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ON-LINE SAMPLE PRETREATMENT OF ENVIRONMENTAL SAMPLES FOR USE WITH ATOMIC SPECTROMETRY

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ON-LINE SAMPLE PRETREATMENT OF ENVIRONMENTAL SAMPLES FOR USE WITH ATOMIC SPECTROMETRY

BY

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Abstract

"On-line sample pre-treatment of environmental samples for the use with atomic spectrometry"

James Robert Murphy

The objective of this study was to develop novel techniques for on-line sample pre-treatment for use with atomic spectroscopy. Preconcentration of the analytes using either complexation of the analyte(s) on an analytical column or by in-situ (in atomiser) trapping on a pre-coated graphite tube has been used. The samples were manipulated using flow injection analysis and detection achieved in most cases by ETAAS. All aspects of the study are supported by reference to the literature.

Using the above approach, a new method has been developed to determine mercury in environmental (sediment) and biological (tuna fish) samples. This approach successfully achieved a sample throughput of 20-30 sample per hour, with a method detection limit of 0.2 ng g^{-1} (3σ) and a precision of less than 10% at the 0.1 $\mu g\ g^{-1}$ level. An interference study was conducted and seven elements (As^{V}, Cd^{II}, Cu^{II}, Ni^{II}, Pb^{II}, Sb^{V} and Se^{VI}) shown to give less than a 5% interference when the interferent concentration was 2 orders of magnitude greater than the Hg. Silver showed a 9% interference when one order of magnitude greater than the Hg.

The technique of "in atomiser trapping" was applied to the determination of As, Bi, Sb and Se, in lake water samples. The hydrides were sequestrated upon an Ir coated graphite tube and the instrumental and chemical parameters optimised for multi-element determinations. Iridium has been identified as the best trapping material (coating) for multi-element determinations. The final method gave detection limits of 0.82, 0.04, 0,26 and 0.29 $\mu g\ l^{-1}$ (500 μl sample loop) for As, Bi, Sb and Se respectively. A characteristic mass of 177 pg for As, 91 pg for Bi, 107 pg for Sb and 90 pg for Se was achieved. Good agreement was obtained with certified and standard reference materials and the method was successfully applied to the determination of As, Bi, Sb and Se in lake water samples.

Six elements (Cd, Cu, Fe, Mn, Ni and Pb) were selected for determination in seawater samples by multi-element on-line column preconcentration. The sample stream was mixed on-line with a buffered solution of diethylammonium dithiocarbamate (DDDC), and the metal-DDC complex retained upon a C_{18} RP silica column. Ethanol was then used to elute the metals from the column directly into the graphite tube. Disappointing results, were obtained due to interference problems, and although more work is required before this approach may be routinely used, it is shown to have potential for the future.

Overall, this study has shown that novel methods employing flow injection methodologies for separation and preconcentration are a viable way to prepare environmental sample for analysis by ETAAS. Further it has demonstrated that multi-element analysis is possible for low levels of analyte despite the presence of troublesome matrices, although further work is required to achieve the ultimate goal of a universal method suitable for all analytes irrespective of the sample type.

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Authors Declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other university award.

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Relevant scientific seminars and conferences were regularly attended at which work was often presented; external institutions were visited for consultation purposes and several papers prepared for publication.

Signed ..... J. Murphy .....

Date ..... 29th May 1998 .....

Chapter One : Introduction

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1.1 Analysis of environmental samples

Environmental science is concerned with chemical, physical and biological changes in the environment and the effect of these changes as perceived by man. Air, water, soil, food and waste are all affected by anthropogenic factors often as a result of human activities. Many of these processes are essentially chemical in nature and thus can be monitored to assess the environmental quality. Analytical methods provide the basis for detailed study.[1] It is the development of such analytical methods that is the basis of this thesis.

The environmental sample to be analysed can be in one of three forms :- gas, solid or liquid and include a multitude of matrices. The possible matrices include atmospheric aerosols and particulate matter, geological samples (*e.g.* soils, rocks), biological samples (*e.g.* blood, tissue and plant foliage) and marine samples (*e.g.* seawaters and sediments) all of which need different sample preparation and pretreatment methods. There are 90 naturally occurring elements (66 being metals or metalloids) on the earth and the chemical form (speciation) of which can vary widely between samples.[2]

All trace elements occur to a varying extent within all components of the environment. The term pollution of the environment is often used to refer to an increase of trace elements relative to the natural levels. This is generally associated with human activities, in particular industrial and agricultural practices which result in widespread trace element contamination.[2]

1.1.1 Trace and ultra trace element determination

The determination of trace elements is necessitated by their importance in the chemical industries and in the environment. While the specific needs of the various areas may all be different, they all have in common the requirement to quantify the presence of trace elements. Therefore the measurement of elements (metals and metalloids) over a range of concentrations from percentage levels through to trace (parts per million) and ultra-trace (parts per billion and below) require the techniques that compromise atomic spectroscopy.[3]

There is some confusion when trying to define exactly what is regarded as trace and ultra trace elements. Howard and Statham,[4] concluded that trying to define in terms of concentration was doomed to failure and stated

"Trace Analysis : An analytical procedure requiring special steps to be taken because of the low concentration of the analyte which is present."

Vandecasteele and Block,[5] define trace element analysis as the determination of concentrations below 100 $\mu g\ g^{-1}$ and ultra trace analysis as the determination of concentrations below 10 $ng\ g^{-1}$, while Fifield and Kealey,[6] define the constituents of a sample as stated below :

Major	> 10%	Trace	1-100 ppm
Minor	0.01-10%	Ultra trace	<1 ppm

For this study, the Fifield and Kealey definition will be used. Quite often the analyte or element of interest is present in the sample at a level which is

below or close to the limit of detection of the instrument being used. In this situation, unless an alternative more sensitive technique can be used, there is a requirement to preconcentrate the analyte. This situation is most likely to occur for aqueous based samples. In the case of solid samples, it may be possible to digest more sample. Various approaches to trace element preconcentration are available, and in addition to preconcentration, it may be necessary to carry out a separation process to remove an interference or to distinguish between the different chemical forms of an element *e.g.* oxidation states.[7]

1.2 Sample pretreatment of environmental samples

Sample preparation is probably the single most neglected area in analytical chemistry. While the level of sophistication of the instrumentation for analysis has increased significantly, a comparatively low technical base for sample pre-treatment often remains.[8] Although great progress has been made in the development of highly selective and sensitive analytical methods, the analytical chemist is increasingly called upon to deal with complex samples. Also lower and lower detection limits are often required. Preconcentration or separation steps may therefore still be necessary even in combination with highly selective and sensitive methods.

If it were possible to identify or quantitatively determine any element or compound by simple measurement, no matter what its concentration or complexity of the matrix, then sample pretreatment techniques would not be needed.[9] However, as this "ideal" situation does not exist, usually because other sample constituents hinder the measurement of the analyte, real world solutions are required.

Since real environmental samples cover the whole range from relatively simple matrixes *e.g.* determining alkali and alkaline earth metals in drinking water to complex matrices *e.g.* determining rare earth elements in geological rock samples, there must also be a range of sample pretreatment methods to assist the analytical chemist.

It is normal in atomic spectrometry for the sample to found in one of two forms *i.e.* solid or liquid. The latter case, would seem to be the easiest form in which to handle the sample. However, the need to carry out the determinations at lower and lower levels means that invariably some form of preconcentration is required. On the other hand, if the sample is in the solid form, the normal requirement is to convert the solid material (biological, environmental or geological) into solution. Conversion of a solid matrix into a liquid matrix involves the decomposition and dissolution of the sample. The major problem with preparing samples for trace metal analysis is the risk of contamination.[7]

Where a homogenous sample already exists, it may be subdivided without further treatment. With many solids such as ores, however, crushing and mixing are usually a prior requirement before analysis. In addition, the sample often needs further preparation for analysis, such as drying, ignition and dissolution[6] before the decomposition step.

1.2.1 Sample pretreatment for solid samples

Decomposition of solid samples is an important part of sample pretreatment for atomic spectrometry. Thus a sample dissolution step must often be performed before analysis can take place. Element preconcentration and chemical separation steps may also be required to improve the quality of the

measurement.[11] The solid sample can be digested and brought into solution in a number of ways, *e.g.* wet decomposition (open or closed systems), dry ashing (open or closed) or fusion with a fused salt flux.

Wet decomposition in an open system is one of the oldest techniques used, while wet decomposition in a closed vessel and with microwave heating has become popular, due to the fact that this approach can be automated to handle a large number of samples. The fusion method is of limited use due to the high blank values introduced from the reagents and vessels used, but for some samples, it still is the best and only way.

1.2.1.1 Wet decomposition for solid samples

Wet decomposition methods involve the addition of mineral and or oxidising acid(s)[7] to acid resistant PTFE (polytetrafluoroethylene), PFA (perfluoralkoxy vinyl ether) or quartz vessels, containing the sample. Most inorganic and some organic materials will dissolve in the acids, and often the sample matrix is destroyed, which reduces or eliminates any possible interferences due to the matrix. The acids used in the digestion mixture have to be of a sufficiently high purity to minimise the blank value. Sample dissolution by concentrated acids results from a number of factors. These include the high proton concentration, the oxidising action of the acid (if any) and in some instances specific reactions or strong complexation with the acid.[11]

For wet decomposition in open vessels, systematic errors can occur due to the fact that some elements will volatilize during the decomposition and contamination can occur due to the open vessel. This can be avoid by using a

pressure digestion system, *i.e.* a wet decomposition method but utilising closed digestion vessels.[10]

Compared with the open vessel system,[10] the closed system has several advantages :-

i) no volatilisation losses,

ii) no contamination from external sources,

iii) shorter reaction times and improved decomposition due to higher temperatures, (above boiling point of digestion acid)

iv) reduced reagent consumption, and

v) nitric acid can be used as a general purpose digestion reagent. It is also available in a high purity state, therefore reduced blank values are obtained.

1.2.1.2 Microwave assisted digestion for solid samples

The microwave dissolution technique makes it possible to speed up the acid dissolution process due to the rapid heating ability of microwave systems. Microwaves can be applied to open systems, but it is more advantageous to use closed microwave systems, which allow higher temperatures to be reached and prevents the loss of the analyte during the dissolution process.[10] Potentially, this type of digestion system can be easily automated allowing for a higher throughput of samples than for conventional hot plate based digestion methods. With certain systems,[7] the pressure and temperature inside the digestion bomb can be measured, helping to ensure the optimal digestion conditions are reached and maintained.

1.2.1.3 Dry ashing for solid samples

Dry ashing in open vessels is the simplest digestion method, whereby the sample is placed in a muffle furnace in the presence of air at 400-800°C to remove the organic constituents. This method can be subject to the loss of certain elements *e.g.* As, B, Ca, Cd, Cr, Cu, Fe, Hg, In, Pb, Se, Te and Zn.[12] However, due to its rather limited applications, dry ashing has largely been replaced by other methods.

Low temperature ashing,[11] can eliminate some of the problems associated with normal dry ashing. The decomposition is performed in the presence of oxygen molecules at a low pressure (1-5 Torr). The excited singlet state oxygen, which has a lifetime of about one second, is produced by a radio frequency field (13.5 MHz). Once, produced, it is then rapidly passed over the sample, which is surrounded by a cooling jacket to help retain any volatiles that may be formed. The method has been successfully used for the hydride forming elements but Hg is usually lost. The method is free from contamination as the oxygen can be cleaned before use, but the method can take days for a complete digestion of the sample.

1.2.1.4 Closed system combustion for solid samples

When the determination of volatile elements is required, combustion with oxygen in a closed system may be chosen, as the elements are absorbed in a suitable solvent after combustion.[10]

The apparatus suggested by Schoniger[13] consisted of a glass flask with ground glass stopper, which had attached to it, a Pt gauze basket which held the

sample. Solid samples are wrapped in low ash filter paper. A small volume of absorbing solution is placed in the flask. During combustion the flask is inverted to absorb the volatiles in the absorbing solution.

1.2.1.5 Fusion of solid samples

For certain substances, such as silicates and mineral oxides,[7] liquid decomposition methods attack the sample very slowly or not at all. Therefore, stronger and more potent methods are required which use fused salt media or fluxes as the digestion agents. The fluxes will decompose most substances in the high temperature range of 300-1000°C.

Using this approach, the sample is in the form of a very fine powder which is then mixed with an excess of flux in a graphite or platinum crucible. The crucible is then placed in a hot muffle furnace until the "melt" is complete. The production of a clear melt signals a complete decomposition. After cooling the melt is dissolved. Basic fluxes for attacking acidic material include carbonates, hydroxides and borates, while acid fluxes such as pyrosulfates can be used. If an oxidising flux is required, sodium peroxide is used. Acidic and basic fluxes are dissolved in basic or acidic medium respectively.[10] However, the final aqueous solution has the disadvantage of having a high salt concentration which may affect the final measurement.

1.2.2 Sample pretreatment techniques for liquid samples

The formation of a precipitate or the chelation of a metal from an aqueous solution can provide a simple means of isolating a selected analyte from the bulk

matrix of a sample. These chemical techniques can in many cases provide analyte enrichment as well as isolation. Extra advantages such as the minimisation or elimination of matrix effects and the lowering of detection limits can allow the application of less complex instrumentation or methods in the final measurement step and may allow for automated analysis.[10] The most important off-line preconcentration techniques for metal ions are liquid - liquid extraction and ion exchange. Other techniques include chelation and co-precipitation of the desired metal analyte.[10] Such preconcentration and separation techniques are aimed at exploiting differences in physico-chemical properties between the various compounds in the mixture / sample.[9] Volatility, solubility, charge, molecular size, shape and polarity are the most useful in this respect. A change in phase, as occurs during distillation, or the formation of a new phase as in precipitation, can provide a simple means of isolating a desired component. Usually, however more complex separation procedures are required for multi-component samples. Most depend upon the selective transfer of materials between two immisicble phases.[9]

Many analytical measurements are subject to interferences from other constituents in the sample. Newer methods increasingly employ instrumental techniques to distinguish between analyte and interference signals. However such distinction is not always possible and sometimes a selective chemical reaction can be used to mask the interference. If this approach fails, the separation of the analyte from the interfering component will become necessary. Where quantitative measurements are to be made, separations must be quantitative or give a known recovery of the analyte.[6]

A report by Leyden and Wegscheider,[14] gives a good overview of some of the many aspects of preconcentrating and determining trace elements in aqueous

solution, while an extensive review by Torre and Marina,[15] explores the state of the art of sample pretreatment with ligand loaded complexing resins, characterising and studying the applications of such resins.

1.2.2.1 Solvent extraction for liquid samples

Solvent extraction is one of the most commonly used separation and preconcentration techniques. The basic requirement for a solute to be extractable from an aqueous solution is that it is uncharged or can form an uncharged ionic compound. A complex is formed with the element of interest, in the aqueous solution, which will partition into an insoluble or sparingly soluble solvent.[11] Charge neutrality reduces the electrostatic interactions between the solute and water, and hence lowers the solubility of the solute in water. Extraction into a less polar organic solvent is facilitated if the species is not hydrated, or if the co-ordinated water is easily displaced by hydrophobic co-ordinating groups such as bulky organic molecules.[10]

The reason for the wide use of extraction in combination with flame atomic absorption spectrometry (FAAS) is the improvement in sensitivity arising partly from the increased element concentration, but partly because nebulization of organic solvents can produce a more finely dispersed aerosol,[16] and the solvent acts as secondary fuel. With electrothermal atomic absorption spectrometry (ETAAS), however, the use of organic extracts instead of aqueous solutions does not, as a rule, enhance the atomic absorption signal. Nevertheless, the hybrid method (ETAAS with solvent extraction) has the advantage of higher sensitivity than that of the FAAS method. The work of Volynsky *et al.*[16] discusses the

problems of rational combination of the solvent extraction of trace metals with determination by ETAAS.

The most important experimental variables in the extraction of metals are the organic solvent, the pH of the aqueous solution, the chelating agent and the use of masking agents.[10] When the substance of interest is extracted, a solvent denser than water is often required. However, when the interferences are extracted and the substance of interest remains in the water, a solvent less dense than water is preferable. This then allows for easy removal of the phase containing the analyte of interest from the separating funnel. Sometimes the metal ions are re-extracted from the organic phase into the aqueous phase at an appropriate pH,[17] but it is often also possible to measure the metal concentration directly in the organic phase.

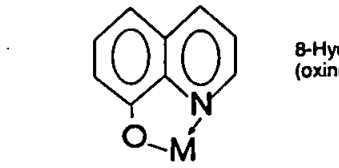
There are three types of chemical compounds that fulfil one or more of the requirements for efficient solvent extraction :-

i) *Neutral molecules* :- These are essentially neutral covalent molecules / complexes and are found naturally in waters,[10]

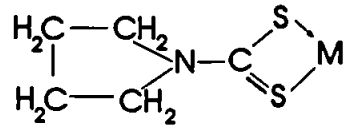
ii) *Ion associated complexes* :- These complexes are only possible if the charge is neutralised by chelation or by association with other ionic species of opposite charge. A further requirement is that at least one of the ions contains a large hydrophobic group,[10] and

iii) *Uncharged metal complexes* :- Such as metal complexes with organic ligands *e.g.* 8-hydroxyquinoline (8-HOQ), ammonium pyrrolidinedithiocarbamate (APDC)[18] and diethyldithiocarbamate (DDC).[19] Also a mixture of complexing agents (APDC and DDC)[20,21] can be used to improve the complex stability and broaden the effective working range.

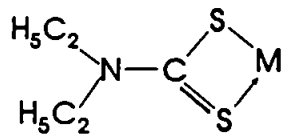
The use of organic ligands which form uncharged metal complexes is the most important route for obtaining chemical compounds which can be solvent extracted from aqueous solutions. The element chelates formed must be apolar to be efficiently transferred into the organic phase. If the chelating molecules fully balance the charge of the ion then transfer to the organic phase should occur.[11] The co-ordination arrangements of several of the most commonly used complexing agents are shown in *Figure 1.1*. For clarity, only one of the chelating groups are shown around the central metal.



8-Hydroxyquinoline
(oxine; 8-quinolinol)



Ammonium pyrrolidine
dithiocarbamate (APDC)
(ammonium ions displaced
by metal in figure)



Diethylammonium
diethyldithiocarbamate
(diethylammonium group
displaced by metal
in figure)

***Figure 1.1* : Common metal ion chelating complex used in solvent extraction**

1.2.2.2 Ion exchange techniques for liquid samples

Ion exchange techniques are well suited for both the separation of ions of opposite charge (cation - anion separations[22]) and for the separation of ions of like charge (for matrix - trace metal separations[23] and mixtures of trace metals[24]). In an ion exchanger, functional groups are immobilised on some type of solid substrate, thus providing the potential for either batch extraction of ions from solution or for using the ion exchange material in a packed column for off-line and on-line applications. Ion exchange resins based on organic polymers are also frequently used. These consist of a polystyrene polymer crosslinked with divinylbenzene and treated to introduce the ionic functional groups. The functional group introduced on to the polymer, will determine which type of exchanger is produced.[10] Examples are given in *Table 1.1*. The general properties and techniques, and some of the many applications of the ion exchange resins are described in detail elsewhere.[25]

Type of Exchanger	Functional group	
Strong cation	sulfonic acid groups	$-SO_3H$
Weak cation	carboxylic acid groups	$-CO_2H$
Strong anion	quaternary ammonium groups	$-N(NH_3)_3Cl$
Weak anion	amine groups	$-NH_2Cl$

Table 1.1 : **Examples of functional groups used with ion exchange resin**

An ion exchange resin operates on an ion association basis, and selectivity and partitioning are a frequent problem. When the sample contains a large excess of alkali metals ions, these ions could compete for the sites on the exchanger material. In situations were the alkali metals are not of interest, a chelating ion exchange resin can be used.[14] These resins contain functional groups which form chelates with the metal ions. A commonly used material is Chelex-100,[26,27] which contains iminodiacetic acid functional groups with a chemical behaviour very similar to ethylenediaminetetraacetic acid (EDTA). The range of metals that can be complexed with this material is large and the complex behaviour can be predicted by analogy with EDTA.[10] Iminodiacetic acid groups are not the only chelating materials attached to a solid support material. Another commonly used material, is an organic chelating agent used in solvent extraction system, namely 8-hydroxyquinoline which can be bonded onto a glass or silica support.[28]

1.2.2.3 Evaporation of solvent from liquid samples

The simplest method[10] for preconcentrating a liquid sample is to evaporate off the solvent leaving behind the analyte of interest which can be reconstituted in a smaller volume of liquid. This method was used by Thompson *et al.*[29], to preconcentrate a suite of 30 metals for simultaneous multi-element determination by inductively coupled plasma-atomic emission spectrometry (ICP-AES), thus facilitating the determination of a wider range of major and trace metals than would have been possible by direct nebulization in the ICP-AES.

1.2.2.4 Removal of volatile elements from liquid samples

Where the sample is more volatile than the matrix, or where it can be converted to be so, then this property can be used to good effect in a separation procedure.[11] Elements which can be separated by this type of procedure are mercury and the hydride forming elements (As, Bi, Ge, Pb, Sb, Se, Sn and Te).

Mercury is a special case in that it has a significant vapour pressure at room temperature. Thus unlike other metals, when ionic mercury is reduced to its metallic state, the mercury becomes a vapour which can be flushed from the remaining sample matrix by a stream of inert gas. The reductant used can be either tin^{II} chloride or sodium tetrahydroborate. The mercury vapour can then be measured directly by cold vapour atomic absorption spectrometry (CVAAS) or atomic fluorescence spectrometry (AFS) or it can be preconcentrated or amalgamated on a gold trap such as fine gold metal gauze, or as a gold coating on a solid support material. After preconcentration, the mercury can be quantitatively removed from the gold by rapid and reproducible heating and the resulting mercury pulse can be measured as before.[11]

For the analysis of elements such as As, Bi, Ge, Pb, Sb, Se, Sn, and Te which form volatile covalent hydrides,[11] a similar procedure can be used. First, the element reacts with the sodium tetrahydroborate to produce the hydride, which can then be flushed out of the sample matrix and measured by heated quartz tube / furnace hydride generation atomic absorption spectrometry (QF-HGAAS) or by in situ trapping where the hydride is concentrated upon a precious metal coated graphite tube. This subject of hydride generation has been covered comprehensively in a book by Dedina and Tsalev,[30] and in situ trapping has been reviewed by Matusiewicz and Sturgeon.[31]

1.2.2.5 Precipitation of trace metals from liquid samples

Most precipitation methods will concentrate a group of metals and great effort has been put into finding specific precipitation agents to remove single metals from the matrix whilst leaving the others (*i.e.* to prevent co-precipitiation) behind so as not to interfere with the measurement.

For trace metal determinations,[10] the solubility of many common precipitates is too high to allow quantitative precipitation of the ions of interest at concentrations less than a few $\mu g\ g^{-1}$ (*i.e.* down to trace and ultra trace levels). For example, nickel in this concentration range, cannot be precipitated quantitatively with dimethylglyoxime.[14] However, it is possible to achieve quantitative results at these low concentrations by adding a co-precipitant, which will not only be precipitated itself but will aid the recovery of the ion of interest. The co-precipitation agent is usually a metal sulphide or hydroxide.[32]

1.3 On-line versus Off-line sample pretreatment techniques

Despite the progress made in analytical instrumentation in the past few decades, the development of basic analytical techniques in the chemical laboratory has been rather slow and incompatible with requirements of the computer age. The sample pre-treatment stage, which often involves manual separations such as extraction, sorption, precipitation and distillation, quite frequently form the weakest link of the entire analytical procedure, not only in terms of efficiency but also in reliability and sample / reagent consumption. It is therefore mandatory that more effort be directed towards the development of efficient automated on-line sample pre-treatment techniques which could remove

the bottleneck. The flow injection technique has proved to be an effective tool for achieving such goals. Originally considered to be merely a technique for automated serial assays, flow injection analysis has gradually evolved into a powerful technique for substituting tedious manual separation procedures by producing stronger contacts between chemistry and the analytical instrument. In fact, this has now become one of the most active fields in automated continuous flow analysis, persistently stimulating further interests in revolutionising conventional operations in the analytical laboratory.[33]

In real analytical situations, the sample introduction is an extension of sample preparation. As a consequence, the selection of a suitable sample introduction technique can depend heavily on available and effective sample preparation procedures.[34]

1.3.1 Off-line (Batch) sample pretreatment techniques

The analytical techniques used for sample pretreatment are mainly off-line or batch (as mentioned in *Section 1.3*) which have the disadvantage of being laborious, time consuming, operator intensive and often prone to contamination due to the many steps and the large amounts of reagents used. Sample preparation techniques where the sample has to be converted from a solid to a liquid are mainly handled using off-line methods, although on-line microwave digestion methods using open focused microwaves system may be used.

1.3.2 On-line sample pretreatment techniques

The traditional batchwise mode of sample pretreatment and handling of solutions is being replaced by continuous flow operation on a microscale. Flow injection (FI) methods of separation and preconcentration exhibit some extremely favourable features over their batch or even continuous flow counterparts. The general features are summarised below :- [25,33]

i) higher sample throughput,

ii) high enrichment efficiencies for preconcentration systems,

iii) low sample and reagent consumption,

iv) high reproducibility typically in the range 1-3% RSD,

v) simple automated operations allow implementation with continuous systems *e.g.* ICP-AES and FAAS, but also with discontinuous systems *e.g.* ETAAS, but the coupling may be more complicated,

vi) improved control and data handling by computers, and

vii) less operator input.

But in the specific case of FI methods with atomic spectrometry detection, the primary advantages[34,35] over a conventional system (*i.e.* direct nebulization) are :-

i) only a relatively small volume of sample is needed to achieve a signal comparable with continuous nebulization,

ii) because of the transient nature of the signal, exponential decay in the wash out process starts much sooner than with continuous sample

introduction. Consequently, the signals decays to baseline more rapidly than for the continuous sample introduction, and

iii) microsample handling combined with greatly enhanced sample throughput.

A feature which is considered a drawback of FI separations is the incomplete mass transfer between phases, which is usually considered unacceptable in batch separation methods. However, the incompleteness in physical and chemical processes is not a problem as flow injection analysis (FIA) systems are basically a technique for reproducible monitoring analytical signals under thermodynamically non-equilibrated conditions, and does not deteriorate the precision or sensitivity of the proposed FI techniques.[33]

Originally, FIA systems were designed for sample introduction to spectrochemical and electrochemical assays. However, due to its versatility, FIA is finding many different application in the area of atomic spectrometry, as highlighted by two books by Fang,[36,37] and reviews by many other workers.[38-40]

The first important discovery in the automation of laboratory process was made by Skeggs[41] in 1957, when for the first time the chemical reactions / processes took place in conduits instead of the usual discrete vessels. But the reactions were performed under equilibrium conditions, as air bubbles separated the flow to ensure homogenous mixing of the sample.

This segmented flow injection system was then superseded by a concept introduced by Ruzicka and Hansen,[42-43] using a non-segmented approach which has become the cornerstone of FIA systems. This area includes on-line sample pretreatment, which will be covered in more detail in the following section.

1.3.2.1 On-line microwave digestion

As mentioned in *Section 1.2.1.2*, microwave digestion techniques are potentially faster than conventional pressure wet digestion methods, but there is still a risk of contamination when transferring the digested sample from the microwave bomb into another vessel prior to the measurement step. If an on-line system could be used, then this risk would be negated and the whole process would be speeded up.

Microwave heating would be expected to be ideal for such continuous flow applications since it has the capability to rapidly heat liquids within a non-conductive tube *e.g.* PTFE.[44] However, such applications are not straightforward as indicated by Tsalev *et al.*[45] Work by Burguera and Burguera[46] has produced on-line microwave systems with FAAS and ETAAS.[47] The samples were mixed with the mineralization acids, before entering the PTFE reaction coil, which was situated in a domestic oven. For the FAAS application, the digested mixture was then sent directly to the nebuliser for measurement, but for the ETAAS application the mixture was sent through a gas diffusion cell to remove any gases produced during the mineralization step. The sample then passed through an ice trap to cool the sample down and condense the reaction vapours before final injection of a sample aliquot into the furnace.

1.3.2.2 On-line liquid-liquid extractions

Liquid-liquid extraction is one of the most frequently used separation techniques in analytical chemistry. Despite its indisputable effectiveness in the removal of interfering matrices, and the preconcentration of trace analytes, its popularity has been somewhat impaired under batch operation conditions due to the tediousness of the operation, which may also induce complications in trace analysis through contamination. A further nuisance is the solvent vapours released into the laboratory.[48]

The on-line extraction systems are readily automated, while the closed system greatly minimises contamination risks as well as the release of solvent vapours. An additional advantage not normally gained with other FIA sample pretreatment systems is that for solvent extraction, there is a much higher mass transfer between the two phases. This leads to higher sensitivities, lower sample consumption and better precision.[48] For an on-line system, the three most important factors[49] are the phase segmentation, extraction and phase separation. Phase segmentation divides the stream into alternating different phases, the extraction phase allows the two liquids to come together and allow the analyte to reach a state of thermodynamic equilibrium (usually in an extraction coil) and then finally the phase separation where the two phases are split, *i.e.* the organic phase is used for the measurement while the aqueous phase goes to waste. This process has been discussed in more detail by Fang.[48]

Backstrom and workers[50-51] use a two stage continuous flow extraction system for sample work up before ETAAS determination of heavy metals. The metals were first extracted as APDC / DDC complexes into Freon 133, which was then back extracted into a dilute Hg^{II} aqueous solution where the Hg forms a

stronger complex with the complexing agents therefore removing the metals from the organic phase and transferring them back to the aqueous phase.

1.3.2.3 On-line column preconcentration techniques

Non-chromatographic separations based on ion exchange and adsorption have been used extensively for enhancing the selectivity and sensitivity of analytical methods. Although most procedures involve some kind of continuous flow operation, they are mostly off-line batch procedures. Automation of sorption separation and preconcentration procedures is therefore a topic which has received much interest.[52]

The technique is inherently easier to operate than other separation methods due to the overall simplicity of the equipment needed. An additional benefit over other separation techniques is its versatility, due to the wide range of different column materials, complexing agents and eluents that can be utilised. However, there are some restrictive features[52] which are characteristic of such techniques which have to be kept in mind when using the method :-

i) the packed on-line columns generate extra flow impedance, depending upon the size of the column and the particle size of the packing material, hence higher grade pumps are required when compared to other methods,

ii) preconditioning of the column is necessary after each elution, a sequence which is not required in other separation methods.

In FIA, on-line separations by sorption has always been connected with using columns containing the sorption material. The first attempt using a micro-

column and FAAS was made by Olsen *et al.*[53] in 1983, who described a chelating ion-exchange method to preconcentrate Cd, Cu, Pb and Zn on a Chelex-100 microcolumn. This was then followed by Ruzicka and Arndal in 1989,[54] who developed an on-line sorbent extraction preconcentration method using a C_{18} bonded silica column and diethyldithiocarbamate as the chelating reagent. The first ETAAS on-line sorbent extraction preconcentration method, again using a C18 bonded silica column and diethyldithiocarbamate as the chelating reagent was published by Fang *et al.* in 1990.[55] This whole subject area is discussed in more detail in *Section 1.6.*

There are many different packing materials that can be used, although they can be sub-divided into six main groups :-

i) Chelating ion exchangers (Chelex-100, CPG-8Q)

ii) Reverse phase columns (C_{18} on silica support)

iii) Polymeric sorbents (Amberlite XAD)

iv) Anion Exchangers (Dowex1-X8)

v) Cation Exchangers (Bio-rad AG50W-X8)

vi) Others (Alumina)

Chelating ion exchange columns :

The most frequently used off-line packing materials for preconcentration are chelating ion exchangers,[52] Chelex 100 being a typical material. Despite the success in batch preconcentration methods, and favourable properties in complexing a large number of heavy metals, such materials do not fully meet the requirements for an ideal packing material for on-line applications. In a detailed

study of the swelling properties of the resin, Fang *et al.*[56] provide some guidelines for its usage in on-line columns.

