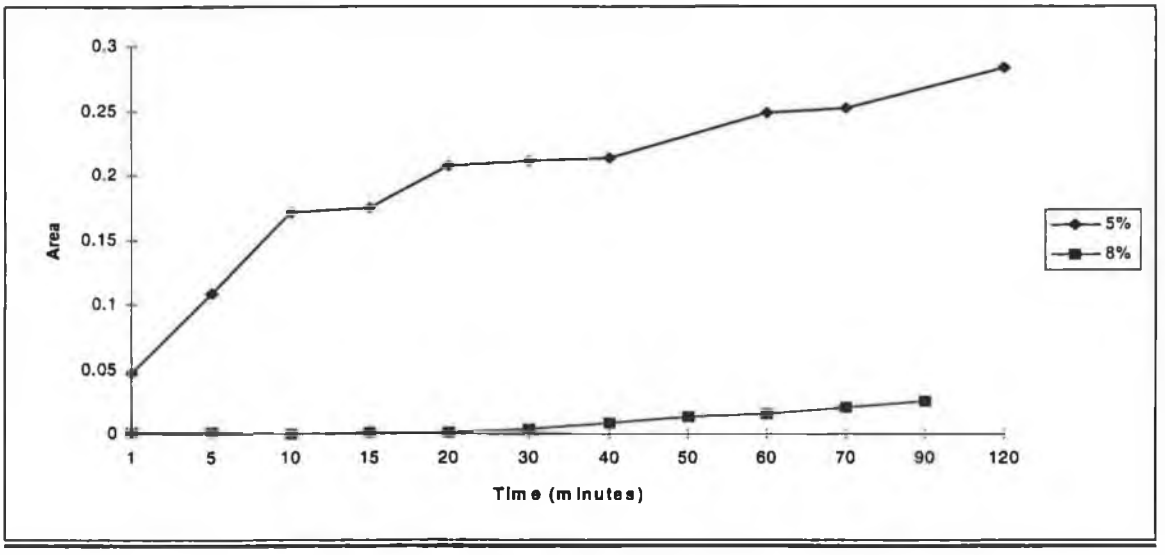

Fig 5.8

Graph of peak areas integrated between 770 - 790 cm^{-1} versus time when a 10ppm fluoranthene solution was enriched onto 5w/v % and 8w/v % PIB coating concentrations over a period of 120 minutes.

From an examination of the plots of peak area versus time for fluoranthene enriched at both 10 and 100ppm onto the various polymer coating concentrations it was concluded that 5w/v % PIB in decalin was the optimum coating solution.

Lower PIB polymer concentrations, 1w/v % and 2w/v %, gave non-detectable results for the 10ppm fluoranthene solution and erratic results for the 100ppm fluoranthene solution. This was probably due to water interference. The higher PIB polymer concentration, 8w/v %, resulted in a longer enrichment time and lower peak area response. This meant it would be less sensitive at detecting the fluoranthene peak than the 5w/v % coating.

5.3.5.2. Reusability

For the enrichment effect of a 5% PIB in decalin solution coated on ZnSe to be of a practical significance it must be possible to reuse the same coating a number of times to make different measurements of an analyte concentrations as it would not be practical to be re-coating the crystal between measurements. To achieve this effect a cleaning procedure had to be developed to remove the fluoranthene from the crystal coating. Various aqueous/acetonitrile combinations were tried and eventually a 30 minutes soak in 200cm^3 of 100% ACN was observed to be the most effective. The ability of a single coating to be reused was investigated by checking how reproducible the enrichment process was at two separate concentrations 10ppm and 100ppm.

5.3.5.2.1. 10ppm Fluoranthene

A 5% PIB coated crystal was prepared and $2cm^3$ of 10ppm fluoranthene were added to the sample trough. A number of spectra were recorded and the resulting areas were plotted against time. This was followed by a 30 minute soak in ACN. This procedure was repeated a further two times and the plots compared.

Time (minutes)/ Peak Area	Run 1	Run 2	Run 3
1.00	0.0008	0.0020	0.0010
3.00	0.0010	0.0059	0.0029
5.00	0.0050	0.0076	0.0136
7.00	0.0166	0.0119	0.0191
10.00	0.0482	0.0294	0.0380
12.00	0.0674	0.0427	0.0506
15.00	0.0771	0.0649	0.0643
17.00	0.0942	0.0734	0.0734
20.00	0.0992	0.0896	0.0868

Table 5.4
Table of peak areas for the peak integrated between 770 – 790cm^{-1} recorded when a 10ppm fluoranthene solution was enriched onto a 5w/v % PIB polymer coating over a period of 20 minutes and the coating was then clean using a soak for 30 minutes in ACN. This procedure was carried out three times.

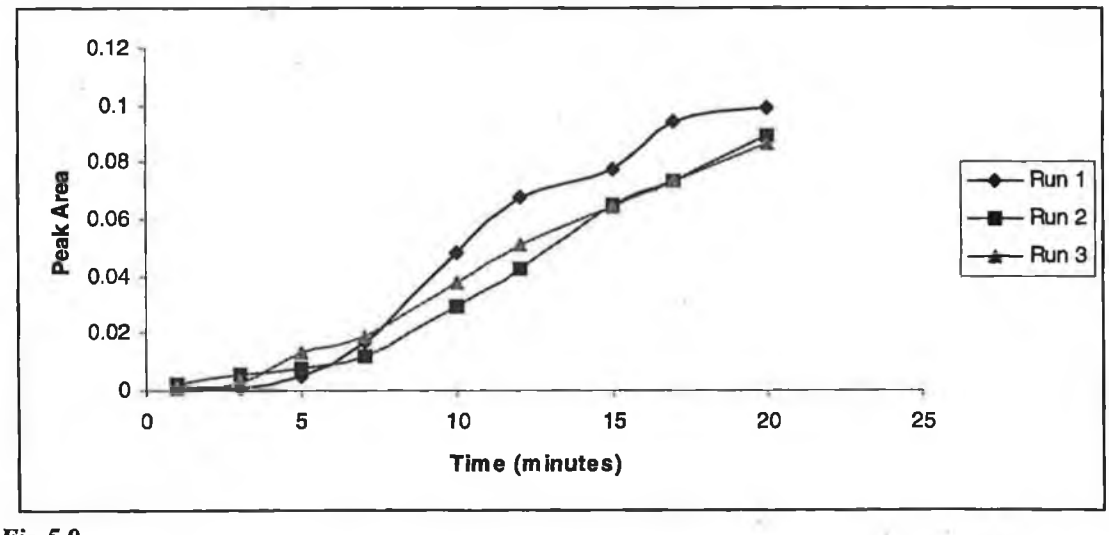

Fig 5.9
Plot of peak areas versus time (minutes) for the peak integrated between 770 – 790cm^{-1} recorded when a 10ppm fluoranthene solution was enriched onto a 5w/v % PIB polymer coating over a period of 20 minutes and the coating was then clean using a soak for 30 minutes in ACN. This procedure was carried out three times.

5.3.5.2.2 100ppm Fluoranthene

This procedure was repeated for a 100ppm solution and the results again plotted (see table 5.5 and fig. 5.10).

Time (minutes)/ Area	Run 1	Run 2	Run 3
1.00	0.0204	0.0279	0.0084
3.00	0.0774	0.1058	0.0759
5.00	0.1976	0.2890	0.2225
7.00	0.3558	0.5085	0.4148
10.00	0.6448	0.8386	0.7606
12.00	0.8823	1.1093	0.9950
15.00	1.1891	1.4330	1.3213
17.00	1.3710	1.6514	1.5364
20.00	1.6241	1.9186	1.7738

Table 5.5

Table of peak areas for the peak integrated between 770 – 790cm^3 recorded when a 100ppm fluoranthene solution was enriched onto a 5w/v % PIB polymer coating over a period of 20 minutes and the coating was then clean using a soak for 30 minutes in ACN. This procedure was carried out three times.

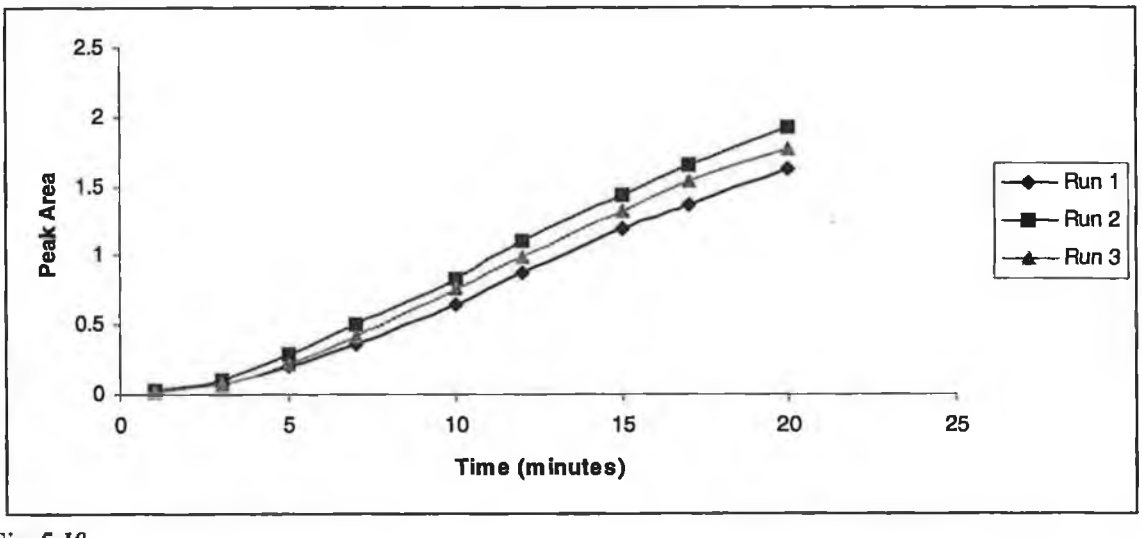

Fig. 5.10

Plot of peak areas versus time (minutes) for the peak integrated between 770 – 790cm^3 recorded when a 100ppm fluoranthene solution was enriched onto a 5w/v % PIB polymer coating over a period of 20 minutes and the coating was then clean using a soak for 30 minutes in ACN. This procedure was carried out three times.

It can be concluded from these plots that reusing the same coating on a crystal for a number of observations has no detrimental effect on the polymer coated crystals ability to enhance the response of the analyte in question. The variation seen in these results are possibly due to the fact that the procedures for loading samples onto the crystal had not been optimised fully. This procedure will be discussed in more detail later on.

5.3.5.3. Physical coating procedure

There are a number of ways of coating the ZnSe crystal with a polymer solution. The two techniques investigated were termed a spread technique and a spin technique. The difference between these techniques is based on the way the polymer solution is spread on the surface of the crystal. As ATR/FTIR is based on the interaction of Infrared light and analyte in a region micrometres above the crystal surface the uniformity of a coating in this region has large effects on the ability of that coating to enhance the response of the analyte.

5.3.5.3.1. Spread Coating Technique

A spreading technique was developed whereby as many variables as possible were kept constant so as to ensure that the technique was as reproducible as possible. The technique used was, 0.2cm^3 of the 5 % w/v PIB in decalin were placed at one end of the ZnSe crystal surface. This was then spread evenly over the entire surface using a pasture pipette and left to dry overnight. The evaluation of the coating procedure was done by loading both the blank and the sample in a consistent fashion. 2cm^3 of the blank solution, 90/10 H_2O/ACN or 2cm^3 of 100ppm fluoranthene solution were placed in the sample trough

and a series of readings were taken over time and a plot of area versus time was constructed for the 100ppm fluoranthene solution. Two enrichment procedures were conducted for each coating using a 100ppm fluoranthene solution, the crystal and coating were cleaned between sample runs by using the cleaning procedure outlined above. Once the two runs had been conducted the coating was removed and a fresh coating applied. Three separate coatings were carried out. (see tables 5.7, 5.8, 5.9 and figs 5.11, 5.12 and 5.13).

Time / Area	Run #1	Run #2	Average
1.00	0.0911	0.1057	0.0984
3.00	0.2736	0.3066	0.2901
5.00	0.4971	0.5495	0.5233
7.00	0.7630	0.8167	0.7899
10.00	1.1282	1.2225	1.1754
12.00	1.3718	1.4522	1.4120
15.00	1.7347	1.7871	1.7609
17.00	1.9408	1.9386	1.9397
20.00	2.2010	2.1652	2.1831

Table 5.7

A table of peak areas for the peak integrated between 770 – 790cm^3 recorded when a 100ppm fluoranthene solution was enriched onto the first 5w/v % PIB polymer coating, which had been coated using a defined procedure. Areas were recorded over a period of 20 minutes and the coating was then cleaned using a soak for 30 minutes in ACN between two runs.

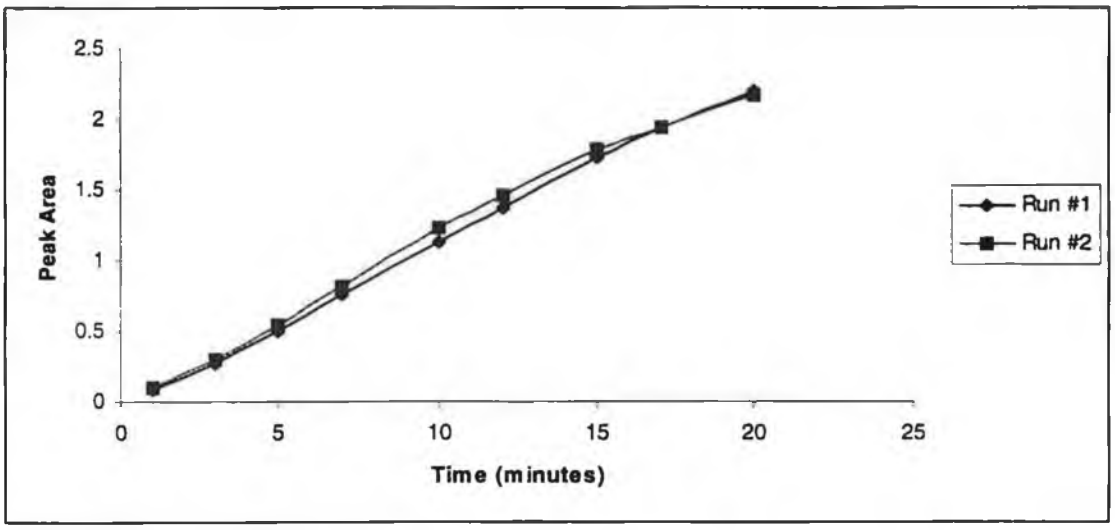

Fig 5.11

Plot of peak areas versus time (minutes) for the peak integrated between 770 – 790cm^3 recorded when a 100ppm fluoranthene solution was enriched onto the first 5w/v % PIB polymer coating, which had been coated using a defined procedure. Areas were recorded over a period of 20 minutes and the coating was then cleaned using a soak for 30 minutes in ACN between two runs.

Time (minutes) / Area	Run #1	Run #2	Average
1.00	0.1846	0.1944	0.1895
3.00	0.5194	0.5511	0.5353
5.00	0.8712	0.8776	0.8744
7.00	1.2503	1.3125	1.2814
10.00	1.7351	1.7857	1.7604
12.00	2.0176	2.0468	2.0322
15.00	2.319	2.3411	2.3301
17.00	2.4773	2.5039	2.4906
20.00	2.7053	2.7252	2.7153

Table 5.8

Table of peak areas for the peak integrated between 770 – 790cm^3 recorded when a 100ppm fluoranthene solution was enriched onto the second 5w/v % PIB polymer coating, which had been coated using a defined procedure. Areas were recorded over a period of 20 minutes and the coating was then cleaned using a soak for 30 minutes in ACN between two runs.

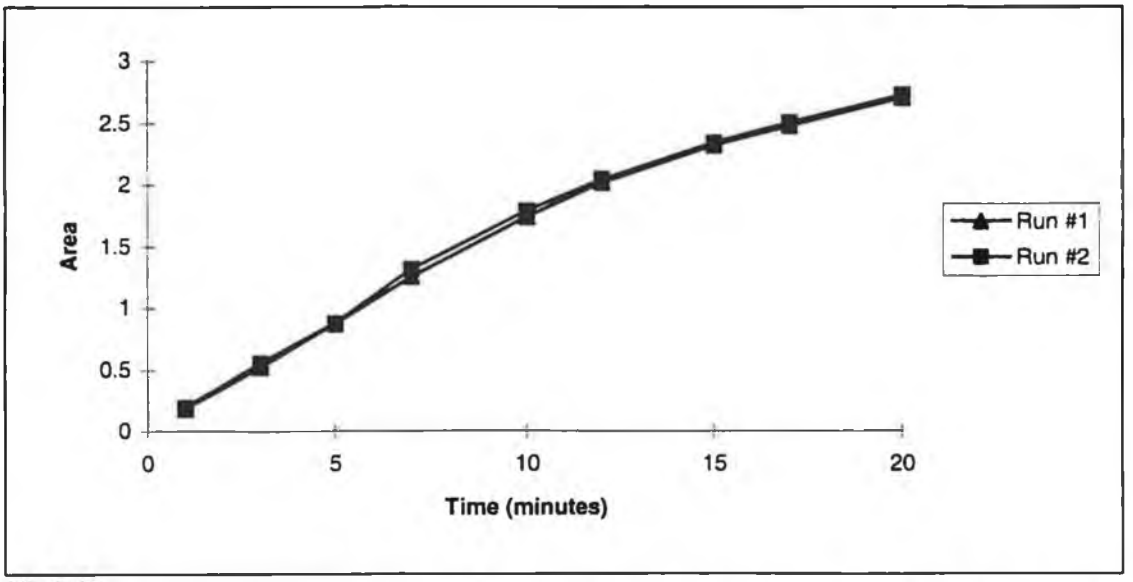

Fig 5.12

Plot of peak areas versus time (minutes) for the peak integrated between 770 – 790cm^3 recorded when a 100ppm fluoranthene solution was enriched onto the second 5w/v % PIB polymer coating, which had been coated using a defined procedure. Areas were recorded over a period of 20 minutes and the coating was then cleaned using a soak for 30 minutes in ACN between two runs.

Time (minutes) / Area	Run #1	Run #2	Average
1.00	0.0025	0.0097	0.0061
3.00	0.0396	0.0425	0.0411
5.00	0.1127	0.1142	0.1135
7.00	0.2347	0.2563	0.2455
10.00	0.5304	0.5254	0.5279
12.00	N/A	0.7203	0.7203
15.00	1.0093	1.0202	1.0148
17.00	1.209	1.1990	1.204
20.00	1.4492	1.4464	1.4478

Table 5.9

Table of peak areas for the peak integrated between 770 – 790cm^3 recorded when a 100ppm fluoranthene solution was enriched onto the third 5w/v % PIB polymer coating, which had been coated using a defined procedure. Areas were recorded over a period of 20 minutes and the coating was then cleaned using a soak for 30 minutes in ACN between two runs.

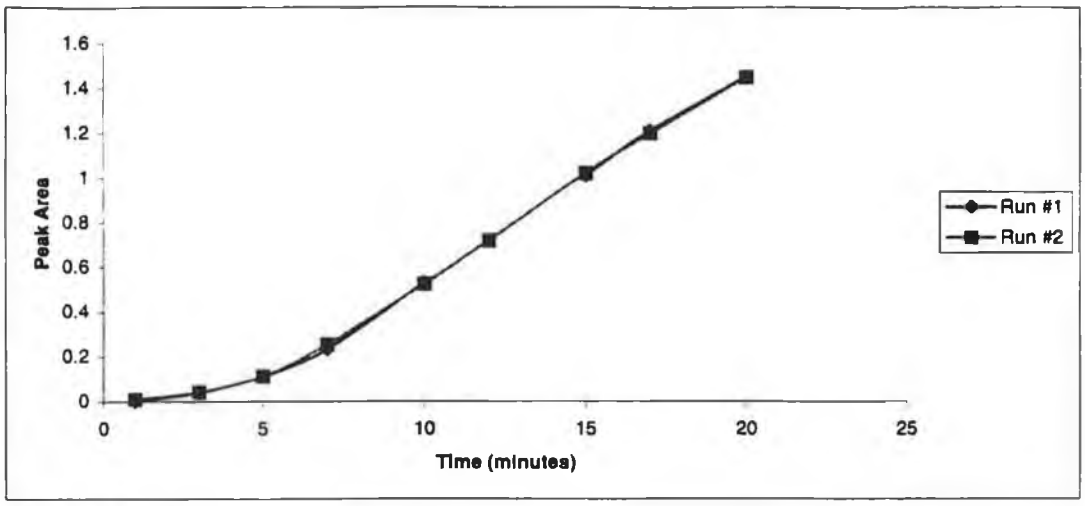

Fig 5.13

Plot of peak areas versus time (minutes) for the peak integrated between 770 – 790cm^3 recorded when a 100ppm fluoranthene solution was enriched onto the third 5w/v % PIB polymer coating, which had been coated using a defined procedure. Areas were recorded over a period of 20 minutes and the coating was then cleaned using a soak for 30 minutes in ACN between two runs.

From these plots the averages were calculated for the runs carried out on the separate coatings. A plot was constructed of these averages and the standard deviations at each point calculated to assess how consistent the spreading technique was (see table 5.10 and fig 5.14).

Time (minutes)	Spread #1	*Spread #2*	*Spread #3*	*Std Deviation*
1.00	0.0984	0.1895	0.0061	0.0917
3.00	0.2901	0.5353	0.0411	0.2471
5.00	0.5233	0.8744	0.1135	0.3808
7.00	0.7899	1.2814	0.2455	0.5182
10.00	1.1754	1.7604	0.5279	0.6165
12.00	1.4120	2.0322	0.7203	0.6563
15.00	1.7609	2.3301	1.0148	0.6596
17.00	1.9397	2.4906	1.2040	0.6455
20.00	2.1831	2.7153	1.4478	0.6365

Table 5.10

Table of the average peak areas for the response of 100ppm fluoranthene enriched onto three separate coatings using a 5w/v % PIB polymer with a defined spread coating technique.

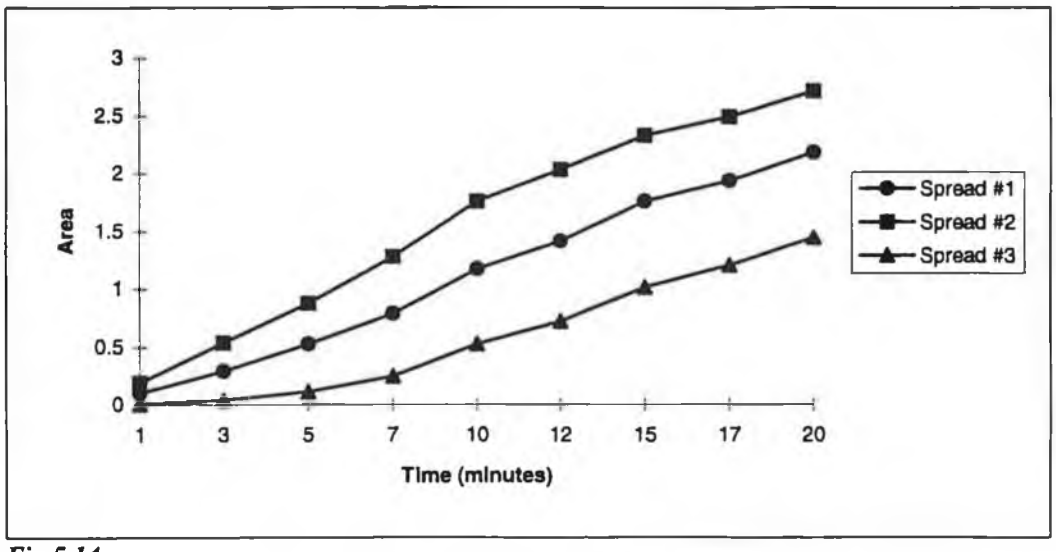

Fig 5.14
Plot of the average results for 100ppm fluoranthene enriched onto three spread coatings using the standard spread coating technique.