The most common material used for on-line ion exchange column is quinolin-8-ol functional groups azo immobilised on controlled pore glass, (8-Q-CPG).[57] This material has excellent mechanical properties due to the immobilisation of the functional groups on an easily accessible porous glass surface. However, the fine particles can cause some problems due to high back pressures but this can be solved by using larger particle size packings.[52]

Reversed Phase Columns :

Non-polar or medium polar sorbents may be used to collect metal complexes of low polarity from aqueous solution by reversed phase adsorption. The metal complexes are subsequently eluted by a suitable solvent such as methanol or ethanol. The technique, termed sorbent extraction or solid phase extraction, has been used in batch mode for sample cleaning and preconcentration purposes.[52]

A number of well known chelating agents, in conjunction with non-polar or medium polar sorbents have been used for sorbent extraction methods *e.g.* sodium diethyldithiocarbamate (NaDDC),[58-59] diammonium diethyldithiocarbamate (DDDC)[55] and ammonium pyrrolidinedithiocarbamate (APDC),[60-61] which form stable complexes with important heavy metals. Common matrix elements *e.g.* Na, Ca, Mg and K are not complexed with the chelating agents therefore they are not retained and flow to waste with the rest of the bulk matrix.

Polymeric Supports :

While these materials do not carry ion exchange groups, they are nevertheless useful adjuncts or alternatives to ion exchange resins in certain applications. These polymer adsorbents are hard insoluble spheres of porous polymer of high surface area, and are available in a variety of polarities and surface characteristics.[25]

1.3.2.4 On-line co-precipitation techniques

Precipitation is one of the oldest separation techniques used in classical chemical analysis. However, its importance in modern methods has declined due to the development of more versatile and efficient techniques, such as solvent extraction and ion exchange which can be easily automated. Conventional batch methods are time consuming, labour intensive and require great operator skill. The manual procedures are usually long and involve many steps which introduce a greater risk of contamination.[62]

On-line systems have been developed but they had to overcome the difficulties of continuous manipulation of a heterogeneous system which could create blockages, a major problem in a FIA system.

Again the low levels of analyte may cause problems and so co-precipitation systems have been introduced, where the precipitated is collected in a knotted reactor (with or without a filter). As a result of this development, separation and preconcentration procedures by precipitation, originally requiring hours of operation and a few hundred millilitres of sample and reagent in the batch mode may now be completed in less than a minute with an automated FIA system, while consuming a few millilitres of sample and reagent. The risk of contamination is

also drastically reduced when using the FI system, which in turns improves the reliability and the precision of the determinations.[62] The technique has successfully been coupled to FAAS,[63] and to ETAAS.[64] The method was adapted from a batch process,[63] where the analyte(s) of interest were co-precipitated quantitatively with iron^{II} hexamethyleneammonium hexamethylenedithiocarbamate (HMA-HMDTC) and the resulting complex was collected on the inside of a knotted PTFE tube. The complex was then dissolved using isobutyl methyl ketone (IBMK) and introduced directly into the flame atomic absorption spectrometer. In the ETAAS method,[64] the precipitate was dissolved in 60 μl of IBMK, and stored in a PTFE tube before being propelled into the graphite tube by a stream of HMA-HMDTC.

1.3.2.5 On-line vapour generation techniques

As mentioned in Section 1.2.2.4, volatile elements *e.g.* hydride forming elements can be removed from the aqueous sample matrix by reaction with sodium tetrahydroborate, and then the gaseous hydrides can be transferred from the aqueous sample into a gaseous acceptor stream via a gas-liquid separator. These methods have the advantage of removing the potential interferences from the analyte of interest, but can also preconcentrate the analyte, allowing lower detection limits than for direct analysis of the sample.

Batch procedures are carried out in a reaction chamber, and then the covalent hydrides once formed are swept out of the vessel by a stream of carrier gas straight into the heated quartz furnace[66] or graphite tube for in situ trapping.[67] High sensitivities for the batch methods have been obtained but at the expense of large sample and reagent consumption.[65]

Automated hydride generation methods have been developed which generate the hydride by reaction of an acidic sample with a reductant solution of sodium tetrahydroborate. The hydride is removed via a gas liquid separator (GLS) using a stream of inert gas usually Ar, and swept into the quartz furnace or into a graphite tube for in situ trapping. These methods will be discussed in more detail in *Section 1.5*. Reaction conditions for effecting the gas liquid separation may be optimised separately and often greatly enhance the selectivity of the determination. The non equilibrium conditions of the FIA process can be exploited to enhance favourably the selectivity of the hydrides through kinetic discrimination in the formation of the interfering hydride.[65]

The literature is full of on-line vapour generation papers where the workers have used hydride generation but combined it with various different pretreatment stages. Nielsen *et al.*[68] preconcentrated As^{III} by co-precipitating it first with $La(OH)_3$, collecting the precipitate in a knotted reactor. It was eluted from the reactor with 1mol l^{-1} HCl and transported to a manifold where the hydride generation reactor took place, and then on to quartz furnace atomic absorption spectrometry (QF-AAS) at 900°C for the final measurement step.

Carrero and Tyson,[69] simultaneously retained Se^{IV} and tetrahydroborate on a strong anion exchange column and produced the hydrogen selenide by passing a slug of HCl through the column with subsequent detection by QF-AAS. This method offers an improvement over conventional HGAAS techniques in terms of sensitivity and the reduction / elimination of cation interferences.

1.4 Cold Vapour Atomic Absorption Spectrometry

The determination of mercury is possible by the cold vapour atomic absorption (CVAAS) technique, since this technique is based upon the unique properties of this element. Mercury can exist in the atomic state at ambient temperatures. It also exhibits an appreciable vapour pressure (0.0016 mbar at 20°C) and the mercury vapour is stable and monoatomic.[70] Hence when mercury is produced in its elemental form by reduction of its compounds, the vapour can be entrained in a stream of air or an inert gas and measured by the atomic absorption of the cold vapour without the need for a flame atomiser.[71]

The most successful procedure for the determination of trace levels of mercury was first proposed by Poluektov and co-workers[71] in 1964 who discovered that when tin^{II} chloride was added to a mercury sample before aspiration into a flame, there was an increase in the signal of 1 or 2 orders of magnitude compared to when no tin^{II} chloride was added. This method was further investigated by Hatch and Ott,[72] in 1968. The mercury was reduced to the free metal in an acidic solution of tin^{II} chloride. A stream of air or inert gas was bubbled through the solution which then passed the atomic vapour to an absorption cell mounted in the radiation beam of an atomic absorption spectrometer. The detection limit for this method was 0.02 $\mu g\ l^{-1}$, which was three orders of magnitude lower than the flame method.[71]

Initially tin^{II} chloride was the most widely used reductant[73] but some workers have used sodium tetrahydroborate.[74-76] The major differences between the two reductants is that sodium tetrahydroborate ($NaBH_4$) is the stronger reducing agent and a large quantity of hydrogen is produced, as a by-product of the reaction. This hydrogen helps remove the mercury from the solution, whilst

when using the tin^{II} chloride method, a constant stream of air must be bubbled through the solution to liberate the mercury. A further advantage of using sodium tetrahydroborate, is that the reduction reaction occurs quicker than with the tin^{II} chloride, which means that there is less contact time between the vapour and the inner surfaces of the system. This is important since the loss of mercury can occur by adsorption, a major problem in mercury determinations. Also a larger sample throughput can be achieved.[71] If lower detection limits than those achieved using AAS are required, an amalgamation method or atomic fluorescence (AF) detection can be used.

Several authors have described the collection of mercury vapour by amalgamation on silver,[77] gold,[77-78] or a gold / platinum gauze.[79] This technique eliminates kinetic interferences due to a different vaporisation rate or a different distribution function of the mercury between the liquid and the vapour phases. The sensitivity is also increased by more than an order of magnitude because the collected mercury can be rapidly released into an absorption cell by heating the collector.[79] The detection limits of this procedure are typically better than 0.1 ng of Hg.[71]

When using the cold vapour generation method with atomic fluorescence spectrometry (AFS), there is minimal background interference and scatter, *i.e.* no flame noise or absorption. Argon must be used as the carrier gas since air quenches the fluorescence of mercury,[80] and for similar reasons, tin^{II} chloride must be used as the reducing agent, as hydrogen also produces a quenching effect.[73] Thompson and Godden,[81] used a commercial AA instrument, but with the mercury vapour contained within a transparent argon shield instead of a normal fluorescence cell, and achieved a detection limit of 0.02 ng of Hg.

The batch method of cold vapour atomic absorption spectrometry has been converted to an on-line method using flow injection methodology by various workers.[76,82-84] This methodology has the advantages of full automation, high speed of analysis, minimum sample consumption and the ability to use exact sample volumes.[85] Also, the use of an on-line system allows the pretreatment steps to be performed on the sample immediately before the measurement step, *e.g.* on-line oxidation of the organomercury in the sample,[82,84] and on-line microwave digestion of samples.[83]

1.5 Hydride Generation Atomic Absorption Spectrometry

It has been known for over 100 years that As and other elements from Groups IV, V and VI of the Periodic Table react with "nascent" hydrogen, to form volatile, covalent hydrides (*e.g.* AsH_3, SbH_3, BiH_3, H_2Se, H_2Te, GeH_4, SnH_4 and PbH_4). This fact has been used to aid the separation and enrichment of such analytes from the matrix with a reduction in interferences.[71]

In 1969, Holak86 published the first paper determining As by hydride generation atomic absorption spectrometry (HG-AAS). The nascent hydrogen was generated by adding Zn to the HCl acidified sample solution, and collecting the arsine in a liquid nitrogen cooled trap. After the reaction had gone to completion, the arsine was released by warming the trap and passing a stream of nitrogen through to sweep the arsine in to an argon / hydrogen diffusion flame to measure the atomic absorption.

However the metal / acid reaction had a number of disadvantages which hindered its use. The metal was not available in a very pure form and hence variable blank readings were obtained. Only a very small fraction of the hydride

was released from the reaction, (about 8%)[87] while 90% remained trapped in the zinc precipitated sludge at the bottom of the vessel. In addition, only a limited number of elements could be analysed (*e.g.* Zn could only be used in the analysis of As, Sb and Se).[71]

The applications of hydride generation increased when sodium tetrahydroborate was used as the source of nascent hydrogen. With sodium tetrahydroborate ($NaBH_4$), most of the hydride forming elements could be determined. Initially, the tetrahydroborate was added as pellets, in a similar manner to the metal / acid reactions, but this produced poor results and the reaction was difficult to control as an alkaline zone formed around the pellet, causing poor reproducibility. When the sodium tetrahydroborate was added in solution form,[88-89] the reaction was easier to control and more reproducible. The reductant was stabilised by making it in an alkaline (NaOH) solution.

Coupled with flow injection (FI) technology, the whole process from sample introduction, via hydride generation to the final measurement step, has been automated. Traditionally, HG-AAS has been performed by passing the volatile hydrides into an atomiser cell, where decomposition of the hydride takes place. The atomiser cell can either be a flame[90] or more commonly a heated quartz tube.[91] The quartz tube can be heated with a flame or electrically. Compared with a flame, a quartz tube offers the advantage of higher sensitivity and for As and Se, negligible spectral background and an improved signal to noise ratio.[71] Hydride generation AAS has been reviewed in detail by Dedina and Tsalev.[30]

1.5.1 "In atomiser" trapping for hydride forming elements

The technique of "in atomiser trapping" utilises the graphite tube as both the preconcentrating medium and the atomisation cell. This technique has a number of advantages for the atomisation of hydride forming elements over the use of conventional heated quartz cells. The generated hydride is trapped within the graphite atomiser, generally upon a coating of Zr,[92-93] Ir,[94] Pd,[95] Ir-W[96] and Ir-Zr[96] or even on the untreated graphite tube surface.[97] The subject of in-situ trapping of hydride forming elements within a graphite furnace has recently been reviewed by Matusiewicz and Sturgeon.[31]

The use of the graphite atomiser to trap the analyte species and then decompose the hydride, provides two clear advantages :-

i) it ensures a clean and rapid separation from the matrix, prior to the atomisation step, and

ii) allows far greater control over the atomisation conditions and thus generating improved peak shapes. This is due to the fact that in atomiser trapping gives conventional ETAAS peaks, with the complete atomisation taking place within 2-5 s instead of 10-15 s, typical for quartz cell atomisers for a similar mass of analyte.

In addition, greater reproducibility is attained when compared to a quartz cell. As the analyte is preconcentrated on the tube surface, less sample volume is required compared to continuous hydride generation coupled with the quartz cell. This results in higher sensitivities and lower detection limits. However, larger

sample volumes can also be handled due to the clear separation between trapping and atomisation intrinsic in the preconcentration process.

As the analyte is trapped within the atomiser, any kinetic effects affecting the hydride generation and decomposition processes (within the quartz cell atomiser leads to extended peak widths), can be minimised as the trapping time is independent of the atomisation process. Performing the atomisation within the graphite atomiser means that careful control of the atomisation parameters is possible leading to improved reproducibility of the atomisation process and reduced interferences.[98]

Hydride generation techniques are prone to two sources of interference, firstly, those that occur during the hydride generation step and secondly, atomisation interferences in the quartz cell. Considerable work has been expended in trying to understand and overcome these interferences.[99]

However, in atomiser trapping can overcome such disadvantages as the technique is less susceptible to the kinetics associated with hydride formation and decomposition.[100] Only the species that are transported to the graphite furnace can interfere. In principle, there are two possible interference mechanisms ; suppression of either the trapping stage or a reduction in the atomisation efficiency of the analyte.[99] Only the hydrides have been found to interfere in this technique. Various workers,[100-102] have concluded that the mutual interference is markedly lower than in an external heated quartz tube. It has been shown that the interferences do not take place either in the liquid phase or during atomisation. The interferences are due to competition for the deposition on the active sites,[103] *i.e.* due to suppression of trapping efficiency. Consequently this interference should be expected to be depend on the total mass of interferent trapped in the furnace rather than on the interferent concentration in the sample. In atomiser

trapping could also be an effective tool for reducing interferences in the liquid phase since trapping should allow sample dilution or the slower addition of the reducing agent, both of which reduce matrix interferences, without reducing sensitivity.[99]

1.6 Column preconcentration

The introduction of flow injection techniques for solution manipulation in atomic spectrometry has greatly enhanced the performance of the latter.[104] The principle drawback of conventional separation procedures is the fact that they are often time consuming and frequently require large sample volumes. With the implementation of on-line flow injection techniques for separation and preconcentration, such drawbacks are, however, no longer valid, while the beneficial effects are further enhanced.

The improvements are such that the separation and preconcentration operations for atomic spectrometry, once often the "bottleneck" of the entire procedure, are now completely compatible with the efficient atomic spectrometric measurement sequence. Therefore, large enhancements in the performance of atomic spectrometric techniques are possible at sample consumption levels and sampling frequencies typical of conventional atomic spectrometric methods without preconcentration treatments.[104]

1.6.1 Development of flow injection on-line column preconcentration with flame atomic absorption spectrometry detection

In 1983, Olsen *et al.*[53] reported for the first time, the use of an on-line column preconcentration system for the determination of trace amounts of heavy metals in seawater by flame atomic absorption spectrometry (FAAS). The method was originally proposed to enhance the sensitivity of the measurement of cationic trace elements in very diluted aqueous samples. Each sampling cycle consists of two separate operations, preconcentration and elution.

First, a large sample volume *e.g.* 5 ml was injected by the first valve into the carrier stream and propelled through the cation exchange column (Chelex-100). Then a small volume of eluting reagent (*e.g.* 50 μl of 1M HNO_3) was injected by the second valve, liberating the preconcentrated analyte (x 100) into the detector. This method allowed Pb to be determined down to 10 ppb and Cd and Zn as low as 1 ppb with a sample throughput of 30 - 60 per hour.

The main advantage of using on-line preconcentration is that all samples and standards are subjected to exactly the same treatment from injection to the moment of detection, and the same ion exchange column is used for all samples and standards. As the geometry of the flow and the ensuring chemical reactions are strictly controlled and reproducibly maintained in the FI system, it is not an absolute prerequisite that quantitative adsorption of the analyte is achieved, but the subsequent elution must be quantitative, otherwise carryover from one sample to the next will occur.[105]

This first paper was quickly followed with more advanced designs of manifold which used different resins,[56,106-107] time based rather than volume based injections,[106,108] and most importantly, counter current operation,[56,108] of the

microcolumn. This manifold design has two advantages[105] over the original design :-

i) the matrix material is flushed to waste, and does not to pass into the detector. Background correction is therefore not required and there is no or little blocking of the instrument lines, and

ii) the ion exchange column is regenerated before the next preconcentration step, and operated in the counter current mode. The chance of the column becoming blocked is thus effectively reduced. This can be a problem with Chelex-100 which undergoes volume changes when converted from the acidic to the alkaline form.

1.6.2 Development of flow injection on-line column preconcentration with electrothermal atomic absorption spectrometry detection

Flow injection sample introduction for ETAAS systems is less straightforward than for FAAS, owing to the discontinuous nature of ETAAS operations, and this has caused the slower development of related techniques.[109]

In 1989, a paper by Beinrohr *et al.*[110] described a design for an on-line microcolumn preconcentration technique for ETAAS, using a column packed with an ion exchange material to determine Pb in a sodium chloride matrix. The method required manual manipulation of sample flows as well as sample introduction. In 1990, a paper by Fang *et al.*[55] discussed the successful coupling of a flow injection on-line column preconcentration system with electrothermal atomic absorption spectrometry. The method developed used sorbent extraction of the lead diethyldithiocarbamate on to a C_{18} column and elution by ethanol into a

collection tube for manual insertion into the furnace for measurement. Full automation of a flow injection on-line column preconcentration system was developed in 1991, by two different groups[58,60] using different approaches.

Porta *et al.*[60] used a miniature C_{18} column mounted at the tip of the autosampler arm. A modification to the tubing line of the autosampler, by adding an external pump, which allowed either the flow of the sample through the column or the normal flow operation of the autosampler. Sperling *et al.*[58] integrated the FI preconcentration manifold system to the ETAAS autosampler arm. The normal autosampler tubing was disconnected, and the delivery tube from the manifold was attached to the autosampler arm. This assembly allowed the preconcentration operation to occur in the manifold. With the arm in the injection position permitted the eluent to be injected into the furnace, and with the arm in the rinse position , permitted all excess solutions to flow to waste.

1.6.3 Advantages of FI-ETAAS online preconcentration

The advantages of FI on-line preconcentration were first demonstrated using FAAS to provide an increase in sensitivity at sampling rates comparable with normal FAAS determinations.[53] The on-line sample pretreatment operations with FAAS in terms of sample throughput and simplicity cannot be compared with ETAAS due to the detector's discontinuous nature of operation, the coupling of FI with ETAAS does have some specific advantages.[111]

The specific features of ETAAS operation which pose special requirements on the design and operation of the flow injection systems (different from those of an FAAS system) are described below.[112] Also the differences between time and volume loading of the sample are discussed.

1.6.3.1 Coupling of flow injection systems with ETAAS

As the normal operation of a graphite furnace atomiser is batch mode with discrete sampling, it does not lend itself to coupling with continuous flow operation such as flow injection.[112] Therefore the preconcentration operations are carried out in one of two ways :-

i) in parallel with the furnace operation *i.e.* one sample is processed in the FI system while the other sample is being atomised in the furnace, or

ii) sequentially *i.e.* the FI system remains on standby while the sample is atomised in the furnace.

A higher sample throughput is obtained with the parallel operation, but two independent control systems are need for this mode of operation.

1.6.3.2 Sample introduction

With normal ETAAS operations, the liquid sample is directly injected into the furnace, and the maximum sample volume (typically 70-100 μl) depends upon the tube design and the solvent being injected.[112] With ethanol as the solvent and using a tube fitted with a L'vov platform, this maximum volume can decrease to about 50 μl.[52]

The volume of eluent used to totally desorb the analyte off the column is usually greater than the total permissible volume that can be analysed by the furnace. Therefore in order to overcome this problem, special zone sampling

procedures,[112] have been developed to introduce the most concentrated fraction of the elute in to the furnace. Such procedures include :-

i) multiple injections with intermediate drying,[60]

ii) heart cut or zone sampling which introduces the most concentrated fraction of the elute into the furnace and discards the rest,[55]

iii) quantitative phase transfer of analyte within manageable volumes by miniaturisation of separators and reduction of dispersion by air flow transportation of concentrates,[113] and

iv) deposition of concentrated aerosol by high pressure nebulization.[114]

As the measurement cycle of the FI-ETAAS method is significantly longer than in a typical FI-FAAS method, this feature makes it easier to make the two operations *i.e.* measurement and preconcentration steps, more compatible. Thus sample loading and the elution rates are slower compared with flow rates in FI-FAAS, therefore a better phase transfer can be achieved between the column and the sample solution, which leads to overall improved performance of the coupled system.[112]

1.6.3.3 Matrix removal and washing procedures

Since ETAAS is more sensitive to matrix interferences than FAAS, (even with background correction), the introduction of even a small amount of matrix cannot be tolerated.[112] Therefore in ETAAS preconcentration methods, washing steps become very important to remove any matrix from both the tubing and from the column. This problem was highlighted by Fang *et al.*[55] who used the method

of analyte addition to correct for high background signals after the washing steps (which occurred in the same direction as loading) had failed to remove the interferents. Sperling *et al.*[58] found that these problems could be overcome by washing the column with dilute nitric acid in the reverse direction to loading.

With on-line preconcentration system, some degree of matrix removal is achieved and this allows for a simple measurement by ETAAS without the need for chemical modification and sophisticated background corrections.[111]

1.6.3.4 Improved detection limits

Due to the closed reaction system and the possibility of on-line purification of reagents,[55,115] the very low blank levels for ultra trace determination are more advantageous with ETAAS than with FAAS. This leads to lower detection levels. Since for many analytes the limiting factor in detection power lies in the background level rather than the sensitivity, this advantage may be exploited to achieve better detection limits than those obtainable under batch operation conditions.[112]

1.6.3.5 Signal measurement

In FI-FAAS methods, the peak height measurements of the transient signals are directly related to the dispersed concentrate zones. In FI-ETAAS methods, the whole concentrate zone used for the measurement is injected into the atomiser and dried before atomisation. Therefore this method does not rely upon the distribution of the analyte in the concentrate zone as long as the total

mass remains constant. This permits some degree of fluctuation in the sampling position of the concentrate when zone sampling techniques are used.

1.6.3.6 Time based and Volume based sample loading

In FI preconcentration systems, the sample volume being processed may be determined either by fixing the time interval,[56,116] for sample introduction (loading) into the preconcentration system, under a defined sample flow rate, or by using a carrier stream to displace a fixed sample volume, defined by the volume of the sample loop.[53,117] The former approach is referred to as time based sampling and the latter, volume based sampling.[33,118]

Time based sampling is more straightforward to operate, since there is no need to prefill the sample loop before injection into the carrier stream. The amount of sample processed is determined by the sample flow rate and the time. Consequently, time based method are more efficient than volume based methods, but they are more dependant upon the stability of the of the pump system and higher quality pumps are needed to maintain a constant and stable flow. This is particularly true for systems which incorporate packed columns which can create large flow impedances. Volume based sampling is much less dependant on flow stability, providing that the sample loops are filled completely with sample before injection into the carrier stream.[33,118]

1.7 Aims of Study

The main objective of this study, as indicated by the thesis title, has been to explore the possibilities of on-line sample pre-treatment of environmental samples for subsequent analysis by atomic spectrometry and in particular electrothermal atomic absorption spectrometry.

Sample pretreatment for atomic spectrometry covers a large number of topics from simple dissolution methods to complicated extraction methods and on-line digestion techniques. Since the main thrust of this work was to look at on-line sample pretreatment methods for ETAAS, the first major problem to be overcome was the linking of a discontinuous detector to a continuous flow system. To overcome this problem, the conversion of commonly used off-line separation and preconcentration methods to on-line methods such as solvent extraction, sorbent extraction, microwave digestion, co-precipitation and vapour generation were to be investigated.

The detection system to be used, *i.e.* ETAAS provides extremely low detection limits for a wide range of metals. This study therefore also examined methods of matrix removal and preconcentration. If the matrix interference well known to be a problem when using ETAAS could be eliminated, the full detection capability of the ETAAS could be utilised for analysis of environmental samples resulting in a selective and precise method with very low detection limits.

Thus three main aims may be identified for this study :-

i) to develop a simple and robust method to determine total mercury in environmental and biological samples by cold vapour atomic absorption spectrometry. In addition, to consider the possibility of using both off and on-line digestion procedures.

ii) to study the possibilities for the multi-element simultaneous determinations of the hydride forming elements by in atomiser trapping. Further to consider the oxidation states of the elements and determine the best way to achieve maximum sensitivity.

iii) to study the possibilities of multi-element simultaneous determinations of ultra trace metals in natural samples such as seawater by on-line column preconcentration.

Chapter Two :

Instrumentation - Theory and Applications

Chapter Two : Instrumentation - Theory and Applications

2.1 Introduction

This chapter details the instrumentation used in the studies presented in this thesis. The first section contains a basic overview of the theory behind atomic spectrometry, although the focus is on atomic absorption spectrometry and how this is put into practice in modern instruments.

The following section describes in more detail the various components of a modern analytical instrument, with particular attention being paid to the components of the electrothermal atomic absorption spectrometers used in this work, *i.e.* the Perkin Elmer SIMAA 6000 and the Model 4100ZL. These two systems have various components in common, *e.g.* the transversely heated graphite atomiser (THGA) furnace and the lamps used (single and multi-element) hollow cathode lamps (HCL) and electrodeless discharge lamps (EDL), although the optical design of the spectrometers and the detector systems are different. These differences will be explained in more detail. The final section of this chapter describes the flow injection analysis system (FIAS 400) used for all the sample introduction and manipulation procedures prior to the measurement step.

2.2 Overview of the theory of atomic spectroscopy

Atomic spectroscopy is used for the qualitative and quantitative determination of up to 70 metals and metalloids from the Periodic Table,[119] as shown in *Figure 2.1.* The term atomic spectroscopy covers three different

techniques, atomic emission, atomic absorption and atomic fluorescence. The work in this thesis utilises mostly atomic absorption, but the basics of all three techniques are described below in *Section 2.2.1.*

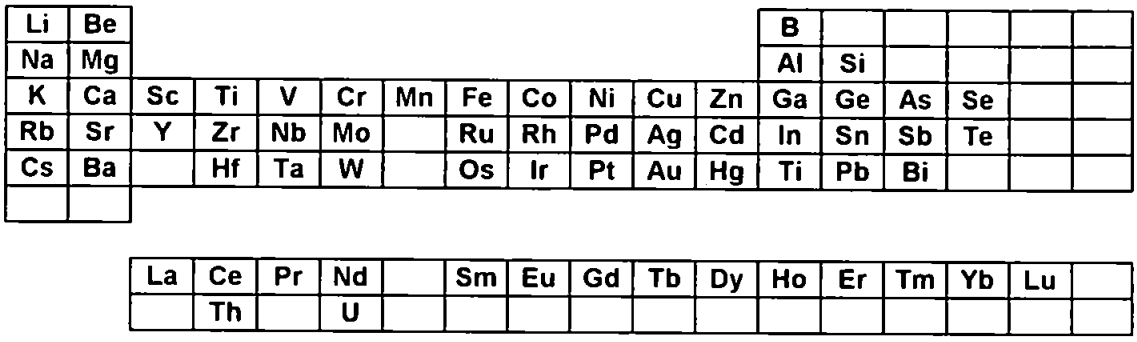


Figure 2.1 : **Periodic table showing elements determined by atomic absorption spectrometry**[119]

Most samples to be analysed by atomic spectroscopy come in one of two forms, liquid or solid. The former is the more common and is relatively easy to manipulate. The liquid can easily be introduced via a nebuliser into a flame or pipetted into the tube, in the case of electrothermal atomisers (ETA). For solid samples, a digestion step is usually required to get the analyte out of the solid matrix and into a solution ready for analysis.

However, spectroscopic determinations have to be made on the atomic species, and this can only be achieved on a gaseous medium in which the individual atoms are well separated from one another.[120]

2.2.1 Basic principles of atomic spectroscopy

When an atomic vapour containing free atoms of an element in the ground state is illuminated by a light source that radiates light of a frequency characteristic of the element present in the vapour, radiation will be attenuated at certain frequencies.[85]

It was found that the amount of light passing through a layer of uniform thickness of a homogenous medium is dependent upon the thickness (d) of this layer and the ratio of the intensity of the transmitted light (I) to the intensity of the incident light (I_0) is independent of the radiant intensity.[121]

$$I = I_0 \exp(-x.d) \qquad (Eqn\ 2.1)$$

The proportionality factor (x) is a measure of the power of attenuation of the layer and is termed the absorption coefficient, but if the irradiated medium is not a single substance but a solution of an absorbing substance in a non-absorbing medium, the absorption coefficient is proportional to the concentration (c).

$$x = k.c \qquad (Eqn\ 2.2)$$

For analytical purposes, this can be re-arranged in terms of absorbance (A) and thus absorbance is proportional to concentration.

$$A = \log (I_0 / I) = k.c.d \qquad (Eqn\ 2.3)$$

one lower energy (or ground) state to a higher energy state.[122-123] More detailed accounts are given elsewhere,[121,124-125] although the basic principles are described below.

Atomic Emission Spectrometry (AES) : This term applies to the measurement of light emitted by an atom (as it returns directly to a lower or ground energy state) after the absorption of energy.[126] A source of energy *i.e.* heat from a flame, promotes the outer electron of the atom from the ground state into an excited state. The excited atom then relaxes to the ground state and releases its excess energy as a photon of visible or ultraviolet (UV) light.[127]

AES is a particularly sensitive technique for the determination of volatile elements with low excitation energies such as the alkali and alkaline earth metals. The technique's sensitivity is partly due to the relatively low degree of ionisation induced by the cool flames.[128]

Atomic Absorption Spectrometry (AAS) : This term is applied to the measurement of light absorbed by an atom in the ground state. In a gaseous medium, the atoms are capable of absorbing radiation of wavelengths characteristic to the electronic transitions[125] and, this radiation at certain frequencies is attenuated. The absorption of radiation excites an outer electron from the ground state to the excited state, and the degree of absorption is a quantitative measure of the concentration of ground state atoms in the vapour.[128] After a few microseconds or less, the excited atoms relax to their ground state by transferring their excess energy to the atoms or molecules in the medium.[127] As only atoms in the ground state will respond in this way, the conditions used for volatilisation and decomposition of the sample to produce an atomic vapour must

induce the minimum of ionisation. This can be achieved where the temperatures remain below 3000 K,[128] *i.e.* in a flame or in an ETA.

Atomic Fluorescence Spectrometry (AFS) : This is the term applied to the measurement of radiation re-emitted from an excited atom as it returns to the ground or lower energy state. In atomic fluorescence, radiation from a line source is used to excite atoms to a higher electronic state. The fluorescence radiation is emitted as the excited atoms return to the ground state.

This fluorescent radiation is characteristic of the atoms which have absorbed the primary radiation and is emitted in all directions. It may be measured in any direction other than in a direct line with the radiation source, which ensures that the detector will not respond to the unabsorbed radiation from the source. The intensity of the fluorescent emission is directly proportional to the concentration of the absorbing atoms but it is diminished by collisions between excited atoms and other species within the flame, a process known as quenching.[128]

There are six types of fluorescence, of which resonance fluorescence is the most intense and is most often used for quantitative analysis.[129] The other types of fluorescence are described elsewhere.[129-130]

The three radiative process for atomic emission, atomic absorption and atomic fluorescence are shown in *Figure 2.2*. The horizontal lines in *Figure 2.2* represent the different energy levels in an atom. E_o is the term used for the lowest energy level (ground state). At room temperature, all of the atoms of a sample are in the ground state.[131] E_i and E_j refer to higher energy levels which are both higher in energy than E_o. A solid vertical line represents a transition involving an

absorption or an emission of radiation. The wavy line refers to a non-radiative transition.