From an examination of these plots two conclusions were made.

The first conclusion was for the individual spread coatings (see figs 5.11, 5.12 and 5.13). It was that the samples could be loaded onto the 5% PIB coating in a reproducible manner i.e. enrichment occurred in a reproducible manner.

The second conclusion was based on the average peak areas for the three separate coatings (see fig 5.14). It can be seen that even though great care was taken during the actual spread coating large variations were seen between the different coatings. The reason for this was the difficulties encountered during the coating of the crystal leading to variations in the coating thickness, which resulted in big differences in responses for the 100ppm fluoranthene standard.

5.3.5.3.2. Spin Coating Technique

The second type of coating technique examined was a spin technique. This is the same as the spread technique except for one important procedure. Instead of spreading the coating with a pasture pipette the fixed volume of coating is placed on the ZnSe crystal and the crystal is spun at a very high speed using a Laurel spin coating device. The crystal was placed on a table in the device and held in position by vacuum. The device then spins this table at very high speeds up to 1600 rpm.

Through experimentation using glass slides an optimum coating technique was developed. This involved spreading $5cm^3$ of coating solution in a zigzag fashion across the surface and then spinning this at a fixed speed for 5 minutes. The excess solution was spun off and a uniform 5w/v % PIB coating remained and the decalin was allowed to evaporate over night. The speed at which the ZnSe crystal was spun was optimised.

Optimising Coating Spin Speed

Four different spin speeds were investigated, 1000 rpm, 800 rpm, 640 rpm and 500 rpm.

1000 rpm

1000 rpm was deemed to be too fast as although the glass slide could be held in place by the vacuum system this was not the case for the heavier ZnSe crystal resulting in the crystal spinning off and it being damaged. A new crystal was used for the rest of the experimental work.

800 rpm

The crystal was coated with 5% PIB in decalin as described above and it was spun at 800 rpm for 5 minutes. This resulted in a smooth uniform coating of the crystal. $2cm^3$ of the 100ppm fluoranthene solution were then loaded as described previously and the spectrum recorded. The crystal was then washed as described previously and the 100 ppm fluoranthene loading was repeated (see table 5.11 and fig 5.15).

Time (minutes)/ Area	**Run #1**	**Run #2**	**Average**
1.00	0.5912	0.4004	0.4958
3.00	0.8044	0.9046	0.8545
5.00	1.1492	1.1402	1.1447
7.00	1.4391	1.3592	1.3992
10.00	1.7334	1.5952	1.6643
12.00	1.9450	1.8141	1.8796
15.00	2.0239	1.9273	1.9756
17.00	2.0588	2.0657	2.0623
20.00	2.1052	2.0776	2.0914

Table 5.11

Table of peak areas for the peak integrated between 770 – 790cm^3 recorded when a 100ppm fluoranthene solution was enriched onto the 5w/v % PIB polymer coating, which had been coated using a spin speed of 800rpm. Areas were recorded over a period of 20 minutes and the coating was then cleaned using a soak for 30 minutes in ACN between two runs.

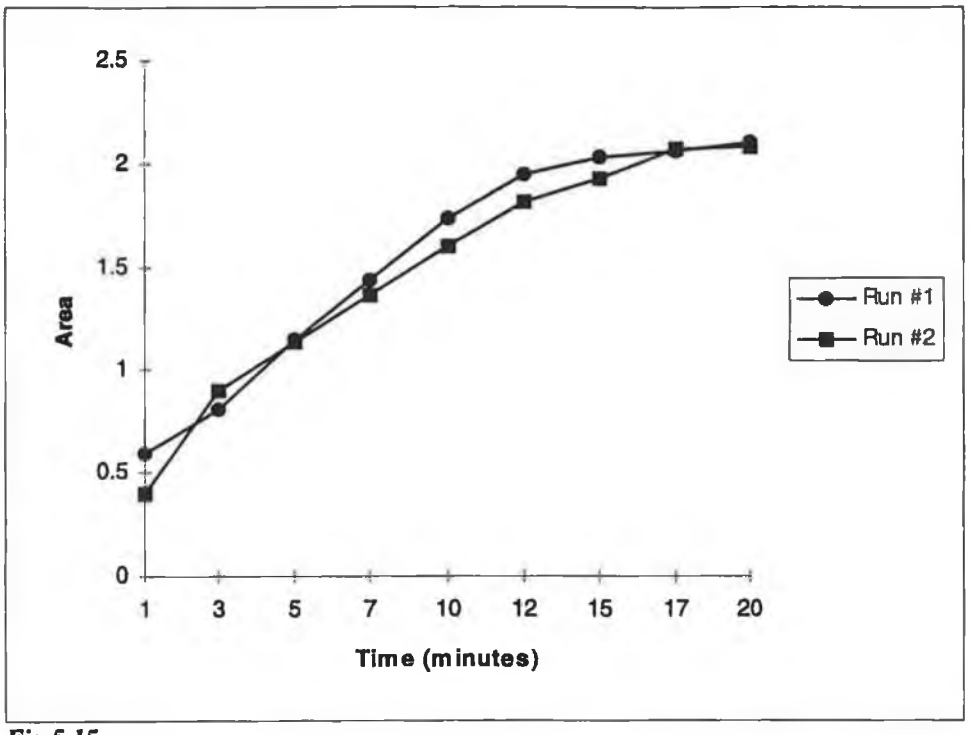

Fig 5.15

Plot of peak areas versus time (minutes) for the peak integrated between 770 – 790cm^3 recorded when a 100ppm fluoranthene solution was enriched onto the 5w/v % PIB polymer coating, which had been coated using a spin speed of 800rpm. Areas were recorded over a period of 20 minutes and the coating was then cleaned using a soak for 30 minutes in ACN between two runs.

600 rpm

The coating procedure was then repeated using a spin speed of 600rpm (see table 5.12and fig 5.16).

Time (minutes) / Area	Run #1	Run #2	Average
1.00	0.2519	0.1674	0.2097
3.00	0.6006	0.5031	0.5519
5.00	0.9338	0.8402	0.8870
7.00	1.2498	1.1785	1.2142
10.00	1.7055	1.5966	1.6511
12.00	1.9432	1.8703	1.9068
15.00	2.2576	2.1762	2.2169
17.00	2.3958	2.3105	2.3532
20.00	2.5677	2.5279	2.5478

Table 5.12

Table of peak areas for the peak integrated between 770 – 790cm^3 recorded when a 100ppm fluoranthene solution was enriched onto the 5w/v % PIB polymer coating, which had been coated using a spin speed of 600rpm. Areas were recorded over a period of 20 minutes and the coating was then cleaned using a soak for 30 minutes in ACN between two runs.

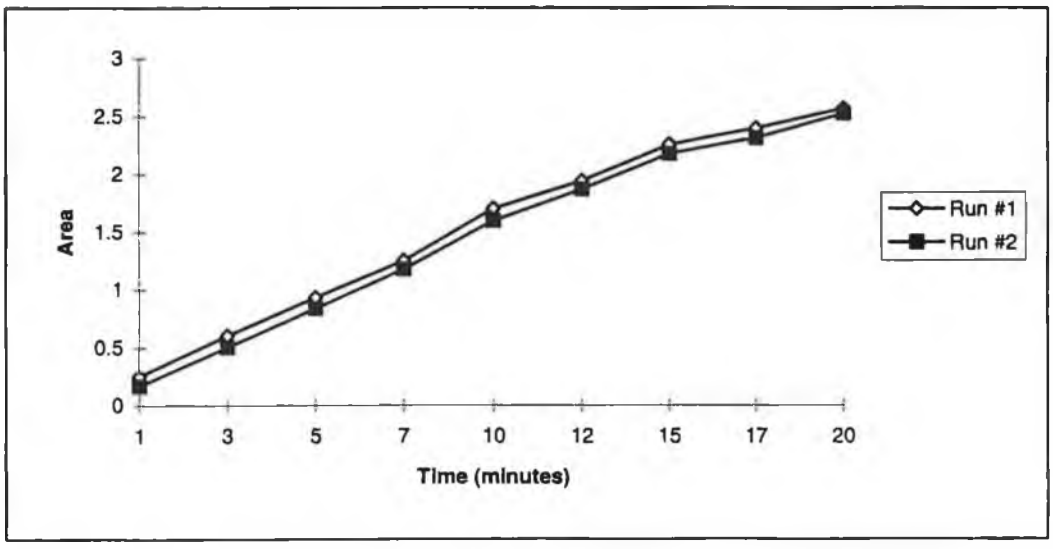

Fig 5.16

Plot of peak areas versus time (minutes) for the peak integrated between 770 – 790cm^3 recorded when a 100ppm fluoranthene solution was enriched onto the 5w/v % PIB polymer coating, which had been coated using a spin speed of 600rpm. Areas were recorded over a period of 20 minutes and the coating was then cleaned using a soak for 30 minutes in ACN between two runs.

It was concluded that the optimum speed for spin coating the ZnSe crystal for the 100ppm fluoranthene standard was 600rpm.

600rpm was chosen because the response for fluoranthene was greater than for the 800rpm coating (see fig 5.15 and 5.16).

A slower speed of 500rpm was also investigated but it was obvious from a visual examination of the crystal that it did not give a uniform coat.

The 600rpm coating procedure was repeated two more times (see tables 5.13, 5.14 and fig 5.17, 5.18).

Time (minutes) / Area	Run #1	Run #2	Average
1.00	0.1990	0.2010	0.2000
3.00	0.5761	0.4960	0.5361
5.00	1.0077	0.8701	0.9389
7.00	1.4232	1.2183	1.3208
10.00	1.8836	1.6826	1.7831
12.00	2.1374	1.9917	2.0646
15.00	2.3166	2.3194	2.3180
17.00	2.4743	2.4520	2.4632
20.00	2.5371	2.6162	2.5767

Table 5.13

Table of peak areas for the peak integrated between 770 – 790cm^3 recorded when a 100ppm fluoranthene solution was enriched onto the 5w/v % PIB polymer coating, which had been coated using a spin speed of 600rpm for the second time. Areas were recorded over a period of 20 minutes and the coating was then cleaned using a soak for 30 minutes in ACN between two runs.

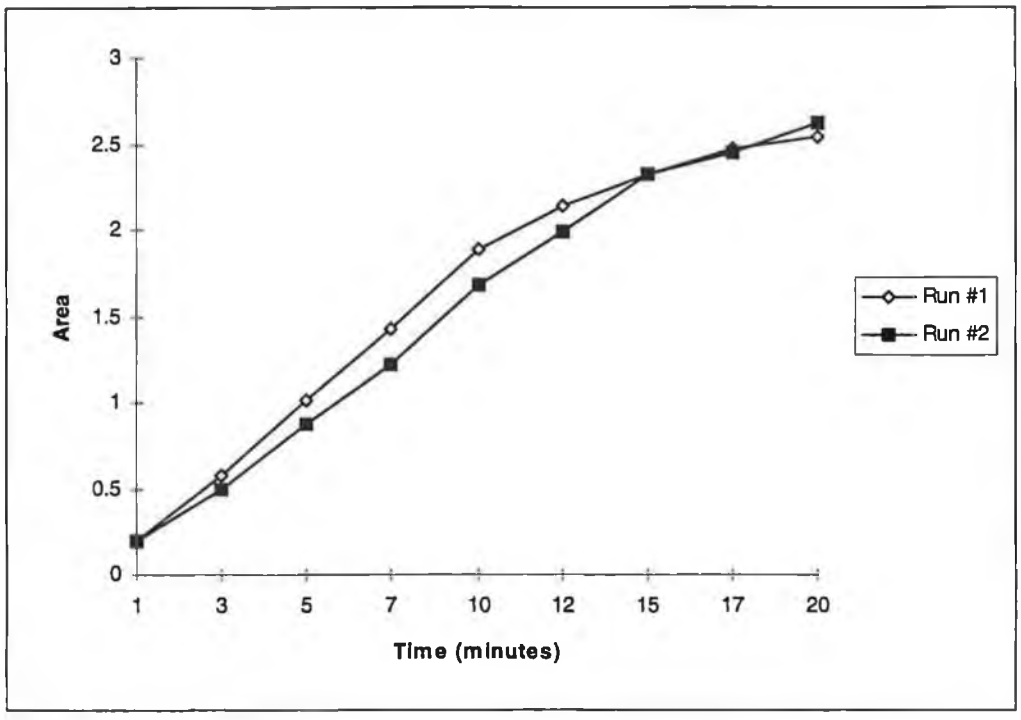

Fig. 5.17

Plot of peak areas versus time (minutes)for the peak integrated between 770 – 790cm^3 recorded when a 100ppm fluoranthene solution was enriched onto the 5w/v % PIB polymer coating, which had been coated using a spin speed of 600rpm for the second time. Areas were recorded over a period of 20 minutes and the coating was then cleaned using a soak for 30 minutes in ACN between two runs.

Time (minutes) / Area	Run #1	Run #2	Average
1.00	0.3446	0.4305	0.3876
3.00	0.7414	0.7125	0.7270
5.00	1.1161	0.9731	1.0446
7.00	1.3992	1.3320	1.3656
10.00	1.6977	1.5909	1.6443
12.00	1.8678	1.8770	1.8724
15.00	1.9767	2.0352	2.0060
17.00	2.1431	2.2122	2.1777
20.00	2.3552	2.3639	2.3596

Table 5.14

Table of peak areas for the peak integrated between 770 – 790cm^3 recorded when a 100ppm fluoranthene solution was enriched onto the 5w/v % PIB polymer coating, which had been coated using a spin speed of 600rpm for the third time. Areas were recorded over a period of 20 minutes and the coating was then cleaned using a soak for 30 minutes in ACN between two runs.

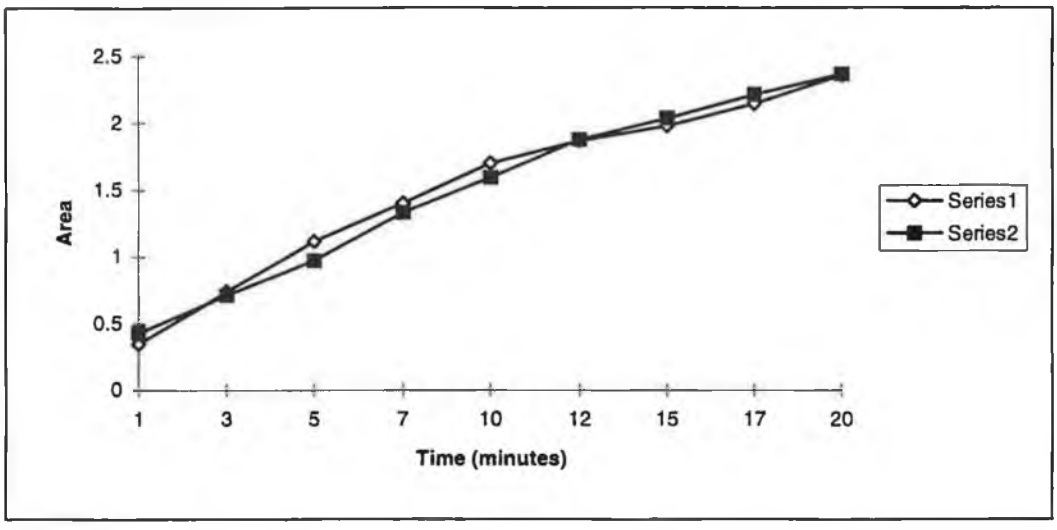

Fig 5.18

Plot of peak areas versus time (minutes) for the peak integrated between 770 – 790cm^3 recorded when a 100ppm fluoranthene solution was enriched onto the 5w/v % PIB polymer coating, which had been coated using a spin speed of 600rpm for the third time. Areas were recorded over a period of 20 minutes and the coating was then cleaned using a soak for 30 minutes in ACN between two runs.

From these plots the averages were calculated for the runs carried out on the separate coatings. A plot was constructed of these averages and the standard deviations at each point calculated to assess how consistent the spin coating technique was and compare it to the spread coating technique (see table 5.15 and fig 5.19).

For the reuse of individual coatings the spread technique lead to much more consistent results this may possibly be due to the fact that the coating on the crystal was slightly thicker and was therefore less likely to give problems due to interferences from water. The spread technique also requires much less 5% PIB solution, as only $0.2cm^3$ are required to coat the crystal whereas the spin technique requires $5cm^3$.

When the spin coating technique is compared overall with the spread technique in terms of reproducibility (see fig 5.14 and 5.18), the spin technique is a more consistent procedure with standard deviations for the result of the 100ppm fluoranthene loaded for 20 minutes on three separate coatings being 11.79% compared to 63.65% for the spread technique.

Time (minutes) / Area	Spin#1	Spin #2	Spin #3	Std. Deviation
1.00	0.2097	0.2000	0.3876	0.1056
3.00	0.5519	0.5361	0.7270	0.1060
5.00	0.8870	0.9389	1.0446	0.0803
7.00	1.2142	1.3208	1.3656	0.0778
10.00	1.6511	1.7831	1.6443	0.0782
12.00	1.9068	2.0646	1.8724	0.1025
15.00	2.2169	2.3180	2.0060	0.1592
17.00	2.3532	2.4632	2.1777	0.1440
20.00	2.5478	2.5767	2.3596	0.1179

Table 5.15

Table of average peak areas for 100ppm fluoranthene loaded onto a 5w/v % PIB coating using three different spin coatings that were coated at 600rpm.

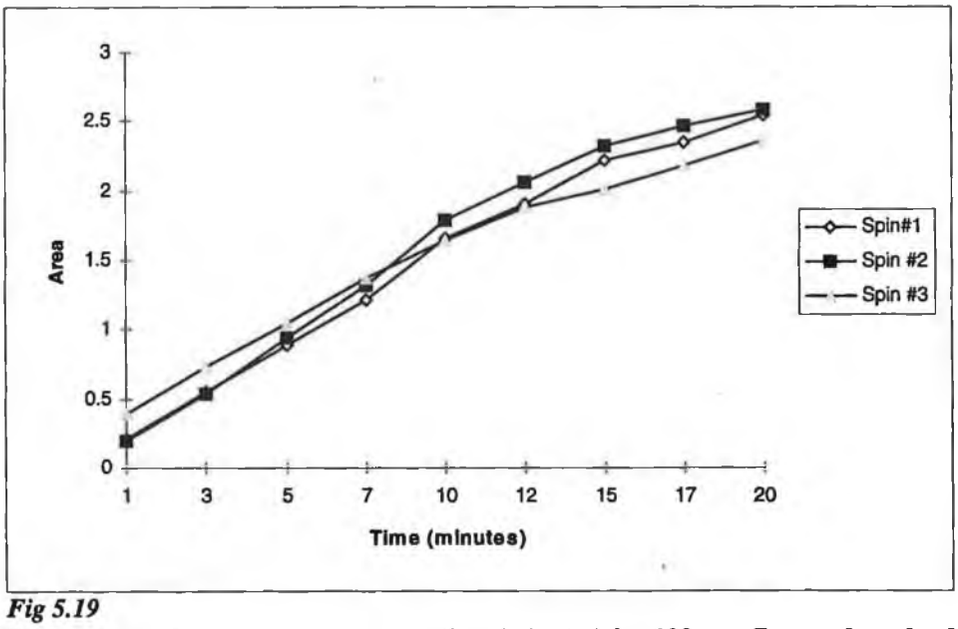

Fig 5.19

Plot of the peak area averages versus time (minutes) for 100ppm fluoranthene loaded onto a 5w/v % PIB coating using three different spin coatings which were coated at a spin speed of 600rpm.

5.3.6. Linearity Study

It had been established that coating the crystal with a polymer lead to a system which could be constructed to give an enhanced response for fluoranthene in a reasonably consistent fashion. The next thing that needed to be investigated was how linear the response of the analyte was in terms of concentration and time for extraction. A series of eight standards were prepared in 90/10 ACN/H_2O ranging in concentration from 10 to 100ppm. These standards were each enriched onto the coated ATR crystal and spectra were recorded at various times (see table 5.16 and fig 5.20).

Fluoranthene Conc. (ppm)/ Time (Minutes)	**10.78**	**21.56**	**32.34**	**43.12**	**53.90**	**64.68**	**75.46**	**107.8**
1.00	0.0021	0.0360	0.0626	0.0515	0.1086	0.1919	0.1683	0.1427
3.00	0.0371	0.1051	0.1414	0.2900	0.3240	0.6395	0.4717	0.4206
5.00	0.0916	0.1650	0.2571	0.4771	0.5798	0.9113	0.8242	0.8610
7.00	0.1407	0.3511	0.3968	0.6271	0.7710	1.2189	1.1007	1.2514
10.00	0.2168	0.4892	0.5913	0.9029	1.1975	1.6510	1.6651	1.7528
12.00	0.2614	0.6141	0.7450	1.1537	1.4589	1.8679	1.9765	2.0249
15.00	0.3783	n/a	1.0348	1.4286	1.8149	2.1124	2.2186	2.2740
17.00	0.4083	0.8649	1.1567	1.5705	1.9102	2.1658	2.3237	2.3967
20.00	0.5199	0.9979	1.3309	1.7526	2.1362	2.2371	2.4204	2.4555

Table 5.16

Table of peak areas for a range of concentrations of fluoranthene enriched onto a 5 % PIB coating which had been coated using the spin coating technique. The area results quoted were measured by integrating the enrichment spectra between 770 and 790 cm^{-1}.

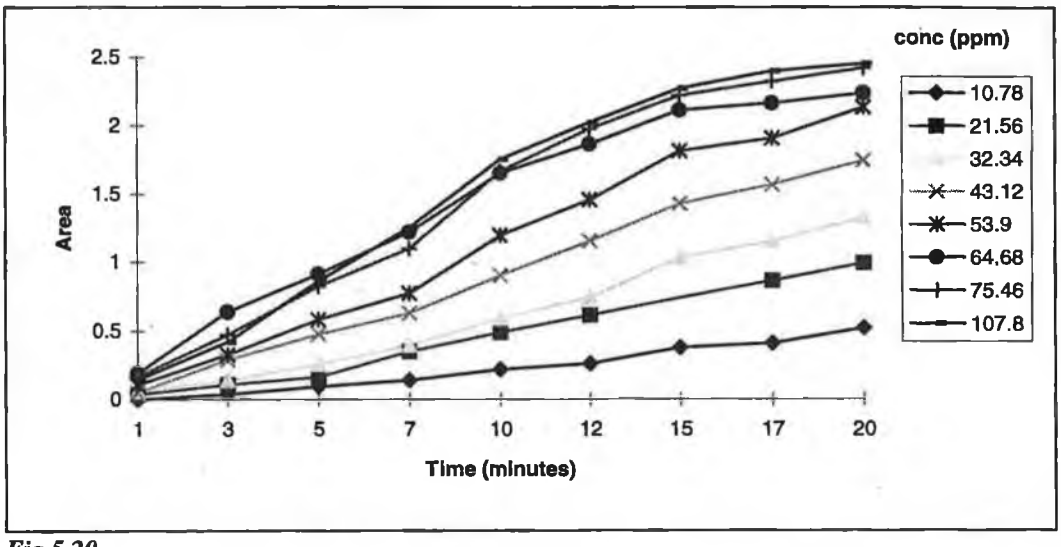

Fig 5.20

Plot of peak area versus time (minutes) for different concentrations of fluoranthene, ranging from 10 to 108ppm, enriched on a coating of PIB at 5% v/v in decalin using a spin speed of 600 rpm

From this data set two types of correlation were examined.

The first was the correlation of concentration and enrichment over the 20 minute time period (see table 5.17 and fig 5.21).