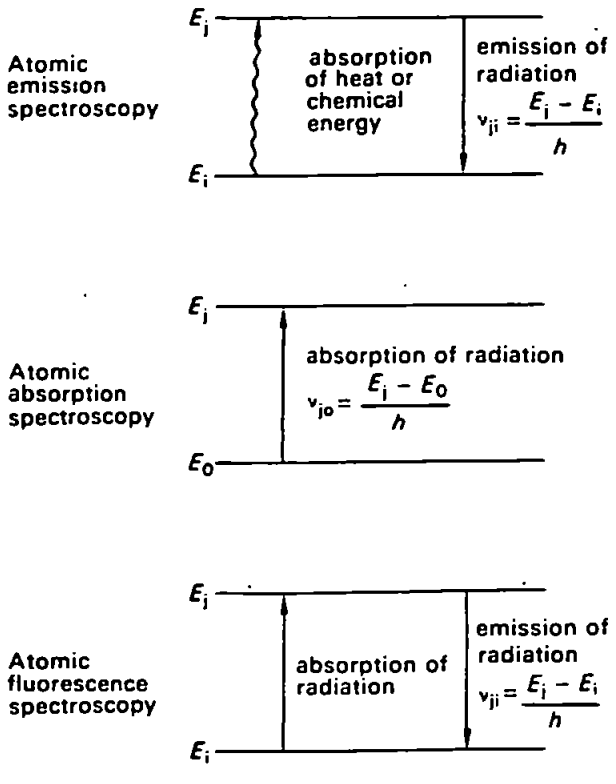


Figure 2.2 : **Summary of radiative processes for AES, AAS and AFS[126]**

The energy of radiation absorbed or emitted is quantised according to Planck's equation,

$$E = h.\nu \qquad (Eqn\ 2.4)$$

The frequency of the light is related to its wavelength by

$$\lambda = c / \nu \qquad (Eqn\ 2.5)$$

therefore on re-arrangement of Eqn 2.4 and 2.5

$$E = h (c / \lambda) \qquad (Eqn\ 2.6)$$

where

E = energy difference between the two energy levels in the atom

h = Planck's constant (6.626×10^{-34} J s)

ν = frequency of the radiation

λ = wavelength of light

c = speed of light (2.998×10^{8} m s^{-1})

The spectral line intensities depend upon the relative populations of the ground or lower electronic state and the upper excited state. The relative populations of the atoms in the ground or excited state can be expressed in terms of the Boltzmann distribution law.[131]

$$N_1 / N_0 = g_1 / g_0 \exp(-\Delta E/KT) \qquad (Eqn\ 2.7)$$

where

N_1 = number of atoms in the excited state

N_0 = number of atoms in the ground or lower state

g_1 and g_0 = the number of energy levels having the same energies as the excited and ground energy levels respectively,

k = Boltzmann constant (8.314 J K^{-1} mol^{-1})

T = Temperature in Kelvin

Since the wavelength of the resonance line is inversely proportional to its energy of excitation (*Eqn 2.6*) the number of excited atoms increases

exponentially with increasing wavelength. Also the exponent is inversely proportional to the absolute temperature, which means that the increase in the relative number (N_1 / N_0) of excited atoms with increasing temperature is exponential. It is found that N_1 is always small compared to N_0 *i.e.* the number of atoms in the excited state can be ignored relative to the number of atoms in the ground state at temperatures below 3000 K and the wavelengths less than 500 nm.[121]

2.3 Components of a modern atomic absorption spectrometry instrument

An atomic absorption spectrometer consists of a number of basic components as illustrated by the Model 4100ZL electrothermal atomic absorption spectrometer, shown schematically in *Figure 2.3.* Each instrument has a light source, which directs the light of a specific wavelength to the atomisation cell, in this case an electrothermal atomiser (ETA), where the light is absorbed by the gaseous atoms. The light is then directed by a series of mirrors to the monochromator, where the light of the correct wavelength is selected, and directed onto the detector (PMT) for the measurements to be made.

2.3.1 Single and simultaneous electrothermal atomic absorption spectrometers

The Model 4100ZL instrument is a single element spectrometer, but the SIMAA 6000 is a true multi-element instrument.[134] The differences between the two systems are :-

i) in the detectors, the SIMAA uses diode arrays instead of a PMT, and

ii) the monochromator of the Model 4100ZL is replaced by a polychromator to handle the extra resonance source lines.

Figure 2.4 shows the optical path and the various components of a SIMAA 6000 instrument. A paper by Radziuk *et al.*[133] gives a good overview of the design and the implementation of the SIMAA 6000, and a review by Harnly[134] shows the characteristics and the performance of the current simultaneous instruments and looks forward to the future for such instruments.

2.3.2 Light sources used in atomic absorption spectrometers

The light source used for AAS is required to provide the element specific radiation, which is of a narrow resonance profile with little or no background light, and the lamps should be stable and have a reproducible output with a sufficient intensity to provide a high signal to noise ratio.[135]

Two different light sources were used during the work, i) single and multi-element hollow cathode lamps were used for the majority of the elements determined and ii) electrodeless discharge lamps for the hydride forming

elements, as a more intense radiation line could be obtained than that from the hollow cathode lamps. A more detailed account of the operation and construction of HCLs and EDLs may be found in many standard texts.[119-120, 125]

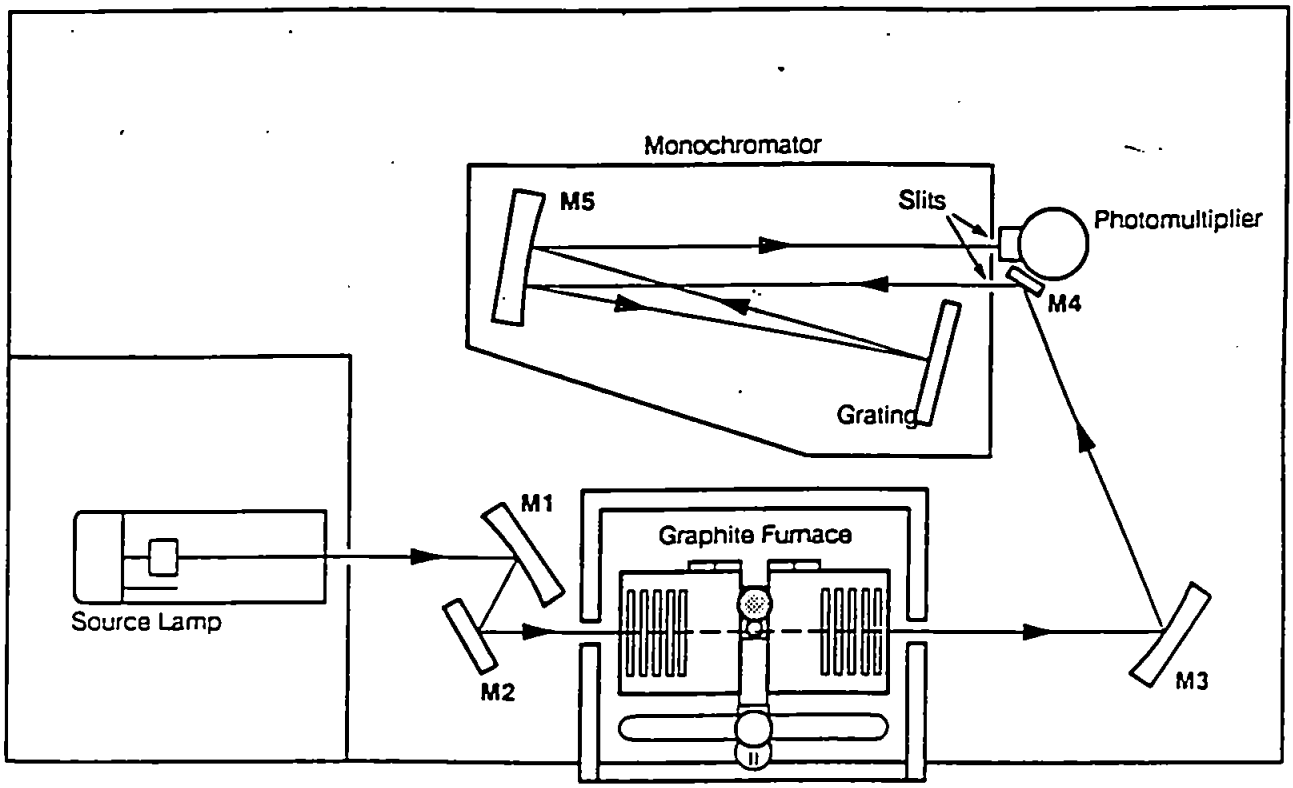


Figure 2.3 **: Diagram of the various components for a modern electrothermal atomic absorption spectrometer (Model 4100ZL)[144]**

2.3.3 Atomisation cell

Although atomisation cells can be either a flame, a quartz cell or a plasma, the discussion in this section will focus on the modern graphite tube used in the electrothermal atomiser (ETA).

The Stabilised Temperature Platform Furnace (STPF) concept was developed to improve the precision and the accuracy of analysis by ETAAS. The

concept makes proper use of the existing equipment to maximise the analytical signal and reduce any potential interferences during the measurement. It was first proposed by Slavin *et al.*[136] in 1981. An overview of the concept is given in two papers by Carnrick and Slavin,[137-138] where they discussed the modern graphite furnace and included a discussion on the STPF concept.

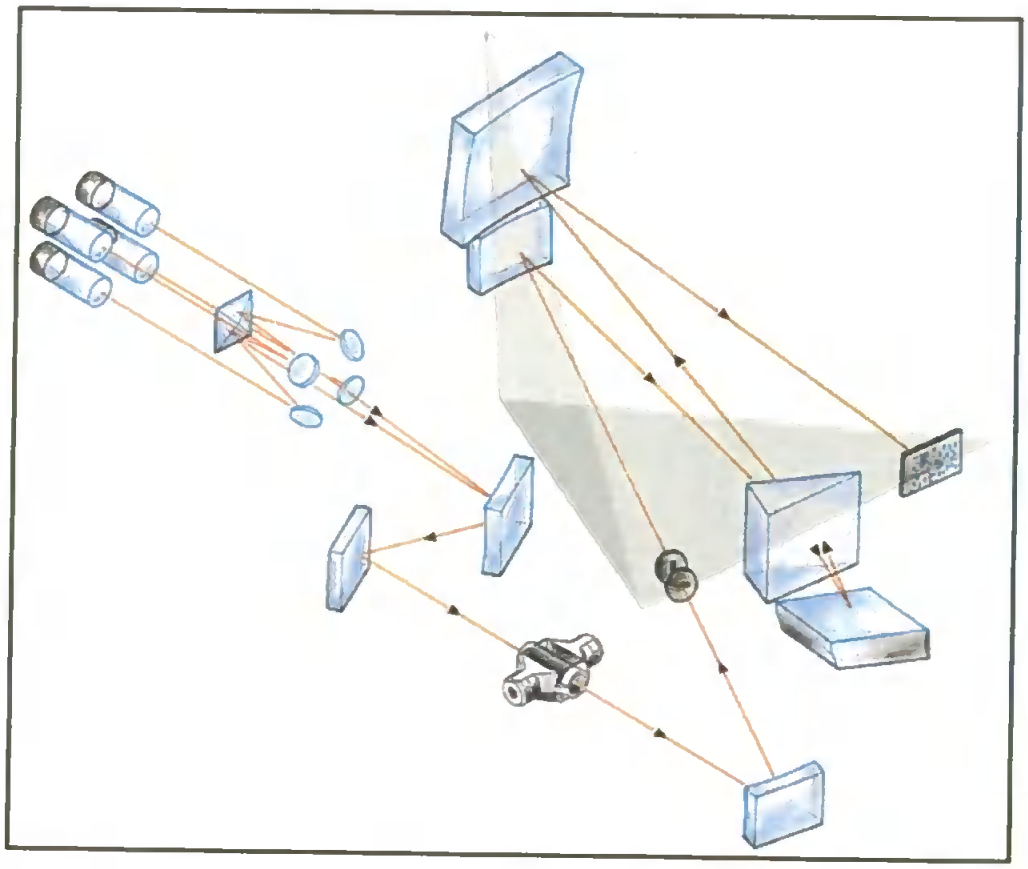


Figure 2.4 **: Optical and component diagram of a simultaneous electrothermal atomic absorption spectrometer showing the light path from source to detector (SIMAA 6000)**[139]

There are seven main conditions, all of which must be implemented to benefit from any improvements the concept can provide over conventional furnace analysis.[139] The seven conditions (listed below) apply to either the spectrometer, the atomiser or to its operation under optimised conditions, and will be discussed under the relevant sections in this chapter.

Fast Instrument electronics

Signal Integration

Accurate background correction

Rapid furnace heating

L'vov platforms and pyrolytically coated graphite tubes

Gas stop during atomisation

Use of matrix modifiers

The experiments in this study were concerned with matrix removal therefore, matrix modifiers were not used, as only the analyte was injected into the tube, but all the other conditions were used.

In the graphite tube, the sample (usually a liquid) is converted into the atomic vapour needed for the measurement following a three or four step temperature program. First, any liquid is removed in one or two drying steps by slowly heating the tube to a temperature just above the boiling point of the liquid.

The next step is an ashing step where any matrix present (inorganic or organic) is broken down and driven off by ramping the tube up to a temperature high enough to remove the matrix (approx. 300-1200°C, depending upon the

analyte) but not high enough so that the analyte is also driven off. This ashing process may be enhanced by the use of a matrix modifier.

Matrix modification is a procedure for reducing or eliminating volatilisation and vapour phase interferences. A reagent (*e.g.* NH_4NO_3, $Mg(NO_3)_2$ and $(NH_4)_3PO_4$)[140] is added to alter the physical and chemical properties of the injected sample. The matrix modifier serves to convert either the analyte into a less volatile form or the concomitants into a more volatile form. Either measure serves to bring about more effective separation of the analyte element from concomitants during thermal pretreatment.[141]

Now, ideally only the analyte of interest is left within the tube which is then rapidly heated in a maximum power atomisation step to the individual analytes atomisation temperature. The analyte is vaporised and the atomic species are formed above the tube surface and in the path of the light source where absorption takes place.

2.3.3.1 Electrothermal atomisers

The graphite furnace used by both instruments is a transversely heated graphite atomiser (THGA).[139] This type of furnace allows the tube to be heated (electrically) along the full length of the tube *i.e.* at right angles to the measurement beam and not from each end, so there is no temperature gradient along the length of the tube (*Figure 2.5*).

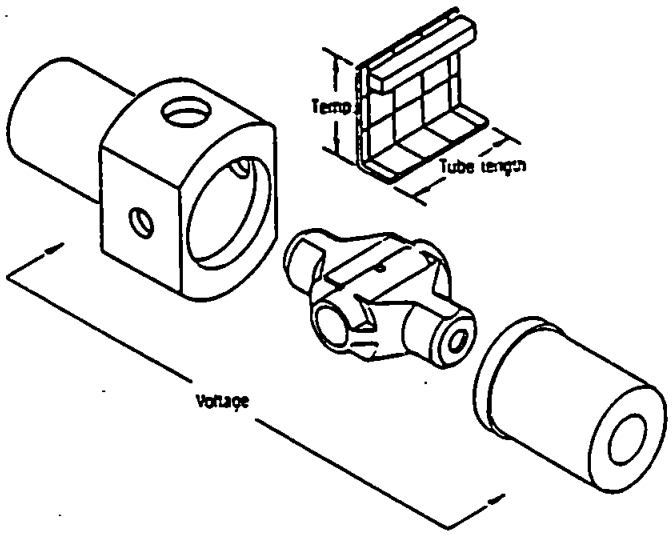


Figure 2.5 : **A THGA graphite tube plus contacts and showing the temperature profile of tube during heating**

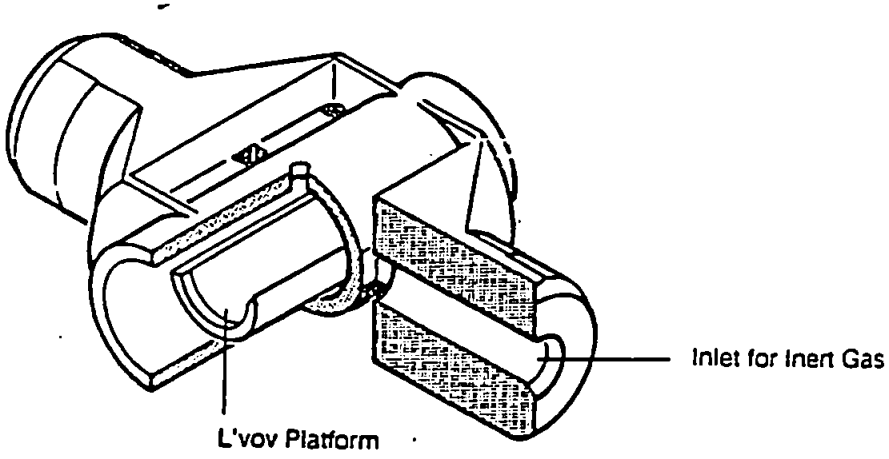


Figure 2.6 : **Diagram of a THGA graphite tube showing integral L'Vov platform and the position of the inlet for inert gas**

When graphite becomes very hot, it is permeable to metal atoms, and so to prevent diffusion of the atoms during analysis, early workers covered the inner

surface of the tube with tantalum foil to stop the metal atoms diffusing into the graphite. This metal coating was later replaced by a pyrolytic graphite layer. This pyro-coating of the tube was performed by passing a stream of hydrocarbons such as methane through the graphite tube at temperatures over 2000°C.[142]

A dense, non-permeable and oxidation resistant pyro-graphite layer was deposited on the graphite tube. It now appears that a layer of pyrolytically deposited carbon gives the best tube characteristics, including improved sensitivity and detection limits for refractory elements and improved tube lifetime due to the greater resistance to oxidising agents and acids.[71]

The graphite tubes used for all experiments in this study were made from pyro-coated graphite which contained a pre-formed L'Vov platform (*Figure 2.6*). The platform is required to delay the atomisation of the analyte as long as possible. During that delay, the graphite tube and the inert gas are directly heated by conduction, while the L'Vov platform is primarily heated indirectly by radiation from the tube walls. This produces a time lag which allows the system to approach thermal equilibrium before the analyte is atomised.[139,144]

As a consequence of the delay in the L'vov platform reaching the correct temperature, atomisation occurs in an environment in which the temperature is not changing so rapidly and thus the peaks obtained are more reproducible.[127] During all but the atomisation step, there is a full Ar gas flow (250 ml min^{-1}) into the tube from the two inlets (*Figure 2.6*) helping to remove the waste products.[127]

There is no gas flow during the atomisation step in order to increase the length of time the atoms are present in the light path. These three factors of rapid furnace heating, gas stop during atomisation and the L'Vov platform are all part of the STPF concept.

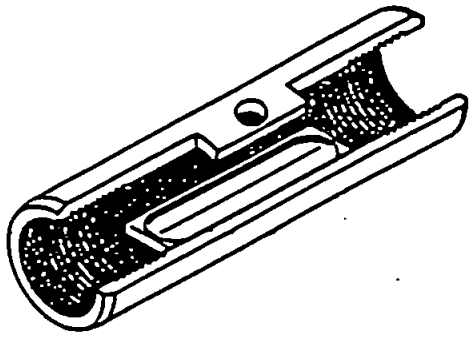


Figure 2.7 : **Diagram of a HGA (Perkin Elmer design) graphite tube showing integral L'vov platform**

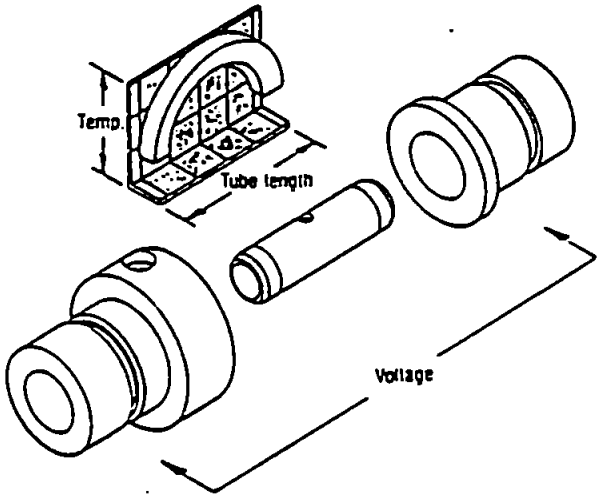


Figure 2.8 : **A HGA graphite tube plus contacts and showing the temperature profile of tube during heating**

2.3.3.2 THGA versus HGA configurations

The original graphite tube furnace was developed by L'Vov. The furnace tube was 10 cm long and internally lined with a tantalum foil. The sample was placed onto a graphite electrode, dried, and then introduced with the electrode into the graphite tube. The tube was heated by resistance heating an the sample was atomised by a direct current arc. The whole arrangement was mounted in a sealed argon chamber with quartz windows to allow the radiation to pass through the system.[71]

Massmann[143] simplified the whole furnace design, with a 5 cm long graphite tube, but the tube was a heated by passing a high current (500 A) at a low voltage (10 v) through the tube. This arrangement permitted a very fine temperature adjustments could be made which facilitated the selection of the optimum temperature for each individual element. The furnace had to be continuously flushed with an inert gas stream to prevent the ingress of air.[71]

Virtually all proprietary graphite furnaces are based upon the Massmann design, *i.e.* they are tube furnaces heated by resistance heating. However, all the designs differ in the tube dimensions, in programmability, in flexibility and in operating conveniences.[71] *Figure 2.7* shows a Heated Graphite Atomiser (HGA) Perkin Elmer design with an integrated L'Vov platform.

This original design was improved, but it was still known to have several limitations in its design. One of these limitations being the temperature distribution along the graphite tube (*Figure 2.8*) which was not uniform due to the contact of the tube with the water cooled contact cylinders.

Even with using the L'Vov platform and a pre-atomisation cool down step, the non-isothermal temperature distribution leads to memory effects for refractory

elements, gas phase interference, matrix and analyte condensation at the cooler ends of the tube.[144]

To overcome these problems, the transversely heated graphite atomiser (THGA) was developed. This design evolved from the side heated constant temperature furnace as described by Frech *et al.*[145] This new design provides a uniform temperature distribution over the entire length of the tube *i.e.* the ends of the tube are at the same temperature as the centre of the tube (*Figure 2.5*). Under these conditions, the formation of free atoms is optimum and recombination of atoms to molecules, the loss of atoms and condensation at cooler tube ends, and memory effects are effectively avoided.[144]

2.3.4 Optical Systems

2.3.4.1 Single or double beam optical layout

The layout of the optical system in a modern AA instrument, can be one of two configurations :- either single or double beam.

In a single beam instrument, the light from the source passes through the atomisation cell and then passes directly to the monochromator and hence onto the detector. In the double beam instrument, the light from the source is split into two by a rotating sectored mirror[146] or a beam splitter.[135] One beam then goes through the atomisation cell, while the other beam is sent around the atomisation cell. The beams of light are then recombined after the atomisation cell, and then as in the single beam instrument pass into the monochromator and onto the detector. The ratio between the alternative beams is then compared and a correction made for any fluctuations in the signal.[135] The double beam instrument

has the advantage over the single beam instrument in that it allows for the correction of any HCL fluctuations (warm-up, drift and signal noise) which leads to improved precision in the final meausrement.[147]

Both instruments used in this study have a single beam / single path optical design, but the systems are described as having a double beam measurement system[139,144] due to :-

i) The AC modulated radiation source and magnetic field allow the measurement of the background and analyte signals consecutively every 18 ms, and

ii) The background and analyte signals are measured at exactly the same wavelength.

2.3.4.2 Monochromators

The device which isolates monochromatic light of a particular wavelength from polychromatic light[148] is known as a monochromator. The function of the monochromator is to isolate the resonance line of the light source from the non-absorbing lines close to it, that may have been caused by the fill gas or by the atomisation source.[146] Various monochromator designs exist such as Czerny-Turner,[149] Ebert Mounting[150] and Littrow.[150] The configuration used in the Model 4100ZL ETAAS instrument[144] is a Littrow monochromator with a reflecting grating blazed at two wavelengths 236 nm and 597 nm for operation in the UV and visible regions respectively. It is shown schematically in *Figure 2.3.*

2.3.4.3 Polychromators

As the SIMAA 6000 is a multi-element instrument, a monochromator is of no use as many different wavelengths have to be monitored during the measurement cycle, therefore the monochromator is replaced by a polychromator.

A polychromator is basically a monochromator which has had the exit slit removed so that the complete spectrum is focused in the image plane. This spectrally dispersed polychromatic radiation can then fall upon the detector (in this case a diode array) which allows the measurement over the whole spectrum.[151] In this instrument (SIMAA 6000), the light is reflected through the entrance slit, off a collimating mirror and then through a quartz prism and onto an echelle grating. The echelle grating disperses the parallel radiation of incidence light into a greater number of interference orders. The light then comes off the grating back through the prism and then reflects off a camera mirror and then directly onto the solid state detector which is located on the focal plane.[139] This optical pathway is shown clearly in *Figure 2.4*. The system can also be used as a monochromator, with the camera mirror slewing until the light falls onto the correct detector.

2.3.5 Detectors

The use of photographic plates and gas filled photocells as detectors has become obsolete,[146] since photomultiplier tubes[146,149-150] have become almost universal in atomic spectrometry instruments. However, the newer instruments are now starting to use charge transfer devices and diode array detectors which potentially offer the ability to measure all the wavelengths simultaneously.[150-151]

2.3.5.1 Photomultiplier tubes

A photomultiplier tube (PMT) contains a photosensitive cathode, an anode and a series of special emitting cathodes called dynodes. The cathode and all the anodes are sealed inside a vacuum tube, with a transparent window above the photosensitive cathode to allow the radiation to enter the tube.[126,152]

The wavelength range covered by each PMT depends upon the composition of the photocathode.[153] The most common material used is Ga-As which covers almost all the range from As at 193.7 nm to Ca at 852 nm with reasonable sensitivity.[126] The Model 4100ZL uses a broad band photomultiplier with a UV transmitting window.[144]

2.3.5.2 Diode array detectors

A photodiode allows the full spectral range to be measured, unlike the PMTs which only have specific ranges. A photodiode is a single crystal silicon chip. When spectrally dispersed light falls on the diode, it produces a photocurrent which is proportional to the intensity and duration of light. This then discharges the connected capacitor[154] and hence the measurement can be made.

The detector used on the SIMAA 6000 is a specially designed cluster of 61 diodes spread over the focal plane, with 38 primary lines (and number of important secondary lines) being covered.[139] A paper by Radziuk *et al.*[155] reviews the design, the performance and the results for the diode array detector used in the SIMAA 6000.

2.3.6 Data handling and electronics

Two of the requirements of the STPF concept are fast instrument electronics and signal integration. The fast electronics are needed to follow the absorbance profile, which can last for 5 or 7 seconds, but to produce a smooth profile, a background corrected signal is measured every 16 to 20 milliseconds. Also, to ensure the profile is correctly measured, an accurate baseline must be measured before the absorbance profile is measured.[139]

Signal integration is needed to give better and more precise analytical measurements of the analyte peaks. As the rate of vaporisation of the analyte depends upon its matrix, if the standard and sample are in different matrixes then peak height measurements are going to vary which will cause errors. Integrated absorbance values can correct for any variances in the rate of vaporisation. An added benefit of this, is the freedom from matrix interferences which can mean that a lower atomisation temperature is needed, and this can extend the linear range of the calibration graph.[139]

2.3.7 Background correction methods

The occurrence of absorbance and scatter from molecular species can be overcome by the use of various background correction methods. Three of the most commonly used are described here,[147] but the main emphasis is on Zeeman effect background correction as this is the system used by both instruments used in this study. More detailed descriptions of background correction methods can be found in a book by Welz.[141]

2.3.7.1 Continuum (Deuterium) background correction

In the deuterium background correction system, a rotating mirror allows only one beam, either from the HCL or the deuterium lamp, to pass through the sample atomic vapour at any one time. The broad band non-specific background absorption can then absorb radiation from both light beams. The detector then measures the background signal only (deuterium lamp) and subtracts this from the analyte signal and background signal (element HCL) to give a corrected absorbance signal due only to the element of interest.[85,156]

The three main problems with this approach are :-[156]

i) the deuterium emission profile must be optically aligned with the element specific emission line, *i.e.* they must have identical optical pathways, and

ii) the background absorption specific profile is considered uniform across the monochromator slit, *i.e.* it has no structure, and

iii) when the level of background absorption increases above 0.4 absorbance, then the electronic subtraction of the background signal becomes less accurate.

2.3.7.2. Smith - Hieftje background correction

This method of background correction is of especial use when there is a strong molecular interference *e.g.* phosphate on Se at 196.0 nm. If the hollow cathode lamp is run at its normal operating current, then atomic and molecular absorption by the analyte and interferents will occur in the atomiser. If however,

the lamps is pulsed periodically at much higher currents, self absorption in the lamp prevents virtually all the atomic absorption in the atomiser, but leaves the molecular absorption. The corrected atomic signal may therefore be gained by subtraction. This method unfortunately leads to accelerated wear of the lamp.[85,156]

2.3.7.3 Zeeman effect background correction

The splitting effect of a magnetic field on a spectral line was first discovered by the Dutch physicist Zeeman in 1897.[141] The Zeeman effect background correction is part of the STPF concept. When the Zeeman effect is used, it tends to make the furnace measurements more independent of the matrix of the sample.

Zeeman background correction can be divided into six different configurations. The configurations are determined :-

i) by the position of the magnet (*Direct or Inverse*). In Direct Zeeman, the magnet is placed around the light source and for Inverse Zeeman, the magnet is placed around the atomisation cell,

ii) in which plane the magnet acts upon the excited atom (*Transverse or Longitudinal*). When the magnetic field is applied perpendicular to the radiation (Transverse Zeeman), when the magnetic field is parallel to the radiation (Longitudinal Zeeman), and

iii) by the type of power supply (*alternating or direct current*) which is determined by the requirements of the instrument.[157]

In the simplest case of the Zeeman effect, the single spectral line is split into three components; a central π component whose energy and frequency is unchanged with respect to the original level, and two σ components of slightly higher and lower energy respectively. These are called the $\sigma+$ and $\sigma-$ and are to the left and right of the original spectral line, *i.e.* at higher and lower frequencies (*Figure 2.9*). The extent of the shift depends upon the applied magnetic flux density. Simultaneous to this splitting of the spectral lines, the radiation is also polarised depending upon the direction of the applied magnetic field.

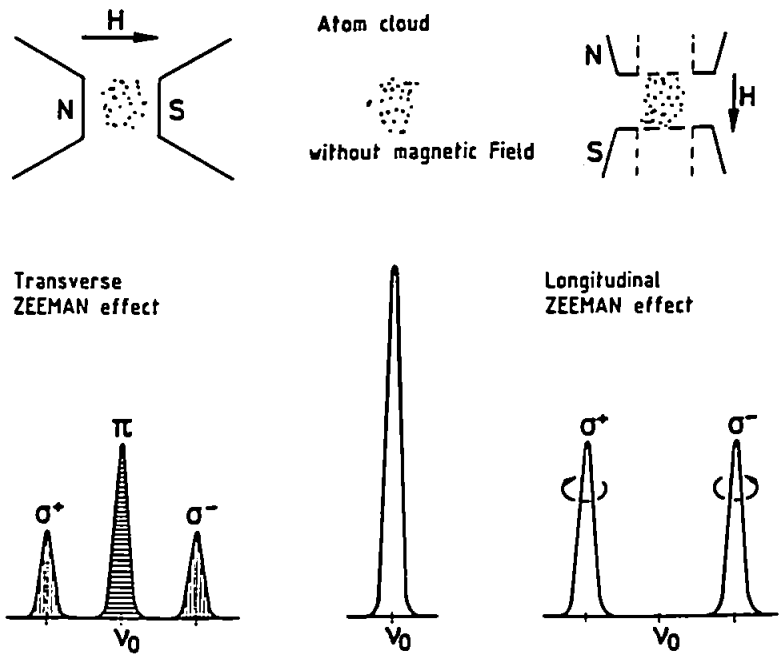


***Figure 2.9* : Zeeman diagrams showing π and σ components for Transverse and Longitudinal Zeeman arrangements**[139]

If the radiation is observed in a direction perpendicular to the magnetic field or if the magnetic field is applied at right angles to the radiation beam, the π component is polarised in a direction parallel to the magnetic field, while the σ

components are polarised in a plane perpendicular to the magnetic field. This is the Transverse configuration (*Figure 2.9*).