This demonstrates that at lower concentration enrichment is occurring in a linear fashion but at higher concentrations, above 50ppm saturation is approached before 20minutes is reached and no more analyte can be extracted into this region.

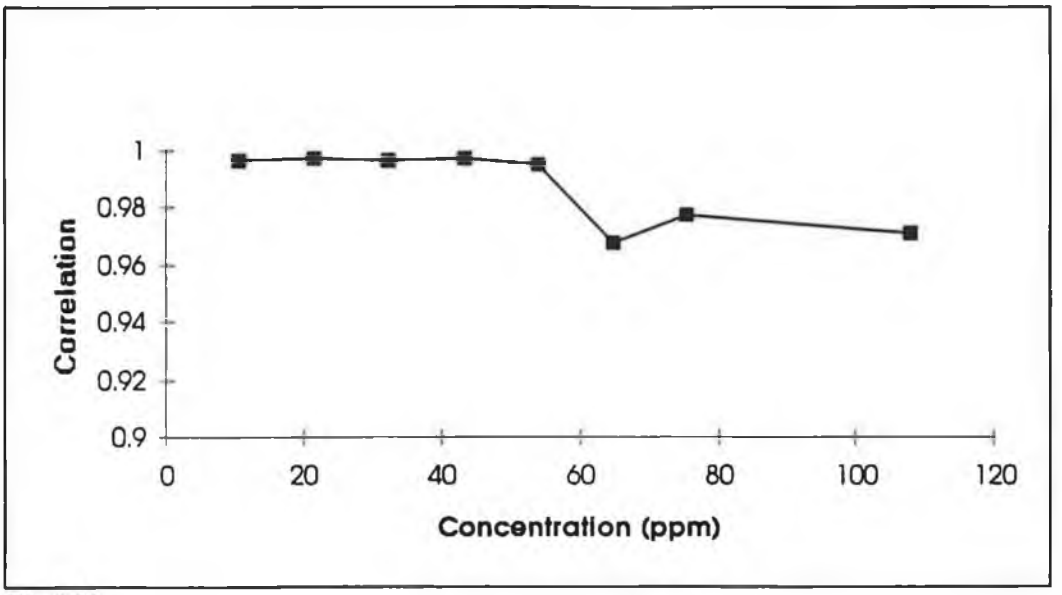

Fig 5.21

Plot of the correlation results for the enrichment of different concentrations of fluoranthene onto a 5 w/v % PIB coating over a 20 minute time period.

Concentration of fluoranthene(ppm)	Correlation for enrichment time of 20 minutes
10.78	0.9967
21.56	0.9969
32.34	0.9967
43.12	0.9974
53.90	0.9950
64.68	0.9679
75.46	0.9775
107.80	0.9709

Table 5.17

The correlation coefficients for the enrichment of the various concentrations of fluoranthene over the 20 minute time period.

The second type of correlation examined was the correlation between time and enrichment for the various concentrations examined (see table 5.18 and fig 5.22).

This demonstrates that a minimum time period is required before a linear response is achieved i.e. before a plot of peak area versus concentration will give a linear response. The minimum enrichment period was deemed to be 7 minutes, this was probably due to small peak sizes at early enrichment times.

Time	Correlation
1.00	0.8225
3.00	0.7812
5.00	0.9086
7.00	0.9352
10.00	0.9390
12.00	0.9380
15.00	0.9168
17.00	0.9316
20.00	0.9127

Table 5.18

Table of the correlation coefficients for the concentration range of fluoranthene at specific times during the enrichment process.

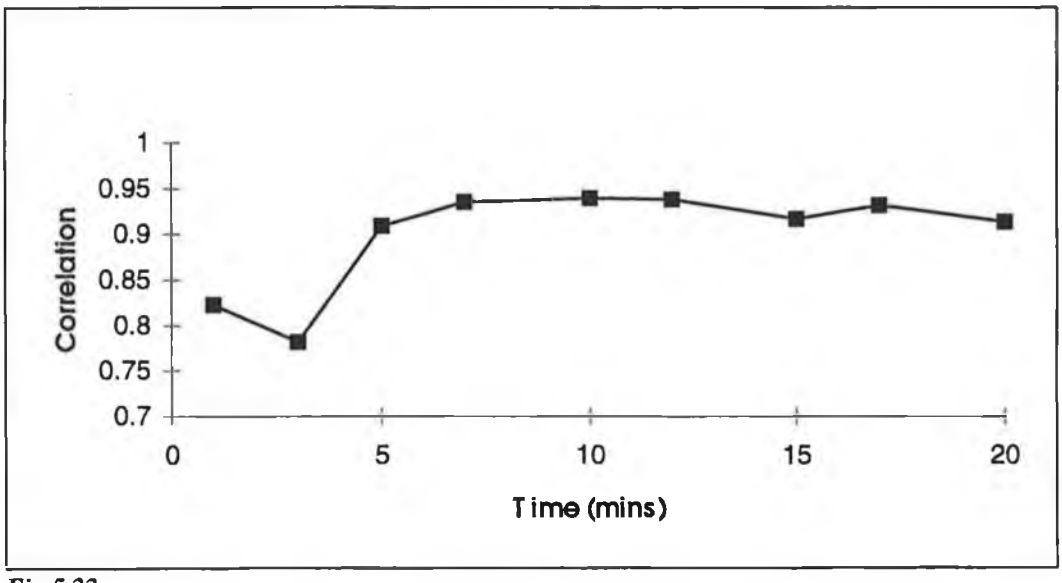

Fig 5.22

Plot of correlation of the peak areas for the range of concentrations of fluoranthene at specified times during the enrichment process.

5.3.7 Limit of Detection

To assess the viability of this coated ATR/FTIR system it was necessary to calculate the minimum level that could be detected. This was done using two procedures. The first was what was termed a static system whereby $2cm^3$ of sample was loaded onto the ATR/FTIR trough and the area recorded when it reached a maximum. The second was a dynamic system whereby a solution of standard was allowed flow over the trough, using a peristaltic pump, at a very slow flow rate $0.5cm^3$/minute and again areas where measured until a maximum was reached.

5.3.7.1. Static System

Initially 100ppb fluoranthene standard was placed on an open trough sitting in the IR beam. This proved to be unsuccessful because the standard was prepared in a 90/10 H_2O/ACN this resulted in a considerable amount of evaporation. This was then repeated with a glass seal placed above the trough, This was left for 24 hours and again nothing was detected. This was then repeated with both a 500ppb and 1ppm standard and left for 24 hours nothing was detected in either case. The extraction procedure is based on an equilibrium across the interface between the membrane and the aqueous sample. It appears in this static mode at low concentrations the equilibrium is reached below the limit of detection. The limit of detection for fluoranthene using the specified 5% PIB membrane is approximately 10ppm (see table 5.16).

5.3.7.2. Dynamic System

A series of standards were prepared 1ppm, 500ppb and 200ppb in 1000cm^3 solutions in 90/10 H_2O/ACN and left flow at 0.5cm^3/minutes over the coated crystal for a specified amount of time. The peak areas were recorded at 1, 10, 15 and 24 hours (see table 5.19 and fig 5.23). A blank of H_2O/ACN 90/10 was run for 24 hours to see if anything interfered at the region of the spectrum of interest. Nothing significant was seen. Noise was measured at 0.0024 area units.

Time (hrs)/ Concentration (ppm)	1 ppm	500 ppb	200 ppb
1	0.0084	0.0007	0.0005
10	0.0320	0.0060	0.0009
15	0.1535	0.0235	0.0010
24	0.1659	0.0883	0.0083

Table 5.19
Table of peak areas for fluoranthene loaded at different concentrations at a flow rate of 0.5cm^3/min onto a 5w/v % PIB coated crystal for various times ranging from 1 to 24 hours.

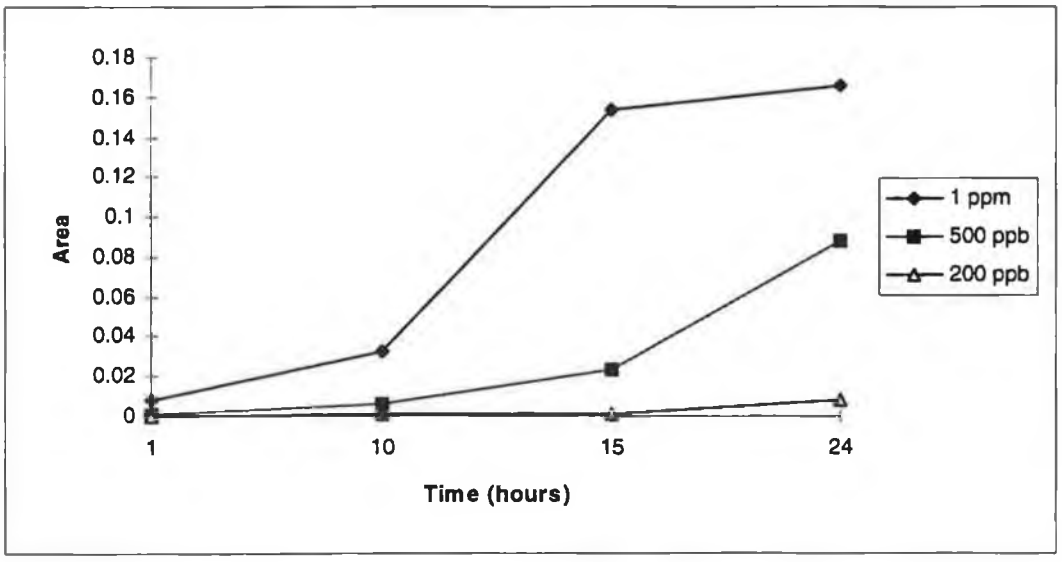

Fig 5.23

Plot of area of fluoranthene peak area versus time (hours) for fluoranthene loaded at different concentrations at a flow rate of 0.5cm^3 onto a 5w/v % PIB coated crystal for various times ranging from 1 to 24 hours.

This was also attempted at 100ppb but nothing above noise was detected after 24 hours. Therefore it was concluded that a limit of detection for fluoranthene extracted onto the surface of this particular coating was 200ppb, this was achieved by loading a solution at 0.5cm^3 /min for 24 hours.

5.4. Conclusions

The possibility of measuring polycyclic aromatic hydrocarbons in the low ppm range was demonstrated using a polymer coated ATR FTIR. The results show that the nature and concentration of the polymer coating have significant effects on the ability of the polymer to extract the analyte. Polyisobutylene, medium weight, was chosen as the most suitable

polymer. 5%w/v PIB in decalin solution was concluded to be the most suitable coating solution.

The type of coating technique used also has a significant effect on the reproducibility of the sample loading. Spin coating was chosen as the preferred technique even though the spread technique had certain advantages. The spin coating gave more consistent results than the spread technique.

The ability of the 5% PIB coating to be reused numerous times was also shown and an optimum wash protocol between uses was devised.

It was concluded from the construction of a concentration versus peak area data set that at lower concentrations enrichment is occurring in a linear fashion but at higher concentrations, above 50ppm saturation is approached before 20minutes is reached and no more analyte can be extracted into this region. It was also concluded that a minimum enrichment period of 7 minutes was required before peak areas could be measured in such away as to allow the construction of a linear calibration curve.

The static L.O.D. was measured to be approximately 10ppm for fluoranthene. A dynamic system was developed which reduced this significantly to 200ppb.

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Chapter 6.0

An Evaluation of Polymer Coated ATR/FTIR as a Technique For Analysing Four PAHs Simultaneously in Aqueous Solutions.

6.1. Introduction.

The study of PAHs often involves the use of complex extractive procedures followed by difficult chromatographic separations. These analytical procedures may require, for example, gradient, HPLC with both UV and FL detectors or GC-FID/MS.

Techniques based on IR spectroscopy are being seen as a viable alternative to these techniques. These techniques have the ability to analyse simultaneously a number of analytes. This is based on the fact that IR spectroscopy gives both qualitative and quantitative information about a sample.

The development of polymer coated ATR FTIR or FEWS has meant that it is now possible to analyse certain types of samples without necessarily using complex and laborious extractive preparative procedures [1]. It is has been shown that a selective extractive process occurs at the interface of the sample and the polymer interface. This selective enrichment then allows the analytes of interest to quantitated.

Much of the work carried out in the field of quantitative ATR-IR has been based on the examination of CHCs in the aqueous environment [1,2].

Recently a coated fiber optic based system was used to quantitate TCE and 1,1,1 – trichloroethane and toluene in aqueous solutions in the concentration range 20 to 300 ppm [3]. In this case the FOCS in the near IR region were used and quantitation was achieved through the application of linear chemometric algorithms.

Quantitative ATR MIR based systems offer certain advantages over the NIR systems in that examination of the region from 1000 cm^{-1} and 600 cm^{-1} can lead to the assigning of individual peaks to compounds thus allowing quantitation without the need for complex

multilinear techniques. These bands with few exceptions are due to fundamental vibrations of a molecule being investigated. The intensity of an absorption band decreases by a factor of 10 – 100 in going from the fundamental to the first overtone [4].

The sensitivity of this technique has greatly improved with the use of tuneable diode laser sources and of fiber based waveguides [5].

The aim of this project was to investigate the use of a coated ATR FTIR, which had been developed previously (see Chapter 5) as a method for the analysis of four PAHs simultaneously. The coated ATR FTIR system was applied to the analysis of naphthalene, acenaphthene, fluorene and phenanthrene. These compounds were chosen as they are the lower MW PAHs and thus have been shown to be difficult to extract quantitatively using standard extractive techniques.

The compounds were first examined individually and their enrichment profiles examined; they were then examined simultaneously in various solutions.

6.2. Experimental

6.2.1. Chemicals

The chemicals used are as defined in section 5.2.1.1.

6.2.2. Materials

The polymer coating, which was selected in chapter 5, was used for all experiments in this section. This was a 5w/v% solution of PIB in decalin coated onto a ZnSe crystal using a spin coating technique at a spin speed of 600 rpm.

6.2.3. Instrumentation

The instrumentation used is defined in section 5.2.1.2.

6.2.4. Methods

6.2.4.1. The sample loading technique

The sample loading technique optimised in chapter 5 was used here.

$2cm^3$ of background solution, in this case 70/30 H_2O/ACN, were allowed to equilibrate on the surface of the ZnSe crystal coated with 5w/v% PIB in decalin using a spin speed of 600rpm. The solution was allowed equilibrate for 50 minutes and the spectrum recorded. The spectrum was recorded by co-adding four scans between 4000 and 600 cm^{-1} at a resolution of 1 cm^{-1}. This was then taken to be the background spectrum. This solution was then removed and $2cm^3$ of the sample of interest added to the sample trough. Spectra were collected in the same way as the background spectrum. Spectra were collected periodically over a twenty-minute period.

6.2.4.2. The spectrum generation technique.

The absorbance spectra were calculated using the GramsAnalyst software on the PE2000 instrument. This was achieved by subtracting the spectrum for the background from the sample spectrum and integrating the peak or peaks of interest. The details of how the integration was achieved are discussed in detail in section 6.3.2.

A plot of area versus time was then constructed and the quantitative ability of the system was then assessed.

6.2.4.3. Spiking standard and sample preparation for simultaneous analysis.

Stock solutions of the four PAHs, naphthalene, acenaphthene, fluorene and phenanthrene, were prepared in ACN at a concentration of 4 mgs/cm^3. From this a 50ppm mixed solution was prepared which contained all four PAHs. This was done by taking 1.25cm^3 of each stock solution adding 25cm^3 of ACN and diluting this to 100cm^3. The 100ppm spiked individual solutions were prepared by taking 0.125cm^3 of a stock solution and diluting this to 10cm^3 with the 50ppm mixed solution. The 5ppm and 10ppm spiked solutions were prepared by diluting the above solutions 1 in 10cm^3 with 70/30 H_2O/ACN.

6.3. Results and Discussion

6.3.1. The effects of ACN / H_2O Composition

The initial idea was to enrich the four PAHs naphthalene, acenaphthene, fluorene and phenanthrene from purely aqueous solution. Due to the fact that these compounds are not miscible with water it was decided to introduce ACN to keep the compounds of interest in solution. At the rather high concentrations examined in this study, 5 –100ppm, it was necessary to add up to 30% ACN to keep the compounds in solution. The presence of such a large amount of ACN in the aqueous solution will obviously hinder an extractive process that is based on non-polar interactions. A comparison of an enrichment of naphthalene into the 5w/v% PIB coated waveguide in two separate solutions was made, one solution with the minimum amount of ACN needed to keep naphthalene in solution, 5% ACN and another using 30% ACN. The 30% ACN solution was found to be the minimum amount needed to keep all four PAHs in solution at a concentration of 100ppm (see table

6.1 and fig 6.1). The results were generated and integrated by using manual adjustment for the start and end points of the peak. Integration will be discussed in detail in section 6.3.2.

Time (mins) / Solvent Composition (%)	70/30 H_2O/ACN	95/05 H_2O/ACN
1	0.2048	0.8325
3	0.5284	1.6492
5	0.7336	1.9989
7	0.9349	2.1557
10	1.1854	2.2729
12	1.2977	2.2794
15	1.4329	2.2826
17	1.4706	2.2344
20	1.5506	2.1969

Table 6.1
Table of peak areas recorded when 100ppm naphthalene was enriched onto a 5w/v% PIB coated ZnSe crystal. Naphthalene was enriched from two separate solutions 70/30 H_2O/ACN and 95/5 H_2O/ACN.

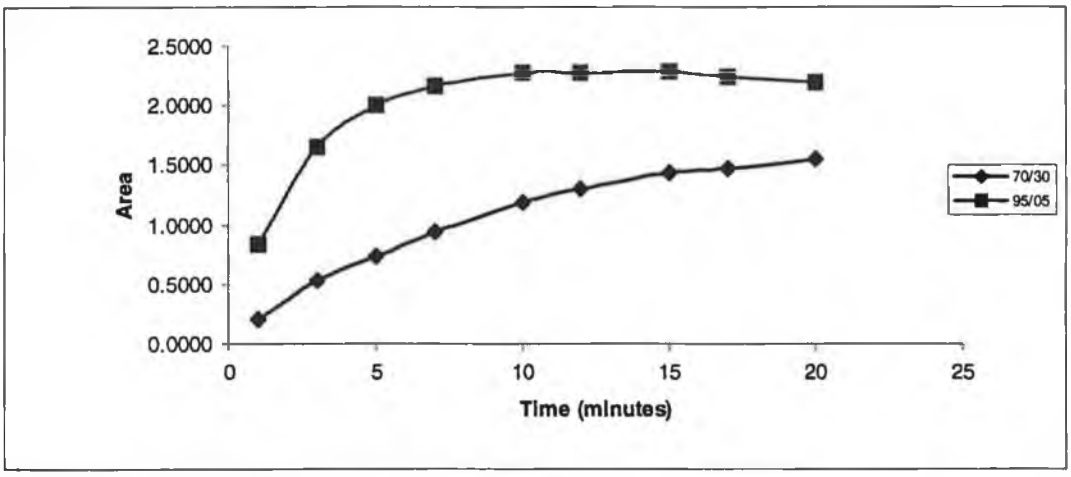

Fig 6.1
Plot of peak area versus time (minutes) for peak areas recorded when 100ppm naphthalene was enriched onto a 5w/v% PIB coated ZnSe crystal. Naphthalene was enriched from two separate solutions 70/30 H_2O/ACN and 95/5 H_2O/ACN.

From an examination of these plots it is clear that the amount of ACN has a significant effect on the enrichment profile. The 95/05 H_2O/ACN solution allowed the naphthalene enrich onto the polymer surface at a faster rate than the 70/30 H_2O/ACN solution and thus

the peak area maximum was much higher and an equilibrium was reached in a much shorter time. However as mentioned previously a solution composition of 70/30 H_2O/ACN was chosen as it was necessary to keep the four PAHs in solution at this concentration.

These profiles indicate that if the sensitivity of the system could be improved in the future, and thus allow lower percentages of ACN to be used, or if the system was used to investigate gaseous samples then enrichment could take place at a faster rate.

6.3.2. Integration Techniques using 100ppm Naphthalene.

A number of ways of integrating the absorbance peak of interest were investigated. It was believed that this integration would have an effect on the ability of the system to relate peak area to concentration. This is critical in assessing the ability of the system to quantitate the various compounds investigated. The initial study was conducted using naphthalene as it gave only one peak in the region of interest, 1000 cm^{-1} to 600 cm^{-1} and the response of this peak is quite large. It was therefore considered one of the most suitable peaks to study (see fig 6.2).

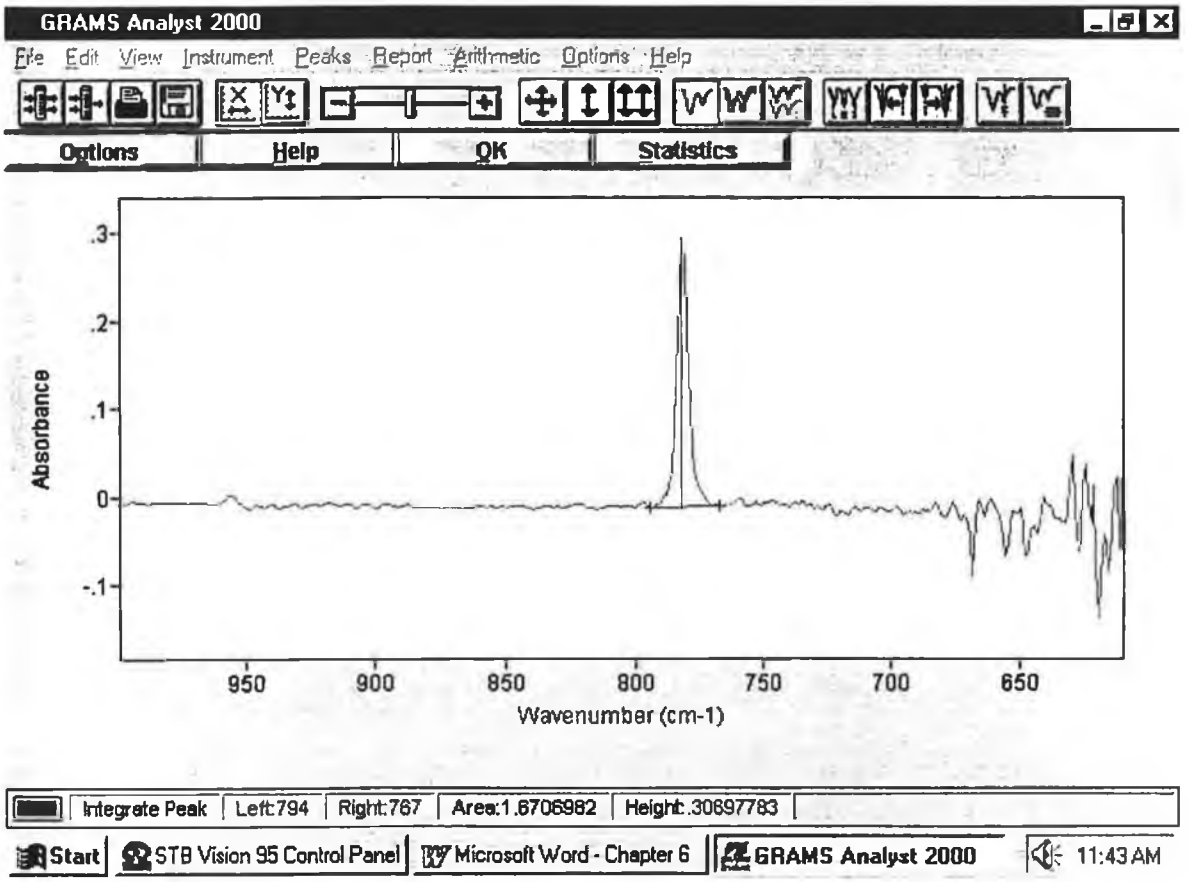

Fig 6.2

The diagram shows the absorbance spectrum for naphthalene between 600cm^{-1} and 1000cm^{-1} with the peak at 783cm^{-1} integrated. 100ppm naphthalene was enriched from a 70/30 H_2O/ACN solution that was placed on a 5w/v% PIB coated ATR crystal.

A number of standards were prepared for naphthalene ranging in concentration from 10 to 100ppm in 70/30 H_2O/ACN and they were then loaded onto the coated crystal and the spectra recorded as defined in section 6.2.4. This data was then subjected to a number of processes.

6.3.2.1. Manual Integration

The first method of integrating these results involved subtracting the background spectra to give the absorbance spectrum and manually selecting the start and end of the peak of interest. Table 6.2 lists the peak areas generated by this method.

Conc. (ppm)/ Time (minutes)	9.86	29.58	49.3	69.02	98.02	Correlation
1	0.0227	0.0722	0.1028	0.1357	0.2048	0.996350
3	0.0383	0.1496	0.2839	0.3608	0.5284	0.997781
5	0.0709	0.2182	0.3548	0.5231	0.7336	0.999625
7	0.0827	0.2349	0.4789	0.6810	0.9349	0.997793
10	0.1079	0.3148	0.5886	0.7758	1.1854	0.998167
12	0.1236	0.3584	0.6591	0.9629	1.2977	0.994500
15	0.1234	0.3954	0.7127	1.0729	1.4329	0.990889
17	0.1106	0.4221	0.7680	1.0939	1.4706	0.998260
20	0.1202	0.3916	0.7994	1.1486	1.5506	0.997384

Table 6.2
Table of peak areas recorded when various concentrations of naphthalene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. Manual integration was carried out for each peak and the correlation coefficients were calculated at the various enrichment times.

From this data a plot was constructed which demonstrates the enrichment profile for naphthalene using the manual peak integration technique (see fig 6.3).

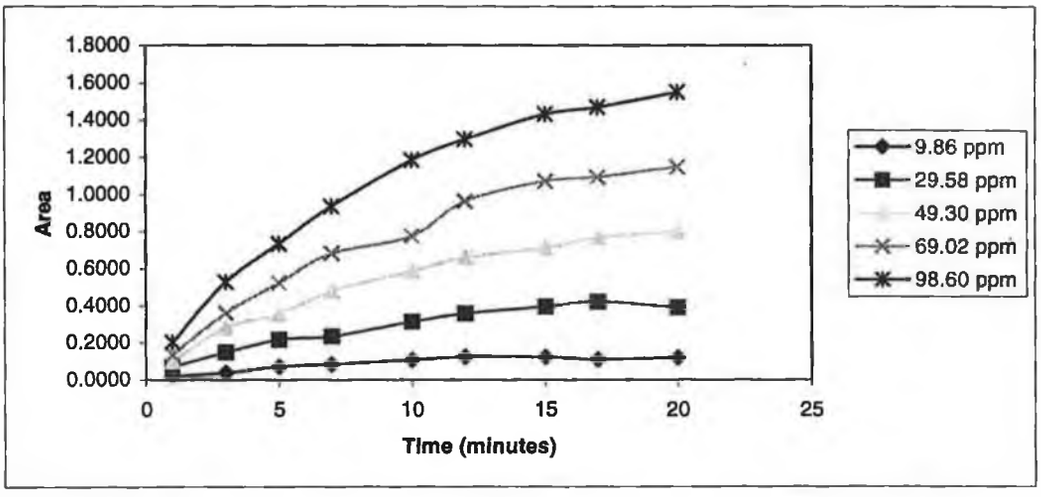

Fig 6.3

Plot of peak area versus time (miniutes) for the peak areas recorded when various concentrations of naphthalene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. Manual integration was carried out for each peak.

From this plot it was concluded that naphthalene was enriched into the polymer layer in a linear fashion and that it would be possible to use these results as a working standard curve for the concentration range of 10 to 100ppm.

6.3.2.2. Fixed Wavelength Integration

The second type of integration investigated was termed fixed wavelength integration. In this case the integration was carried out as described in section 6.3.2.1. except the start and end wavelengths for each peak were predetermined. The wavelengths chosen were such that the largest peak would integrate properly. That is they were set so as the naphthalene peak at 730cm^{-1} for the 100 ppm standard enriched for 20 minutes was integrated. Therefore the start and end wavelengths were chosen to be 770 and 794 cm^{-1}. The same data used in section 6.3.2.1 was then reintegrated using the above procedure (see table 6.3).

Conc. (ppm)/ Time (minutes)	9.86	29.58	49.3	69.02	98.02	Correlation
1	0.1040	0.1299	0.1254	0.1802	0.2594	0.942813
3	0.0963	0.2425	0.3038	0.3546	0.5368	0.985131
5	0.1248	0.3369	0.4286	0.6064	0.7451	0.989422
7	0.1391	0.3808	0.5271	0.7436	0.9411	0.994654
10	0.1418	0.4398	0.6602	0.8516	1.1809	0.997194
12	0.1787	0.4844	0.7040	0.9778	1.3043	0.998409
15	0.2184	0.5278	0.7947	1.0867	1.4329	0.998727
17	0.1887	0.5817	0.8024	1.0778	1.5030	0.996890
20	0.1666	0.5435	0.8904	1.1967	1.6690	0.999209

Table 6.3

Table of peak areas recorded when various concentrations of naphthalene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. Integration was carried out for each peak over a fixed wavelength period of between 770 and 794cm^{-1}. The correlation coefficients were calculated at the various enrichment times.

From this data a plot was constructed which demonstrates the enrichment profile for naphthalene using the fixed wavelength integration technique (see fig 6.4).

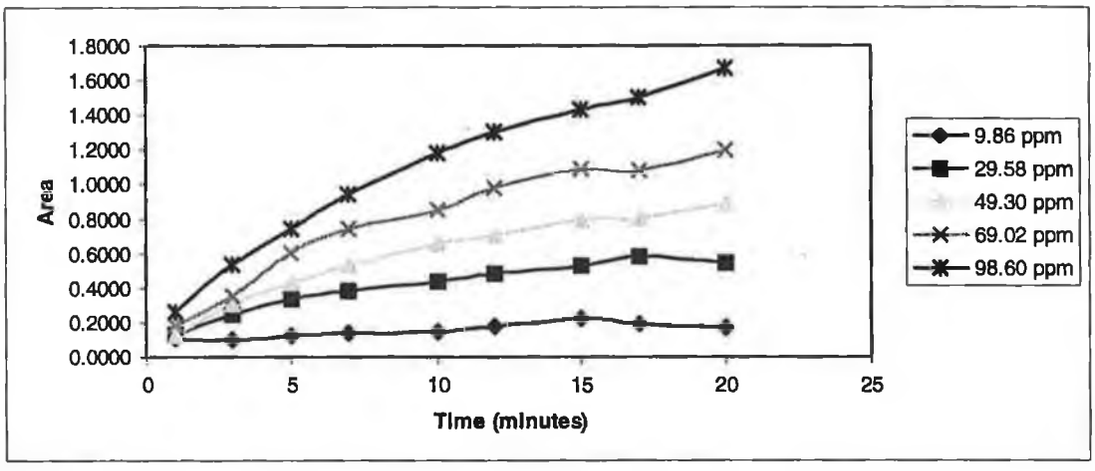

Fig 6.4

Plot of peak area versus time (minutes) for the peak areas recorded when various concentrations of naphthalene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. Integration was carried out for each peak over a fixed wavelength period of between 770 and 794cm^{-1}.

It was concluded that this integration technique would also be acceptable to use for the construction of a working calibration curve from this data. A workable standard curve was defined as one with a correlation coefficient above 0.990 calculated for the range of

standards at a certain time. In this case a minimum enrichment time of 7 minutes was required before a workable standard curve could be constructed. The reason for this appears to be because of errors introduced into the system by using a fixed wavelength period for integration of the smaller peaks at early times.

6.3.2.3. The first derivative technique.

The third type of data manipulation investigated was the use of the first derivative spectrum. Derivative spectroscopy provides a means for presenting spectral data in a potentially more useful form than the normal data [6].

This technique is commonly used in quantitative spectroscopy as it can allow for unresolved peaks to be integrated and it can negate any shifts in baseline as it emphasizes changes in slope. The method used to compute the derivative in the GramsAnalyst software is the quadratic polynomial suggested by Savitzky and Golay[7]. Using the polynomial and a five point moving window, the first derivative is given by the equation 6.1.

$$dy/d\lambda = [1/10\,\Delta\lambda]\,[-2y_{i-2} - y_{i-1} + y_{i+1} + 2y_{i+2}] \quad \textbf{Eq 6.1}$$

The absorbance spectrums are generated as outlined in section 6.2.4.2. This spectrum is then differentiated using the polynomial outlined above (see Eq. 6.1). The height of the peak was then noted (see fig 6.5 and table 6.4).

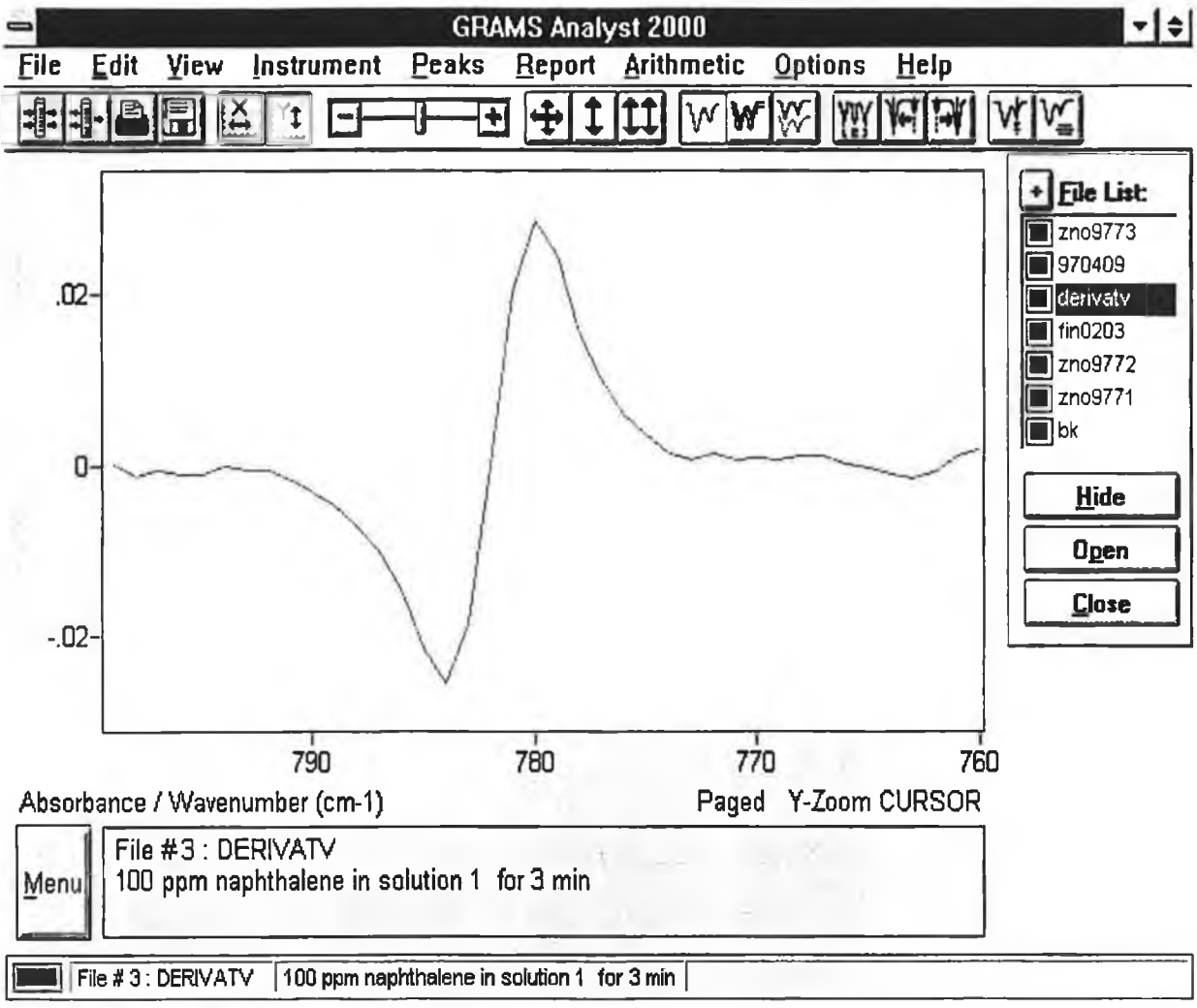

Fig 6.5

The diagram shows the first derivative spectrum calculated for naphthalene between 760cm^{-1} and 800cm^{-1}. 100ppm naphthalene was enriched from a 70/30 H_2O/ACN solution that was placed on a 5w/v% PIB coated ATR crystal for 3 minutes.

Conc. (ppm)/ Time (minutes)	9.86	29.58	49.3	69.02	98.02	Correlation
1	0.0052	0.0120	0.0252	0.0316	0.0141	0.48388
3	0.0064	0.0195	0.0366	0.0166	0.0461	0.77636
5	0.0108	0.0244	0.0387	0.0479	0.0652	0.99757
7	0.0176	0.0289	0.0443	0.0618	0.0792	0.99745
10	0.0148	0.0376	0.0530	0.0678	0.0995	0.99693
12	0.0152	0.0374	0.0596	0.0761	0.1069	0.99898
15	0.0161	0.0451	0.0678	0.0932	0.1208	0.99685
17	0.0156	0.0451	0.0752	0.0967	0.1293	0.99653
20	0.0182	0.0458	0.0732	0.1008	0.1373	0.99970

Table 6.4

Table of peak height recorded when various concentrations of naphthalene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. The spectra were recorded and the first derivatives calculated for each peak using Grams Analyst software and the peak height measured. The correlation coefficients were calculated at the various enrichment times.

Table 6.4 contains the peak heights and correlation information for the first derivative spectra generated from the data used in section 6.3.2.1. A plot was then constructed from this data which demonstrates the ability of the first derivative spectra to reflect the enrichment of naphthalene into the coated surface. (see fig 6.6).

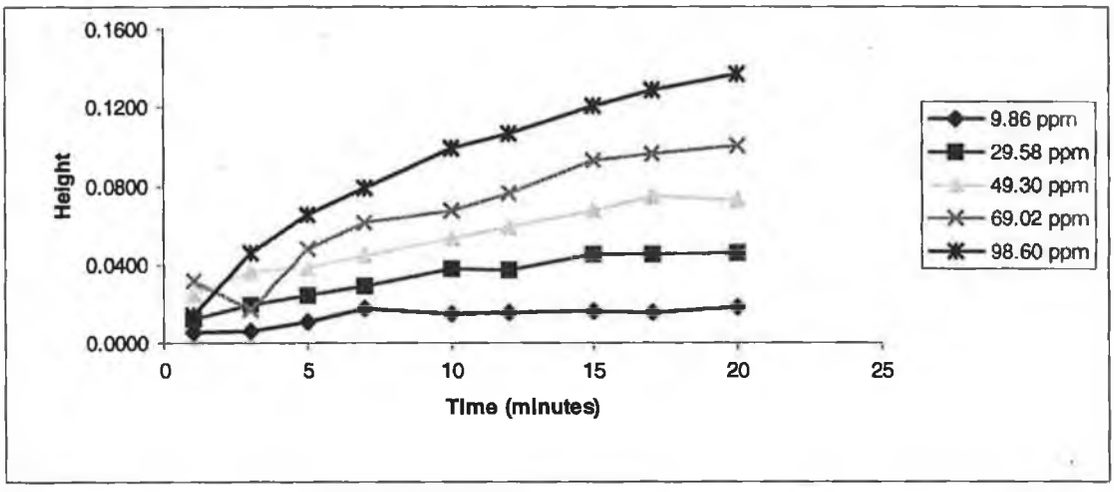

Fig 6.6
Plot of peak heights versus time (minutes) for the peak height recorded when various concentrations of naphthalene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. The spectra were recorded and the first derivatives calculated for each peak using Grams Analyst software and the peak height measured.

It was concluded from this plot that the first derivative of the absorbance spectrum gave good correlation for the standards at the later time but poor correlation at the initial times when the peak size was small and suffered interference by noise peaks. The minimum enrichment time required for a workable standard curve was 5 minutes.

6.3.2.4. Kubelka Munk Algorithm with Manual Integration

In this case the absorption spectrum were generated as described in [section 6.2.4.2.] however instead of integrating the data at this point the data was subjected to an algorithm, the Kubelka Munk[KM] algorithm. This algorithm was designed for reflectance spectra because even after the spectra have been converted to absorbance spectra they do not always obey Beer's law [7]. When the spectra are converted to KM units they behave linearly with concentration. The conversion used is described by equation 6.2

$$F[R] = [1 - R]^2 / 2R \quad \textbf{Eq. 6.2}$$

It must be noted that if a transmission spectrum has any totally absorbing bands [0% T] they are truncated at 0.0655 % T to avoid division by zero.

Once the spectra were converted to KM units the naphthalene peak at 783 cm^{-1} was integrated by manually selecting the start and end wavelengths (see fig 6.7).

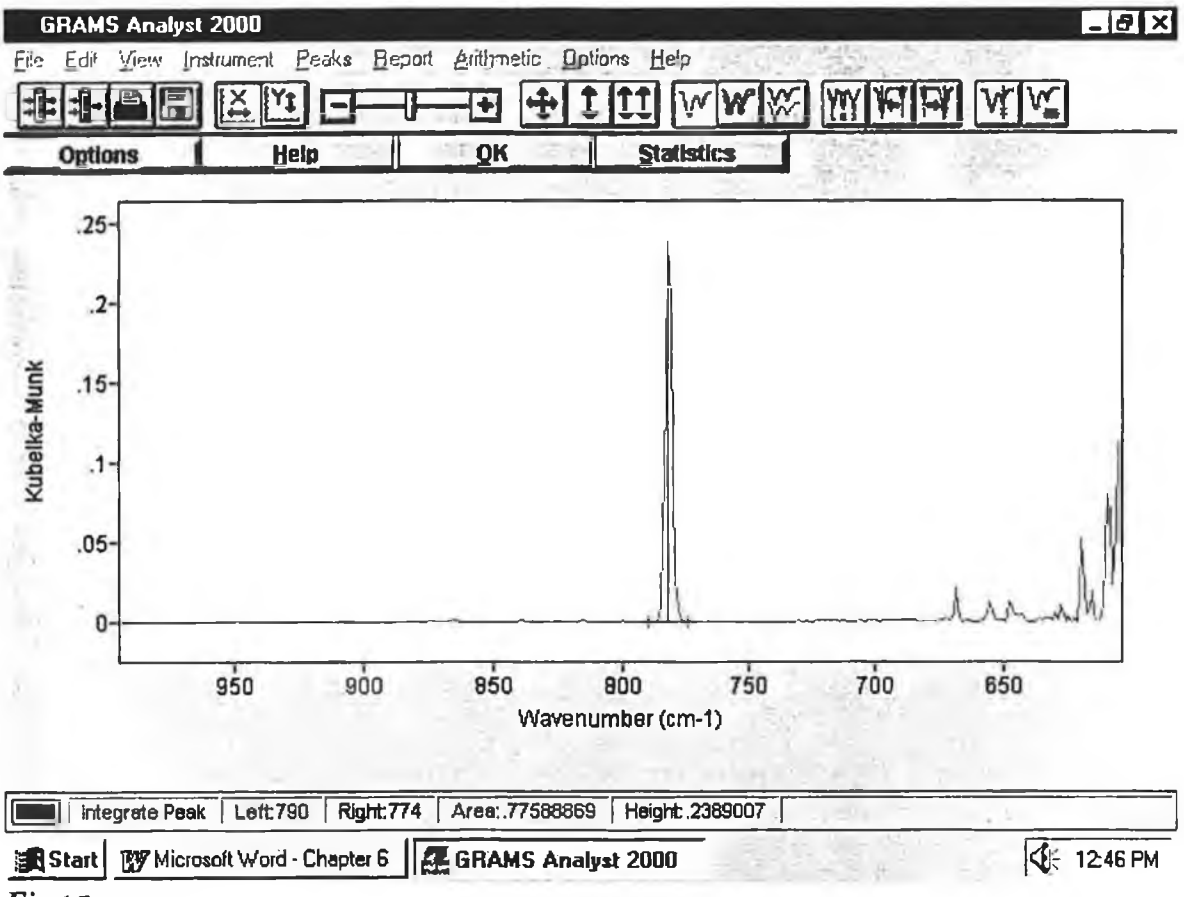

Fig 6.7

The diagram shows the spectrum calculated for naphthalene between 600cm^{-1} and 1000cm^{-1} after the Kubelka Munk algorithm had been performed. Manual integration was then conducted on the peak at 783cm^{-1} and the peak areas recorded for a 100ppm naphthalene solution enriched onto a 5w/v% PIB coating.

This method was then applied to the same absorbance spectra used in section 6.3.2.1.(see table 6.5) and a plot of area versus time in minutes constructed (fig 6.8).

Conc. (ppm)/ Time (minutes)	9.86	29.58	49.3	69.02	98.02	Correlation
1	0.0003	0.0000	0.0000	0.0001	0.0008	0.584024
3	0.0015	0.0199	0.0067	0.0128	0.0343	0.761881
5	0.0001	0.0139	0.0270	0.0510	0.0946	0.981826
7	0.0000	0.0099	0.0529	0.1096	0.1804	0.981983
10	0.0023	0.0247	0.1005	0.1669	0.3238	0.97844
12	0.0064	0.0321	0.1303	0.2335	0.4204	0.980888
15	0.0087	0.0555	0.1879	0.3413	0.5550	0.988096
17	0.0087	0.0664	0.2161	0.3842	0.6552	0.986897
20	0.0221	0.0745	0.2623	0.4487	0.7759	0.984288

Table 6.5

Table of peak area recorded when various concentrations of naphthalene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. The spectra were recorded and the Kubelka Munk calculated for each peak using Grams Analyst software and the peak area were integrated manually. The correlation coefficients were calculated at the various enrichment times.

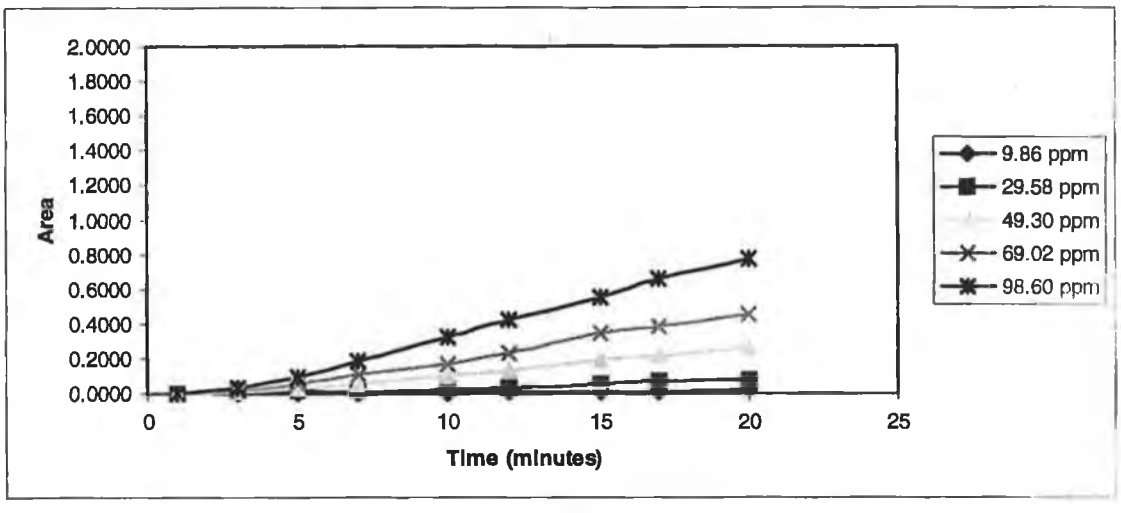

Fig 6.8

Plot of peak area versus time (minutes) for the peak area recorded when various concentrations of naphthalene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. The spectra were recorded and the Kubelka Munk calculated for each peak using Grams Analyst software and the peak area were integrated manually.