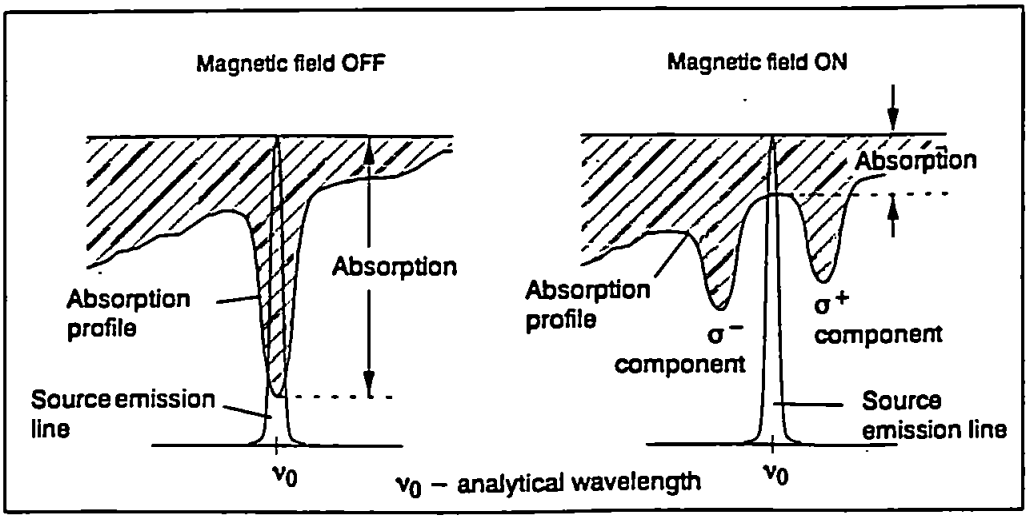


***Figure 2.10* : Background correction with the longitudinal Zeeman effect arrangement[139]**

When the direction of observation is parallel to the magnetic field, or the magnetic field is parallel to the radiation beam, this is the Longitudinal configuration (*Figure 2.9*), and this has the effect of removing the π component from the spectrum and only the circularly polarised σ+ and σ- components are observed.[157]

During each measurement cycle, there are two light phases, and the magnetic field is on for one and off for the other. With the magnetic field on, the analyte signal is split into the two shifted σ components and the "unseen" π component. The two σ components cannot absorb the energy at the analytical

wavelength, and the π component, not having an effect in the longitudinal direction, also cannot absorb energy at the analytical wavelength (*Figure 2.10*).[139]

Any attenuation of radiation at the analytical wavelength is thus due only to the background (structured or continuous).

With the magnetic field off, any attenuation of the radiation at the analytical wavelength is due to absorption by the single analyte signal in addition to any attenuation due to the background (structured and / or continuous). This procedure of rapidly alternating measurements of [analyte and background] and [background only], at exactly the same wavelength during the measurement cycle is sufficient to minimise errors due to drift (*Figure 2.10*).[139] A more detailed description of the application of Zeeman background correction is given in a book by Welz.[141]

2.4 Sample introduction and manipulation using automated flow injection system

This section describes the automated flow injection system (FIAS 400) system and the sample pretreatment stages performed in this study. A general description of the FIAS and the experimental procedures adopted will be given in this section, while the specific information for each different experiment will be given in the respective chapters. Each of the two configurations *i.e.* single element determinations (FIAS with Model 4100ZL) and multi element determinations (FIAS with SIMAA 6000) will be described, including basic default conditions, diagrams of the configuration and the procedures used for the sample manipulation.

2.4.1 Flow injection analysis system

All sample manipulations, from the pumping of the sample from the autosampler to the injection of the sample into the graphite tube were handled by the flow injection analysis system (FIAS 400), and the AS-90/91 autosamplers.

The FIAS 400 consists of two peristaltic pumps, one valve (either a five or an eight port). When used for cold vapour / hydride analysis, a gas valve and flow meter are also required. The FIAS unit for all the experiments in this study was controlled by the computer attached to the spectrometer. The two pumps are fully controllable from 20 to 120 rpm (in single steps), and can be used with a wide range of different internal diameter tubing which gives rise to precise control over the flow rates from 0.5 ml min^{-1} to 12 ml min^{-1} for typical applications.

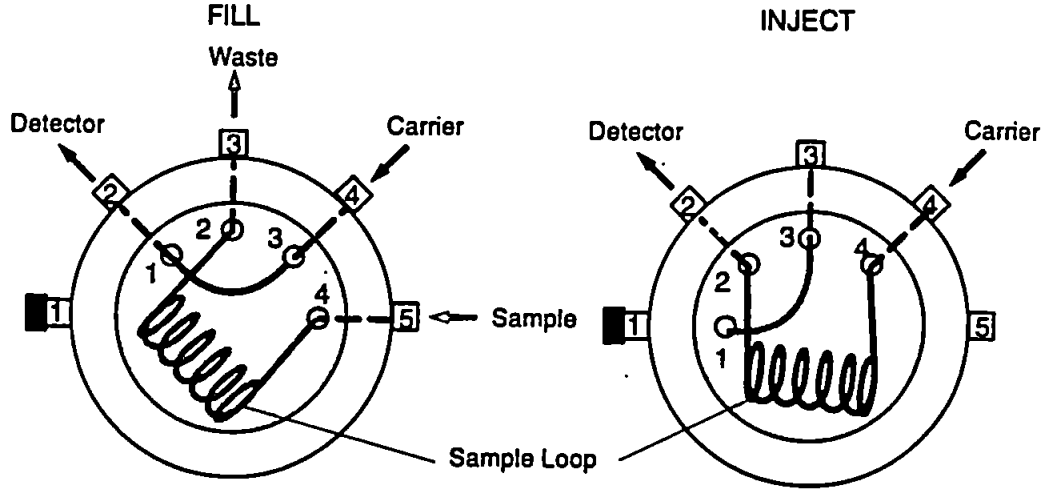


Figure 2.11 : **A schematic diagram of the five port valve showing the "Fill" and "Inject positions" when used for mercury and hydride forming element determinations**

The basic five port valve was used in the cold vapour / hydride forming elements (CV / HFE) determinations, while the eight port valve was used for the on-line column preconcentration studies when more control and flexible manipulation was required for the on-line methodologies. Both valves have only two positions, "Fill" and "Inject", as shown by the five port valve in *Figure 2.11*.

A flow of purge gas (argon) into the manifold is used to flush the mercury vapour / hydride into the atomisation cell. The gas valve allows adjustment of the gas flow from 0 to 250 ml min^{-1}.

For cold vapour and hydride analysis, a manifold kit containing mixing blocks and the gas liquid separator (GLS) was used, along with an electrically heated mantle to hold the quartz cell. The two autosamplers, Perkin Elmer AS-90 and AS-91, were used with different trays, which allowed a variety of different sample volumes (large 50 ml and small 15 ml) to be held ready for analysis.

2.4.1.1 Furnace flow injection system coupling

The physical connection between the furnace and the FIAS 400 depended upon the analyte being analysed. The simplest coupling was that used for the determination of mercury (Chapter 3). The outlet of the gas liquid separator (GLS) was simply connected to the inlet of the quartz tube with a piece of PTFE tubing. Once the mercury vapour had been generated, the argon gas flushed the mercury from the GLS, through the tubing into the quartz cell. The measurement step started as soon as the valve has been switched to the "Inject" position.

For the in atomiser trapping of the hydride forming elements (HFE), the coupling required the use of the sampler arm to move the delivery tube in and out of the furnace. The PTFE capillary of the AS-72 autosampler was disconnected from the autosampler arm and was replaced by a PTFE capillary with a quartz tip. The quartz tip was connected to the sampler arm and the other end of the PTFE tube was connected to the GLS. The quartz tipped capillary was carefully adjusted so as to inject the hydrides over the heated platform of the THGA without touching the platform or the tube walls. The optimum distance between the quartz tip and the heated platform surface was about 1.5 mm.

The coupling for the on-line preconcentration work was slightly more difficult, as a defined volume of ethanol was to be injected into the tube. The set up of the delivery tube was identical to that of the in atomiser trapping work except that the delivery tube was connected to one of the valve ports and not to the GLS.

To prevent dispersion of the eluent slug, the distance between the graphite tube and the eight port valve was kept to a minimum, which meant the FIAS 400 unit was elevated from its normal position, to a position were the top of the valve was slightly below the graphite tube and so the eluent "slug" had a slight upward slope to the tube. The smallest possible diameter PTFE tubing was used, to minimise the total internal volume of the delivery tube. This optimisation procedure is discussed further in *Section 5.3.4.2.*

The eight port valve allowed the use of a defined sample loop, therefore all of the eluent could be injected into the furnace[56,113] and not just the most concentrated sub sample as previously used by other workers.[55]

2.4.2 Configuration of flow injection system and ETAAS for mercury determination

In Chapter Three, the FIAS 400 was set up with the five port valve and the manifold kit, where the gas liquid separator was attached directly to the quartz cell as shown in *Figure 2.12*. The Model 4100ZL had the furnace head removed and an electrically heated mantle containing the quartz cell, was placed in the light path instead.

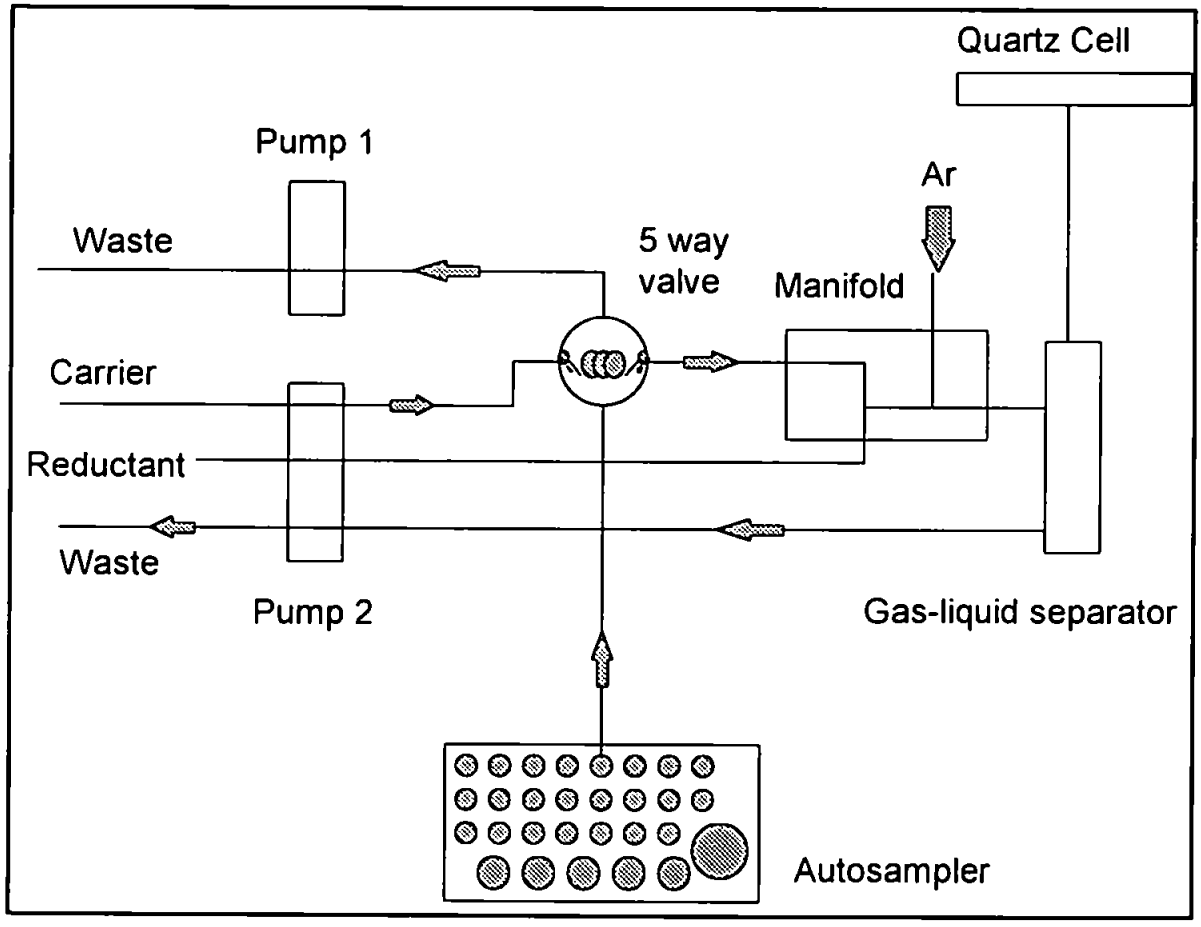


Figure 2.12 : **Flow injection system configuration for mercury determination by cold vapour atomic absorption spectrometry**

Both instruments were controlled via an Epson EL2 computer with the Perkin Elmer FIAS-Furnace Version 7.21 software operated in the FIAS only mode. This allowed the software to control the FIAS functions *i.e.* the speed of the pumps and the switching of the valve. The software also controlled the temperature of the mantle and the quartz tube.

2.4.2.1 Operating procedure for mercury determination

The FIAS 400 uses a three step program in order to fulfil the two separate operations of loading and then injecting the known sample volume into the carrier stream. The default FIAS program is shown in *Table 2.1*.

Step No	Time / secs	Pump 1 / rpm	Pump 2 / rpm	Valve	Read
Prefill	20	100	120	Fill	
1	10	100	120	Fill	
2	20	0	120	Inject	Read

***Table 2.1 :* Default flow injection system program for mercury determination**

Solution	Pump Tubing colour code	Pump Tubing i.d. / mm
Carrier	blue / yellow	1.52
Reductant	red / red	1.14
Waste from GLS	white / black	3.18
Waste from valve	blue / yellow	1.52

***Table 2.2 :* Default set up conditions for mercury determination**

In the prefill step, a time of 20 seconds was used to allow all the solution present in the tubing to be flushed to waste by the new solution drawn in from the reservoirs.

In Step 1, a sampling time of 10 seconds was used during which the mercury sample / standard solution was drawn into the sample loop (500 µl) with the five way valve in the "Fill" position. Meanwhile the carrier and reductant solution were continuously passed through the manifold to the gas liquid separator.

In Step 2, an injection time of 20 seconds was employed to allow the reaction to go to completion. The five way valve was then switched into the "Inject" position, pump No.1 stopped, leaving pump No 2 to pass the carrier stream through the sample loop, which introduced the 500 µl volume of sample and transported it to the first section of the manifold. At this convergence point, the carrier stream and sample merged with the reductant. The reaction mixture then continues through the reaction coil together with the gaseous mercury vapour and hydrogen gas produced as a by-product. The mixture then flowed into the second section of the manifold where it meets the argon carrier gas prior to entering the gas-liquid separator. The mercury vapour was then transported with the argon carrier gas to the top of the gas-liquid separator, through a 0.45 µm PTFE membrane filter (Schleicher + Schuell, Dassel, Germany) and into the quartz cell placed in line with the mercury hollow cathode lamp. The filter was placed between the gas-liquid separator and the quartz cell to prevent any aerosol droplets from reaching the cell which could decrease the precision of the measurement.

Sequence	Operation
A	Run FIAS steps 1 to 1
B	Run furnace step1 with FIAS step 2
C	Move AS arm into furnace for FIAS steps 3 to 3
D	Move AS arm out of furnace
E	Stop FIAS pumps
F	Run furnace steps 2 to 4

Table 2.3 : **Sequence control program for hydride determination**

2.4.3 Configuration of flow injection system and ETAAS for multi element determinations

The next two sections describe the two different configurations used with the FIAS 400 and the SIMAA 6000 for in atomiser trapping and on-line column preconcentration.

2.4.3.1 "In atomiser" trapping of hydride forming elements

For the study described in Chapter Four, the FIAS 400 was set up with a five port valve and the manifold kit. However, one end of the delivery tube was connected to the autosampler arm and the other end was attached to the top of the gas liquid separator. This coupling is described in more detail in *Section 2.4.1.1.*

Both instruments were controlled by a computer running Perkin Elmer AA Winlab software, operating in the flow injection-furnace mode. The rotation speed

of the pumps, their stop / go intervals, the actuation of the injection valve and the interaction of the autosampler arm were controlled by the sequence program shown in *Table 2.3*. The program is made up of simple statements which issue a command to the FIAS or the furnace, or in some cases, both. The default furnace and FIAS programs are shown in *Tables 2.4* and *2.5*, respectively. The furnace temperatures in steps 1-3 are chosen automatically by the software when the elements to be determined are selected. Step 4 is always 2300°C when Ir is being used as the trapping agent.

Step	Temperature / °C	Ramp Time / s	Hold Time / s	Gas Flow / ml min^{-1}
1	250	1	60	0
2	250	1	20	250
3	2000	0	5	0
4	2300	1	3	250

***Table 2.4 :* Default furnace program for the determination of Bi and Se**

Step No	Time / s	Pump 1 / rpm	Pump 2 / rpm	Valve
Prefill	20	100	0	Fill
1	10	100	0	Fill
2	5	100	80	Fill
3	20	0	80	Inject

***Table 2.5 :* Default flow injection system program for hydride generation**

2.4.3.2 Operating procedure for multi-element hydride generation

The combined FI procedure and synchronisation of the atomiser time / temperature program was controlled by the sequence in *Table 2.3.* The whole preconcentration and furnace operation took 2 minutes and consisted of seven individual steps. The preconcentration process started with the sample loop being filled with fresh sample, and ended with the sequestration of the hydride upon the tube and finally the atomisation and measurement step in the graphite tube. The furnace operation started with the trapping step, followed by a conventional furnace program of drying / ashing, atomisation and a clean out step. The atomiser ran for 88 seconds which included a 60 second trapping time and the FIAS ran for 55 seconds. Before the first replicate of any new sample, the Prefill Step (Pump No 2) pumped fresh sample into the sample loop from the sample reservoir expelling any previous sample left in the tubing to waste. This prefill step was automatically omitted for replicate determinations on the same sample. The FIAS set up is identical to *Figure 2.12* except the quartz cell is replaced by a graphite tube.

Sequence A : Sample solution pumped (FIAS Step 1, pump No 1) from the autosampler vessel to fill the 500 µl sample loop, excess sample to waste.

Sequence B : Furnace Step 1, the tube is preheated to 250 or 300 °C, *i.e.* the trapping temperature. In parallel, FIAS Step 2 (pump No 2) pumped fresh carrier and reductant solutions into the manifold, and the argon gas swept the GLS clean, pump No 1, completed the sample loading.

Sequence C : The autosampler arm moved the quartz pipette tip from the standby position into the graphite tube, (tip approximately 1.5 mm from the platform surface). FIAS Step 3 switched the valve from the "Fill" to the "Inject" position, and the sample was mixed with the carrier stream followed by the tetrahydroborate solution and argon gas and passed through a reaction coil. The mixture entered the GLS where the hydride vapour was swept into the graphite tube by the argon gas flow, and the waste was pumped out. Between the GLS and the delivery tube, was a PTFE membrane filter to prevent excessive moisture from entering the graphite tube. Furnace Step 1 maintained the tube at the defined trapping temperature.

Sequence D : The autosampler arm moved back to its standby position.

Sequence E : Both pumps on the FIAS were stopped to reduce reagent and sample consumption.

Sequence F : The atomiser time / temperature program (*i.e.* Steps 2 to 4) ran to completion. The second step was a drying step to remove excess hydrogen and any water vapour before the atomisation step (Step 3). The clean-out step (Step 4) cleaned the tube before the next sample was trapped. A maximum temperature of 2300°C was used to prevent the iridium coating from being removed.

2.4.3.3 Multi-element on-line column preconcentration

For the study described in Chapter Five, the FIAS 400 was set up with the eight port valve, but connected to the SIMAA 6000 as in *Section 2.4.3.1*, although

the delivery tube was connected directly to the eight port valve and not to the gas-liquid separator.

Both instruments were controlled by a computer running Perkin Elmer AA Winlab software, operating in the flow injection-furnace mode. The rotation speed of the pumps, their stop / go intervals, the actuation of the injection valve and the interaction of the autosampler arm was programmed and controlled by the sequence program shown in *Table 2.6*.

Sequence	Operation
A	Run FIAS steps 1 to 4
B	Move AS arm into furnace for FIAS steps 5 to 5
C	Run FIAS steps 5 to 5
D	Move AS arm out of furnace
E	Stop FIAS pumps
F	Run furnace steps 1 to 5

***Table 2.6* : Sequence control program for on-line column preconcentration**

The program consisted of simple statements which issue a command to the FIAS or the furnace or in some cases, both, which allows a greater control of the whole preconcentration process. The default furnace and FIAS programs are shown in *Tables 2.7* and *2.8* respectively. The furnace temperatures in steps 3 and 4 were chosen automatically by the software when the elements to be determined were selected.

Step	Temperature / °C	Ramp Time / s	Hold Time / s	Gas Flow / ml min^{-1}
1	110	0	45	250
2	130	5	75	250
3	400	10	20	250
4	2300	0	5	0
5	2400	1	2	250

Table 2.7 : **Default furnace program for the determination. The pyrolysis temperature is determined by Cd and the atomisation temperature by Ni.**

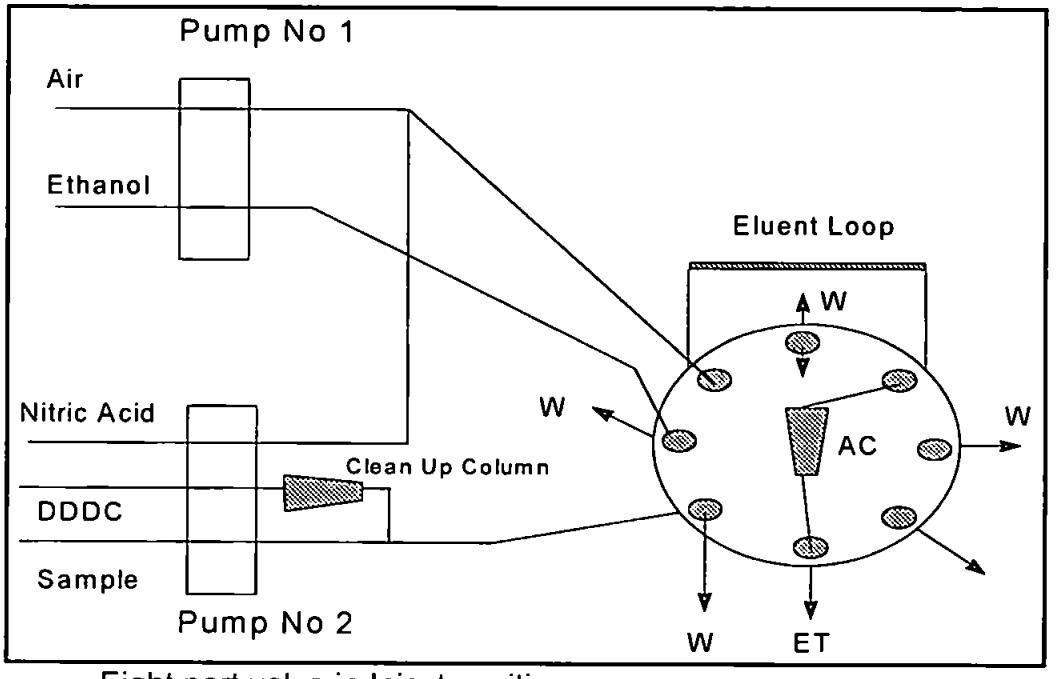
Step No	Time / s	Pump 1 / rpm	Pump 2 / rpm	Valve
Prefill	60	0	20	Inject
1	60	0	20	Fill
2	20	0	20	Inject
3	40	60	0	Inject
4	10	10	0	Fill
5	90	20	0	Inject

Table 2.8 : **Default flow injection system program for on-line column preconcentration**

2.4.3.4 Operating procedure for on-line column preconcentration

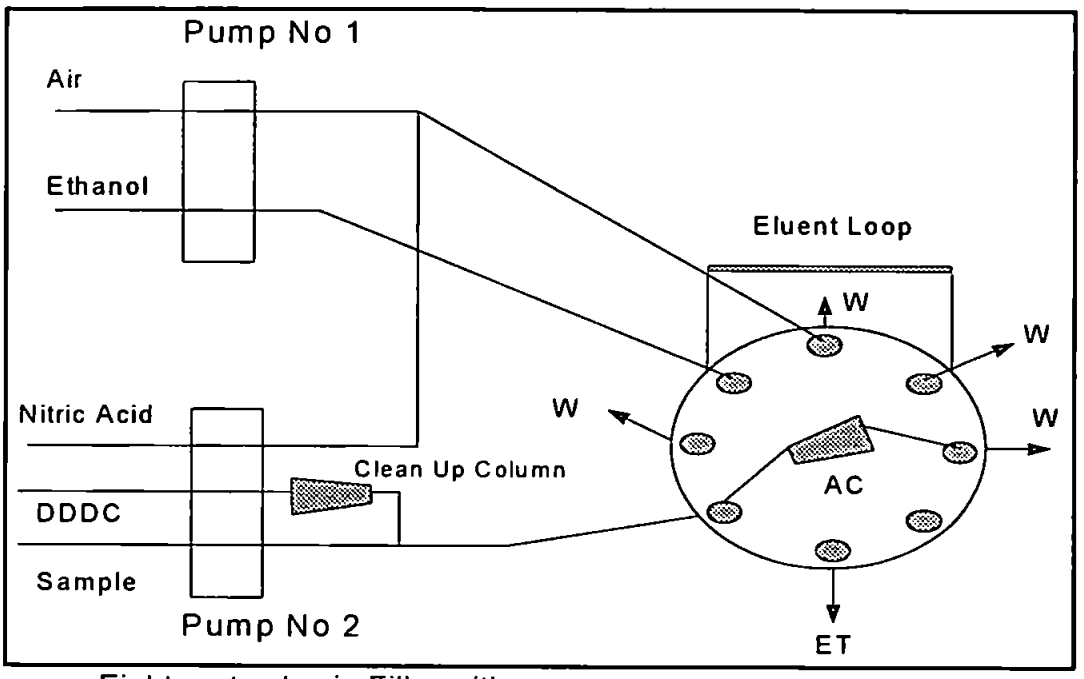
The combined FI procedure and synchronisation of the atomiser time / temperature program was controlled by the sequence in *Table 2.6.* The whole operation (seven individual steps) took 7 minutes, from the prefill step and

finished with the measurement step. The atomiser ran for 150 seconds with two minutes drying time to remove the ethanol without spluttering, and the FIAS ran for almost four minutes. Before the first replicate of any new sample, the Prefill step (Pump No 2) pumped fresh sample / complexing agent and acid washing solution from the reservoirs through the tubing, expelling any previous sample to waste. This prefill step was automatically omitted for replicate determinations on the same sample. A schematic diagram of the FIAS set up is shown in *Figure 2.13* a+b for the multi-element column preconcentration operation.



Eight port valve in Inject position
W = Waste, AC = Analytical Column, ET = To ETAAS

Figure 2.13a : **Flow injection system configuration on-line multi-element preconcentration by electrothermal atomic absorption spectrometry**



Eight port valve in Fill position
W = Waste, AC = Analytical Column, ET = To ETAAS

Figure 2.13b : **Flow injection system configuration on-line multi-element preconcentration by electrothermal atomic absorption spectrometry**

Sequence A : With the valve in the "Fill" position, the first four FIAS steps were started. In Step1, the preconcentration step, the sample and complexing agent (Pump No 2) were pumped through the column while the acid washing solution flowed to waste. After the preconcentration time, the valve moved to the "Inject" position and the acid washing solution (Step 2) was pumped through the column to remove any matrix that may have been left on the column or in the tubing. In the third step, Pump No 1 stopped and Pump No 2 propelled air across the column to dry it and expel any solutions left in the delivery tube to waste. The fourth step involved the

valve moving to the "Fill" position and the ethanol was pumped (Pump No 1) to fill the sample loop.

Sequence B : The autosampler arm moved the quartz pipette tip from the standby position into the graphite tube.

Sequence C : FIAS Step 5 switched the valve to the "Inject" position. The ethanol was propelled (Pump No 1) by a slow steady stream of air, from the sample loop through the column desorbing the metal - DDC complex and injecting it into the furnace.

Sequence D : The autosampler arm moved back to its standby position.

Sequence E : Both pumps on the FIAS were stopped to reduce reagent and sample consumption.

Sequence F : The atomiser time / temperature (*i.e.* steps 1-5) ran to completion. Two drying steps were used to ensure that the ethanol was evaporated off slowly without spluttering. The pyrolysis step was included to remove the organic complexing agent before the atomisation and measurement step.

Chapter Three :

Determination of Mercury

Chapter Three : Determination of Mercury

3.1 Introduction

This chapter describes the development of an off-line microwave digestion sample pre-treatment procedure for the determination of total mercury in environmental and biological samples by flow injection cold vapour atomic absorption spectrometry (FI-CVAAS).

The chapter is split into four main sections. The introduction section gives an overview of the background behind determining mercury in real samples and looks at some of the problems that were identified and resolved before the work was completed. The second section is the experimental section detailing the reagents, the instrumentation used, (if not already been discussed in Chapter Two) and the operating parameters and reagents used. The results section details the many different stages used in optimising the system, and the validation of the method using appropriate certified reference materials (CRMs). It also contains the various procedures used to analyse the real samples, and includes a brief investigation into some of the known interferences when using sodium tetrahydroborate as the reducing agent for this type of analysis. The final part of the chapter is the conclusion section, which discusses the merits of the proposed system and the conclusions for this stage of the work.

3.1.1 Introduction to the determination of mercury

The determination of mercury in the natural environment has long been recognised as important when assessing environmental quality. The rapid growth in agricultural and industrial activity has resulted in chemical pollution. In this context mercury is one of the most toxic elements and can produce serious and irreversible damage.[158] However, the accurate determination of mercury in real samples is not without its difficulties and precautions are required at all stages of the analysis. Relatively poor analytical sensitivity and contamination during the analytical procedure combine to give unreliable mercury concentrations.[76]

As mentioned in Section 1.4, the analytical technique for determining mercury is based upon its unique properties. Mercury can easily be reduced to the metal from its compounds by $SnCl_2$ or $NaBH_4$. The mercury vapour may be entrained in a stream of inert gas or air and measured by atomic absorption of the cold vapour without the need of an atomiser or a flame.[159]

3.1.2 Problems associated with the determination of mercury

When reviewing the current literature associated with the determination of mercury, various problems were identified. These problems included achieving sufficiently low detection limits, choosing the best analytical methodology, and digestion process to correctly analyse the environmental and biological samples for total mercury content.

3.1.2.1 Detection limit

The direct determination of mercury by atomic spectrometry can be problematic. For example, the determination of mercury by flame atomic absorption spectrometry (FAAS) is limited to high concentrations of mercury owing to the poor detection limit offered by the technique (200 $\mu g\ l^{-1}$), whilst the use of electrothermal atomic absorption spectrometry (ETAAS), although offering better detection limits (2 $\mu g\ l^{-1}$), suffers from matrix interferences since the high volatility of mercury restricts the ashing temperature.[71]

One of the most common analytical approaches for the determination of total mercury at lower concentrations is cold vapour atomic absorption spectrometry (CVAAS) as mentioned in *Section 3.1.1.* This approach offers a detection limit[85] in the order of 0.008 $\mu g\ l^{-1}$ and is based upon the reduction of inorganic mercury by tin^{II} or tetrahydroborate. Due to the low detection limit, CVAAS was selected as the measurement technique for use in this study. Flow injection analysis (FIA) methodology was also employed to automate the sample introduction to the CVAAS, thereby improving sample throughput as well as accuracy and precision.