It was concluded from this plot that the response was lower and not as linear as previously seen. This is confirmed by the lower correlation seen for certain times when this is compared to previous results .

6.3.2.5. Kubelka Munk Algorithm with Integration between fixed wavelengths of 770 and 790 cm^{-1}.

In this case the data manipulation was followed as described in section 6.3.2.4 except the start and end wavelengths for the peak were set at 770 and 790 cm^{-1} respectively. This procedure was carried out for the same absorbance spectra in used in section 6.3.2.1. and the results and correlations are listed in table 6.6.

Conc. (ppm)/ Time (minutes)	9.86	29.58	49.3	69.02	98.02	Correlation
1	0.0137	0.0091	0.0091	0.0145	0.0211	0.692936
3	0.0053	0.0003	0.0119	0.0169	0.0419	0.910912
5	0.0060	0.0032	0.0291	0.0554	0.0973	0.965583
7	0.0029	0.0157	0.0522	0.1090	0.1806	0.981477
10	0.0035	0.0285	0.1078	0.1657	0.3218	0.980180
12	0.0076	0.0342	0.1302	0.2321	0.4184	0.980872
15	0.0090	0.0569	0.1877	0.3413	0.5538	0.988306
17	0.0107	0.0665	0.2164	0.3844	0.6556	0.986468
20	0.0141	0.0746	0.2636	0.4500	0.7753	0.986214

Table 6.6

Table of peak area recorded when various concentrations of naphthalene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. The spectra were recorded and the Kubelka Munk calculated for each peak using Grams Analyst software. The peak area were then integrated using a fixed wavelength period of between 770 and 790cm^{-1}. The correlation coefficients were calculated at the various enrichment times.

From this data a plot was constructed which demonstrates the enrichment profile for naphthalene using the fixed wavelength integration after the Kubelka Munk algorithm was carried out (see fig 6.9).

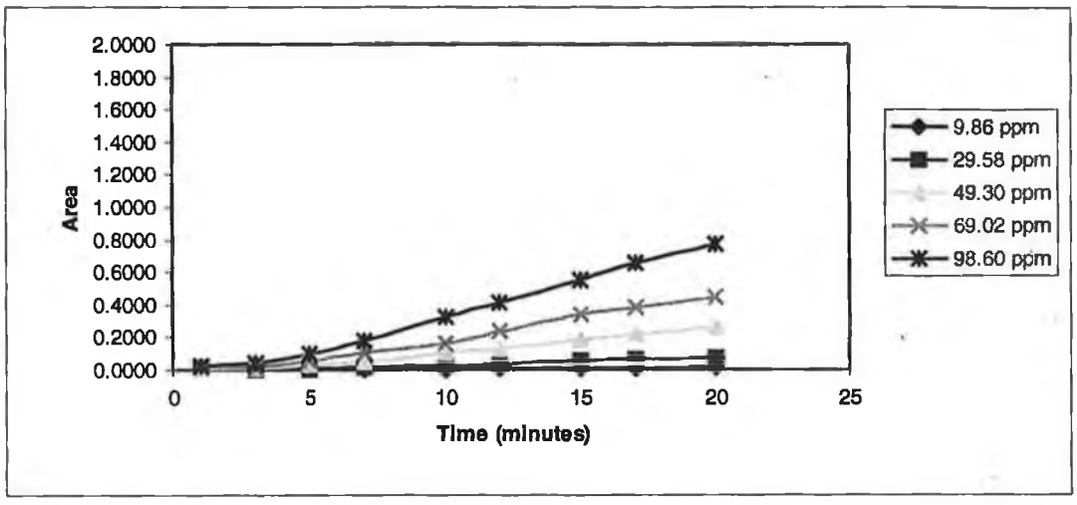

Fig 6.9

Plot of peak area versus time (minutes) for the peak area recorded when various concentrations of naphthalene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. The spectra were recorded and the Kubelka Munk calculated for each peak using Grams Analyst software. The peak areas were then integrated using a fixed wavelength period of between 770 and 790cm^{-1}.

It was concluded that very little difference was seen in the performance of this manipulation and the previous section 6.3.2.4.

6.3.2.6. Comparison of correlation data for the various integration techniques.

The correlation data collected for the various methods used in section 6.3.2. was then plotted so that the effectiveness of the various methods could be compared (see table 6.7 and fig 6.10).

Method / Time (mins)	A. Manual integration	B. Fixed Wavelength Integration	C. First Derivative with Peak Height	D. Kubelka Munk Algorithm with manual integration.	E. Kubelka Munk Algorithm with fixed wavelength integration
1	0.996350	0.942813	0.48388	0.584024	0.692936
3	0.997781	0.985131	0.77636	0.761881	0.910912
5	0.999625	0.989422	0.99757	0.981826	0.965583
7	0.997793	0.994654	0.99745	0.981983	0.981477
10	0.998167	0.997194	0.99693	0.97844	0.980180
12	0.998391	0.998409	0.99898	0.980888	0.980872
15	0.998042	0.998727	0.99685	0.988096	0.988306
17	0.998260	0.996890	0.99653	0.986897	0.986468
20	0.997384	0.999209	0.99970	0.984288	0.986214

Table 6.7
Correlation results over time for the various methods used on the data set for the naphthalene standards.

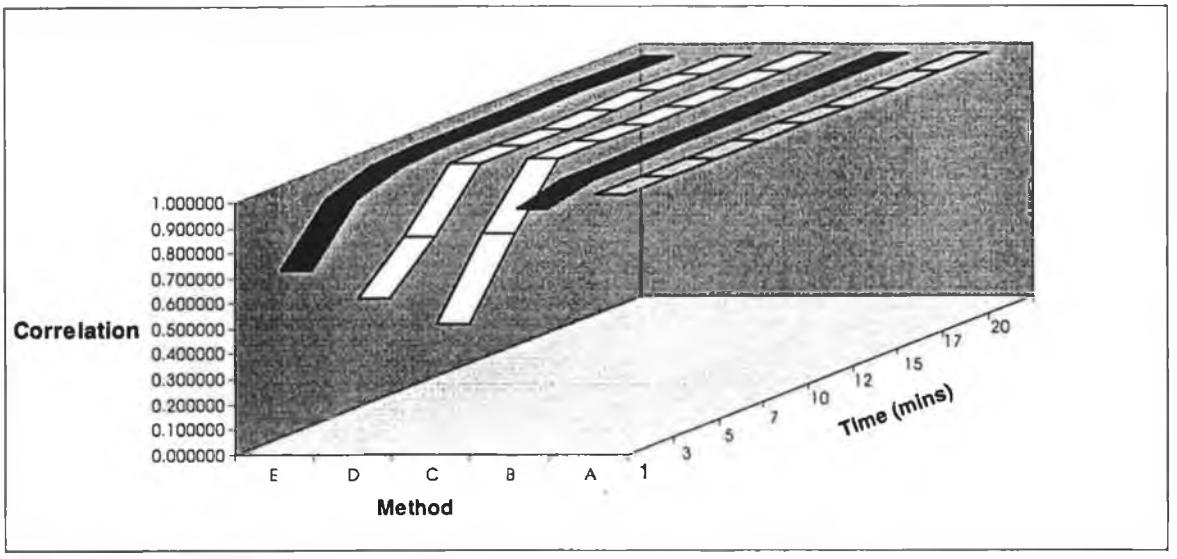

Fig 6.10
Plot which compares the correlation data over time for the various methods used to integrate the data generated for the naphthalene standards.

The plot of the various correlations for the various integration methods over time for naphthalene (see fig 6.10) demonstrates that over the range of data collected that Method A gave the best correlation of response versus concentration. This means that the integration gave the most linear response for the different concentrations over the range of times enrichment spectra were gathered i.e. from 1 minute to 20 minutes.

Method B also gave good correlation results except for the initial times when the peak size was at it smallest and the fixed wavelength method would have assigned more noise values to the individual peak than would have been deemed correct.

Method C gave excellent correlation for the later times but again when the peak size was at its smallest the correlation was poor.

This trend was also noted for the KM methods, however even at the later times the correlations did not compare well with the other methods. This maybe due to the fact that the algorithm reduced the overall peak area considerably (see fig 6.8 and fig 6.9).

It was therefore concluded that for quantitative analysis over a wide range of peak sizes that Method A was the most appropriate.

6.3.3. Application of the quantitative technique to other PAHs.

This quantitative method was then applied to a number of PAHs and the linear relationship between concentration and peak area was assessed. These PAHs were acenaphthene, fluorene and phenanthrene.

6.3.3.1. The enrichment profile for acenaphthene

The next PAH after naphthalene that was assessed was acenaphthene. A number of standards over the concentration range 10 to 100ppm were enriched onto the 5w/v% PIB coated ATR guide and the peak areas calculated using the manual integration technique defined in section 6.3.2.1. In this case however two peaks were present in the region of interest (see fig 6.11).

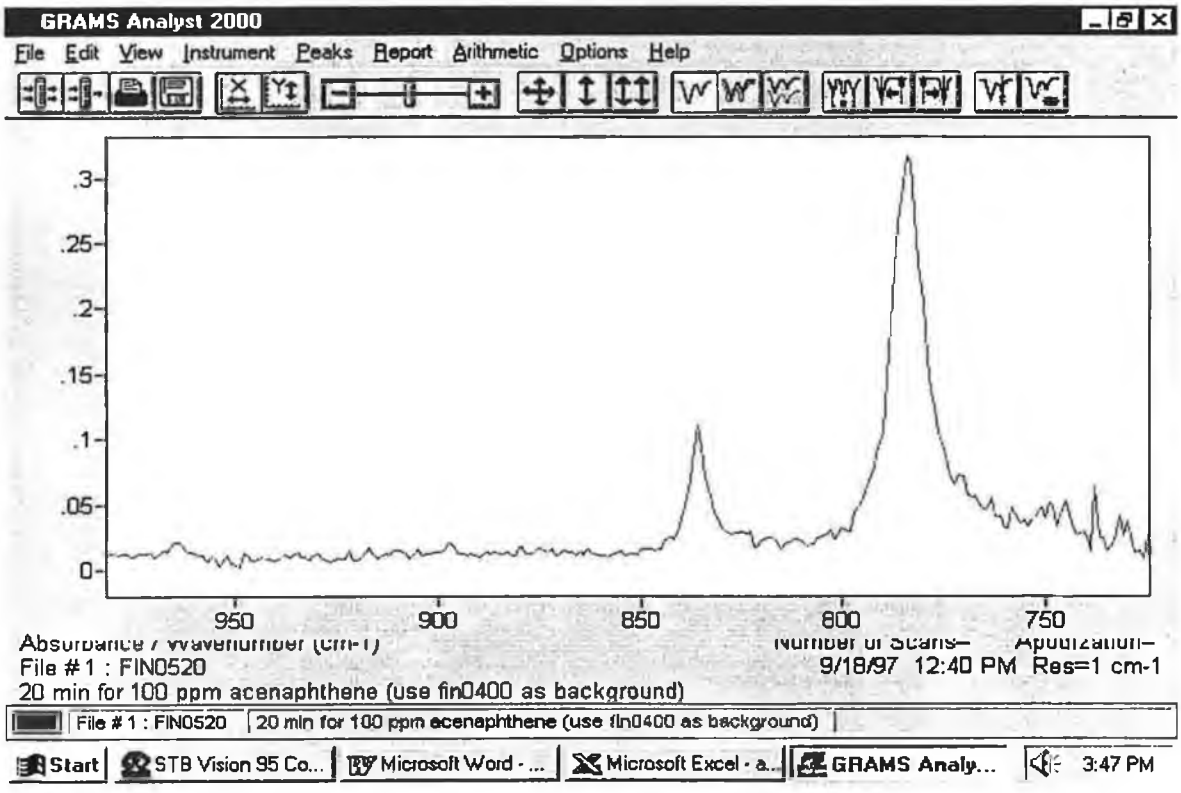

Fig 6.11

The diagram shows the absorbance spectrum for acenaphthene between 700cm^{-1} and 1000cm^{-1}. Two peaks are seen for acenaphthene one at 783cm^{-1} and the other at 834cm^{-1}. 100ppm acenaphthene was enriched from a 70/30 H_2O/ACN solution that was placed on a 5w/v% PIB coated ATR crystal.

Conc. (ppm) / Time (minutes)	10.6	21.19	52.96	74.71	105.96	Correlation
1	0.0119	0.0279	0.0563	0.0745		0.99562
3	0.0046	0.0947	0.3597	0.1944	0.3214	0.76898
5	0.0127	0.1361	0.4435	0.3363	0.6995	0.93991
7	0.0492	0.2382	0.5343	0.5515	0.9241	0.97826
10	0.0782	0.3630	0.7930	0.7958	1.4255	0.97339
12	0.1099	0.4740	0.8350	0.9153	1.8017	0.96545
15	0.1265	0.4703	0.9935	1.2430	1.9586	0.99338
17	0.1556	0.6243	1.1966	1.3840	2.2173	0.98579
20	0.2056	0.7676	1.3094	1.6043	2.6007	0.98479

Table 6.8

Table of peak areas recorded when various concentrations of acenaphthene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. Manual integration was carried out for the first peak at 783cm^{-1} and the correlation coefficients were calculated at the various enrichment times.

From this data a plot of area versus time was constructed for acenaphthene for the peak at 783 cm^{-1} (see fig 6.12).

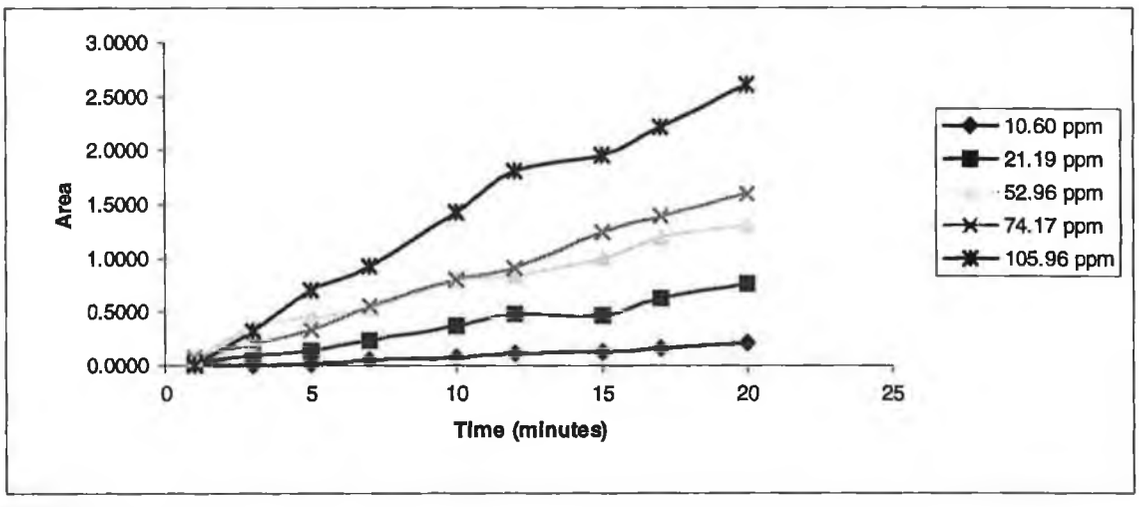

Fig 6.12

Plot of peak area versus time (minutes) for the peak areas recorded when various concentrations of acenaphthene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. Manual integration was carried out for the first peak at 783cm^{-1}.

This procedure was then repeated for the second peak for acenaphthene at the wavelength 834 cm^{-1} (see table 6.9 and fig 6.13).

Conc. (ppm) / time (mins)	10.6	21.19	52.96	74.71	105.96	Correlation
1	0.0091	0.0171	0.0027	0.0050		-0.67421
3	0.0154	0.0267	0.0369	0.0324	0.0163	0.03497
5	0.0319	0.0373	0.0302	0.0710	0.0926	0.89360
7	0.0215	0.0421	0.0762	0.0807	0.1490	0.97036
10	0.0320	0.0423	0.0938	0.1428	0.2531	0.97861
12	0.0249	0.0528	0.1519	0.1711	0.2397	0.98814
15	0.0492	0.0495	0.2027	0.2163	0.3305	0.98180
17	0.0669	0.1098	0.2343	0.2599	0.3575	0.98975
20	0.0899	0.1452	0.1732	0.1957	0.4256	0.90737

Table 6.9

Table of peak areas recorded when various concentrations of acenaphthene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. Manual integration was carried out for the second peak at 834cm^{-1} and the correlation coefficients were calculated at the various enrichment times.

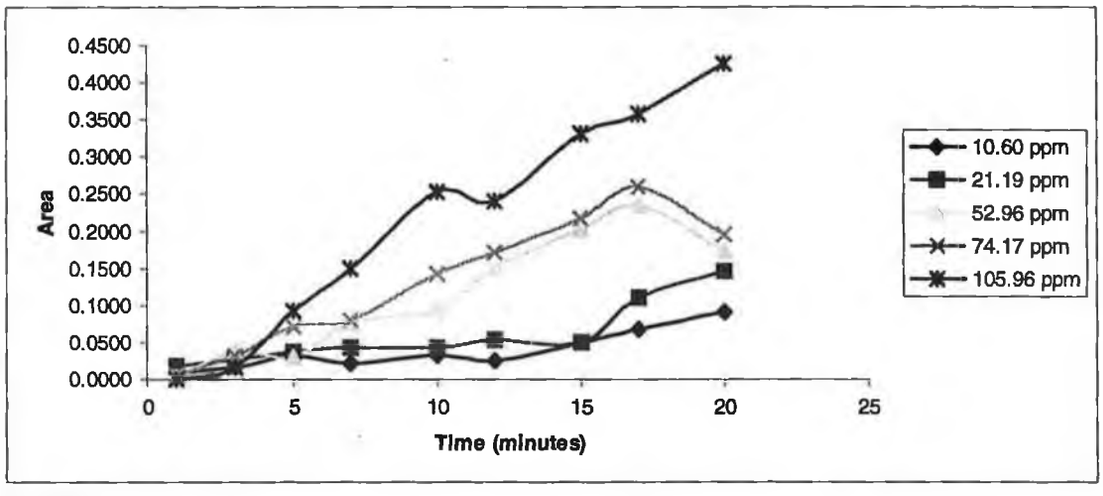

Fig 6.13

Plot of peak area versus time (minutes) for the peak areas recorded when various concentrations of acenaphthene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. Manual integration was carried out for the second peak at 834cm^{-1}.

It is obvious from comparing the two sets of data for acenaphthene peaks that the first peak gives much better correlation between area and concentration. However the reason for including the data for the second peak is that it is necessary to know the ratios of these

peaks for accurate quantitation of acenaphthene when naphthalene is present (see section 6.3.4.3.).

6.3.3.2. The enrichment profile for fluorene

The next compound to be investigated using the technique defined in section 6.3.2. was fluorene. Fluorene has one absorbance peak in this region at 740 cm^{-1} and it is this peak which was used to correlate area with concentration for this particular compound (see fig 6.14).

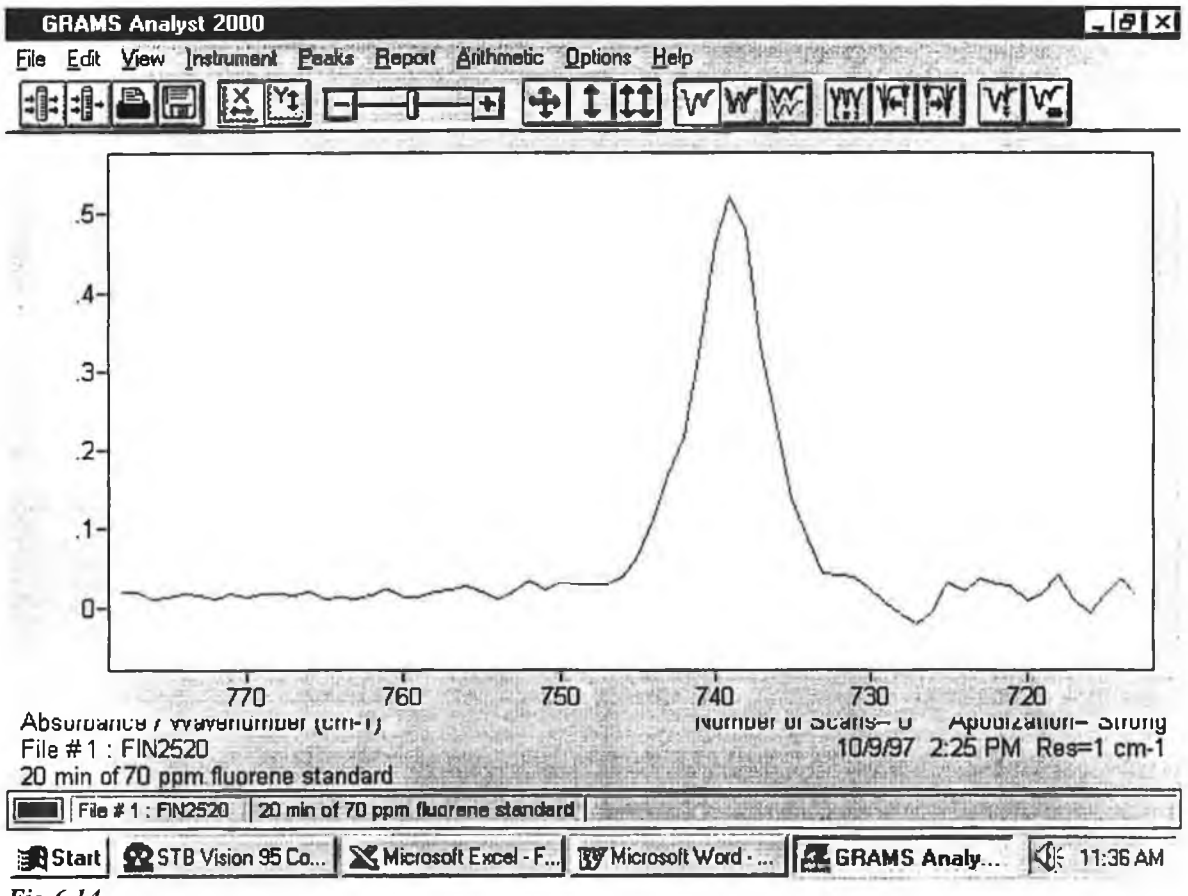

Fig 6.14

The diagram shows the absorbance spectrum for fluorene between 700cm^{-1} and 800cm^{-1}. 70ppm fluorene was enriched from a 70/30 H_2O/ACN solution that was placed on a 5w/v% PIB coated ATR crystal.

A series of data points were gathered using various concentrations of fluorene enriched onto the coated ZnSe crystal for 20 minutes (see Table 6.10).

Conc. (ppm) / Time (mins)	10.00	30.00	50.00	70.00	100.00	Correlation
1	0.0367	0.1219	0.0249	0.0993	0.2594	0.76059
3	0.0652	0.1707	0.3529	0.2523	0.9090	0.88901
5	0.1271	0.2565	0.4745	0.7818	1.2436	0.98845
7	0.1933	0.3685	0.7994	1.0058	1.6670	0.98998
10	0.1913	0.4675	1.1936	1.2998	2.1809	0.98454
12	0.1644	0.7302	1.5582	1.6719	2.3583	0.97866
15	0.3140	0.9486	1.7512	2.1072	2.7229	0.98692
17	0.3399	1.0468	2.1048	2.4045	2.9860	0.97251
20	0.3665	1.2496	2.4963	2.7002	3.4528	0.96762
Correlation	0.96065	0.98911	0.99749	0.99639	0.98916	

Table 6.10

Table of peak areas recorded when various concentrations of fluorene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. Manual integration was carried out for the peak at 740cm^{-1} and the correlation coefficients were calculated at the various enrichment times.

From this data a graph was constructed of area versus time for the various concentrations of fluorene enriched onto the surface of the coated crystal (fig 6.15).

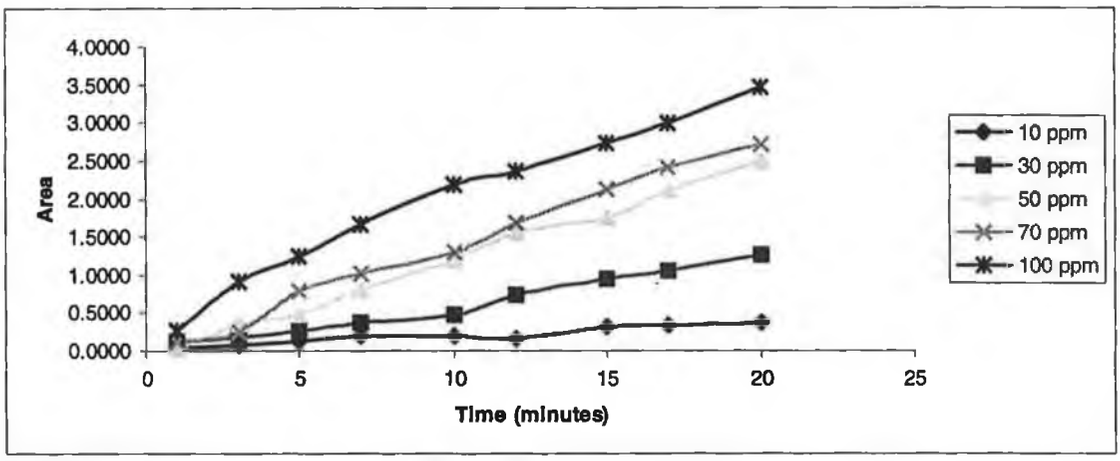

Fig 6.15

Plot of peak area versus time (minutes) for the peak areas recorded when various concentrations of fluorene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. Manual integration was carried out for the peak at 740cm^{-1} and the correlation coefficients were calculated at the various enrichment times.

This graph demonstrates that at certain enrichment times, e.g. 15 minutes that a linear relationship exists between concentration and area for fluorene. The lower concentrations

gave poor enrichment as they appear to be too close to the limit of detection. This may also be due to the actual shape of the infrared stretch at 740 cm^{-1} and its position, as below this wavelength large interferences are present, possibly due to water.

6.3.3.3. The enrichment profile for phenanthrene.

The fourth compound investigated using this procedure was phenanthrene. This compound has three peaks in the region of interest between 1000 and 600 cm^{-1}(see Fig 6.16).

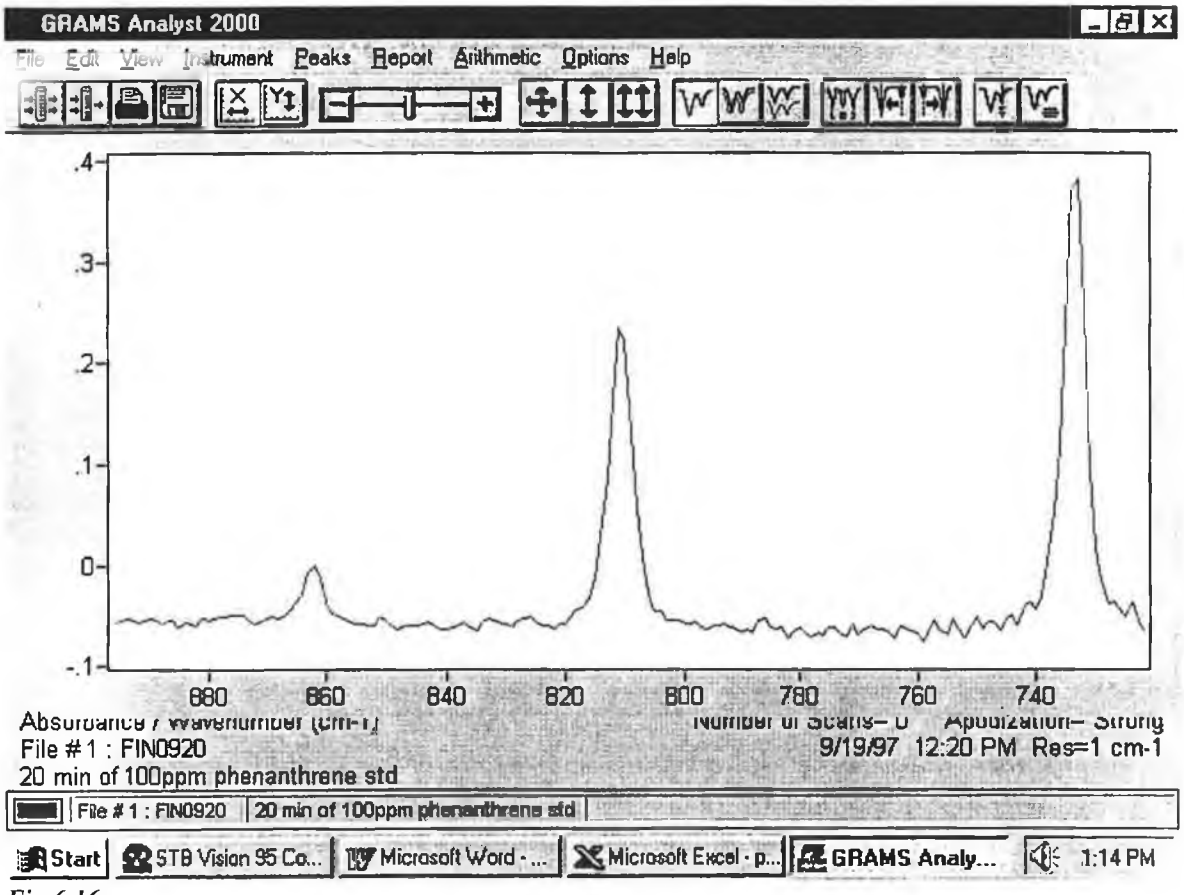

Fig 6.16

The diagram shows the absorbance spectrum for phenathrene between 700cm^{-1} and 900cm^{-1}. There are three peaks for phenanthrene in this region 732, 810 and 861cm^{-1}. 100ppm phenanthrene was enriched from a 70/30 H_2O/ACN solution that was placed on a 5w/v% PIB coated ATR crystal.

Each peak area was calculated based on the procedures outlined in section 6.3.2.1 A series of standards were prepared between 10-100ppm and enriched onto the coated ATR

waveguide. Tables were prepared from this data (see tables 6.11,6.12 and 6.13) . A plot of peak area versus time was constructed for each peak (see fig 6.17,6.18 and 6.19).

Conc. (ppm) / Time (mins)	10.2	30.59	50.99	71.39	101.98	Correlation
1	0.0023	0.0548	0.0262	0.0135	0.0908	0.65152
3	0.0035	0.1291	0.2429	0.1752	0.2997	0.88676
5	0.0192	0.1458	0.2523	0.3382	0.6656	0.98189
7	0.1683	0.2374	0.4301	0.4497	0.9109	0.95374
10	0.2063	0.5023	0.6063	0.8866	1.2392	0.99388
12	0.2425	0.6245	0.7726	1.0648	1.5570	0.99369
15	0.2318	0.7016	0.9372	1.1574	1.7161	0.99210
17	0.2894	0.7913	1.2096	1.4650	1.8006	0.98202
20	0.3957	0.9268	1.4218	1.7822	1.9261	0.95954

Table 6.11

Table of peak areas recorded when various concentrations of phenanthrene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. Manual integration was carried out for the peak at 732cm^{-1} and the correlation coefficients were calculated at the various enrichment times.

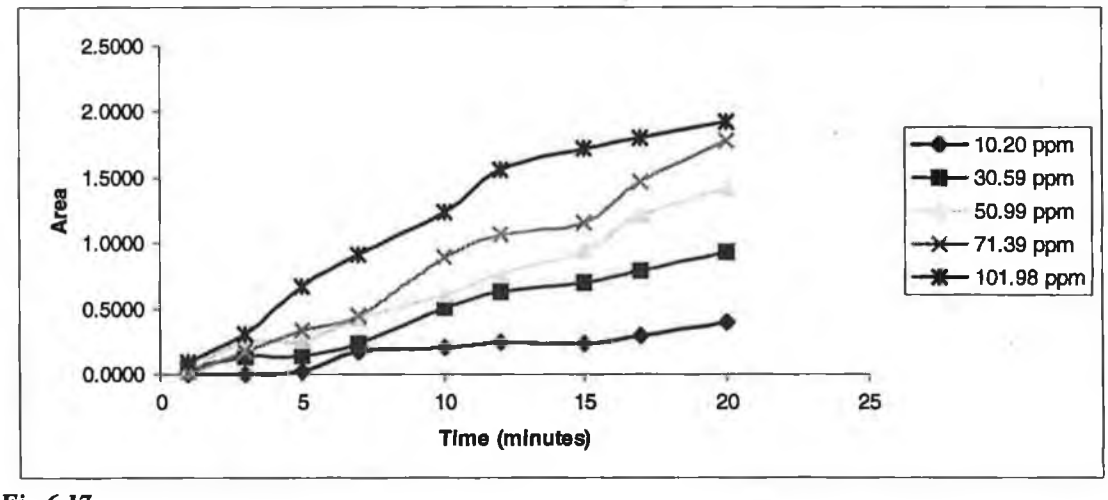

Fig 6.17

A plot of peak areas versus time (minutes) for the peak areas recorded when various concentrations of phenanthrene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. Manual integration was carried out for the peak at 732cm^{-1}.

Conc. (ppm) / Time (mins)	10.2	30.59	50.99	71.39	101.98	Correlation
1	0.0023	0.0044	0.0272	0.0532	0.1039	0.96828
3	0.0034	0.0543	0.0964	0.2074	0.2210	0.96325
5	0.0354	0.1199	0.1943	0.3123	0.4291	0.99770
7	0.0271	0.1686	0.2951	0.4542	0.6767	0.99941
10	0.0601	0.2237	0.4641	0.6342	0.9227	0.99877
12	0.0831	0.2444	0.5155	0.8035	1.0811	0.99572
15	0.0976	0.4310	0.7762	0.9742	1.2546	0.98779
17	0.1264	0.4647	0.9220	1.1560	1.3955	0.97834
20	0.1896	0.6042	1.1320	1.2500	1.4067	0.94081

Table 6.12

Table of peak areas recorded when various concentrations of phenanthrene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. Manual integration was carried out for the peak at 810cm^{-1} and the correlation coefficients were calculated at the various enrichment times.

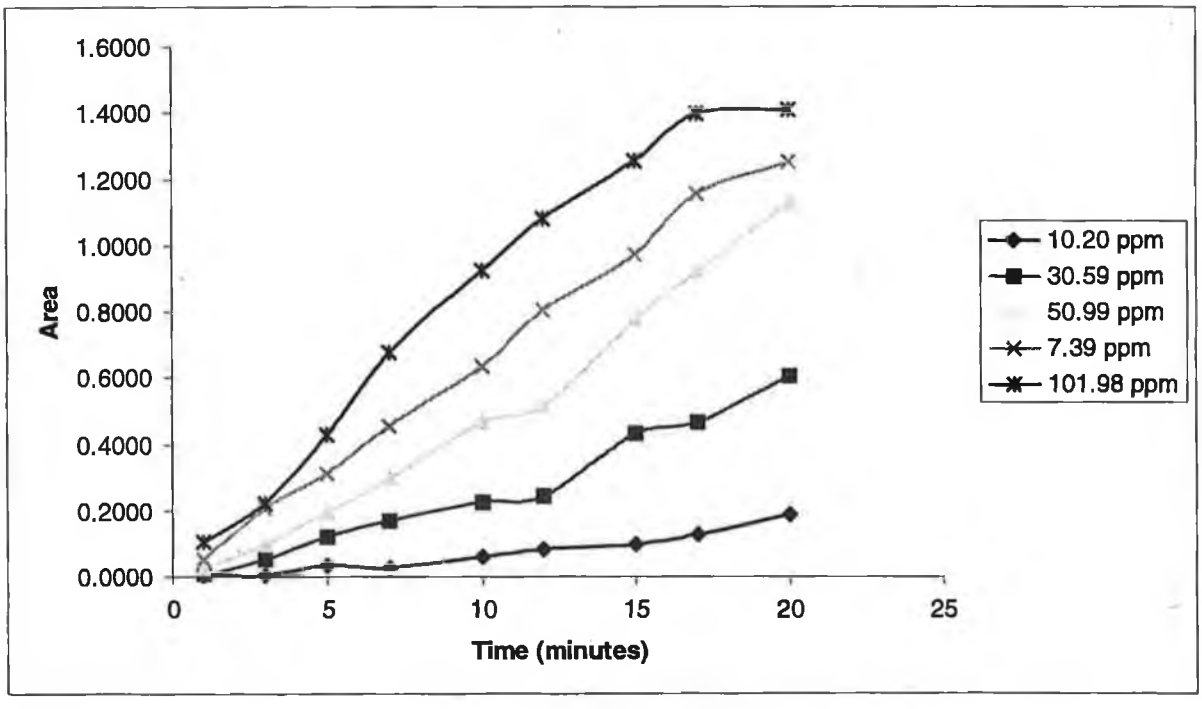

Fig 6.18

A plot of peak areas versus time (minutes) for the peak areas recorded when various concentrations of phenanthrene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. Manual integration was carried out for the peak at 810cm^{-1}.

Conc. (ppm) / Time (mins)	10.2	30.59	50.99	71.39	101.98	Correlation
1	0.0020	0.0000	0.0021	0.0000	0.0061	0.58797
3	0.0024	0.0075	0.0061	0.0183	0.0505	0.90117
5	0.0007	0.0025	0.0230	0.0322	0.0619	0.97735
7	0.0019	0.0296	0.0432	0.0854	0.1236	0.99183
10	0.0022	0.0328	0.0626	0.0954	0.1634	0.99461
12	0.0357	0.0552	0.0716	0.1224	0.1667	0.98384
15	0.0332	0.0687	0.1107	0.1394	0.2336	0.98951
17	0.0295	0.0742	0.1291	0.1676	0.2409	0.99916
20	0.0416	0.0714	0.1169	0.1707	0.2422	0.99618
Correlation	0.87843	0.96418	0.97763	0.98125	0.97505	

Table 6.13

Table of peak areas recorded when various concentrations of phenanthrene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. Manual integration was carried out for the peak at 861cm^{-1} and the correlation coefficients were calculated at the various enrichment times.

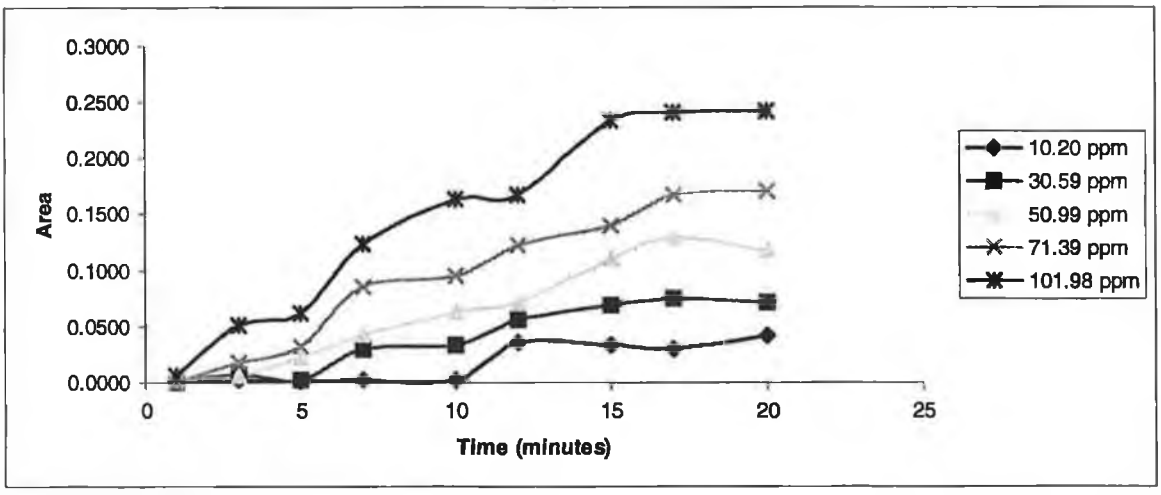

Fig 6.19

A plot of peak areas versus time (minutes) for the peak areas recorded when various concentrations of phenanthrene, ranging from 10 to 100ppm, were enriched onto a 5w/v% PIB coated ZnSe crystal. Manual integration was carried out for the peak at 861cm^{-1}.

From these plots and the calculated correlations it was concluded that for phenanthrene that there is a linear relationship between these peak areas and concentration. It must be noted that even though the largest response was seen for the peak at 732 cm^{-1}, there was some interference from noise which affected the results. Also the response varied for the

third peak at 861 cm^{-1} this was due to the actual smaller size of this peak and the resulting problem with noise.

6.3.4. Simultaneous analysis of the four PAHs

6.3.4.1. The optimum enrichment.

The correlation data for the range of standards enriched onto the ATR waveguide for the series of compounds was examined (see table 6.14). From the set of data a 10 minute enrichment was found to be the optimum enrichment time for the 5w/v% PIB coated ATR waveguide for the group of compounds studied in the 10 –100ppm concentration range. This indicates that for the working calibration curves to be constructed for these compounds the 10 minute enrichment time was chosen as the point for data to be gathered, as it was the one that gave the highest correlations for the various standards investigated.

PAH	Naphthalene	Acenaphthene		Fluorene	Phenanthrene		
Time (mins)	783 cm^{-1}	783 cm^{-1}	834 cm^{-1}	740 cm^{-1}	732 cm^{-1}	810 cm^{-1}	861 cm^{-1}
1	0.996350	0.99562	-0.67421	0.713276	0.65152	0.968281	0.587974
3	0.997781	0.76898	0.03497	0.882105	0.88676	0.963253	0.901167
5	0.999625	0.93991	0.89360	0.988312	0.98189	0.997705	0.977355
7	0.997793	0.97826	0.97036	0.992475	0.95374	0.999414	0.991826
10	0.998167	0.97339	0.97861	0.988163	0.99388	0.998766	0.994611
12	0.998391	0.96545	0.98814	0.975303	0.99369	0.995723	0.983836
15	0.998042	0.99338	0.98180	0.982589	0.99210	0.987793	0.989506
17	0.998260	0.98579	0.98975	0.97099	0.982021	0.97834	0.999164
20	0.997384	0.98479	0.90737	0.96425	0.95954	0.94081	0.996185

Fig 6.14

Table of correlation data for four PAHs examined at a range of concentrations, 10-100ppm, enriched onto a 5% PIB coating of ATR waveguide over a period of 20 minutes. The 10 minute results are highlighted as it was found to be the optimum enrichment time over the range of compounds and their various wavelengths.

6.3.4.2. Calculation of the line equations.

From the calibration data already gathered for these compounds, based on the 10 minute enrichment time in section 6.3.3., the slope of the line, which relates concentration to area, was calculated for each compound. The equation for this line took the form of **Y = MX +** C. Where Y is the concentration in ppm, X is the peak area, M is the slope of the line and C is a constant relating to the intercept with the Y axis (see table 6.15)

Compound	Wavelength	Equation
Naphthalene	734 cm^{-1}	Y = 81.87175x + 2.4
Acenaphthene	734 cm^{-1}	Y = 74.3177X + 1.7
	834 cm^{-1}	Y = 423.83 X + 5.2
Fluorene	740 cm^{-1}	Y = 46.513X + 0.3
Phenanthrene	732 cm^{-1}	Y = 90.1806X – 9.0
	810 cm^{-1}	Y = 104.8860X + 4.6
	861 cm^{-1}	Y = 571.06X + 12.0

Table 6.15
Table of line equations calculated based on the 10 minute enrichment profiles for each individual PAH at their various wavelengths.

6.3.4.3. Difficulties with simultaneous analysis.

Due to the complex nature of the absorbance spectrum for the simultaneous enrichment of these four compounds two difficulties were noted (see fig 6.20). These must be overcome if quantitation of the compounds was to be achieved.

The first is the fact that both naphthalene and acenaphthene have absorbance peak at 783 cm^{-1} . In real or spiked samples that contain both of these compounds the peak at 783 cm^{-1}

will have a contribution from both compounds. Acenaphthene also has an absorbance peak at 834 cm^{-1} and therefore if the ratio of this peak to the 783 cm^{-1} peak is known for acenaphthene it will be possible to predict the area for naphthalene at 783 cm^{-1}.

The data collected for the enrichment of acenaphthene was examined and based on the data for 30 to 100 ppm enriched for 7 to 20 minutes in section 6.3.3.1. An average ratio for the 834 cm^{-1} to 783 cm^{-1} was calculated. The reason these data points were chosen was due to the peak size. These points were all deemed to be large enough to be included in an averaging of the ratio. This ratio was 1:6.6496.

Example : Calculation of Naphthalene Concentration.

The areas for the 50ppm mixed spike sample containing all four PAHs were taken as an example. The rest of the data can be seen in table 6.17.

Wavelength	Area
783 cm^{-1}	1.3950
834 cm^{-1}	0.1116

Table 6.16
Table of peak area results for the peaks at 783cm^{-1} and 834cm^{-1} recorded when a 50ppm mixed spike sample containing all four PAHs was enriched onto a 5w/v% PIB coating. The peak at 783cm^{-1} has contributions from both acenaphthene and naphthalene.

Acenaphthene contribution to 783 cm^{-1} peak is $0.1116 \times 6.6496 = 0.7421$

Naphthalene contribution to 783 cm^{-1} peak is $1.3950 - 0.7421 = 0.6529$

Predicted naphthalene concentration is $Y = MX + C$ $\quad Y = 8.8715X + C$

$Y = 0.6529 \times 81.8715 + 2.4$

$Y = 55.82$ ppm

The actual spiked concentration for naphthalene was 49.30 ppm

The ratio method of calculating naphthalene's concentration was therefore deemed to be satisfactory.

The second difficulty encountered was the fact that the 740 cm^{-1} peak for fluorene and the 732 cm^{-1} were not resolved. It was necessary to drop a vertical between these peaks to predict their areas at certain concentrations (see fig 6.20).