3.1.2.2 Analytical Methodology

Zhu *et al.*[76] developed a method which could determine total mercury at sub ng ml^{-1} levels in environmental samples by CVAAS using sodium tetrahydroborate as the reductant. The analysis of the samples was performed on-line but the pre-digestion stage was performed off-line. Other papers have reported the determination of mercury by CVAAS in biological materials[75,160] and

sediments[161-162] and give a valuable insight into the procedures used, highlighting the pitfalls to avoid in the analytical method under investigation.

Three relevant papers to this study have been published by various workers in Germany. These concerned the use of automated flow injection systems for the on-line decomposition of mercury in urine following the addition of $KMnO_4$ and bromate / bromide reagent[82] and the on-line microwave digestion of mercury by CVAAS in blood and urine samples.[83,163] These studies highlighted several problems that might occur with the use of automated on-line flow injection systems, such as evolution of gases during digestion, short reaction time and incomplete digestion of organic matter. It was therefore decided to use an off-line digestion technique instead of an on-line technique as this would simplify the procedure.

Since CVAAS was chosen as the method of analysis, there are two primary pre-requisites to be satisfied, if it is to be used successfully and volatilisation losses are to be avoided. These are :-

i) sufficient oxidation of the organic matter present in the sample to liberate the mercury from the matrix, and

ii) the quantitative production of a single labile mercury species (*e.g.* Hg^{II}) which can afterwards be reduced quantitatively to Hg^0 for spectrometric evaluation. If these conditions are not achieved, serious underestimation of the total mercury concentration may result.[75]

It is generally agreed that oxidative conversion of all forms of mercury in environmental and biological samples to Hg^{II} is necessary prior to reduction to elemental mercury.[83,163-164] Sodium tetrahydroborate is often preferred as the

reducing agent because it offers rapid liberation of the mercury vapour from the liquid phase, and is effective with organic compounds that are not reduced to the metal by tin^{II} chloride.[75] Therefore, sodium tetrahydroborate was used as the reductant since it fulfilled the two primary requisites slightly better than the tin^{II} chloride.

3.1.2.3 Digestion techniques and mixtures

The direct determination of mercury in environmental and biological materials may be problematic as a result of the matrix effects encountered, particularly with solid samples. The accurate determination of mercury in these materials usually requires the decomposition of the matrix and the conversion of mercury to the inorganic divalent form. Two types of decomposition method have been employed for this purpose, *i.e.* wet digestion and dry ashing.[165-166] Dry ashing is not suitable for mercury determinations because of the low boiling point of mercury and its associated compounds. Consequently wet digestion using either relatively low temperatures (<100°C) or higher temperatures (230°C) with an appropriate selection of oxidising acid mixtures have been employed successfully. However such methods can be quite lengthy because of the many steps required and may be prone to contamination. Thus wet digestion methods have increasingly been succeeded by microwave assisted digestion methods, which offer rapid sample preparation, increased sample throughput, reduced risk of contamination and reduced reagent consumption. The microwave assisted digestion methods have previously been employed for a number of environmental and biological matrixes.[167-169]

Various workers have used a bewildering combination of strong acids (HCl, H_2SO_4, HNO_3, H_3PO_4 and $HClO_4$), oxidants (H_2O_2, $KMnO_4$, $K_2Cr_2O_7$, and $K_2S_2O_8$), as well as UV irradiation and elevated temperatures in order to ensure complete digestion and separation of the analyte from the parent matrix. The various combinations favoured in the literature are stated in *Table 3.1*.

Reagents	Ref	Reagents	Ref
HNO_3	160,167,169-171	HCl, HNO_3, H_2O_2	167
HNO_3, H_2SO_4, H_2O_2	167,171-172	HNO_3, $K_2S_2O_8$, H_2O_2	172
HNO_3, H_2O_2	172	HNO_3, H_3PO_4, H_2O_2	172
HNO_3,HF, HCl	167	HNO_3 H_2SO_4	171]

Table 3.1 : **Combination of digestion reagents used for microwave decomposition of environmental and biological samples**

The most popular digestion reagent has been nitric acid but a mixture of nitric acid, sulphuric acid and hydrogen peroxide is also favoured. A review by Adeloju *et al.*[165] evaluated four commonly used wet digestion mixtures for the determination of mercury in biological and environmental matrixes with analysis by cold vapour atomic absorption spectrometry (CVAAS).

The four mixtures evaluated in this study were,

i) nitric acid and hydrogen peroxide,

ii) nitric acid only,

iii) nitric acid and perchloric acid, and

iv) nitric acid and sulphuric acid.

Adeloju *et al.*[165] indicated that a mixture of HNO_3 and H_2SO_4 gave the most accurate and reproducible analytical data when applied to a range of biological and environmental CRMs. The mixture was also suitable for the reliable determination of mercury in soil samples.

The digestion was performed by an off-line microwave digestion technique using the digestion mixtures chosen. Clearly, as the method should ideally be simple to operate, the digestion mixture chosen should be equally applicable to all the samples being investigated.

3.1.3 Experimental Aims

The main aim of this study was to develop a simple method to determine total mercury in environmental and biological samples. The method had to include a digestion procedure that ensured that all the mercury from the sample was available for analysis. The accuracy and precision of the method was validated by analysing appropriate CRMs and by performing an interference study as well as analysing real samples.

3.2 Instrumentation

3.2.1 Furnace and flow injection system configuration

A Perkin-Elmer Model 4100ZL atomic absorption spectrometer in conjunction with a Perkin-Elmer FIAS 400 flow injection analysis system and an AS-90 autosampler were used in this study. The system was operated through an

Epson EL2 computer and the associated Perkin-Elmer software, Version 7.21. An Epson FX-850 printer was used to print the results and signal traces. The spectrometer was set up using the optimised operating conditions shown in *Table 3.2*. The FIAS program is stated in *Table 3.3* and the optimised flow rates and tubing are described in *Table 3.4*.

Lamp	Mercury Hollow Cathode Lamp
Lamp Current	6 mA
Spectral band pass	0.7 nm
Wavelength	253.7 nm
Cell Temperature	100°C
Integration Time	20 seconds
Read Time Delay	none
Baseline Offset Correction	2 seconds

Table 3.2 : **Instrumental operating conditions for electrothermal atomic absorption spectrometer**

Step No	Time / s	Pump 1 / rpm	Pump 2 / rpm	Valve	Read
Prefill	20	100	120	Fill	
1	10	100	120	Fill	
2	20	0	120	Inject	Read

Table 3.3 : **Optimised program for the flow injection system for determination of mercury**

Solution	Pump Tubing colour code	Pump Tubing i.d / mm	Flow Rate / ml min^{-1}
Carrier	blue / yellow	1.52	11.5
Reductant	red / red	1.14	6.4
Waste from gas liquid separator	white / black	3.18	18.8
Waste from valve	blue / yellow	1.52	7.5
Argon gas	-	-	100
Sample intake	-	-	8.1

Table 3.4 : **Optimised pump tubing and flow rates for the flow injection system**

3.2.2 Microwave instrumentation

All of the microwave digestions were performed with an unmodified 750W domestic microwave oven (Tecnolec T200M). The oven power (0-100% power in 25% increments) and time range (0-30 minutes) were set manually. The microwave oven was placed in a laboratory fume cupboard whilst the digestion stage was in progress.

Screw capped 60 ml PTFE digestion vessels (Saviller, Minnesota, USA) were used. These were cleaned prior to use with concentrated nitric acid in the microwave at full power for 60 seconds. The vessels were rinsed thoroughly with distilled water and dried prior to use.

3.2.3 Quartz cell

The quartz cell (length 160 mm x i.d. 7.5 mm) was housed inside an electrically heated mantle with the temperature set at 100°C to prevent any build up of water vapour inside the cell which would interfere with the measurement of the mercury signal.

3.2.4 Atomic Fluorescence instrumentation

In the comparison experiment described in *Section 3.3.5.2*, the system used was a Merlin atomic fluorescence detector with a commercial hydride generator, both supplied by P.S. Analytical, Orpington, Kent. The whole system was controlled by a computer equipped with Touchstone software unit (P.S. Analytical, Orpington, Kent). The hydride generator consisted of a peristaltic pump delivering three streams, (reductant, carrier and blank solutions) to a switching valve and a type B gas liquid separator (GLS). *Table 3.5* gives the optimised conditions for the hydride generator. The detector consisted of a boosted mercury hollow cathode lamp (wavelength 254.7 nm) and a non solar blind (IP 28) photomultiplier tube with a 254 nm interference filter, which detected the focused emission from the atomisation cell which consisted of a cylindrical hydrogen argon flame with a sheath gas of argon.

3.2.4.1 Procedure for the atomic fluorescence measurement

The measurement procedure had four separate steps. The first step was the *delay time* (25 seconds), where the reductant and the blank solution were pumped through the system until they met at the gas - liquid separator (GLS) to establish a baseline signal. The sample solution was pumped to waste during this step.

The next step was the *rise time* (30 seconds) where the valve switched so that the sample and reductant mixed in the GLS. The mercury vapour produced was flushed from the GLS to the detector by a stream of argon gas. The blank solution then flowed to waste. The analytical signal starts to rise to a maximum.

Solution	Reagent	Flow Rate / ml min^{-1}
Blank solution	3% (v/v) Hydrochloric acid	3.0
Carrier solution	3% (v/v) Hydrochloric acid	7.0
Reductant solution	1% (m/v) Sodium tetrahydroborate in 0.5% (m/v) Sodium hydroxide	3.0
Sheath Gas	Argon gas	300
Carrier Gas	Argon gas	300

Table 3.5 : **Optimised conditions for the hydride generator with atomic fluorescence detection**

In the *analysis step* (30 seconds), the conditions were identical to the rise time, and so a steady state signal was obtained. The next step was the *memory step* (60 seconds) where the valve was switched back to its original positions *i.e.* the blank solution and the reductant flowing to the GLS flushing the system and allowing the signal to return to baseline.

Quantification of the analytical signal was calculated by the Touchstone software by taking peak area measurements from the steady state signals obtained.

3.2.5 Reagents

All reagents used were of analytical grade (BDH Laboratory Supplies, Poole, Dorset, UK) unless otherwise stated. Double distilled water was used throughout.

Reductant solution : 0.2% (w/v) $NaBH_4$ in 0.05% (w/v) NaOH

The solution was prepared by first dissolving 0.5 grams of sodium hydroxide pellets and then 2.0 grams of sodium tetrahydroborate in 1000 ml of distilled water. The solution was stirred and then filtered before use. This solution was prepared daily.

Carrier Solution : 3% (v/v) HCl

The solution was prepared by adding 30 ml of concentrated hydrochloric acid to a 1000 ml volumetric flask and making up to volume with doubly distilled water.

Stabilising Solution : 5% (w/v) $KMnO_4$

A stock solution was prepared by dissolving 5.0 grams of potassium permanganate (Spectrosol grade, BDH Ltd) in 100 ml of doubly distilled water. Two drops of this stabilising solution was added to every 100 ml of standard solution. This stock solution could be kept for 1 month in a dark glass bottle.

Standard Mercury Solutions :

The standard solutions of inorganic Hg^{II} were prepared by stepwise dilution from a stock standard solution containing 1001 ± 2 mg l^{-1} Hg as $Hg(NO_3)_2.H_2O$ (BDH Spectrosol). All standards were freshly prepared prior to use.

Diluent Solution : 2% (v/v) HNO_3 + 2% (v/v) H_2SO_4

This solution was prepared by slowly adding 20 ml of concentrated nitric acid and 20 ml of concentrated sulphuric acid to approximately 900 ml of doubly distilled water and then making up to 1000 ml, once the solution had cooled down.

All the sample and standard solutions were made up using this diluent solution. To prevent any loss of mercury through adsorption onto the containers walls, 1 ml of concentrated hydrochloric acid per 100 ml of sample solution was added. The hydrochloric acid concentration was kept to a minimum to prevent the premature reduction of the potassium permanganate.

Digestion Mixture Reagents :

The reagents used in the various digestion procedures were concentrated sulphuric and nitric acids and 30% hydrogen peroxide.

Carrier Gas :

The carrier gas used in all experiments was argon gas with a purity of more than 99.95% (Cryospeed, BOC Limited, Plymouth, UK.)

Certified Reference Materials :

The certified reference materials used in this study were DORM-1 dogfish muscle reference material and MESS-2 estuarine sediment. Both of these CRMs were purchased from National Research Council Canada, Ottawa, Canada.

3.3 Optimising the system

Once the furnace and the flow injection system (FIAS 400) were set up using the default conditions stated in the *Section 2.3.2.1*, the coupled instrument was optimised for the basic parameters, such as reductant / carrier flow rates, sample loop volume and argon gas carrier flow to maximise the analyte signal. Once the basic parameters had been optimised, the system could be evaluated using mercury standard solutions to determine the linearity and the sensitivity of the method for the determination of total mercury.

3.3.1 Optimisation of flow rates

The tubing was put under tension and when the reagents were flowing smoothly through the tubing, the flow rates were determined by measuring the uptake of water at the various pump speeds. The recommended carrier to reductant ratio as stated in the literature[173] is 2 : 1, but the optimised flow rate for this study was 1.8 : 1.

A high (100 $\mu g\ l^{-1}$) mercury standard was used (for maximum response) to determine the optimum gas flow by ensuring that the full peak (signal) was within the 20 second time window. The optimum flow rate was found to be 100 ml min^{-1}

since this gave good symmetrical peak shapes and the tail of the peak returned to the baseline within the time window.

3.3.2 Determination of linearity for mercury by FI-CVAAS

Using the 100 μg l^{-1} mercury standard as an initial maximum, a range of mercury standards (0-100 μg l^{-1}) were analysed to determine the linear range for the method. *Figure 3.1* depicts the calibration graph which shows that there is a good correlation for the line of best fit over the 0-60 μg l^{-1} range, but for standards over 60 μg l^{-1} (*Figure 3.2*), the calibration is not linear and shows a tendency to become parallel to the baseline. *Table 3.6* shows the calibration data over the working linear range.

Standard μg l^{-1} of Hg	Mean Peak Height / A	Std Devn	RSD / %
0	0.005	1.00×10^{-3}	20.0
5	0.041	1.53×10^{-3}	3.7
10	0.091	1.53×10^{-3}	1.7
20	0.182	5.00×10^{-3}	2.7
40	0.336	3.79×10^{-3}	1.1
50	0.415	8.02×10^{-3}	1.9
60	0.480	6.11×10^{-3}	1.3

n = 5

Table 3.6 : **Linear calibration data over the range 0-60 μg l^{-1} for mercury by FI-CVAAS**

3.3.3 Analysis of Certificated Reference Materials

In order to check that the proposed method was valid for real samples appropriate Certificated Reference Materials (CRMs) as described in *Section 3.2.4* were analysed. These were chosen to reflect the matrix and mercury concentration expected in the samples. The two reference materials chosen for this purpose were :-

i) NBS 1646 Estuarine Sediment

Certified concentration of mercury 0.063 ± 0.012 mg kg^{-1}

ii) DORM-1 Dogfish Reference Muscle

Certified concentration of mercury 0.798 ± 0.074 mg kg^{-1}

3.3.3.1 First analysis of Certified Reference Materials

The two CRMs (NBS 1646 and DORM-1) were first analysed by direct calibration after the microwave digestion stage. Only two replicates were run in the first experiment, and from these experiment results, a recovery could be calculated from the known certified values. Blank digested sample were also determined using the same conditions and reagents but minus any CRM sample, and a blank solution of 3% (v/v) HCL were run to check for any contamination in the carrier solution.

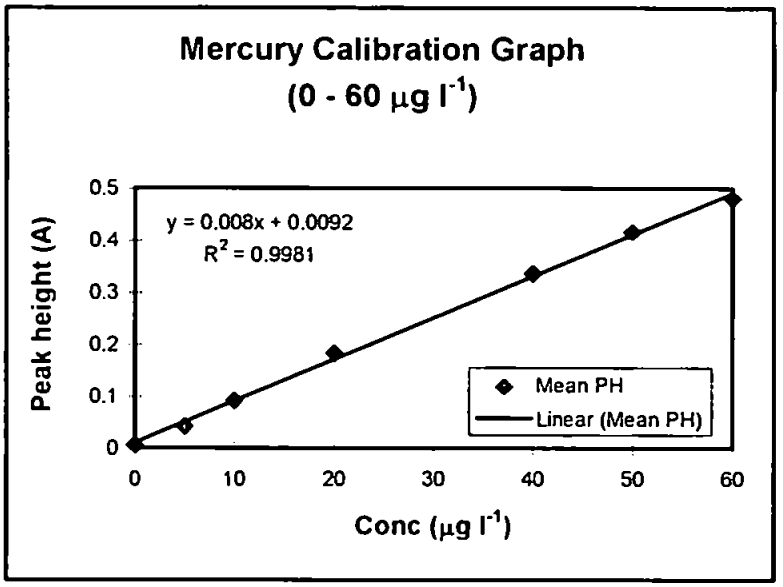


Figure 3.1 : **Linear calibration range (0-60 μg l^{-1}) for mercury by FI-CVAAS**

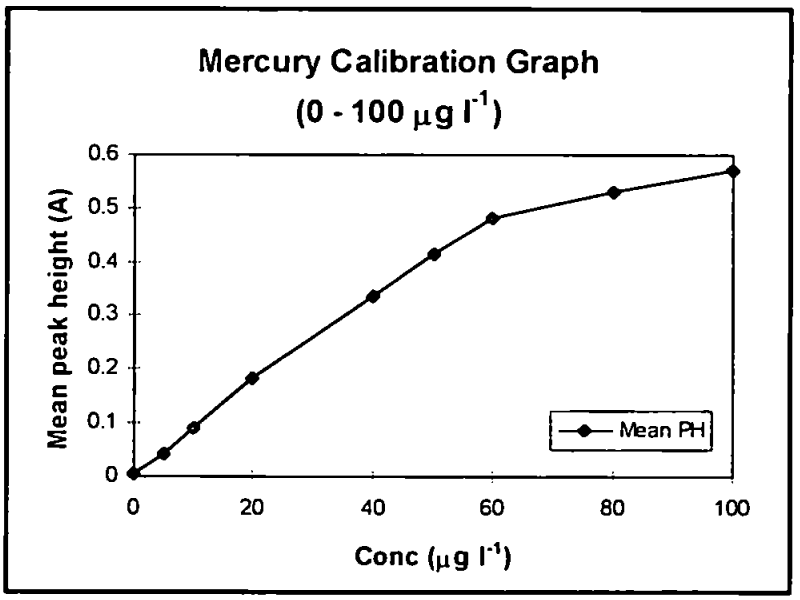


Figure 3.2 : **Complete range (0-100 μg l^{-1}) for mercury by FI-CVAAS**

Initially using the results from *Section 3.1.2.3*, three digestion mixtures were used *i.e.* i) HNO_3 only, ii) HNO_3 and H_2O_2, and iii) HNO_3, H_2SO_4 and H_2O_2. Mixture iii) was rejected for operator safety reasons as when the digestion mixture was added a violent reaction occurred causing loss of sample from the digestion bomb. Mixtures i) and ii) gave reasonable results, but the combined mixture of HNO_3 and H_2O_2 gave lower blank values than nitric acid only. Once, the digestion mixture had been added, the lids were put on the bombs and they were left overnight to allow any organic matter to oxidise before they were irradiated. The results shown in *Table 3.7* are for the digestion mixture of 3 ml conc nitric acid and 1 ml of 30% hydrogen peroxide.

The next morning, the PTFE bombs were irradiated for a total of 4 minutes in 1 minute bursts, with 2 minutes standing time between each bursts. After a two hours cooling down time, the DORM-1 digested mixture was made up to 25 ml in a calibrated flask with the diluent solution, while the NBS 1646 digestion mixture was made up to 10 ml. These volumes were chosen to ensure that the concentration of mercury in the solution would fall within the calibration range.

	Calculated Result mg kg^{-1} of Hg #	**Recovery %**	**Certified Value mg kg^{-1} of Hg +**
DORM-1	0.699 ± 0.010	87.6	0.798 ± 0.074
NBS 1646	0.091 ± 0.008	144.4	0.063 ± 0.012

Mean and one standard deviation
+ Mean and 95% confidence limits

Table 3.7 : **Direct calibration results for mercury in NBS 1646 and DORM-1 certified reference materials**

3.3.3.2 Conclusion

From these results, it can be seen that both CRMs gave results outside the certified ranges. This would indicate that either :-

i) there was a contamination problem,

ii) there was an interference problem, or

iii) that the digestion process was not releasing all the mercury from the sample matrix.

In order to overcome these problems, two different approaches were used. The first approach was to analyse the CRMs by the method of standard additions. The second approach was to try alternative digestion mixtures, previously identified in *Section 3.1.2.3.* The certified values are given for corrected mass, therefore a weight loss experiment was conducted to find the weight loss for each CRM, and therefore corrections could applied to the experimental values so the two sets of values could be directly compared. The % weight loss results are given in *Table 3.8.*

Sample	% Weight Loss
DORM-1	14.65
MESS-2	1.44
NBS 1646	2.54

***Table 3.8 :* Weight loss results for all the certified reference materials**

3.3.4. Analysis of CRMs by Standard Addition

From each unspiked digestion, 4.0 ml of solution was transferred to a clean sample vial and 4.0 ml of a standard solution (either 0, 1.0, 2.0, 10.0 μg l^{-1}) was added. The mean peak height of each sample was then plotted against half the added standard concentration (*i.e.* 0, 0.5, 1.0, 5.0 μg l^{-1}). The direct and standard addition calibration graphs are shown in *Figure 3.3* for NBS 1646 and *Figure 3.4* for DORM-1. A standard calibration graph (*Table 3.9*) was used in the range of 0-10 μg l^{-1} (n = 4 for each standard) for this experiment.

Standard μg l^{-1} Hg	Mean Peak Height / Abs	Std Devn	RSD / %
Blank	0.002	5.8×10^{-3}	38.5
1	0.012	0.0×10^{-3}	0.0
2	0.021	5.0×10^{-4}	2.4
5	0.052	0.0×10^{-3}	0.0
10	0.101	5.0×10^{-4}	0.5

(n = 4)

***Table 3.9 :* Standard calibration graph for mercury**

Due to the limited sample size, the samples were only analysed in duplicate (n = 2). *Table 3.10* shows the standard addition data for both CRMs. *Table 3.11* shows the results for the standard addition method along with the previously obtained direct calibration data for comparison

Addition of Hg $\mu g\ l^{-1}$	Mean Peak Height / A	Std Devn	RSD / %
DORM-1			
0	0.030	7.0×10^{-4}	2.4
1	0.033	1.4×10^{-3}	4.3
2	0.037	0.0×10^{-4}	0.0
10	0.070	7.0×10^{-4}	1.0
NBS 1646			
0	0.007	0.0×10^{-4}	0.0
1	0.012	7.0×10^{-4}	6.2
2	0.016	7.0×10^{-4}	4.6
10	0.053	7.0×10^{-4}	2.7

(n = 2)
Certified value for Hg
DORM-1 0.798 ± 0.074 mg kg^{-1} NBS 1646 0.063 ± 0.012 mg kg^{-1}

Table 3.10 : **Standard addition analysis data obtained from NBS 1646 and DORM-1 Certified Reference Materials**

3.3.4.1 Conclusion to standard addition experiments

The recovery for NBS 1646 was reasonable (120.6 %), but the direct calibration gave results greater than the certified value for mercury, while the result by the standard addition method is only just outside the certified range (*Table 3.11*). In both cases, the gradients are similar indicating that there was no (or a minor) interference present. It was possible that the elevated values may be due to something present in the digested sample mixture enhancing the mercury signal. It was unlikely to be due to the contamination of the vessels or reagents,

as the blank digested samples (HNO_3 and H_2O_2) show little or no signals when analysed.

	Calibration Graph mg kg^{-1} of Hg #	Standard Additions mg kg^{-1} of Hg #	Std Add Recovery / %	Certified Value mg kg^{-1} of Hg *
DORM-1	0.699 ± 0.010	0.418 ± 0.015	84.0	0.798 ± 0.074
NBS 1646	0.091 ± 0.008	0.076 ± 0.010	102.3	0.063 ± 0.012

Mean and one standard deviation
* Mean and 95% confidence limits

Table 3.11 : **A comparison of direct and standard addition results with recovery results for the standard additions experiment**

The recovery for DORM-1 was poor (52.3 %). The direct calibration gave a low result which was about 0.1 mg kg^{-1} lower than the certified result of 0.798 mg kg^{-1}, and the standard addition method showed about half the expected certified value (*Table 3.11*). Again, the gradients of the mercury calibration lines are similar, indicating a minor or no interference present. These results indicated that for the DORM-1 analysis, not all the mercury was being released from the matrix.

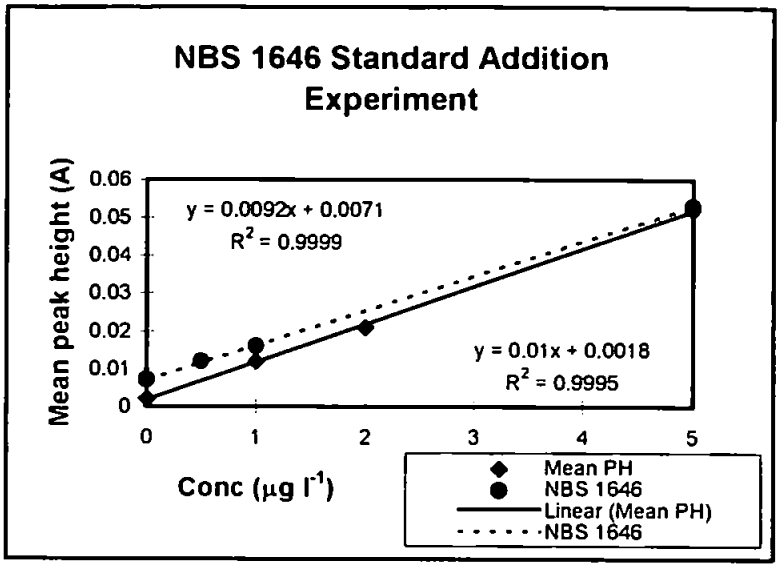


Figure 3.3 : **Standard addition calibration graph for NBS 1646 Certified Reference Material**

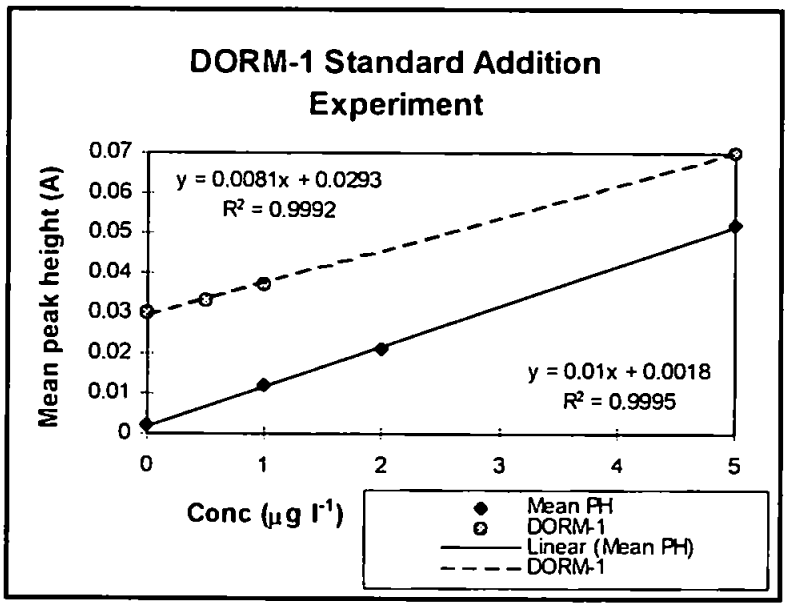


Figure 3.4 : **Standard addition calibration graph for DORM-1 Certified Reference Material**

3.3.5 Further analysis of Certified Reference Materials

3.3.5.1 Analysis of MESS-2 Certified Reference Material

The sediment certified reference material NBS 1646 used in the first part of this study (*Section 3.3.3*), once exhausted was no longer available from NIST. Unfortunately the replacement CRM, NBS 1646a is not certified for mercury. Therefore an alternative sediment CRM, MESS-2 was acquired. This has a certified total mercury value of 0.092 ± 0.009 mg kg^{-1}.

	Practical Result mg kg^{-1} of Hg #	**Certified Value mg kg^{-1} of Hg +**
NBS 1646	0.077 ± 0.016	0.063 ± 0.012
MESS-2	0.085 ± 0.015	0.092 ± 0.009

Mean and one standard deviation
+ Mean and 95% confidence limits

Table 3.12 : **Experimental results for mercury in both sediment certified reference materials**

The previous results obtained using NBS 1646 sediment were very close to but outside the certified range (*Table 3.12*). It was felt that a different digestion mixture would improve the accuracy of the analysis. The new digestion mixture used was 2.5 ml concentrated H_2SO_4, 2.5 ml concentrated HNO_3 and 1.0 ml of 30% H_2O_2 and the irradiation time was 4 minutes. The total volume of the digestion mixture was increased from 4 ml to 6 ml to ensure that all the sample / CRM was covered by the acid mixture. Before the analysis, the sample solutions

were de-gassed (See *Section 3.3.5.2)*. With these new conditions, a result *(Table 3.12)* was obtained which was within the certified range.

3.3.5.2 Analysis of DORM-1 Certified Reference Material

It was noted (*Section 3.3.3.1*) that poor results were obtained following microwave digestion of DORM-1. The problem was thought to originate in one of three areas :-

i) an interference in the quartz cell,

ii) incomplete conversion of Hg^{II} to Hg^{0} in the reaction manifold, or

iii) incomplete digestion of the sample in the closed vessel.

With a closed vessel digestion stage, any gases (*e.g.* nitrogen oxides) produced during the procedure are kept within the vessel and become re-dissolved when the mixture cools down. Since the Model 4100ZL graphite furnace when used with the quartz cell, does not have the background correction facility available, the dissolved gases may cause interference and nitrogen oxides will scavenge the reductant preventing complete reduction of the mercury.[174] To remove this, the digestion mixtures should be de-gassed before analysis in an ultra-sonic bath.[175] This procedure was introduced at the start of this experimental stage.

The incomplete conversion of Hg^{II} to Hg^{0} was thought to be very unlikely since sodium tetrahydroborate is a very effective reductant, and has been used successfully by many other workers.[75,83,163-164] Also when the FIAS system was optimised initially, the calculated characteristic mass (m_o) values for the standards

were in good agreement with the published characteristic mass values,[173] indicating that full conversion of Hg^{II} to Hg^{0} was achieved in the manifold.

The remaining possibility was therefore incomplete digestion within the digestion vessel. To investigate this, the digested mixtures were first analysed by an alternative analytical technique under similar conditions. This was done in order to ensure that the effect was not specific to the instrument used rather than the microwave digestion procedure.

Atomic fluorescence spectrometry (AFS), was selected for this purpose. However, careful consideration was given to the other techniques available, before AFS was chosen.

The five options considered were :-

i) atomic fluorescence spectrometry (AFS).

ii) inductively coupled plasma-mass spectrometry (ICP-MS).

iii) inductively coupled plasma-atomic emission spectrometry (ICP-AES).

iv) electrothermal atomic absorption spectrometry (ETAAS).

v) flame atomic absorption spectrometry (FAAS).

The latter three techniques (iii, iv and v) did not offer the required detection limit to handle the very low mercury concentration in the sample. ICP-MS has a suitable detection limit, but the instrumentation is complex and requires the addition of an on-line scrubber in the gas-line to achieve the required detection limits. This left AFS, which provides suitable detection limits and the advantage of being simple to use. The mercury vapour is produced on-line with sodium

tetrahydroborate as the reductant and hydrochloric acid as the carrier, so the basic chemistry of the AFS system is identical to that of the proposed method.

The instrumentation used for this study is described in *Section 3.2.4*. The system was optimised with a 1 $\mu g\ l^{-1}$ mercury standard. Due to the larger sample size required for each determination, the samples could only be run in duplicate and not triplicate as for the FIA system.