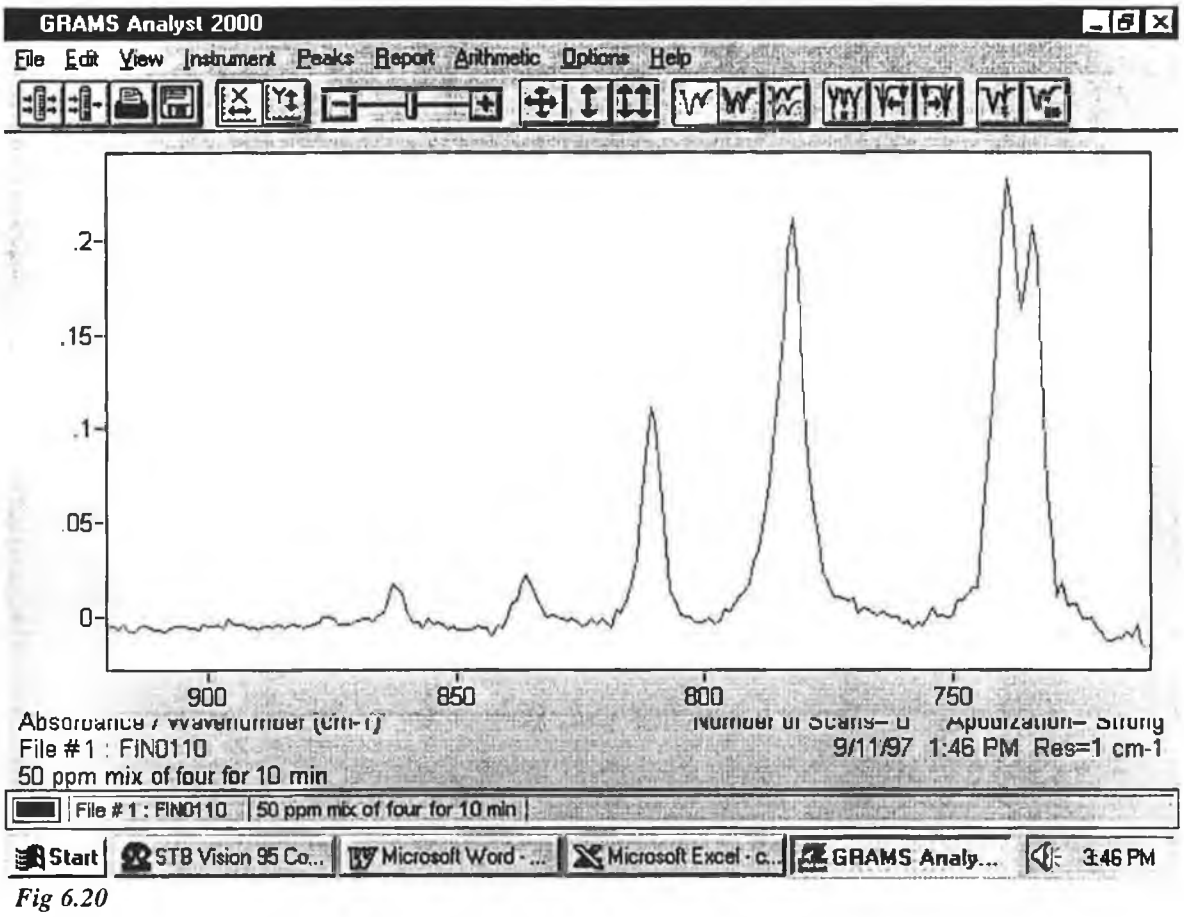

Fig 6.20

The absorbance spectrum for a 50ppm mixture of the four PAHs, naphthalene, acenaphthene, fluorene and phenanthrene enriched onto the 5% PIB coating for an enrichment time of 10 minutes. It is clear that the 740$cm^{-1}$and 732cm^{-1} peak are over lapping at this concentration.

6.3.4.4. Spiking Experiments.

A number of standards were prepared to examine the ability of the calibration system to calculate individual PAH concentrations in the presence of various other PAHs at varying concentrations.

6.3.4.4.1. High Concentration Spiking Experiments

The first group of experiments carried out using the 5% PIB coated ATR waveguide for quantitating the four PAHs simultaneously were carried out at the high concentrations (50ppm). The reason a 50 ppm mixture of the four PAHs was chosen was because it is the middle of the calibration range and therefore should not suffer due to small peak sizes.

50ppm Mixed Sample.

A solution was prepared from the original stock standards for the various individual standard curves, which contained approximately 50 ppm of these compounds in a single solution (see fig 6.17). The actual concentrations used were 49.30, 52.96, 50.00 and 50.99 ppm for naphthalene, acenaphthene, fluorene and phenanthrene respectively. The areas for the individual peaks were measured and from the calibration equations already generated (see table 6.15) the concentrations of the individual PAHs were predicted (see table 6.17). The area for the naphthalene peak was generated using the ratio method outlined in section 6.3.4.3.

It can be concluded from the results in table 6.17 that the calibration curves were able to predict the concentrations of the various PAHs. Phenanthrene at 732 cm^{-1} gave a poor

results. The reason for the poor result maybe due to the fact that in this region noise does have a significant effect on the integration of this peak.

Sample		Naphthalene	Acenaphthene		Fluorene	Phenanthrene		
		783cm^{-1}	783cm^{-1}	834cm^{-1}	740cm^{-1}	732cm^{-1}	810cm^{-1}	861cm^{-1}
50ppm mixed	Actual conc. (ppm)	49.30	52.96	52.96	50.00	50.99	50.99	50.99
	Predicted conc. (ppm)	55.8543	56.8508	52.4994	52.5007	69.6009	60.3679	56.9424
98.02ppm Naphthalene	Actual conc. (ppm)	98.02	52.96	52.96	50.00	50.99	50.99	50.99
	Predicted conc (ppm)	101.6451	56.3566	52.0756	46.5930	60.5739	59.2246	59.8548
105.96ppm Acenaphthene	Actual Conc. (ppm)	49.30	105.96	105.96	50.00	50.99	50.99	50.99
	Predicted conc (ppm)	22.3886	108.2459	96.5777	46.6678	65.1099	56.9906	58.4843
100.00ppm Fluorene	Actual Conc. (ppm)	49.30	52.96	52.96	100.00	50.99	50.99	50.99
	Predicted Conc. (ppm)	78.7276	37.9236	36.2667	95.9742	68.4646	57.2528	59.6264
101.98ppm Phenanthrene	Actual Conc. (ppm)	49.30	52.96	52.96	50.00	101.98	101.98	101.98
	Predicted Conc. (ppm)	46.2055	58.1851	53.6438	63.9859	121.3642	98.6932	103.4838

Table 6.17
Table of concentrations for various spiked samples with the actual concentration in the sample placed above the predicted concentration based on the line equations and calculations outlined previously. The samples were all in the 50-100ppm concentration range.

98.02ppm Naphthalene

The 50ppm solution was then spiked with naphthalene to a concentration of 98.02ppm. The area for this peak was calculated as described above to be 101.65ppm and the other compounds remained at approximately 50ppm which indicates that naphthalene's concentration can be predicted in a range of samples without any significant interference with the other compounds examined (see fig 6.17).

105.96ppm spike of Acenaphthene into 50ppm mixed sample.

The 50ppm solution was spiked with acenaphthene to a concentration of 105.96ppm. The peak areas for all four compounds were then measured and the concentrations calculated (see fig 6.17).

The two peaks that correspond to acenaphthene predicted the spike concentration to be 108.25ppm at 783cm^{-1} and 96.58ppm at 834cm^{-1}. This demonstrates the calibration curves ability to predict acenaphthene concentration in a mixture containing the four PAHs. Significantly the concentration for naphthalene in this spiked sample was reduced to 22.4ppm which indicates that acenaphthene peak can effect the calculation of the naphthalene concentration. The phenanthrene result is poor at 732 cm^{-1} but appears to be reasonable at the two other wavelengths.

100.00ppm spike of fluorene in the 50ppm mixed solution.

The 50ppm mixed solution was spiked with fluorene to a concentration of 100.00ppm. The area for the fluorene was measured and the concentration calculated to be 95.97ppm. The naphthalene concentration was calculated to be 78.73, which indicates that the enrichment of naphthalene in the polymer surface is not independent of the amount of fluorene present (see fig 6.17).

101.98ppm spike of Phenanthrene in the 50ppm mixed solution

The 50ppm mixed solution was spiked with phenanthrene to a concentration of 101.98ppm. Three absorbance peaks are known to be associated with phenanthrene in this region and each was used to predict the concentration of phenanthrene in this solution.

The results for the 734 cm^{-1}, 810 cm^{-1} and 861 cm^{-1} were 121.36ppm, 98.96ppm and 103.48ppm (see fig 6.17). The 734 cm^{-1} peak did not give very accurate results and this is true for all the previous samples as well as this one. One reason for this poor result is the possible interferences as it is noisy in this region. Another reason is the fact that this peak is not resolved from the fluorene peak at 740 cm^{-1}, which makes it difficult to integrate both of these peaks accurately. This could explain the fluorene peak in this particular sample giving a higher then predicted result of 63.98ppm. The predicted concentrations for naphthalene and acenaphthene are approximately 50ppm, which demonstrate that these compounds are not influenced by the concentration of phenanthrene.

The overall conclusion was that it was possible to measure the concentration of four PAHs in the 50-100ppm concentration range simultaneously and accurately to an approximation.

6.3.4.4.2. Low Concentration Spiking Experiments

The second group of experiments was carried out at low concentrations, which were near or at the limit of detection 5 – 10 ppm. Table 6.18 contains the predicted individual concentrations.

Sample		Naphthalene	Acenaphthene		Fluorene	Phenanthrene		
		783cm^{-1}	783cm^{-1}	834cm^{-1}	740cm^{-1}	732cm^{-1}	810cm^{-1}	861cm^{-1}
5ppm mixed	Actual conc. (ppm)	4.93	5.29	5.29	5.00	5.10	5.10	5.10
	Predicted conc. (ppm)	2.93	5.51	8.47	1.10	-5.20	4.67	17.20
9.86ppm Naphthalene	Actual conc. (ppm)	9.86	5.29	5.29	5.00	5.10	5.10	5.10
	Predicted conc (ppm)	15.45	2.79	6.13	4.59	-5.20	11.87	18.74
10.60ppm Acenaphthene	Actual Conc. (ppm)	4.93	10.60	10.60	5.00	5.10	5.10	5.10
	Predicted conc (ppm)	1.71	10.50	12.74	3.14	-9.00	12.26	14.86
10.00ppm Fluorene	Actual Conc. (ppm)	4.93	5.29	5.29	10.0	5.10	5.10	5.10
	Predicted Conc. (ppm)	8.93	N/D	9.31	13.09	-9.00	25.33	42.89
10.20ppm Phenanthrene	Actual Conc. (ppm)	4.93	5.29	5.29	5.00	10.20	10.20	10.20
	Predicted Conc. (ppm)	1.71	9.90	12.24	3.32	-7.58	17.07	21.59

Table 6.18
Table of concentrations for various spiked samples with the actual concentration in the sample placed above the predicted concentration based on the line equations and calculations outlined previously. The sample concentration range was 5 - 10ppm.

5ppm Mixed Sample

The first sample investigated was a standard that was prepared to contain the four PAHs at approximately 5ppm. The actual concentrations were 4.93, 5.29, 5.00.and 5.10 for naphthalene, acenaphthene, fluorene and phenanthrene respectively. The results were calculated and listed in table 6.18. From this it can been seen that these concentrations were below the limit of determination for the calibration curves due to the small size of the 10 minute enrichment peaks for these compounds.

9.86ppm Naphthalene Spike.

The 5ppm mixed solution was spiked with naphthalene to a concentration of 9.86ppm. The areas for the peaks were measured and the concentrations calculated (see table 6.18). Naphthalene concentration was calculated to be 15.44ppm that is not accurate and the results of the other three compounds at 5ppm are also poor. This indicates that all compounds are at concentrations below the limit of detection.

10.6ppm Acenaphthene

The 5ppm mixed solution was spiked with acenaphthene to a concentration of 10.6ppm In this case two separate peaks were used to predict the concentration of acenaphthene 783 cm^{-1} and 834 cm^{-1}. They predicted acenaphthene concentration to be 10.49 and 12.74ppm (see table 6.18). This demonstrates the calibration curve worked for both wavelengths with 783 cm^{-1} being the more accurate of the two.

10.0ppm Fluorene

The 5ppm mixed solution was spiked with fluorene to a concentration of 10.0ppm. The calibration curve predicted the fluorene concentration to be 13.1ppm (see table 6.18). Variations were also seen for the predicted concentrations of the other three compounds with the peak at 861 cm^{-1} predicting a concentration of 42ppm for phenanthrene in the 5ppm mix. This may due to the actual peak size being small leading to variations in % recoveries.

10.2ppm Phenanthrene Spike

The 5ppm solution was spiked with phenanthrene to a concentration of 10.2ppm. The calibration curves for all three peaks present for phenanthrene were very poor (see table 6.18). This is probably due to the fact that this concentration is below the limit of determination for the calibration curve. Variations were also seen in the other three compound concentrations and this again is due to the small peak sizes.
The 5ppm concentration was below the limit of detection for these compounds however it was possible to detect these compounds in most cases at 10ppm.

6.4. Conclusions.

The possibility of analysing four PAHs simultaneously was demonstrated. The four PAHs analysed were naphthalene, acenaphthene, fluorene and phenanthrene. The effects of adding ACN to aqueous solutions were investigated. It was concluded that although 30% ACN was needed to keep the four PAHs in solution even though it was shown to hinder the enrichment process.

The way in which the spectral data generated was processed was also studied. Five separate techniques were applied to the same set of data generated for naphthalene enrichment onto the 5% PIB coated waveguide. These were peak areas from manual integration; peak areas from fixed wavelength integration, peak heights from first derivative spectra, application of Kubelka Munk algorithm to manually chosen peaks and to fixed wavelength peaks. The correlation data for concentration and area/height for the different methods was examined. It was concluded that manual adjustment of the start

wavelength and end wavelength of a peak in an absorbance spectrum without any further processing gave the best correlation. This method was then chosen for the rest of the integration in this chapter.

Enrichment profiles for the other three PAHs were then investigated. It was concluded that good correlation existed between concentration and peak area for these compounds. For simultaneous analysis of these compounds an optimum enrichment time of 10 minutes was chosen. This was based on the correlation data gathered for each individual compound. This data was then used to calculate the line equation for each individual compound. These equation took the form $Y = MX + C$.

Spectral complexities due to the number and nature of peaks being investigated were discussed and a ratio method was devised which enabled naphthalene and acenaphthene to be quantitated.

All this information was then applied to the analysis of mixtures of these four compounds at various concentrations. This was carried out in two separate concentration ranges 50-100ppm and 5-10ppm. It was concluded for the 50-100ppm range that the method could be used to quantitate the four individual PAHs simultaneously. The 5ppm range for most of these compounds proved to be below the limit of determination. Most compounds were detected at the 10ppm concentration, phenanthrene was the exception. It was also concluded that interference effects were difficult to define at this low concentration.

Spiking experiments revealed a complex situation whereby each compound acted in an individual manner. It was concluded that the technique was suitable for predicting the concentrations of the four PAHs at relatively high concentrations, 50-100ppm simultaneously.

The coated ATR/FTIR as an enrichment technique has many advantages over the standard extractive techniques used to estimate the concentration of PAHs in aqueous solutions. No sample preparation is required, it is possible to place the sample directly onto the coating. Thus there is no chance of the analytes being lost during any sample manipulations. No chromatographic separation is required, it is possible to analyse quantitatively for the four PAHs simultaneously.

The main disadvantage of the technique as presented here is the lack of sensitivity. It must be noted that the removal or reduction in ACN content of sample would cause an increase in sensitivity. The use of an optical fiber based system could also enhance the technique considerably.

6.5 Bibliography.

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Chapter 7.0

General Discussion

7.0 General Discussion.

Various techniques were used to extract low MW PAHs from aqueous solutions. These PAHs are relatively volatile and therefore would be the most sensitive to extractive procedures.

It was attempted initially to extract PAHs from spiked water samples using a conventional liquid/liquid extraction. The main advantages of this technique were the fact that large volumes of samples could be used and the fact that no sophisticated instrumentation was required. However the technique proved to be laborious and irreproducible. Recoveries ranged from 88.8% to 55.8% for naphthalene and 126.6% to 63.7% for phenanthrene. The reason given for the techniques poor performance was the fact that a solvent replacement step was required prior to analysis and thus these compounds were susceptible to losses.

An offline solid phase extractive system was assessed for the extraction of these compounds from aqueous samples. The offline solid phase extractive system removed the need for a solvent replacement, which is a major advantage over the liquid extractive process. In this case however large variations were noted for the recovery of the low MW PAHs. % CVs varied from 15.5% for acenaphthene to 2.24% for phenanthrene. The main reason given for these inconsistencies was the fact that the flow rate during sample loading and elution could not be controlled accurately. The lighter PAHs gave less repeatable results than the higher MW compounds this is possible due to the fact that the lighter compounds are more susceptible to washing effects.

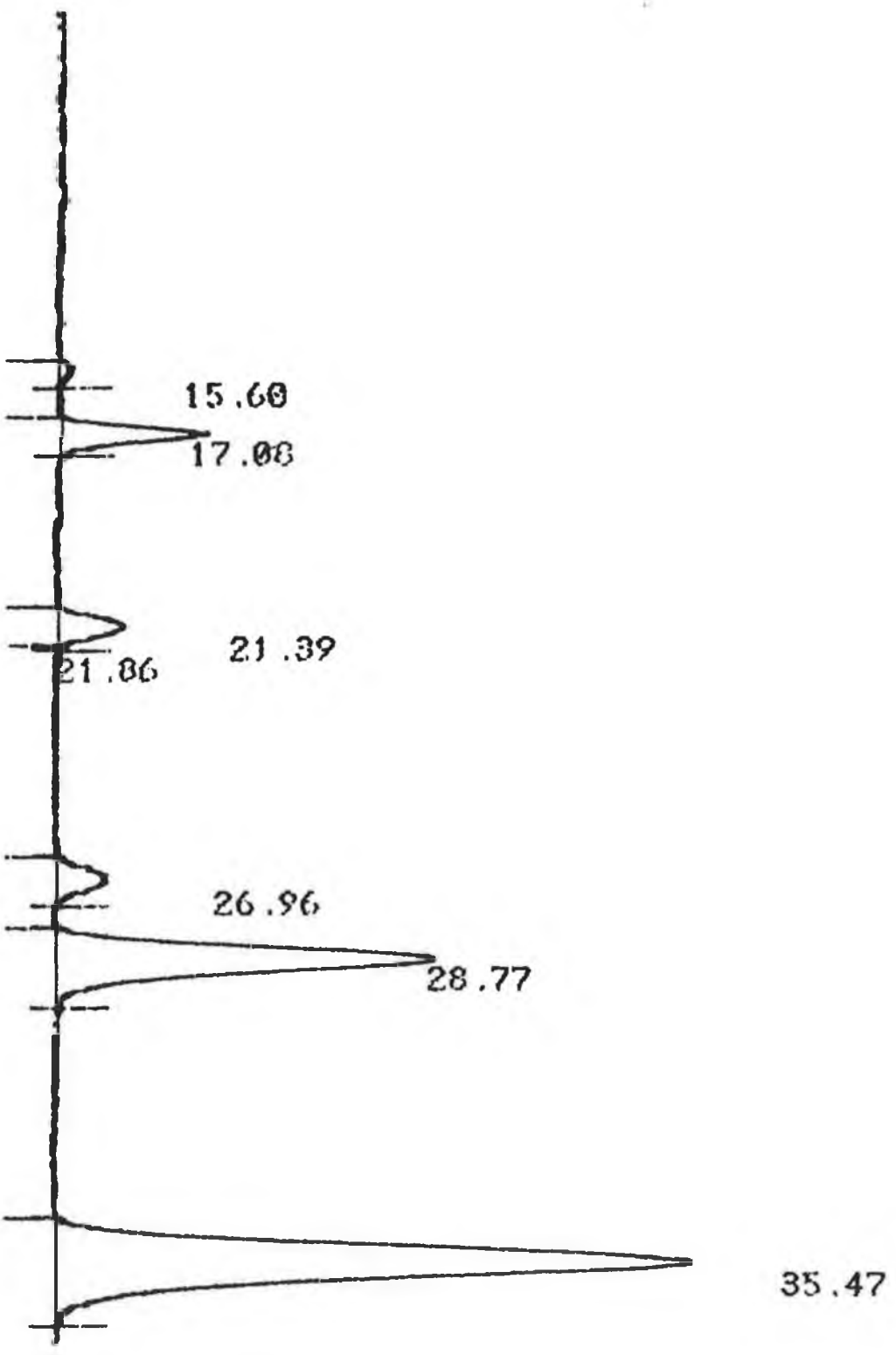
An online solid phase extractive system was developed which proved itself to be reproducible and sensitive when enriching low MW PAHs from aqueous samples. LODs for this system were measured at 0.18 –0.01ppb. The column switching setup made the

enrichment process very easy to use. It was possible to extract directly from the sample onto the precolumn without any need for sample preparation. The loading and elution flow rates were controlled via HPLC pumps leading to consistent enrichment. The main draw back of this technique is the fact that it is only possible to conduct one extraction at a time. Instrumentation has been developed which makes it possible to conduct a number of offline extractions with a controlled flow rate using a vacuum based system.

A novel extractive process known as MSPD was used to extract low MW PAHs from spiked milk samples. The main advantage of this technique was its simplicity, a normally complex and difficult matrix milk was dealt with easily. This was because of the fact that the mixing of the C_{18} material lysed the fat cells and allowed interaction of the analyte with C_{18}. The main disadvantage of this technique was the variation in results noted. The main reason for this being that difficulty was encountered controlling the loading and elution flow rates.

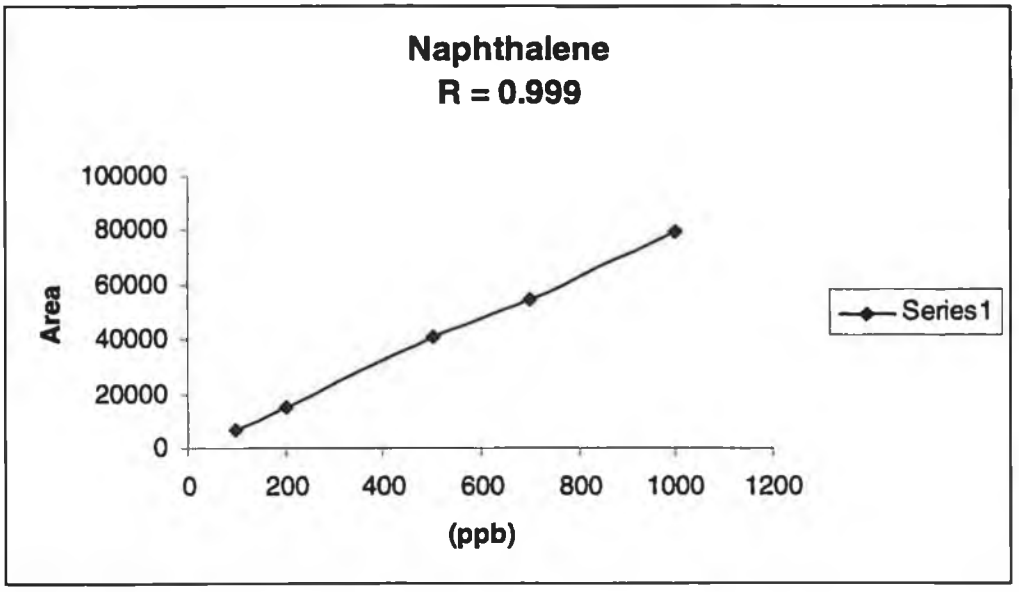
A mid IR sensor was developed using a coated ATR waveguide. This sensor was then used to extract four PAHs simultaneously from an aqueous sample. This proved to be a very effective technique as no sample preparation was required the sample was simply placed on the coating surface and no chromatographic separation was required to quantitate the amount of analyte present. Quantitation was calculated based on the absorbance of specific IR peaks in the 700 to 1000cm^{-1} region. Although not as accurate or reproducible as the HPLC techniques it is still a very promising technique as almost real time information can be gathered from a sample. The main disadvantage of the technique was its lack of sensitivity compared to the chromatographic methods. However this problem could be overcome if the amount of ACN in the sample was reduced.

Future possible work in this area would include moving from the crystal based system described here to an optical fiber based system. These types of systems increase dramatically the number of reflections within the waveguide thus making the system more sensitive. Sensitivity can be further improved by reducing the diameter of the fiber. The speed of enrichment would also improve due to the reduction in polymer layer thickness. The optically active fiber would also make the system very suitable for adaptation to remote sensing instrumentation and thus could used as a real time alarm system for these compounds at low concentrations.