The results of the comparison test between analytical techniques (*Table 3.13*), indicated that the problem originated from the digestion of the sample within the digestion vessel, since both analytical experiments produced similar results. Thus the microwave was not breaking down all the mercury containing compounds to a form that could be analysed by the FI-CVAAS system. To solve this problem the digestion mixture and the irradiation time were re-evaluated to see if a more effective combination could be found.

	FIAS Result mg kg^{-1} of Hg #	AFS Result mg kg^{-1} of Hg #	Certified Value mg kg^{-1} of Hg +
DORM-1	0.660 ± 0.042	0.512 ± 0.089	0.798 ± 0.074

Mean and one standard deviation
+ Mean and 95% confidence limits

Table 3.13 : **Results obtained for mercury in DORM-1 certified reference material by atomic fluorescence and FI-CVAAS**

3.3.6 Final evaluation of DORM-1 digestion procedure

Following the comparison tests, the digestion procedure for the DORM-1 CRM was re-evaluated. Previously the digestion mixture used consisted of concentrated nitric acid and 30% hydrogen peroxide. However, in *Section 3.1.2.3*, concentrated nitric and sulphuric acid with hydrogen peroxide was identified as a suitable digestion mixture, and the results in *Section 3.3.5.1*, prove that it works well. However this digestion mixture proved problematic (*i.e.* excessive and rapid effervescence) when adding the concentrated sulphuric acid to the DORM-1 and sample.

For the digestion of biological samples, a mixture of HNO_3 and H_2O_2 is commonly used by many workers.[161,165,172] This mixture had been tried in an earlier study but was rejected due to a interference problem which affected only the digested samples. However, following the introduction of a de-gassing stage at the end of the digestion procedure, this problem could potentially be overcome.

The analysis was repeated with the digestion mixture of 5 ml concentrated nitric acid and 1 ml of 30 % hydrogen peroxide. The total volume of digestion mixture was again increased from 4 ml to 6 ml to immerse all of the sample. The first variable to be examined was the length of time the sample was irradiated by the microwave. At first, 4 minutes was used and the results obtained were lower than the certified values. This was increased to 5 minutes and results within the certified range were obtained. The sample was irradiated in 30 second bursts instead of the original 60 second bursts, to allow the bomb to be heated gently and to stabilise between irradiation bursts. The results obtained using this

procedure can be seen in *Table 3.14* and the conditions used are summarised in *Table 3.15.*

Irradiation Time	Practical Value mg kg^{-1} of Hg #	Certified Value mg kg^{-1} of Hg +
4 minutes	0.646 ± 0.010	0.798 ± 0.074
5 minutes	0.771 ± 0.028	

Mean and one standard deviation
+ Mean and 95% confidence limits

Table 3.14 : **Results obtained for different microwave irradiation times for the digestion of DORM-1 certified reference material**

	Environmental *i.e.* sediment	Biological *i.e.* fish tissue
Nitric acid	2.5 ml	5 ml
Sulphuric acid	2.5 ml	None
Hydrogen peroxide	1 ml	1 ml
Irradiation Time	4 mins	5 mins

Table 3.15 : **Summary of conditions for the closed vessel microwave digestion procedure of environmental and biological samples**

3.3.7 Interference study for mercury determinations

The last stage of this study examined the effect of a number of potential interferences on the method described above. It is known that the transition

metals and the hydride-forming elements may interfere with the determination of mercury.[176-177] The selectivity of the proposed method was therefore investigated by use of a mercury standard (10 μg l^{-1}) in the presence of the various interferents (Ag, As, Cd, Cu, Ni, Pb, Sb and Se).

The results of the study are shown in *Table 3.16*. It can be seen that silver and selenium had a suppression effect, whilst all the other interferences had a small enhancement effect. However, the levels at which the interference was noted (with the exception of silver) was at least 2 orders of magnitude greater than that of mercury and not considered to be a major problem when determining mercury in environmental and biological samples.

	Added interferent / mg l^{-1}	Hg conc found / μg l^{-1}	Error / μg l^{-1}	Interference effect
Ag(I)	1.0	9.02	- 0.98	Suppression
As(V)	2.0	10.02	+ 0.02	Enhancement
Cd(II)	5.0	10.44	+ 0.44	Enhancement
Cu(II)	2.0	10.32	+ 0.32	Enhancement
Ni(II)	10.0	10.35	+ 0.35	Enhancement
Pb(II)	5.0	10.44	+ 0.44	Enhancement
Sb(V)	2.0	10.44	+ 0.44	Enhancement
Se(VI)	1.0	9.77	- 0.23	Suppression

Table 3.16 : **Results of interference study on the determination of mercury by FI-CVAAS**

3.4 Analysis of environmental and biological samples

The analysis of two different types of samples were evaluated by the method, environmental (sediments) and biological (tuna fish) samples. As indicated by the analysis of the appropriate CRMs, each type of sample required a different digestion mixture as well as a different sample pre-treatment strategy to ensure that any mercury present in the sample remained in the sample and was not lost during the pre-treatment stage.

3.4.1 Analysis of sediment samples

The method previously validated using the certified reference materials, was evaluated for real samples. It was decided to analyse sediment samples with a low mercury content (0.1 mg kg^{-1}) and a high mercury content (2-3 mg kg^{-1}).

The low mercury content sediment samples were collected from the River Avon, Devon at the Saltham estuary. Three different sampling positions were chosen from the estuary. These were :-

i) estuarine river bed within the main flow of the river,

ii) inlet river bank 20 cm above the waterline and

iii) inlet river bed.

The high level mercury content sediment samples were collected from the River Mersey, Liverpool. Again three different sampling sites were chosen and these were :-

i) up river from the power station

ii) directly opposite the power station

iii) down river from the power station

To prevent any contamination, all the high density plastic bottles (Nalgene, Rochester NY, USA.) used for the sample collection were cleaned with 1 mol l^{-1} nitric acid before use and then rinsed three times with double distilled water and dried at room temperature.

Once collected and back in the laboratory, the samples were air dried at 30°C to prevent any mercury escaping from the sample matrix during the drying stage. The sample was then placed in a two tower sieve system consisting of a top sieve of 2.00 mm and a bottom sieve of 710 μm. The top sieve removed any large stones, foreign matter and vegetation. The sample that passed through this first sieve was ground up using mortar and pestle, and placed into the bottom sieve to provide a fine homogenous sample of the river sediment, which could be used for the analysis. Also, before drying, a portion was taken from each sample to perform a weight loss experiment, so that the values obtained could be corrected for moisture loss. The results obtained are given in *Table 3.17.*

Sediment sample	% Weight Loss
Mersey A	53.20
Mersey B	48.93
Mersey C	25.63
Avon A	50.39
Avon B	49.71
Avon C	48.38

***Table 3.17 :* % Weight loss results for all sediment samples**

The samples were left to digest slowly overnight in the hydrogen peroxide acid mixture and then analysed immediately after they were irradiated. A recovery experiment was run on all three of the samples, 1 ml of 100 $\mu g\ l^{-1}$ Hg being added to each sample (spike = 4 $\mu g\ l^{-1}$) and left to soak for 2 hours before the digestion stage.

% Recovery = 100 x [(Spiked Sample - Unspiked) / Spiking level]

3.4.1.1 Digestion procedure

The environmental samples (ca. 0.25 grams) were weighed directly into the digestion vessel and then the digestion mixture was carefully added to the vessel. The digestion mixture used for the environmental samples was 2.5 ml of concentrated nitric acid, 2.5 ml of concentrated sulphuric acid and 1.0 ml of hydrogen peroxide. The hydrogen peroxide was added last and very slowly to prevent the initial reaction from occurring too vigorously. The vessel was left loosely capped overnight to ensure that excessive pressure did not build up when placed in the microwave oven due to the oxidation of any organic matter in the sediment.

The following morning the lid of the bomb was tightened and the bomb placed in the microwave oven for 2 x 30 seconds at medium power. After this time the bomb was inspected to make sure that the seal was still intact. If the seal was damaged the entire digestion procedure was repeated, but if the seal was intact the bomb was irradiated in 30 seconds bursts for a total of 4 minutes. The bomb was then left to cool for approximately 1-2 hours.

The digestion mixture was then carefully transferred to a 25 ml volumetric flask, together with washings from the bomb (2% nitric acid / 2% sulphuric acid solution). Once the solutions had been made up to volume, the volumetric flasks were placed in an ultrasonic bath and left for 30 minutes to de-gas. The samples were then transferred into the autosampler vials ready for analysis. The results obtained from this study are shown in *Tables 3.18 and 3.19*.

Sample	Result [a] mg kg^{-1} of Hg [#]	RSD %	Spiked Result mg kg^{-1} of Hg [#]	Spiked Recovery %
Mersey I	2.99 ± 0.05	1.2	6.92 ± 0.03	98.3
Mersey II	2.36 ± 0.04	1.4	6.01 ± 0.02	91.3
Mersey III	2.02 ± 0.03	1.2	5.81 ± 0.03	94.7

[#] Mean and one standard deviation (n = 6)
Spiking level 4 μg l^{-1}

Table 3.18 : **Total mercury concentration in River Mersey sediments**

Sample	Result [a] mg kg^{-1} of Hg [#]	RSD %	Spiked Result mg kg^{-1} of Hg [#]	Spiked Recovery %
Avon I	0.19 ± 0.05	6.1	3.91 ± 0.04	93.5
Avon II	0.12 ± 0.05	9.1	3.98 ± 0.03	96.5
Avon III	0.10 ± 0.05	8.4	4.38 ± 0.03	107.8

[#] Mean and one standard deviation (n = 6)
Spiking level 4 μg l^{-1}

Table 3.19 : **Total mercury concentration in River Avon sediments**

3.4.2 Analysis of biological samples

The biological samples analysed were canned tuna fish. The tuna fish samples required a different pre-treatment procedure to that for the sediment samples in order to prevent the mercury escaping from the matrix during the drying stage. This pre-treatment stage is detailed in *Section 3.4.2.1*. Once this stage had been completed, the biological samples were subjected to the same digestion procedure as that used for the sediment samples (*Section 3.4.1.1*) although a different digestion mixture was used as detailed in *Table 3.15*.

3.4.2.1 Biological sample pre-treatment

The tuna fish were first drained to remove the brine solution. The sample was then pre-treated by freezing in a domestic freezer at -10°C for 6 hours. After this time, it was transferred to a freezer dryer (Edward's Supermodulyo Model 12K, West Sussex, UK.). The sample was placed in the vacuum chamber, (evacuated to 0.8×10^{-3} millibar) and left there for 48 hours. The condensation trap was set at -50°C.

The sample was then finely ground using a mortar and pestle and stored in a pre-cleaned screw capped plastic bottle (Nalgene, Rochester NY, USA.). Some of the original sample was retained in its original form to allow mass loss experiments to be performed in order to quote the results on a dry mass basis. The results from the weight loss experiments (*Table 3.20*) give a correction factor to take into account any moisture present in the original sample when it was

weighted out for analysis. The biological results obtained are shown in *Table 3.21*.

3.5 Conclusion

The objective of this investigation was to develop a sensitive and accurate analytical method for the determination of total mercury in environmental and biological samples by flow injection cold vapour atomic absorption spectrometry.

Cold vapour atomic absorption spectrometry (CVAAS) was used because of its ease of operation, its low detection limits compared with other techniques, and its suitability for use on-line with a flow injection analysis system. The use of flow injection in conjunction with the CVAAS technique enables the rapid analysis of between 20 and 30 samples per hour. The results of the interference study, although not exhaustive, show that the method is relatively robust with regard to the selected elements, and in most cases gave less than 5% error when their concentration was at least 2 orders of magnitude greater than that of mercury. The method offers a detection limit of 0.2 ng g^{-1} (3x SD of baseline (blank) signal), a relative standard deviation for real samples of less than 10%, and has been validated using two certified reference materials (DORM-1 Dogfish and MESS-2 Estuarine Sediment) with the results agreeing well with the certified values.

Fish sample	% Weight Loss
Tuna 1	66.41
Tuna 2	66.36

***Table 3.20 :* % Weight Loss for tuna fish samples**

Sample	Result [a] mg kg^{-1} of Hg [#]	RSD %	Spiked Result mg kg^{-1} of Hg [#]	Spiked Recovery %
Fish 1	1.00 ± 0.16	1.6	4.72 ± 0.12	93.0
Fish 2	0.92 ± 0.17	3.4	4.56 ± 0.14	91.0

[#] Mean and one standard deviation (n =)
Spiking level 4 μg l^{-1}

Table 3.21 : **Total mercury concentration in tuna fish**

Fundamental to this study was the choice of the best digestion technique. The use of microwave digestion had several advantages over conventional digestion techniques. The digestion stage was completed in under 5 minutes following a pre-digestion stage, where the digest was left to stand overnight to facilitate the breakdown of any organic matter present prior to irradiating the sample. The pre-digestion stage, clearly lengthens the sample preparation step, however the microwave technique is comparable to the conventional wet digestion techniques in terms of time, and is quicker than conventional cold digestion techniques which can take up to a week. The microwave technique is also less demanding upon human resources since it requires less supervision of the digestion process

By coupling the sensitivity of CVAAS to the automated flow injection system, this method offers considerable savings in both time and labour over conventional batch analysis. Although the coupling of CVAAS and FI is not totally novel, most of the previous reports in the literature are for clinical applications involving the measurement of total mercury in liquid samples *e.g.* blood and urine, and not for the analysis of complex matrix environmental samples as reported here.

Chapter Four : Multi element determination of Hydride Forming Elements

Chapter Four : Multi element determination of Hydride Forming Elements

4.1 Introduction

This chapter describes the investigations of multi-element "in atomiser" trapping of hydride forming elements. The chapter is split into four sections. The first section is an introduction to the subject of multi-element analysis techniques and briefly looks at the optimum conditions for hydride generation. The second section is the experimental section detailing the reagents, the instruments used (if not already discussed in *Chapter 2*) and the operating parameters and reagents used. The results section contains the results for each of the three linked investigations performed in this study. The three experiments are summarised below.

Experiment 1 : Initial investigations into multi-element "in atomiser" trapping on an iridium coated graphite tube. Only two elements were tested, bismuth and selenium.

Experiment 2 : Following the success of the first experiment, the next experiment was to see how many and which elements could be analysed together. Three more elements were added, mercury, arsenic and antimony.

Experiment 3 : This final experiment drew together the knowledge from the first two experiments, and applied this to practical applications using lake water samples. The method was validated by analysing appropriate reference

materials. The long term stability of the method, interferences and the choice of trapping material were also investigated.

Finally, the conclusion summarises the results obtained and discusses the advantages and disadvantages of the developed application.

4.1.1 Multi-element analysis

Traditionally, the technique of "in atomiser" trapping with atomic absorption spectrometry detection has been a single element technique due to the nature of the element specific hydride generation chemistry, and methods have been reported for all the hydride forming elements.

However, there are reports of simultaneous multi-element hydride generation determinations with ICP-AES,[178-179] and ICP-MS.[180-181] Sturgeon and Gregoire[182] discussed the use of collection of hydrides on a graphite surface followed by electrothermal vaporisation coupled with ICP-MS. Even with direct introduction of the hydrides into a FI-ICP-AES or FI-ICP-MS system, compromises are required as these systems are based upon measuring the hydrides as they are passed directly into the plasma, and consequently fast transient signals are obtained.

Two applications have been described with AA techniques. A paper by Garbos *et al.*[183] described the determination of two elements (As and Se) together on a Zr coated graphite tube and Kraus[184] described some preliminary research into the determination of As^{V}, Se^{IV}, Sb^{V} and Hg^{II} in lake water.

For sequential single element determination of the hydride forming elements, the instrumental and hydride forming conditions can be fully optimised for each individual element.[185] But no individual set of optimised conditions can

be used for the simultaneously multi-element determinations, therefore a key requirement is achieving compromise conditions. The selection of instrumental parameters is relatively easily achieved by taking the lowest trapping temperature and the highest atomisation temperature from the optimum instrumental conditions for the group of elements to be determined. The hydride generation conditions are very similar for each element, with $NaBH_4$ being used as a common reducing agent and HCL being used as the carrier solution. The chemistry behind the hydride generation is discussed in more detail in *Section 4.1.2.*

4.1.2 Conditions for hydride formation

The issue of the hydride forming chemistries is more difficult and there are two critical areas that have to be considered; reduction of the analytes to the most sensitive oxidation state and the hydride generation chemistry. The latter of these, generating the hydrides, is somewhat easier to control. The preferred reductant is usually sodium tetrahydroborate because it has a fast reaction time (10-30 s), it can be used for all eight of the HFEs and in addition, it can be added in solution form.[159] As long as there is a sufficient excess of tetrahydroborate available to the analyte in the manifold, and the reductant solution remains stable, there are usually few problems. The only problem with using sodium tetrahydroborate as the reductant, is that it is hydrolysed in aqueous solution, so to prevent this hydrolysis, the reagent is stabilised in a solution of sodium hydroxide. The normal acidic carrier stream is hydrochloric acid.

It is well known that the optimum sensitivity in hydride generation is obtained when the analyte is in a low oxidation state. The problem in analysis arises because the analytes to be determined may be present in various oxidation

states (*e.g.* As^{III} + As^{V}, Sb^{III} + Sb^{V}, Se^{IV} + Se^{VI}). The reduction of the analyte to the hydride can occur at different rates depending upon the original oxidation state of the analyte prior to reduction (*e.g.* As^{V} has about 70-80% of the sensitivity of As^{III}, while Sb^{V} is about 50% of the sensitivity of Sb^{III} [159] and Se^{VI} does not form the hydride while Se^{IV} does).[186] Therefore the sensitivity of the element being determined will depend upon the oxidation state of the analyte in the sample. Kraus[184] studied the simultaneous determination of As, Sb, Se and Hg by in-atomiser trapping ETAAS using by careful adjustment of the various chemistries. In the experimental work described here in this thesis, As and Sb were in the higher oxidation states and Hg and Se in the lower oxidation states. The objectives of the work presented in this chapter were to build on this previously unpublished work and assess the performance of the multi-element approach when all the elements were present in their most lowest oxidation states.

4.1.2.1 Optimum oxidation states for hydride forming elements

In the past, workers have used either potassium iodide[187] or a mixture of potassium iodide and ascorbic acid[188] to reduce As^{V} to As^{III}, but more recently, L-cysteine has been favoured[189-191] as it is compatible with flow injection systems and is used with lower reagent (0.5-2.0% (m/v)) and acid concentrations. The advantages of L-cysteine[191] over the other reductants used previously are :-

i) the reduction of As / Sb^{V} to the trivalent oxidation state is faster and more efficient,[189,192]

ii) L-cysteine helps to stabilise the analyte solutions,[189,192]

iii) L-cysteine is used in low (< 1 mol l^{-1} HCl) acid conditions which leads to milder hydride generation conditions resulting in less hydrogen being produced in the manifold,[189-192] and

iv) there is a higher tolerance to interferences.[192]

These seemed to be clear advantages which were felt to be worth examining in this study.

For selenium and tellurium, the highest oxidation state is hardly reduced at all by tetrahydroborate, owing to the extremely slow kinetics of the reduction,[186] therefore if the total Se and Te concentrations are required, a reduction step before the analytical step must be implemented to reduce the hexavalent state to the tetravalent state. For the reduction of Se^{VI} to Se^{IV}, the use of concentrated hydrochloric acid is considered an efficient and successful method,[186,193-194] with the chloride ion being the reductant.

For an ideal multi-element analysis, only one reductant solution would be required. L-cysteine will reduce the As / Sb^{V} to the lower trivalent oxidation state, but it will also reduce Se^{VI} or Se^{IV} to the elemental state,[180] while concentrated hydrochloric acid will reduce Se / Te^{VI} to the tetravalent state, but it has no effect on reducing the As / Sb^{V} to the trivalent state.[193] Clearly an optimum reductant that will meet all the criteria discussed above and reduce the HFEs to their most sensitive oxidation state has yet to be identified.

4.1.3 Aims of the study

Based on a consideration of the chemistry and due to the fact that there appears to be no 'ideal' reductant, a different strategy to that adopted by Kraus[184]

was chosen to investigate simultaneous multi-element determinations. The crucial factor was the choice of reducing agent. To enable optimum reducing conditions to be used, the elements were split into groups according to which reducing agent they required.

Group A contained As, Sb and Bi which used L-cysteine as the reducing agent and Group B contained Bi, Se and Te which used elevated temperatures with concentrated HCl as the reducing agent. Bi was kept with As and Sb due to it also being a Group Vb element.

4.2 Instrumentation

A Perkin-Elmer Model SIMAA 6000 simultaneous multi-element electrothermal atomic absorption spectrometer with a transversely heated graphite atomiser (THGA) with longitudinal Zeeman effect background correction and an AS-72 autosampler were employed for this study. Standard THGA graphite tubes with integrated platforms and Perkin-Elmer EDL 'System 2' electrodeless discharge lamps were used. The optimised instrumental parameters are shown in *Table 4.1* and the optimised THGA program *in Table 4.2.* The temperatures in Steps 2 and 3, depended upon the elements being determined.

Flow injection (FI) hydride generation was performed with a Perkin-Elmer FIAS 400 flow injection system equipped with an AS-91 autosampler. The optimised FIAS 400 program is shown in *Table 4.3.*

Parameter	As	Bi	Hg	Sb	Se	Te
Wavelength / nm	193.7	223.1	253.7	217.6	196.0	214.3
Read Time / s	5	5	5	5	5	5
Read Delay / s	0	0	0	0	0	0
Signal Measurement	Peak area					
Signal Type	Background correction AA					

Table 4.1 : **Instrumental parameters used for simultaneous multi-element electrothermal atomic absorption spectrometer**[141]

Step	Temp / °C	Ramp Time / s	Hold Time / s	Gas Flow / ml min^{-1}
1	Group A 300 Group B 250	1	60	50
2	Group A 300 Group B 250	1	20	250
3	2200	0	5	0
4	2300	1	3	250

Table 4.2 : **Optimised THGA temperature program for determining Group A (As, Bi and Sb) and Group B (Bi and Se) hydride forming elements**

	Time / s	Pump 1 / rpm	Pump 2 / rpm	Valve Position
Prefill	20	100	0	Fill
1	10	100	0	Fill
2	5	100	80	Fill
3	20	0	80	Inject

Table 4.3 : **Optimised program for determining Group A (As, Bi and Sb) and Group B (Bi and Se) hydride forming elements using the flow injection manifold with an Ar gas flow of 150 ml min^{-1}**

Parameter	As	Bi	Hg	Sb	Se	Te
Sample	As^{3+} in 10% HCl	Bi^{3+} in 10 % HCl	Hg^{2+} in 3% HCl	Sb^{3+} in 10% HCl	Se^{4+} in 10-50% HCl	Te^{4+} in 10% HCl
Trapping Temp / °C	400	300	130	400	250	300
Atomisation Temp / °C	2100	1800	1300	2000	2000	2000
Sensitivity / Int Abs (Conc / $\mu g\ l^{-1}$)	0.140 (3 $\mu g\ l^{-1}$)	0.160 (5 $\mu g\ l^{-1}$)	0.090 (20 $\mu g\ l^{-1}$)	0.180 (5 $\mu g\ l^{-1}$)	0.150 (4 $\mu g\ l^{-1}$)	0.350 (10 $\mu g\ l^{-1}$)
Characteristic Mass / pg	50	70	500	60	60	60
Carrier	10% (v/v) Hydrochloric acid					
Reductant	0.2% (m/v) $NaBH_4$ in 0.05% (m/v) NaOH					

Table 4.4 : **Analytical parameters for the hydride forming elements[195]**

4.2.1 Reagents

All reagents were of *pro analysi* grade (Merck, Darmstadt, Germany) unless otherwise stated. Dilutions were made using deionised water (Nanopure, Barnstead, USA).

Carrier solution : 10% (v/v) HCl

This solution was prepared by diluting 100 ml of concentrated hydrochloric acid (Suprapur, Merck, Darmstadt, Germany) to 1000 ml. This solution was prepared fresh when needed.

Carrier solution : 1% (v/v) HCl

This solution was prepared by diluting 10 ml of concentrated hydrochloric acid (Suprapur, Merck, Darmstadt, Germany) to 1000 ml. This solution was also prepared fresh when needed.

Reductant solution : 0.2% (m/v) $NaBH_4$ in 0.05% (m/v) NaOH

This solution was prepared by first dissolving 0.5 grams of sodium hydroxide pellets followed by 2.0 grams of sodium tetrahydroborate (95% assay, Riedel-de Haen, Seelze, Germany) and diluting to 1000 ml with deionised water. This solution was prepared daily and stored in a plastic Nalgene bottle.

Reductant solution : 0.5% (m/v) $NaBH_4$ in 0.5% (m/v) NaOH

This solution was prepared by first dissolving 5.0 grams of sodium hydroxide pellets followed by 5.0 grams of sodium tetrahydroborate (95% assay Riedel-de Haan, Seelze, Germany) and diluting to 1000 ml with deionised water. This solution was prepared daily and stored in a plastic Nalgene bottle.

Argon Gas :

Research grade argon was used for both the flow injection system and the internal and external gas streams for the THGA atomiser system.

Stabilising Solution : 5% (w/v) $KMnO_4$

A stock solution was prepared by dissolving 5.0 grams of potassium permanganate (low in mercury, Merck, Darmstadt, Germany) in 100ml of deionised water. Approximately one or two drops of this solution were added to every 100 ml of sample or standard solution. This solution was kept for up to 1 month in a dark glass bottle.

Metals Standards :

The working calibration standards in the range (0-5.0 $\mu g\ l^{-1}$) were made from stepwise dilution's from the original stock solutions as supplied by the manufacturer. The standards were made up in the appropriate hydrochloric acid carrier solution.

Selenium	1000 mg l^{-1} as SeO_2 in 0.5 mol l^{-1} HNO_3
Mercury	1000 $\mu g\ ml^{-1}$ in 10% HNO_3 (Inorganic Ventures Inc., USA)
Antimony	1000 mg l^{-1} as $SbCl_3$ in 5 mol l^{-1} HCl
Arsenic	1.342 g of As_2O_3 and 0.5 g NaOH in 1000 ml (Fixanal, Germany)
Tellurium	1000 mg l^{-1} as H_6TeO_6 in 0.5 mol l^{-1} HNO_3
Bismuth	1000 mg l^{-1} as $Bi(NO_3)_3.5H_2O$ in 0.5 mol l^{-1} HNO_3

Reducing agent : 1% (m/v) L-cysteine

The reducing agent for Group A elements (As, Bi and Sb) was L-cysteine. A stock solution of 10% L-cysteine in 0.5 mol l^{-1} HCl was made up by adding 10 g

of L-cysteine (Biochemistry, Merck, Darmstadt, Germany) to 4.5 ml of concentrated HCl (Suprapur, Merck, Darmstadt, Germany) and diluting to 100 ml. This solution was kept for 1 week. From this solution, enough L-cysteine was added to make the final concentration in the standard or sample 1% (m/v).

Iridium Trapping Solution : 1000 mg l^{-1} $IrCl_3$

From an original stock solution of 1000 mg l^{-1} $IrCl_3$ (PlasmaChem, USA), 50 µl was injected onto the graphite platform and the furnace program (*Table 4.5*) was started. This procedure was repeated twice. The pre-treatment was sufficient to last the lifetime of the tube, providing that the clean-out temperature did not exceed 2300°C.[195]

Zirconium Trapping Solution : 0.02 mol l^{-1} $ZrOCl_2.8H_2O$

A 0.02 mol l^{-1} solution of $ZrOCl_2.8H_2O$ was prepared by dissolving 0.06445 g of the salt in 10 ml of water. A total of 100 µl was injected onto the platform in two aliquots of 50 µl followed by the temperature program shown in *Table 4.5*. The coating lasted for the lifetime of the tube.[92]

Step	Temp / °C	Ramp Time / s	Hold Time / s	Read	Gas Flow / ml min^{-1}
1	110	10	60		250
2	130	30	50		250
3	1200	30	20		250
4	Ir 2000 Zr 2600	1	3	Yes	250

***Table 4.5* : Thermal pre-treatment program for Ir and Zr coated tubes**

4.3 Initial experimental work into determining Bismuth and Selenium by in atomiser trapping

This section describes the first investigations into the application of 'in atomiser trapping' for the simultaneous measurement of Bi and Se. From the literature[195] it is clear that the individual optimised conditions (*Table 4.4*), for the trapping and atomisation temperatures are very different for each element. Therefore to allow the simultaneous determination, the lowest trapping temperature was selected along with the highest atomisation temperature. The carrier solution and reductant solution are identical and the sample solutions were similar, with Bi requiring 10% (v/v) HCl and Se requiring 10-50% (v/v) HCl. From the sensitivity data[195] (*Table 4.4*), it was thought that a calibration graph in the range 0-5.0 μg l^{-1} could be obtained for both elements. This would then allow the experimental characteristic mass (m_0), sensitivity data and detection limits (instrumental and method) to be compared with the literature values and a judgement could then be made on the validity of the method.

4.3.1 Initial results from Bi and Se analysis

After the initial trials, three main problems were identified which would have to be eradicated before any further optimisation and analytical work could take place. The first problem, was that the Se peak did not return to the baseline within the default time. A second problem was a memory effect which was observed mainly for Se but also to a lesser extent for Bi. The third problem was

the irreproducibility of the three replicates, with the first value always being greater than the other two values.

4.3.2 First problem : Se not returning to baseline

After the read time of 5 seconds, the signal for Se had not returned to the baseline. The first attempt to cure this was to increase the read time from 5 to 6 and then to 7 seconds. Only a slight improvement was seen in the longest read time. The next attempt was to increase the atomisation temperature from 2000°C in steps of 100°C until the maximum clean-out temperature of 2300°C was reached. At 2100°C, the Se signal almost attained the baseline within the 5 seconds read time with a good symmetrical peak shape but this change caused the Bi signal to become slightly more narrow. At 2200°C, there was an improvement, in that the Se signal returned to the baseline, retaining a good peak shape, but still a much narrower Bi signal.

Overall, the higher atomisation temperature of 2200°C, reduced the baseline problem to a minimum, and a compromise was obtained between the best peak shapes for both of the elements. This was possibly to be expected, because Bi has a lower atomisation temperature than Se as indicated in *Table 4.4* of the optimum individual parameters.

4.3.3 Second problem : Se and Bi memory effect

The memory effect was due to Se becoming trapped upon the iridium in the tube but not on the L'Vov platform. During the coating step, some iridium can be

lost from the solution on the platform and become trapped upon one of the other internal surfaces of the THGA, *i.e.* the front and back end contacts. The contacts were taken out and cleaned, but this only reduced the effect slightly indicating that there was possibly another Ir coated internal surface in the HGA. This problem of memory effect could be solved, if the Se could be stopped from leaving the internal confines of the L'Vov platform and the graphite tube. The solution was to have a mini-internal gas flow of 50 ml min^{-1}, to increase the pressure around the tube, leaving only the small inlet hole for the gas to escape. This solution did not totally eliminate the problem, but it was considered to have reduced it sufficiently, so as not to be a problem.

4.3.4 Third Problem : Irreproducibility of the first value

The main cause of the irreproducibility was determined to be the first value, as it was always significantly different to the other two replicate values. A washing step was introduced in order to remove the previous sample from the tubing and wash the tubing before the next sample was introduced. A 15 second wash step was introduced before the prefill step for each sample. Initially, deionised water was used as the wash solution, then 10% (v/v) hydrochloric acid. This did not appear to make any difference in the reproducibility of the samples / blank but with the deionised water, the blank increased slightly. Therefore 10% (v/v) HCl was used in all further experiments as the wash solution.

Finally the prefill step was increased from 15 seconds to 20 seconds, all the flow injection tubing was replaced, and the quartz pipette tip was removed, placed in 50% (v/v) hydrochloric acid, washed with deionised water and then

returned to the mount, where it was rinsed with acetone and then blown dry by passing the argon gas through the delivery tube. A combination of these actions, resolved the reproducibility problem.

4.3.5 Results for Bi and Se analysis

Once, the problems identified above had been resolved or minimised, and using the operating conditions stated in *Tables 2.3 - 2.5*, a calibration graph in the range 0 -5 $\mu g\ l^{-1}$ was constructed. From this graph the Instrumental and Method detection limits could be calculated. The method detection limit (MDL) *(Eqn 4.1)* gives a measure of how sensitive the method is for each experiment, as the calculation takes into account the intercept and the slope from the best line of fit of the calibration graph,[210] while the instrumental detection limit (IDL) *(Eqn 4.2)* enables the instrument to be optimised to maximise the instruments response and hence its sensitivity.

$$\text{MDL} = \{[\text{Blank} + (3 \times \text{Std Devn of Blank})] - \text{Intercept}\} / \text{Slope} \qquad (Eqn\ 4.1)$$

$$\text{IDL (pg)} = (3 \times \text{Std Devn of Blank} \times m_o) / 0.0044 \qquad (Eqn\ 4.2)$$

The experimental calculated data *e.g.* sensitivity, characteristic mass (m_o) and detection limits can then be compared against existing data, to assess the potential of the developed method. A blank solution was also run in replicate (n = 10) to provide adequate statistical data for the standard deviation and mean of the

blank solution, used for the detection limit calculations. The results obtained are summarised in *Table 4.6*.

Parameter	Bismuth	Selenium
Gradient	0.04096	0.05603
Intercept	0.0008	-0.0002
r^2	0.99980	0.99992
SD of Blank (n=10)	0.0008	0.0015
Blank	0.0036	0.0057
Calculated m_0 / pg	55.3	40.9
(Std used)	(3.0 μg l^{-1})	(3.0 μg l^{-1})
Literature m_0 / pg	70	60
Observed Sensitivity / Int Abs	0.189	0.232
(Std used)	(4.0 μg l^{-1})	(4.0 μg l^{-1})
Literature Sensitivity / Int Abs	0.160	0.150
(Std used)	(4.0 μg l^{-1})	(4.0 μg l^{-1})
IDL / pg	30.16	41.83
MDL / μg l^{-1}	0.13	0.19

***Table 4.6* : Analytical figures of merit for the simultaneous determination of Bi and Se[195]**

4.3.6 Conclusion to experiment one

The initial study presented here showed that the simultaneous determination of Bi and Se by hydride generation in atomiser trapping is possible, and the data obtained indicates little degradation of the analytical figures of merit,

compared with those established for optimised single element hydride generation in atomiser trapping.

The developed method for the simultaneous determination of selenium and bismuth is sensitive and the calibration graph is linear within the working range of 0 - 5.0 μg l^{-1}, with good correlation (Bi r^2 = 0.99980 and Se r^2 = 0.99992) of the best straight lines through the six data points (*i.e.* one blank and five standards.) Both analytes have sub μg l^{-1} detection limits (Bi 0.13 μg l^{-1} and Se 0.19 μg l^{-1}) and absolute limits (Bi 30.2 pg and Se 41.8 pg) in the picogram range.

From the experimental results, the proposed method is slightly more sensitive than expected, with the calculated characteristic mass values being lower than the literature values and this is verified by the higher sensitivity readings for a given standard.

4.4 Further investigations into "in atomiser" trapping

4.4.1 Introduction

Following the promising results described in *Section 4.3*, it was decided to try and extend the method to include two other hydride forming elements (As and Sb) and also Hg, to evaluate if all five elements could be determined simultaneously. This study also considered the different oxidation states of the various elements found in the sample.

4.4.2 Experimental conditions

The instrumental conditions, as stated previously in *Section 4.1.3*, are determined by the lowest trapping temperature and the highest atomisation temperature of the elements in the group. From *Table 4.4*, it can be seen that the default trapping temperature would be 130°C and the atomisation temperature would be 2100°C.

From the sensitivity data (*Table 4.4*), the calibration range for As, Bi, Sb and Se would be 0-5.0 $\mu g\ l^{-1}$ and for mercury the range would be 0-50 $\mu g\ l^{-1}$. The same carrier solution was to be used for this experiment, but in order to accommodate the five elements, the overall concentration of the sodium tetrahydroborate was increased to 0.5% (m/v) with a similar increase in the sodium hydroxide concentration to keep the tetrahydroborate stable. The reductant used was therefore 0.5% (m/v) $NaBH_4$ in 0.5% (m/v) NaOH.

4.4.3 Possible and optimum oxidation states

Each of the five elements to be determined can exist in two different oxidation states *e.g.* As^{III} and As^{V}, Sb^{III} and Sb^{V}, Se^{IV} and Se^{VI}, Bi^{III} and Bi^{V}. Hg is a special case since it mainly exists as Hg^{II}, however it can also exist as Hg^{I} in organomercury compounds. All these oxidation states can readily be found in natural samples, with the exception of Bi^{V} which is very rare in the environment. It is also advisable to have a stabilising agent in the mercury solution to prevent the Hg from adsorbing onto the walls of the container. Thus approx. 50 µl of 5% $KMnO_4$ was added to each standard.

Selenium needs to be heated with concentrated HCl to be reduced from Se^{VI} to Se^{IV}, since Se^{VI} will not be reduced by the sodium tetrahydroborate in the reaction manifold. Bismuth only needs acidified conditions to prevent oxidation. This then leaves As and Sb which in both oxidation states will yield hydrides although at different rates and hence different sensitivities. Therefore to make the reaction as sensitive as possible, the As and Sb should be in the trivalent state, which required the addition of L-cysteine. This is now favoured over KI and ascorbic acid, due to the advantages as stated by Tsalev *et al.*[191]

It was noted that if L-cysteine was added to a selenium solution, the selenium was reduced to Se^0, which could then not form the hydride. Thus in order to determine Se and As in the same solution a new procedure had to be developed.

4.4.4 Results

4.4.4.1 Ideal Trapping Temperature

Using an initial trapping temperature of 130°C, no peaks were observed above the background noise. The trapping temperature was increased to 150°C and small signals were observed for all five elements. At this stage, the gas flow during the trapping step was eliminated from the flow injection program as it was thought that the gas flow was preventing the hydrides from being correctly sequestrated in the tube. The trapping temperature was increased to 170°C, and an increase in signals for four out of the five elements could be clearly seen. A

decrease in the Se signal was observed, and it could only just be seen above the background noise.

To see if a further increase in trapping temperature would increase the peak area, a trapping temperature of 200°C was chosen. This increase in trapping temperature did not give the expected results, *i.e.* another increase in peak areas. Therefore it was decided to lower the trapping temperature back to 170°C for the further work.

In order to try and improve the peak areas, a more concentrated standard (10 μg l^{-1} for As, Bi, Sb and Se and 100 μg l^{-1} for Hg) was used in the optimisation stages. In this experiment, no selenium peak was observed and the Hg peak was thin and sharp in shape, but this is understandable as the pyrolysis and atomisation temperatures were much greater than its optimised temperatures. Good symmetrically shaped peaks were observed for As, Bi and Sb. It was clear from these results that it was possible to determine As, Bi and Sb together, but the characteristic mass values were low.

4.4.4.2 Position and length of reaction coil

Following the initial work, the internal gas flow of 50 ml min^{-1} was restored and kept constant through the rest of the experiments, as it was previously shown to reduce the memory effect for some of the analytes. However, it was clear from the results, that the system was not operating well. At first, it was assumed that the reaction time was insufficient for the hydrides to be formed. To solve this, a longer reaction loop (length 300 mm, i.d. 1.0 mm) instead of the normal reaction coil (length 110 mm, i.d. 1.0mm) was introduced between the reductant/carrier

mixing point and the inlet for the argon gas flow. With this new reaction coil, As, Bi and Sb peaks were detected with a thin Hg peak although there was no peak for Se.

A much longer reaction coil was then used to see if the hydrides required a still longer reaction time. This coil was 1000 mm long with an i.d. of 1.0 mm and proved to be much better. Better shaped peaks were detected with larger areas and hence better sensitivities than seen previously. When the characteristic mass (m_0) values were calculated and compared with the literature values (*Table 4.4*) that for Bi was within 20% of the literature value, while these for As and Sb were a factor of 3 higher than the literature values. Se was still not detected, and the Hg peak was poor for the reasons stated previously.

	Bi	Se	Hg	Sb	As
Slope	0.0196	n.d.	0.0023	0.0051	0.0069
r^2	0.9998	n.d.	0.9979	0.9668	0.9932
Intercept	0.0001	n.d.	0.0160	-0.0015	0.0003
m_0 / pg	114	n.d.	976	556	322
Sensitivity / Int Abs	0.0972	n.d.	0.0467	0.0192	0.0202
(Std used)	(5 μg l^{-1})		(20 μg l^{-1})	(5 μg l^{-1})	(5 μg l^{-1})
Blank (n=10)	0.0012	0.0009	0.0004	0.0003	0.0175
SD of Blank (n=10)	0.0005	0.0005	0.0127	0.004	0.0005
IDL / pg	39	n.d.	200	1818	110
MDL / μg l^{-1}	0.13	n.d.	0.13	5.64	2.72

Table 4.7 : **Analytical figures of merit for the simultaneous determination of As, Bi, Hg, Sb and Se by "in atomiser" trapping**

A longer reaction coil was tried (length 2000 mm, i.d. 1.0mm), and there was no improvement in the results, therefore this reaction coil was rejected, as the same results could be obtained within a shorter time period using the previous coil (1000 mm, i.d. 1.0 mm). This was used in all further experiments. The reaction coil had two possible positions to occupy in the manifold, i) between the carrier / reductant inlet and the Ar gas inlet and ii) between the Ar gas inlet and the gas-liquid separator (GLS). The optimum signal for any given standard was maximised when the reaction coil was placed between the Ar gas inlet and the GLS. The reaction coil remained in this position for all further work.

The next stage was to obtain a calibration graph for each of the elements using the following experimental conditions; trapping temperature of 170°C, atomisation temperature of 2100°C, reaction coil (length 1000 mm, i.d. 1.0 mm) and an internal gas flow of 50 ml min^{-1}. The analytical figures of merit for each analyte were calculated in order to determine how the method was developing. The results are shown in *Table 4.7*.

4.4.4.3 Further studies on Selenium

In order to find out why no Se peaks were observed, fresh Se standards were analysed using the new default conditions stated in *Section 4.4.4.2*. A Se peak was detected and a linear calibration graph was constructed. This would indicate that the Se in the five element standard was either being

i) oxidised to Se^{VI} and hence not being detected, or

ii) it is being reduced to Se^{0} and again is not being detected.

Both of these oxidation states will not produce H_2Se from the reaction with sodium tetrahydroborate. To prove this theory, two identical 5 μg l^{-1} selenium standards (Std 1 and Std 2) were made up. To Std 2, 50 μl of 5% $KMnO_4$ was added. A blank solution was also made up as a reference solution. Each standard was analysed at three different time intervals, straight away, after 60 minutes and after 180 minutes. The results are shown in *Figure 4.1*. Initially, the Se was present in both standards, but after 60 minutes, there was decrease in signal for the standard with $KMnO_4$, and by 180 minutes, the signal was down to almost background levels.

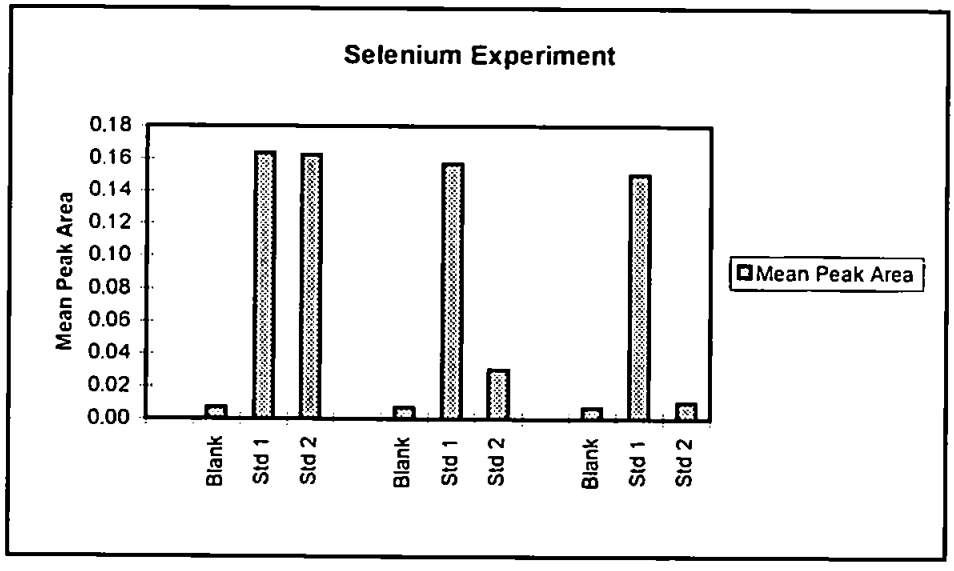


Figure 4.1 : A graph showing the experimental results from the selenium studies at time intervals of 0, 60 and 180 minutes. Standard One 5.0 μg l^{-1} of Se^{IV} and Standard Two 5.0 μg l^{-1} of Se^{IV} and 50 μl of $KMn0_4$, Blank solution 10% (v/v) HC

Half reactions and potentials[196]

$MnO_4^- + 8H^+ + 5e^- \leftrightarrow Mn^{2+} + 4H_2O$ $E^\circ + 1.507$ (Eqn 4.3)

$SeO_4^{2-} + 4H^+ + 2e^- \leftrightarrow H_2SeO_3 + H_2O$ $E^\circ + 1.151$ (Eqn 4.4)

From the half reaction potentials, it can be seen that *Eqn 4.3* has the more positive value of the two, therefore *Eqn 4.3* is the reduction reaction and *Eqn 4.4* is the oxidation reaction. This means the full reaction is Mn^{VII} is reduced to Mn^{II} and to balance the complete reaction, the Se^{IV} must be oxidised to Se^{VI}.

This explains the reason for the small Se peak initially being observed but eventually disappearing as the Se^{IV} was being oxidised to Se^{VI} over a period of time and hence not forming the hydride when the $NaBH_4$ was added.

4.4.5 Arsenic and Antimony contamination

The blank values for As and Sb, showed an increasingly higher signal as the experiment proceeded. It was assumed that there was a contamination problem from one of the reagents, possibly the sodium tetrahydroborate. This was investigated in the next round of experimental work.

4.4.6 Conclusion

It was concluded from this trial that the five selected elements, As, Bi, Hg, Sb and Se could not be determined together using the same experimental

conditions. The first problem encountered was the fact that Hg needed a stabilising agent, and the one chosen $KMnO_4$, had a detrimental effect on Se *i.e.* oxidising the Se^{IV} to Se^{VI}. The second problem caused by Hg was the severe compromises need in the furnace temperatures to enable the successful simultaneous determinations. If Hg was excluded, then the analysis could have worked well with the elements in their current oxidation states, As^{III}, Bi^{III}, Sb^{III} and Se^{IV}.

Further experimental work showed that the potassium permanganate was slowly oxidising the Se^{IV} to Se^{VI} in the solution which explained why a small Se peaks was first observed but after a few hours, the Se peak had completely disappeared.

It was also concluded that for the remaining four elements, there was no single reductant which would enable all the elements to be determined in the most sensitive oxidation states.

4.5 Simultaneous determination of five hydride forming elements by "in atomiser" trapping

This experiment was designed to bring together all the important facts learnt from the previous work, and apply them to a method to determine five hydride forming elements in lake water samples. Various other parameters such as the potential interferences, long term stability of the method and the various possible tube coatings available were also examined.

In this work, As, Bi, Sb, and Se were again determined together with Te, which is in the same group as Se and therefore would be expected to have the

same reducing characteristics. The experimental conditions were derived from the single element conditions. The problem of the elevated blank levels for As and Sb was investigated first, in order to minimise this problem in later studies.

4.5.1 Blank Contamination Problem

During the initial experiments, elevated blank values for As and to a lesser extent for Sb were observed. It was thought that this contamination could be due to the sodium tetrahydroborate as noted by Haug and Liao.[92] Different batches of sodium tetrahydroborate were assessed, but even the 'purest' batch still gave unacceptable blank values which seriously affected the detection limits.

It was considered that the possibilities of 'cleaning up' the sodium tetrahydroborate should be explored. Activated alumina has been used previously to preconcentrate oxyanions[197] and it was assumed that the contamination was in the alkaline sodium tetrahydroborate. If this were the case, then the most likely form of the contamination would be the oxyanions of As and Sb. Initially, an on-line column approach appeared to be the best option as no special treatment would then be needed and the reductant would be cleaned up immediately prior to use, which would keep the method as simple as possible.

A small column containing activated alumina was made from a 1 ml Eppendorf pipette tip, with the activated alumina being held in place by glass wool plugs at either end of the column. The column was inserted between the pump and the manifold in the FI system, and activated by passing 1% (v/v) HCl through it for 5 minutes followed by distilled water for 5 minutes and finally the alkaline

reductant for a further 5 minutes or until the waste solution was alkaline. The column was then ready for use.

Sample ($NaBH_4$) (n = 10)	Arsenic / Int Abs	SD	Antimony / Int Abs	SD
Contaminated Solution	0.0397	5.5×10^{-2}	0.0036	2.2×10^{-2}
Fresh Solution	0.0085	1.8×10^{-3}	0.0027	3.6×10^{-3}
Fresh Solution (After on-line clean up)	0.0051	9.0×10^{-4}	0.0026	2.8×10^{-3}

Table 4.8 **: As and Sb contamination in three different sodium tetrahydroborate solutions**

To test the efficiency of the column, blank measurements were made with and without the clean-up column in the FI system. The integrated absorbance results for As and Sb blank solutions (n = 10) are shown in *Table 4.8*.

The lowest integrated absorbance values were gained with a fresh sodium tetrahydroborate solution made from a new unopened bottle and the column in-line. With the same solution but no column, the results were only slightly greater but experience showed that this value would gradually increase as the stock reagents age increased. The worst values came from an open reagent bottle (approx. 2 months old) stock of sodium tetrahydroborate.

To test the lifetime of the column, continuous blank replicates were run. The capacity of the column was eventually exceeded after 30 replicates, which was long enough to perform a calibration but not long enough to permit the actual

analysis of samples, without re-activating the column. While it was clear that the on-line procedure with activated alumina was effective in reducing the contamination levels, the limited column capacity was a serious disadvantage. Consideration was given to increasing the size of the column, but when the on-line column was used, the reductant flow was reduced from 4.6 to 4.0 ml min^{-1}, and it was deduced that a larger column would reduce the flow even more, which would upset the optimised hydride generation conditions.

As it seemed unlikely that an effective on-line procedure with sufficient analytically useful lifetime would be developed, therefore it was decided to perform the procedure off-line. The contaminated tetrahydroborate was passed through an activated alumina column and collected in a fluorinated ethylene propylene (FEP) bottle prior to the start of analysis. A large column (150 mm x 10 mm i.d.) was made from plastic drain tubing normally supplied with flame AA instruments from Perkin-Elmer. This material was selected as it has a PTFE coating on the internal wall. A plastic restrictor was placed in one end of the tube, with a glass wool plug. Approximately 2 grams of activated alumina was slurried with a small volume of water and carefully poured into the column, ensuring there was no voids or air gaps. The alumina was allowed to settle and once the column was correctly packed, a glass wool plug was inserted above the alumina. The column was pre-treated in an identical way to the small on-line column and was left in alkaline solution when not in use.

The off-line cleaned reductant solution gave similar values for the blank as the on-line cleaned solution, *i.e.* system blank values of 0.005 for As, 0.003 for Sb, but the analytically useful lifetime of the procedure was greatly increased. A volume of 500 ml of 'contaminated' reductant could be cleaned up in two hours by

passing the solution slowly through the column using a pump rate of 5 ml min^{-1}. This volume of reductant was sufficient for 160 analytical cycles and a fresh batch of reductant was prepared daily following re-activation of the column as previously described.

4.5.2 Evaluation of different reducing agents

Technically, from the point of view of the instrumentation used in this study, it is possible to determine As, Bi, Sb, Se and Te simultaneously. However, to have all the elements in their most sensitive oxidation state requires that a single reducing agent should be used to reduce all the higher oxidation state elements down to their lower and more sensitive oxidation states. As yet, no suitable reducing agent has been identified. When the standard electrode potentials of the half reactions of As, Sb, Se and Te were examined, it became clear that no reductant was going to reduce As, Sb, Se and Te simultaneously from their higher oxidation state to the lower oxidation state while not reducing Se or Te to the elemental state.[193]

Two sets of workers have tried to solve this problem by using two stage reduction procedures. Uggerud and Lund[178]used thiourea in combination with concentrated HCl as a multi-element reducing agent. The samples were first heated with 5 mol l^{-1} HCl for 4 hours at 70°C, to reduce the Se and Te to the lower oxidation state, and then the thiourea was added to reduce the As and Sb to their lower oxidation state. This combination appears to work well, but eventually the thiourea also slowly reduced the Se^{IV} and Te^{IV} to their elemental state. Therefore, once the thiourea had been added the analysis had to be completed within 60

minutes. In developing routine, reliable procedures, maximum time constraints are a serious disadvantage and are to be avoided.

Morrow *et al.*[179] attempted to solve the problem by generating the hydrides of the elements which did not require KI as the reducing agent (*e.g.* As, Bi, Se, Ge and Te) and then adding the potassium iodide reductant after the original mixing coil and so generating SbH_3. The sediment samples used were previously digested in an acid mixture, (nitric and hydrochloric acid) which presumably was sufficient to reduce any Se / Te^{VI} to the Se / Te^{IV} oxidation states before hydride generation. The main disadvantage of this system was that to perform it efficiently, all the hydride forming elements must be in their most favourable oxidation states to produce the most sensitive results possible.

In the experiments described here, after an initial examination of the results obtained for As, Bi, Sb, Se and Te, the analytes were split into two groups, the criteria being to select those analytes which could be determined with the same reducing agent and conditions. Therefore one group consisted of As, Bi and Sb (Group A), which used L-cysteine as the reducing agent, and the other group was Bi, Se and Te (Group B), where concentrated HCl at an elevated temperature was used for reduction. While not an ideal solution, by grouping the elements into two distinct sets, this ensured that the elements would be in their most favourable states before the hydride generation reaction. Bismuth was included in both groups since it was already in the correct oxidation state *i.e.* Bi^{III} and therefore did not require any reducing agent.

4.5.3 Tellurium and Selenium

Initially, Te was included in Group B along with Bi and Se. Tellurium requires the same reducing agent as Se, *i.e.* 50% (v/v) HCl, but the optimum temperature for reduction is 100°C for 20 minutes.[195] This temperature is 20°C higher than the optimum Se temperature.[195] It is has been proposed that Se can be lost from solution as the chloride,[194] and hence the temperature of the reduction vessel should be kept as low as possible in order to eliminate any loss.

In the literature, there is disagreement as to the time and temperature required to affect the quantitative reduction of the Se^{VI} to Se^{IV}. Pitts *et al.*[198] claimed that 75°C for 30 minutes in 5-6 mol l^{-1} HCl was sufficient, but Bye and Lund[186] state that using 10 mol l^{-1} HCl, complete reduction can be accomplished in 15 minutes at 60°C. However, Hill *et al.* investigated the kinetics of the reaction[199] and found that with 6 mol l^{-1} HCl at a temperature of 70°C, complete reduction of Se^{VI} to Se^{IV} was achieved in 6 minutes.

The experimental results obtained in this study showed that for an off-line reduction, if the temperature was increased to 100°C for 3 hours to facilitate the reduction of Te, significant losses of Se were observed. The 3 hour reduction time was required to produce the best results for Te. If the temperature was reduced to 80°C, optimum for the selenium reduction, then the results for Te were irreproducible indicating incomplete reduction.

Based on these results, it became clear that finding a set of conditions that would reduce Te and Se completely, but without losses of either Se or Bi would be difficult and that these three elements were incompatible in terms of the proposed simultaneous determination procedure. Consequently, it was decided

to remove Te from Group B. During the course of these experiments, Bi was also monitored, and it was found that with prolonged heating times, the accuracy and reproducibility of the results decreased.

Overall, the final optimised conditions for the Se reduction step were found to be 70°C for 120 minutes in 50% (v/v) HCl and under these conditions reproducible results were obtained for Bi.

4.5.4 Comparison of Ir and Zr trapping materials

Whilst many different trapping coatings have been proposed,[92,94-95,200-201] it seems that Ir and Zr are the most favoured, because these coatings only need to be applied once before the measurements start and the coating lasts for the lifetime of the tube. Both of these materials have been applied successfully to single element analysis,[92,94,195,200] Work by Haug and Liao,[92] indicated that Zr may also be a possible candidate for a trapping agent for simultaneous multi - element hydride trapping. In this study, a comparison was made to assess the suitability of both materials for simultaneous multi - element analysis. The results are shown in *Figure 4.2*.

Using the Zr-coated tube, at a low trapping temperature of 300°C (*Figure 4.2a*), only Bi could be trapped successfully, with no As but some Sb. At a trapping temperature of 600°C (*Figure 4.2b*), a good Sb peak was observed along with a small Bi peak and a very poor, distorted As peak. Therefore at this temperature only Sb can be trapped successfully.

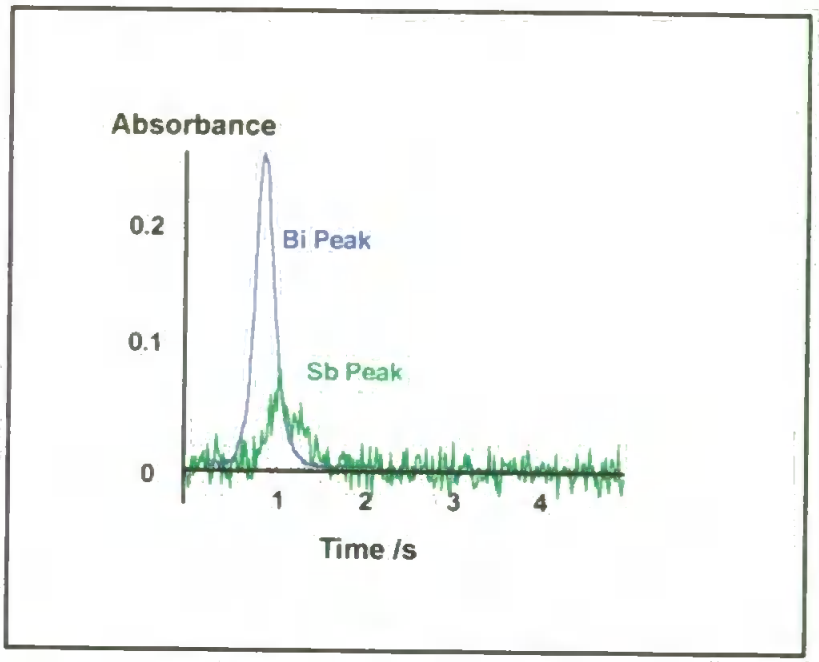


Figure 4.2a : Simultaneous atomic peak signals for Group A elements, As, Bi and Sb, at the 5 μg l^{-1} level, atomisation temperature 2200°C, sample volume 500 μl on a Zr-coated tube with trapping temperature of 300°C.

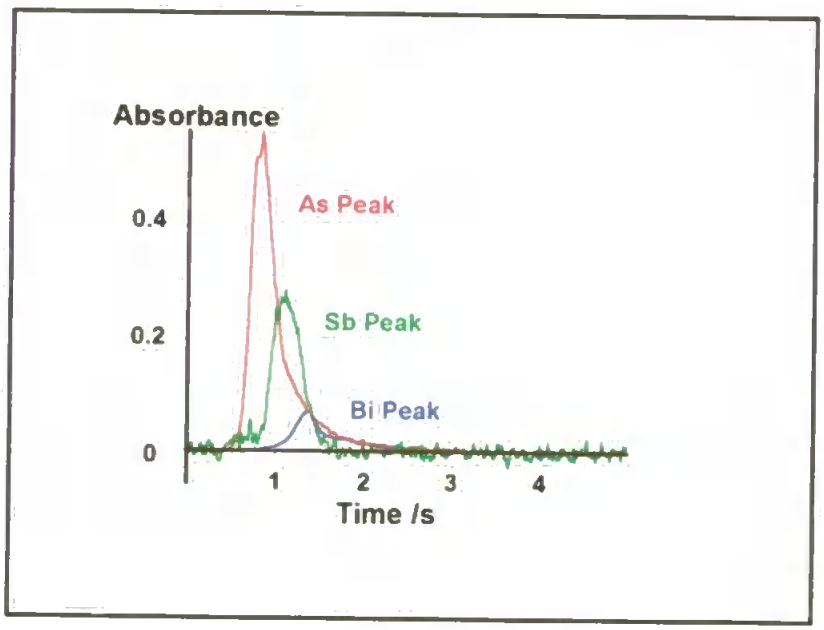


Figure 4.2b : Simultaneous atomic peak signals for Group A elements, As, Bi and Sb, at the 5 μg l^{-1} level, atomisation temperature 2200°C, sample volume 500 μl. Zr- coated tube with trapping temperature of 600°C;

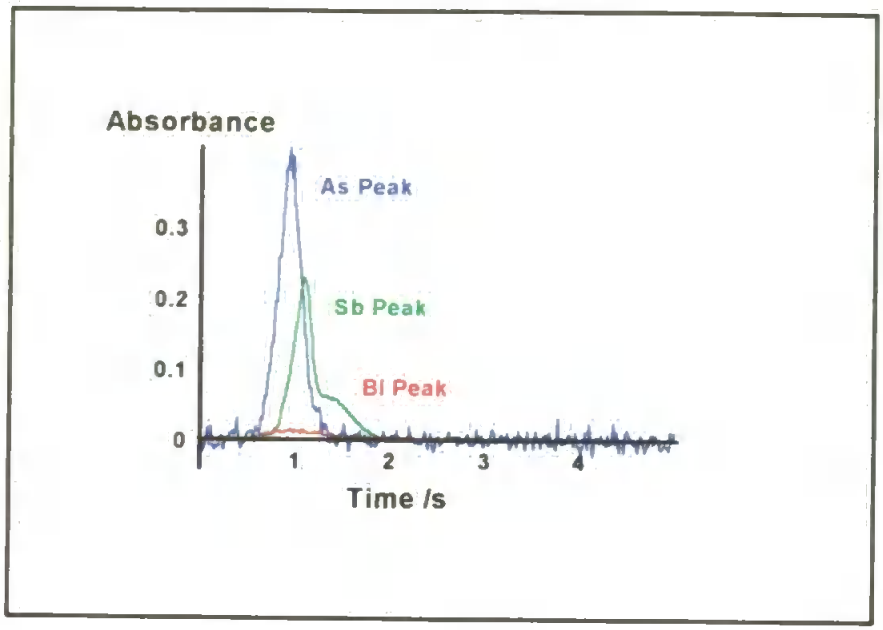


Figure 4.2c : Simultaneous atomic peak signals for Group A elements, As, Bi and Sb, at the 5 μg l^{-1} level, atomisation temperature 2200°C, sample volume 500 μl on a Zr-coated tube with trapping temperature of 800°C.