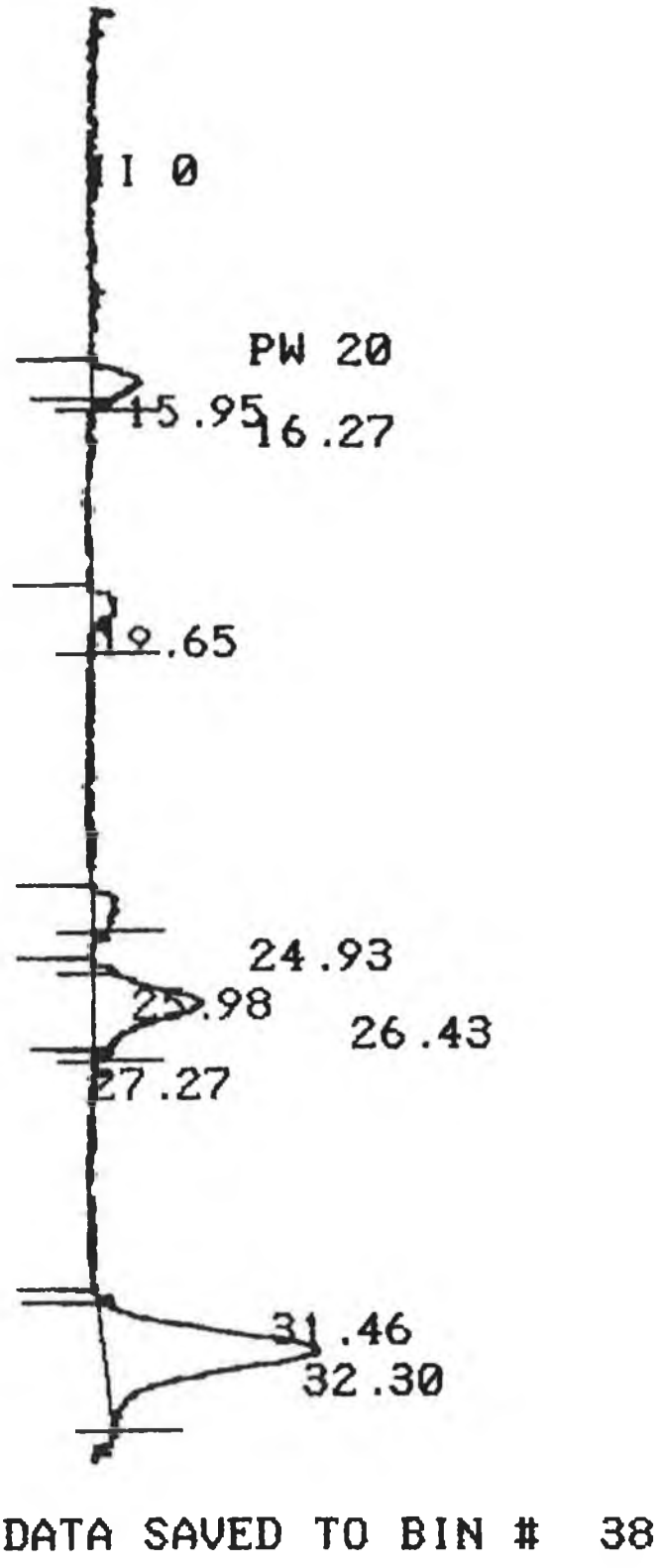
Appendix 1

Chromatogram which shows the optimum separation for the five PAHs, naphthalene, acenaphthylene, acenaphthene, fluorene and phenanthrene.



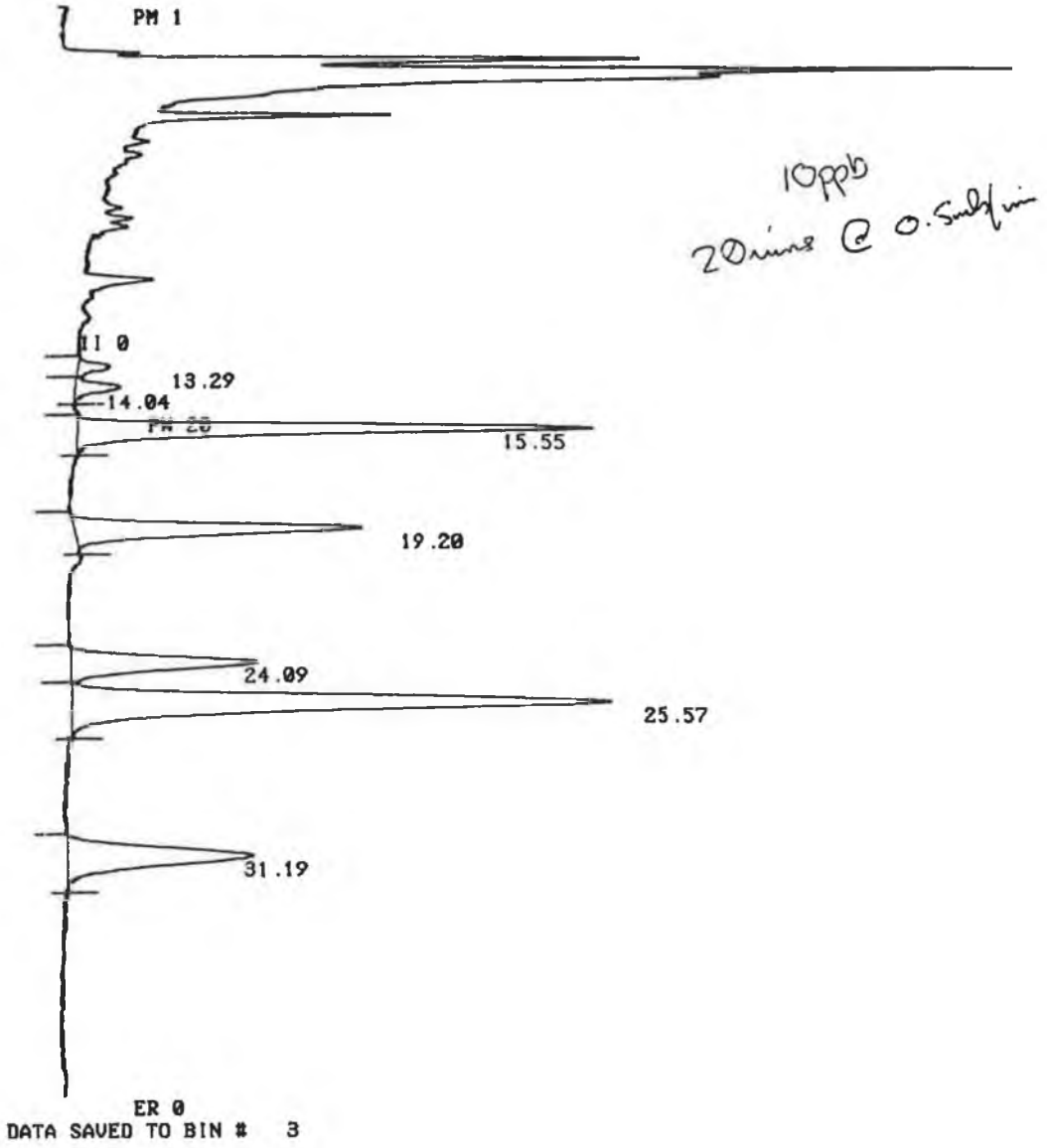
Appendix 2

Plot of area versus concentration for naphthalene with the correlation coefficient R calculated.



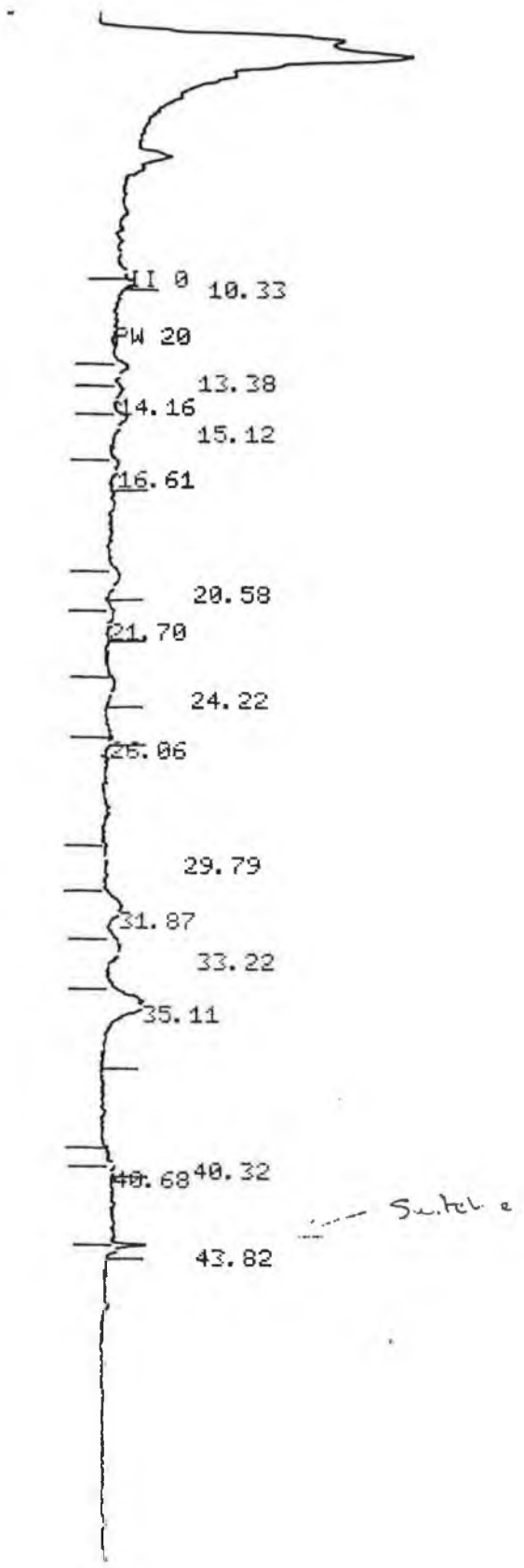
Appendix 3

Chromatogram showing the poor peak shape of acenapthylene and acenaphtene at low concentrations.



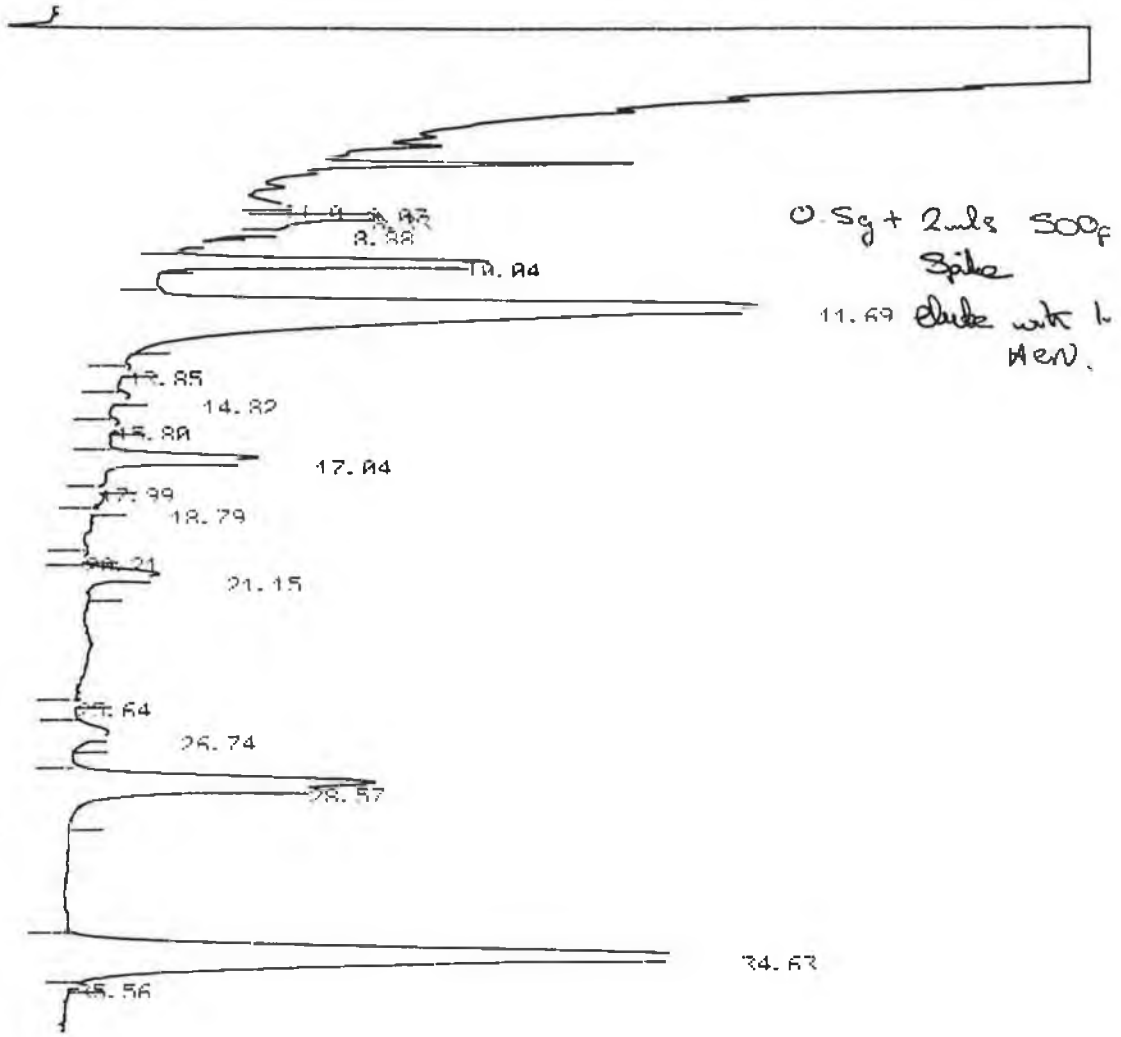
Appendix 4

Chromatogram which demonstrates the enrichment of the five PAHs using online column switching.



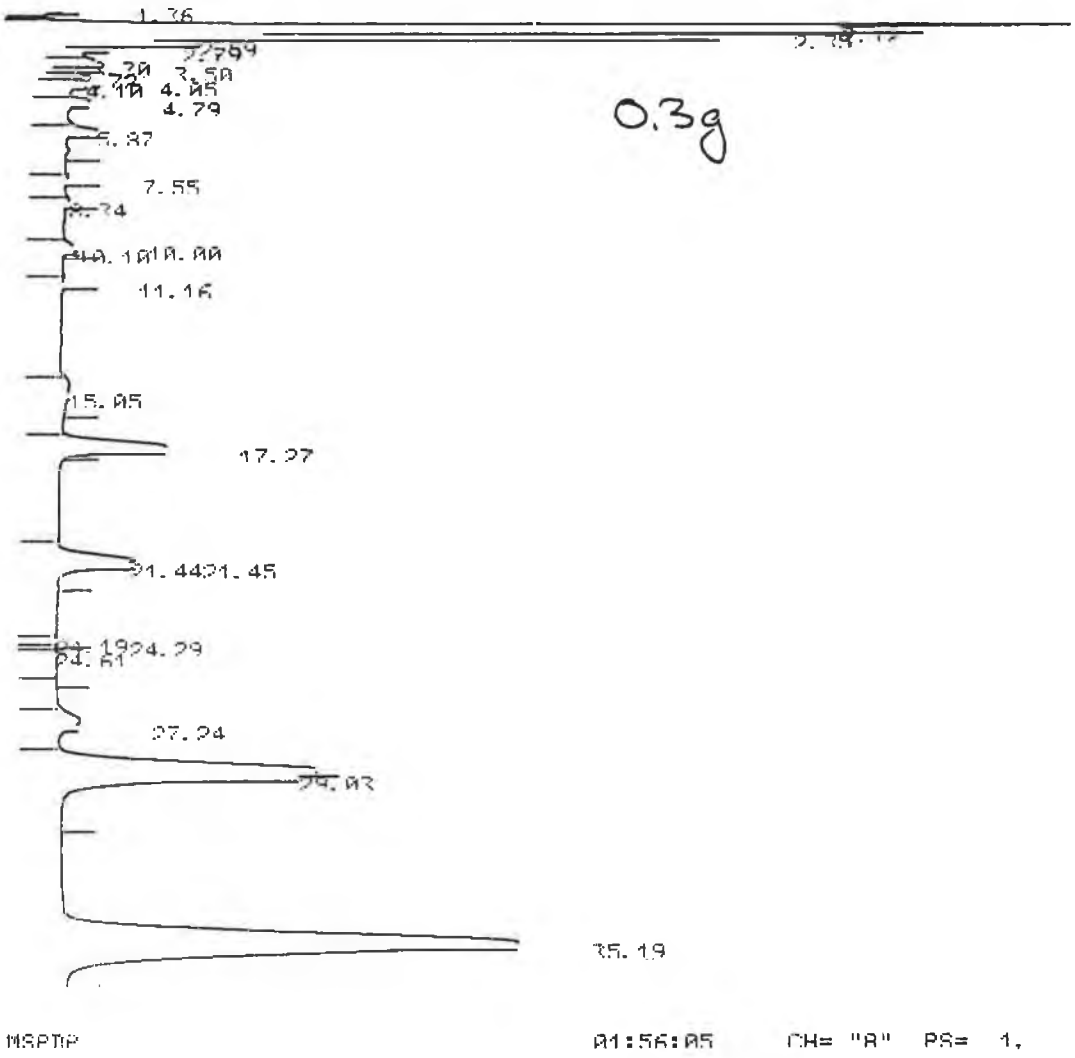
Appendix 5

Chromatogram of a water blank which had been enriched onto the online system.



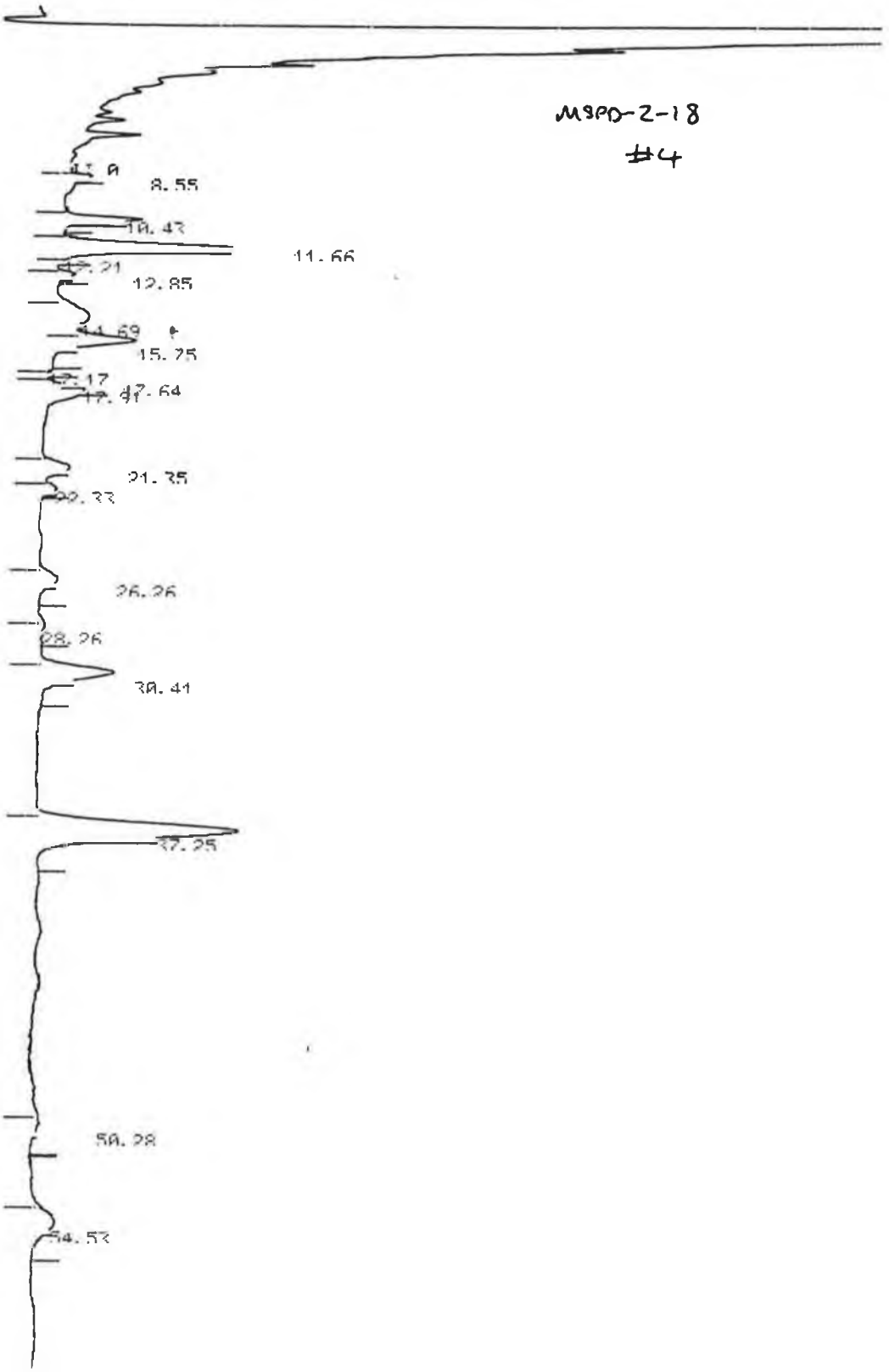
Appendix 6

This chromatogram shows a peak at 25.6 minutes which can interfer with acenaphtene.



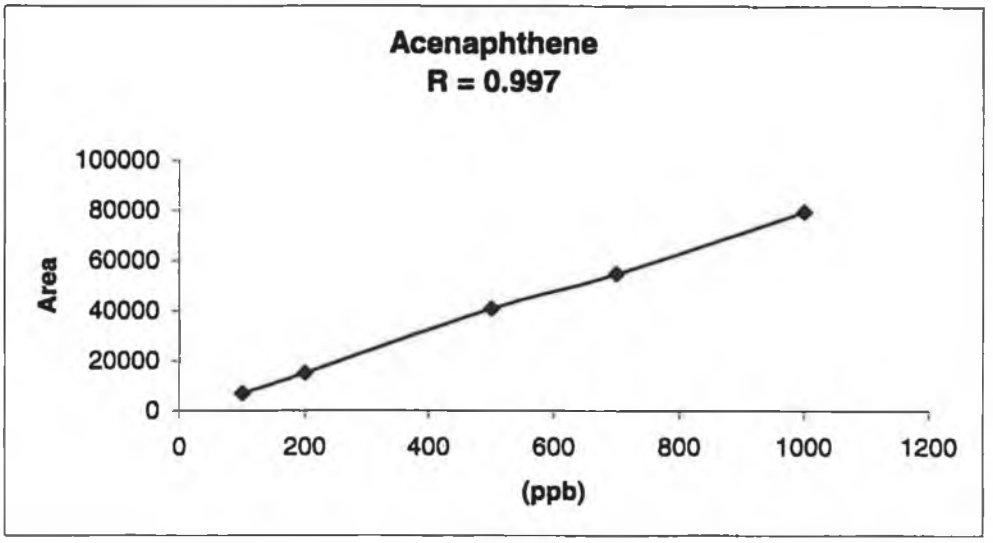
Appendix 7

Chromatogram for the extraction of five PAHs using MSPD at a concentration of 2ppm.



Appendix 8

Chromatogram for a 500ppb mix containing five PAHs which had been extracted using multi solid phase dispersion on C_{18} material. This chromatogram demonstrates the poor peak size for acenaphtene at this concentration.



Appendix 9

Plot of concentration versus area for acenpthene with the correlation coefficient R. This data was used to calculated percentage recoveries for the MSPD experiments.