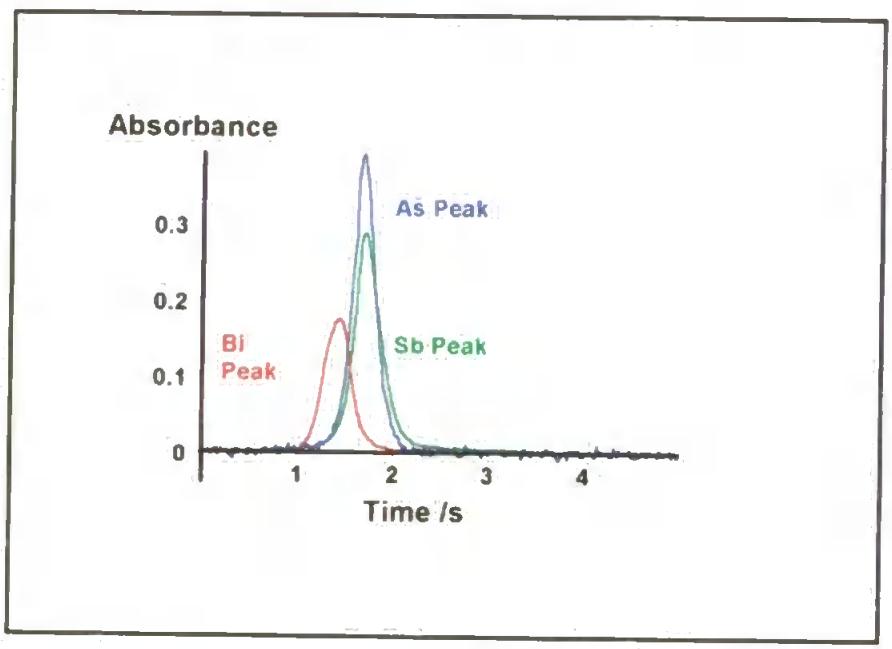


Figure 4.2d : Simultaneous atomic peak signals for Group A elements, As, Bi and Sb, at the 5 μg l^{-1} level, atomisation temperature 2200°C, sample volume 500 μl on an Ir-coated tube with trapping temperature of 300°C.

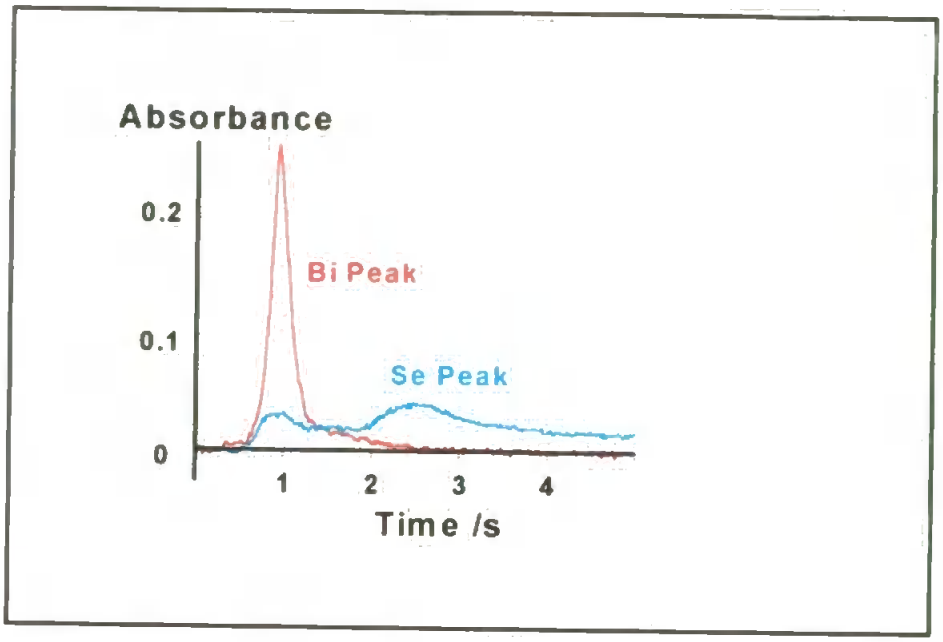


Figure 4.3a : Simultaneous peak signals for Group B elements, Bi and Se at the 5 μg l^{-1} level, atomisation temperature 2200°C, sample volume 500 μl on a Zr coated tube trapping temperature 250°C.

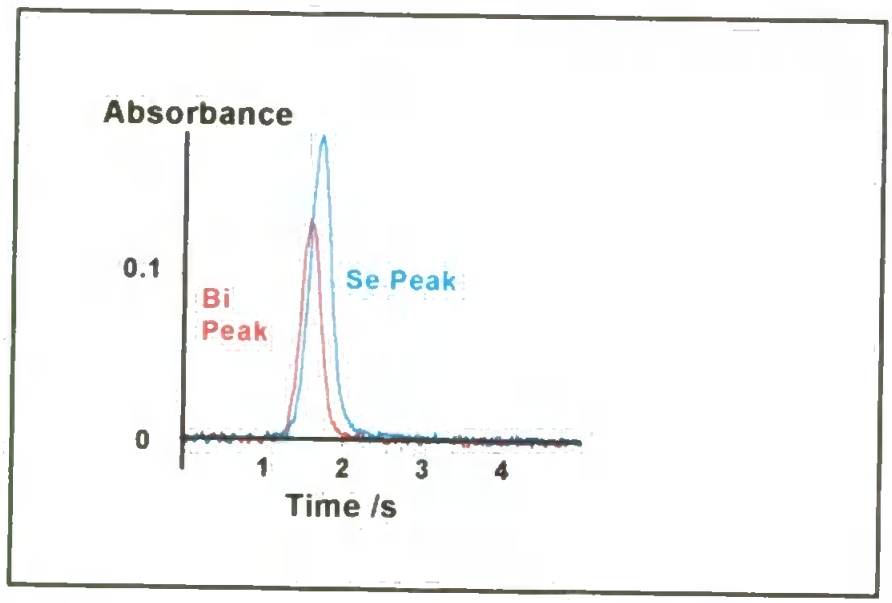


Figure 4.3b : Simultaneous peak signals for Group B elements, Bi and Se at the 5 μg l^{-1} level. atomisation temperature 2200°C, sample volume 500 μl. Ir coated tube trapping temperature 250°C.

At a high trapping temperature of 800°C (*Figure 4.2c*), a good Sb peak was obtained along with a reasonable As peak, but no Bi peak was seen. It was not possible to find a temperature where As, Bi and Sb could be trapped together. Similar results were found for Bi and Se whereby Se could not be trapped using the temperature conditions for Bi (*Figure 4.3a*).

The results presented here were reinforced by the single element optimised conditions found by Haug and Liao,[92] where with a Zr-coating, As has an optimum trapping temperature range of 750-850°C, Bi has a range of 100-500°C and Sb has a range of 500-750°C, with a common atomisation temperature of 2200°C. These are all mutually exclusive. It was concluded that Zr could not be used as an effective trapping material for multi-element analysis for As, Bi, Sb and Se. With an Ir-coating, As, Bi and Sb could be trapped successfully at 300°C (*Figure 4.2d*) while Se and Bi required a trapping temperature of 250°C (*Figure 4.3b*). To the best of the author's knowledge, this is the first reported comparison of the applicability of Ir- and Zr-coated graphite tubes for simultaneous multi-element hydride trapping. Clearly, it can be concluded that Ir is the coating of choice for all elements, both for single and multi-element determinations. The coating procedure is simple, the material (Ir solution) readily available and similar trapping temperatures can be used for all elements, making method development and optimization considerably easier.

4.5.5 Long term Stability tests

In the development of any analytical procedure, it is important to establish the lifetime or the stability of the procedure. It is known that the Ir coating has a

finite lifetime, based upon the previous single element work,[94] it was considered essential to demonstrate the long term stability of the proposed method.

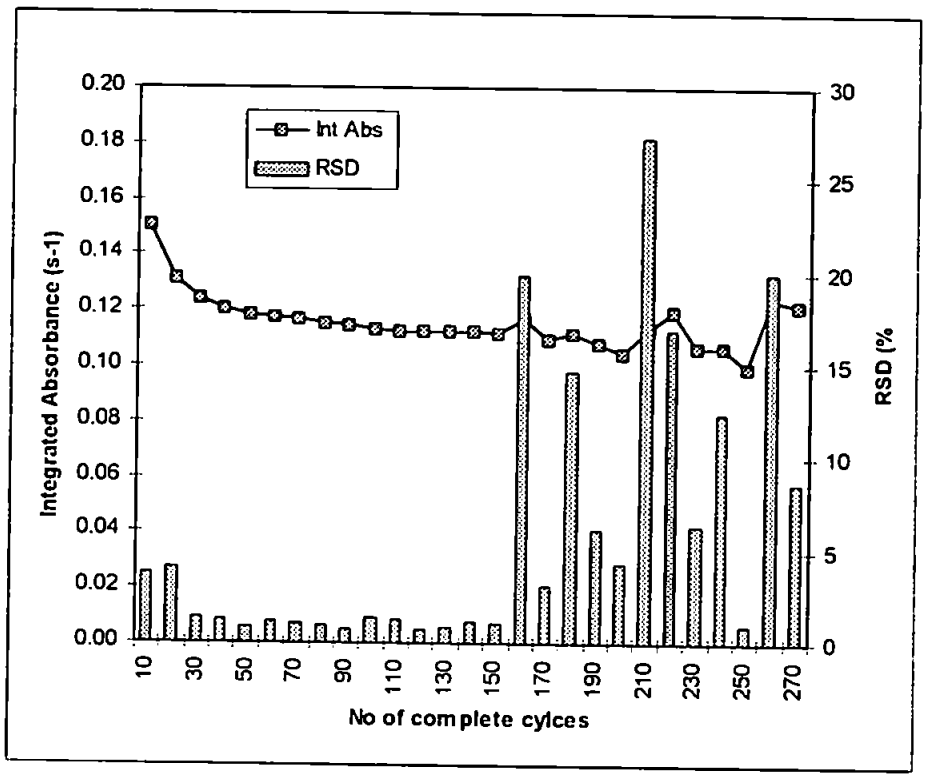


Figure 4.4a : **Long term stability as indicated by the integrated absorbance and precision (%RSD, *n* = 10). Concentration for As 5 μg l^{-1}, sample volume 500 μl, trapping time 60 seconds, trapping temperature 300°C, atomisation temperature 2200°C.**

Lifetime tests were run overnight to check on the stability of the analyte signal in terms of integrated absorbance and the precision of the signal (RSD %) for 10 replicates. A solution of 5 μg l^{-1} of As, Bi and Sb, (Group A), and Bi and Se, (Group B), was used and all other instrumental parameters were as stated in *Tables 4.1 - 4.3.* The results are shown in *Figure 4.4.*

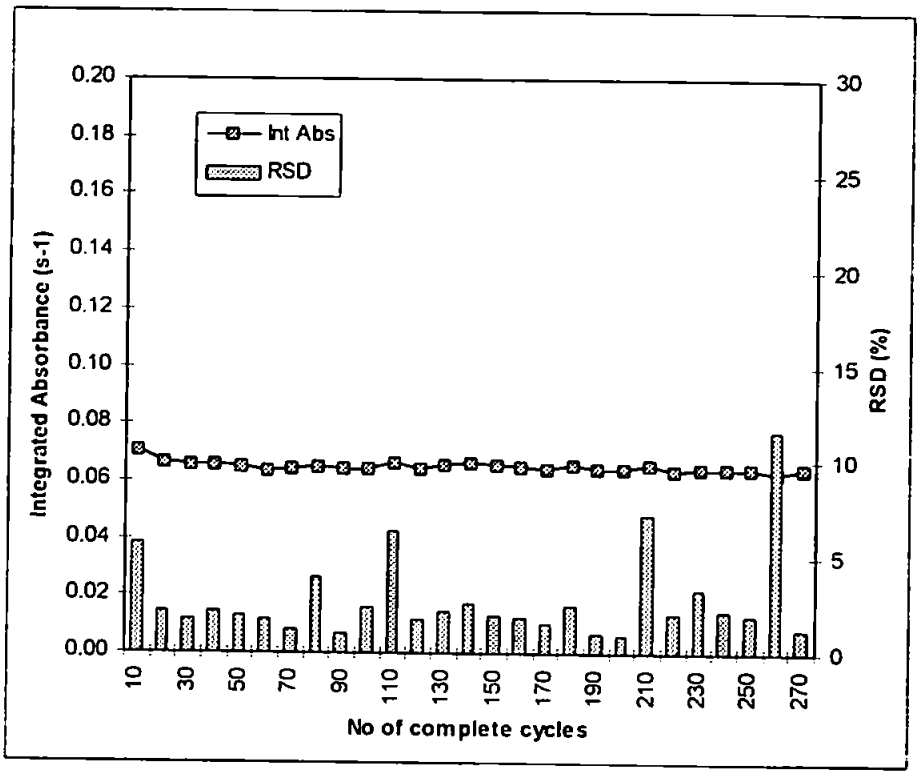


Figure 4.4b : **Long term stability as indicated by the integrated absorbance and precision (%RSD, *n* = 10). Concentration for Bi 5 μg l^{-1}, sample volume 500 μl, trapping time 60 seconds, trapping temperature 300°C, atomisation temperature 2200°C.**

For Group A, initially for all three elements (*Figures 4.4a - 4.4c*), the integrated absorbance was high with good precision, but the mean peak absorbance then dropped down to a constant level for the next 120+ samples. This is expected as the instrument will be the most sensitive just after the graphite tube has been freshly treated with the trapping material for 30 cycles and this "conditioning" effect has been observed before.[202]

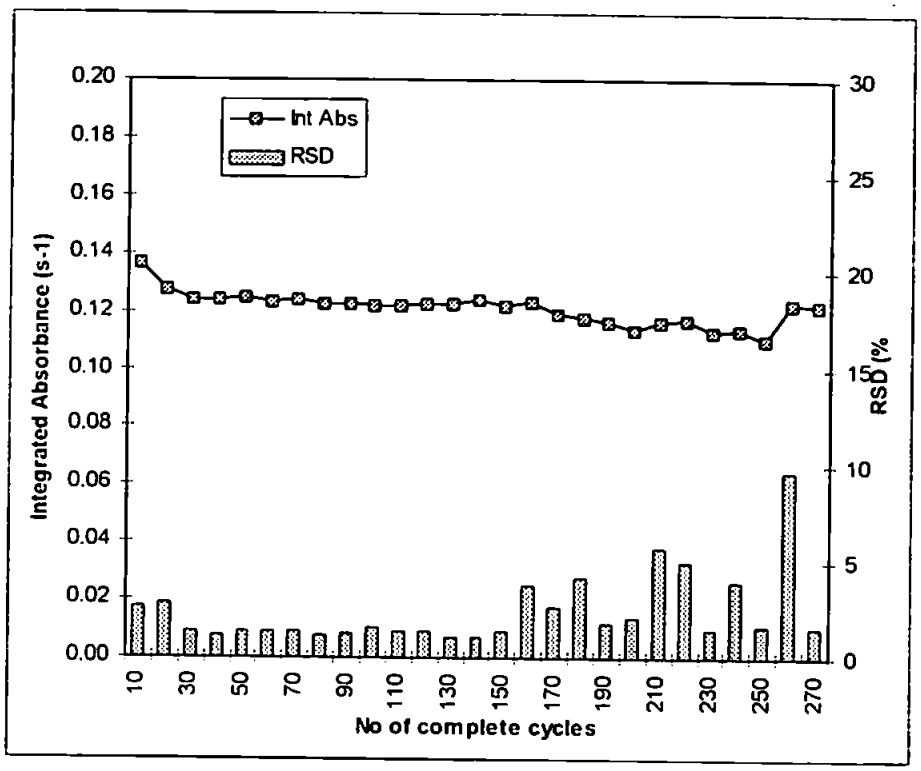


Figure 4.4c : **Long term stability as indicated by the integrated absorbance and precision (%RSD, *n* = 10). Concentration for Sb 5 μg l^{-1}, sample volume 500 μl, trapping time 60 seconds, trapping temperature 300°C, atomisation temperature 2200°C.**

For the first 5 hours *i.e.* 150 cycles, the results are steady and reproducible, but then for As and Sb but not for Bi, the precision deteriorates and the average RSD exceeds 5%. This is thought to be because both As and Sb require L-cysteine as the reducing agent but L-cysteine also acts as a complexing agent[191] for these two analytes. However, under the operating conditions of 1% (v/v) HCl carrier solution, the L-cysteine is not stable for long periods of time, as lower acid carrier concentrations *i.e.* below 0.1 mol l^{-1} HCl are recommended[189] with 1% (m/v) L-cysteine.

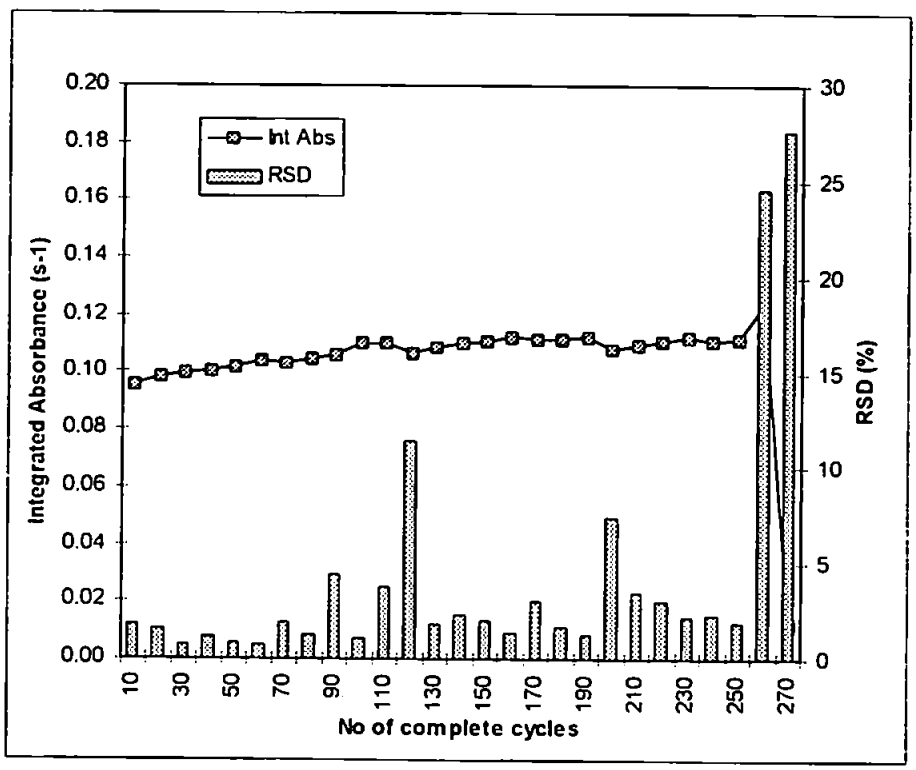


Figure 4.4d : **Long term stability as indicated by the integrated absorbance and precision (%RSD, *n* = 10). Concentration for Bi 5 μg l^{-1}, sample volume 500 μl, trapping time 60 seconds, trapping temperature 250°C, atomisation temperature 2200°C.**

It was found in these studies, that Sb was more stable (*i.e.* gave more reproducible results) in 10% (v/v) HCl carrier solution, than in 1% (v/v) HCl carrier solution. This result was confirmed by Welz and Sucmanova[189] who found that there was no significant difference in absorbance and integrated absorbance values for Sb^{III} using 0.1 or 1 mol l^{-1} HCl carrier, but the stability of the signals was better with the higher acid concentration.

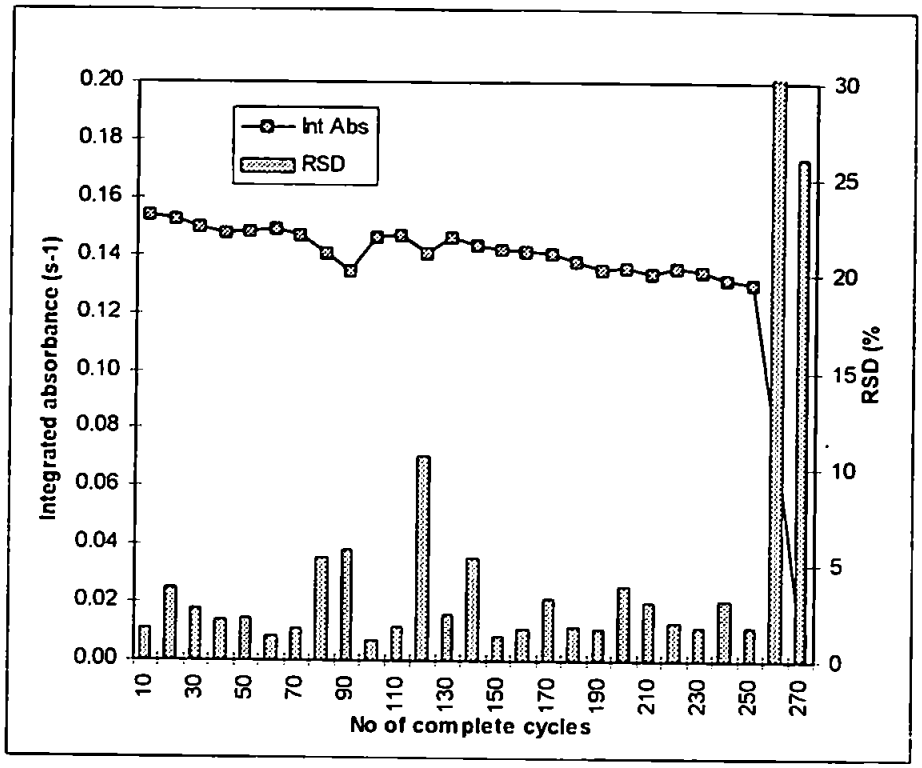


Figure 4.4e : **Long term stability as indicated by the integrated absorbance and precision (%RSD, *n* = 10). Concentration for Se 5 μg l^{-1}, sample volume 500 μl, trapping time 60 seconds, trapping temperature 250°C, atomisation temperature 2200°C.**

Arsenic ^{III} could not be determined satisfactorily with a 10% (v/v) HCl carrier solution, therefore to allow the simultaneous determinations of As^{III} and Sb^{III}, a compromise was reached which used a carrier solution of 1% (v/v) HCl. These issues with respect to the HCl acid carrier concentration and the stability of L-cysteine in the solutions, explain the worsening precision found after 5 hours of operation. It was originally observed by Welz and Sucmanova[189] that the integrated absorbance of an As^{III} standard (10 μg l^{-1}) in 1 mol l^{-1} HCl with no reductant, decreased almost linearly with time to 50% of its original value within 3

to 6 hours. This highlights the instability of the As^{III} standard (which is oxidised to As^{V}) which must be correctly stabilised to prevent poor precision. Bismuth does not appear to be affected by the acid concentration or the L-cysteine and both the precision and the integrated absorbance values remain steady during the full nine hours of the experiment.

For the Group B elements (*Figures 4.4d - 4.4e*), Bi shows a slight increase of integrated absorbance over the nine hours (*i.e.* 270 runs) while the precision is approximately 2% for the whole run. The high RSD values at 260 cycles is due to the reagents running out. This is also indicated by the reduced integrated absorbance. Selenium showed a slight decrease in integrated absorbance over time.

Overall, these results indicated that iridium as the trapping reagent for simultaneous multi-element analysis is reliable, facilitates good precision and is stable for a reasonable working period *i.e.* five hours for As and Sb while for Bi and Se the method is stable for up to nine hours. The reduced analysis time for As and Sb is due to the chemistry of the system rather than the trapping material used. However, the reduced stability / lifetime of the chemistry of the As and Sb situation raises the question as to whether the use of L-cysteine is the optimum reductant for this procedure.

4.5.6 Interference Studies

It is well known that conventional hydride generation suffers from various interferences *i.e.* kinetic, chemical and gas phase effects. Kinetic interferences are due to the different rates at which the hydrides are formed and released from the reaction mixture. These interferences do not occur when the hydride is

collected upon the graphite tube,[159] since the generation of the hydride and subsequent atomisation are separated into discrete stages. The gas phase interferences are caused by other hydride forming elements. The interference is due to the interferent removing the hydrogen radicals required by the hydride for atomisation. This process happens in conventional hydride generation within the quartz cell,[203] but since with "in atomiser" trapping, higher atomisation temperatures are possible and the atomisation mechanism is different to that in a quartz tube atomiser, this type of interference should be eliminated. This has been confirmed by the work of Schlemmer and Feuerstein.[204] Alkali and alkaline earth metals along with Al, Ti, V, Cr^{II} and Mn^{II} do not interfere in the determination of hydride forming elements. The major chemical interferences are caused by the elements in Groups 8, 9, 10 and 11 of the Periodic Table,[159] so only these elements were considered in this study. To confirm this, a study was initiated to see what effect various known interferents would have when the analyte hydrides were measured simultaneously by 'in atomiser trapping'.

A transition metal cation can cause an interference in one of two ways. In the hydride generator, the metal cation will be reduced by the tetrahydroborate and be precipitated as a fine metallic powder[204] therefore using up the tetrahydroborate before the hydride forming element can use it. Alternatively, nickel and metals of the platinum group are hydrogenation catalysts and can therefore absorb large amounts of hydrogen, hence removing the hydrogen from the reaction vessel. These metals can also capture and decompose the formed hydrides.[159,206]

Based on a review of the literature[159,190,178,200,205-206] and previous experience, eight metals were identified as being major interferents and/or could be present in sample matrixes. The eight metals were Ag^{I}, Cd^{II}, Co^{II}, Cu^{II}, Fe^{III},

Ni^{II}, Pb^{II} and Zn^{II}. A 5 μg l^{-1} multi-element standard was analysed (n = 3) in the absence of interferent to give a base response and then increasing amounts of the interferent (0.01, 0.1, 1.0, 10, 100 mg l^{-1}) were added and the new response (n = 3) was normalised with respect to the base response. An interference was defined as any signal which gave a deviation of ± 5% from the standard only response. The levels at which such deviations were observed are shown in *Table 4.9*.

	Group A			Group B	
	As^{III}	Bi^{III}	Sb^{III}	Bi^{III}	Se^{IV}
Cu^{II}	10	1	10	1	0.01
Zn^{II}	0.01	0.1	0.01	1	0.01
Fe^{III}	0.1	0.01	100	0.01	0.1
Co^{II}	10	>100	10	0.1	0.01
Cd^{II}	0.01	1	0.1	0.01	0.01
Ni^{II}	0.01	10	10	0.1	0.1
Ag^{I}	0.1	0.1	0.1	0.01	0.01
Pb^{II}	0.01	0.1	0.1	10	0.01

***Table 4.9* : Results from interference study : Level (in mg l^{-1}) at which the interferent produces a deviation of 5% or more from the 5 μg l^{-1} metal standard**

Out of the four analytes, Se^{IV} was the most affected by the transition metals interferences with 6 out of 8 metals giving an interference at 0.01 mg l^{-1} level, *i.e.* only a 2-fold increase above the original Se concentration. The other two

interferents, Fe^{III} and Ni^{II} gave an interference at the 0.1 mg l^{-1} level, *i.e.*, a 20-fold increase. The interference on As^{III} was from four elements at the 0.01 mg l^{-1} level, Zn^{II}, Cd^{II}, Ni^{II} and Pb^{II}, with Fe^{III} and Ag^{I} at the 0.1 mg l^{-1} level. Arsenic could be determined free of interference in the presence of 10 mg l^{-1} of Co^{II} and Cu^{II}. The least affected analyte was Sb^{III}, with only Zn^{II} interfering at the 0.01 mg l^{-1} level and Cd^{II}, Ag^{I}, Pb^{II} at the 1.0 mg l^{-1} level. Sb^{III} could tolerate up to 10 mg l^{-1} of Cu^{II}, Co^{II} and Ni^{II}, and 100 mg l^{-1} of Fe^{III} were required to give a -5% deviation.

The tolerance of Bi to interference depends upon the group with which Bi is determined. In both Group A and B, exactly the same interference level was obtained for Fe^{III} at 0.01 mg l^{-1} and Cu^{II} at 1 mg l^{-1}. However for Bi in Group A, there is no interference from Co^{II} up to 100 mg l^{-1} but under Group B conditions, Co^{II} interferes at the 0.1 mg l^{-1} level.

The results show that there are many possible interferences for each group of elements, but the major sources are Fe, Cd and Zn, which are often present in natural waters at concentrations greater than the 2-fold increase of concentration need to cause the stated ± 5% deviation from the base response. Therefore, when analysing samples, care must be taken to either reduce the potential interferences whenever possible, or confirm the absence of any interferences. Overall, Group A elements, are more resistant to interfering elements than Group B. This is probably due to the presence of the reductant, L-cysteine, which has been shown to increase the tolerance of As and Sb to interferences during the hydride generation step.[191]

4.5.7 Results

4.5.7.1 Analysis of Certified and Standard Reference Materials

One certified reference material (CRM), High Purity Standard (HPS) CRM No 490915 Trace metals in Drinking water and one Standard Reference Material (SRM), National Institute of Standards and Technology (NIST) SRM 1643c Trace elements in water, were found to contain the analytes of interest.

As the concentrations of the reference materials were outside the calibration range for all the analytes of interest, appropriate dilutions were made, and these were incorporated into the off-line reduction procedure. As both reference materials had similar concentrations, identical dilution factors could be used. For Group A, two separate dilutions had to be made. The As required a 20 times dilution (5 ml of sample to 100 ml) to bring it within range, while Sb and Bi only required a 2.5 times dilution (40 ml of sample to 100 ml). During the dilution step, the appropriate amount (1 ml per 10 ml) of 10% (m/v) L-cysteine was added to each volumetric flask. After the 30 minute reaction period, the analysis was performed. For Group B, 25 ml of sample was added to 25 ml of concentrated HCl, hence a 2-fold dilution. The solution was heated for 120 minutes at 70°C, and once cool, used for analysis. All solutions were analysed under the conditions stated above and the instrumental parameters stated in *Tables 4.1 - 4.3*. The results are shown in *Table 4.10*.

The drinking water CRM was certified for all four elements, and good agreement was achieved with the certified values. The NIST SRM was certified for As and Se, with a recommended but not certified value for Bi and no value was

provided for Sb. Again good agreement between the found and certified values was achieved, with all results falling within the certified range of two standard deviations.

	HPS Drinking water CRM No 490915		SRM 1646c Trace elements in water	
	Certified Value + / µg l^{-1}	Found # / µg l^{-1}	Certified Value + / µg l^{-1}	Found # / µg l^{-1}
As	80.00 ± 0.40	80.8 ± 2.1	82.1 ± 1.2	80.9 ± 0.5
Bi	10.00 ± 0.05	9.7 ± 0.4	12	12.6 ± 0.2
Sb	10.00 ± 0.05	11.1 ± 1.2	n.d.	n.d.
Bi	10.00 ± 0.05	9.2 ± 0.0	12	11.7 ± 0.0
Se	10.00 ± 0.05	9.7 ± 0.0	12.7 ± 0.7	12.1 ± 0.0

Mean and one standard deviation
+ Mean and 95% confidence limits

Table 4.10 : **Results for the analysis of HPS Drinking water Certified Reference Material No 490915 and Standard Reference Material 1646c Trace elements in water**

4.5.7.2 Analysis of Lake Constance water samples

Two different water samples were taken from the northern shore of the Bodensee (Lake Constance), and analysed for all four elements. The results are shown in *Table 4.11*. Only results for As were obtained as the other three elements were below the method detection limit. These results were compared

with those obtained for samples previously analysed by the Bodensee Water Works, Sipplingen, Germany, (typical values in *Table 4.11*), using a single element continuous flow procedure.[207] The total As values show good agreement, with the total Sb and Se concentrations being below the detection limits of this procedure.

	Lake Water 1 / μg l^{-1}	Spiked Result / μg l^{-1}	Spiked Recovery %	Lake Water 2 / μg l^{-1}	Spiked Result / μg l^{-1}	Spiked Recovery %	Typical values [(a)] / μg l^{-1}
As	0.97	1.86	89.0	1.40	2.46	106.5	1.28
Bi	< 0.12	0.81	81.2	< 0.12	1.10	109.7	-
Sb	< 0.26	0.89	88.5	<0.26	0.95	95.3	0.118
Bi	< 0.04	0.96	96.0	< 0.04	1.03	102.5	-
Se	< 0.29	0.79	79.0	< 0.29	0.83	83.4	0.161

[(a)] Values obtained from a single element instrument.[208]

***Table 4.11* : Lake Constance water analysis results**

The recoveries are good (all within ± 10 %) with the exception of Se which was low, presumably caused by the interferences noted in *Section 4.5.6*. No comparison values were available for Bi for these lake water samples. The samples were also spiked with a known multi-element standard so a % recovery could be calculated. The spiking level was 1 μg l^{-1} for all elements (As, Bi, Sb and Se)

% Recovery = 100 x [(Spiked Sample - Unspiked) / Spiking level]

4.5.7.3 Analytical Figures of Merit for the simultaneous determination of Group A (As, Bi and Sb) and Group B (Bi and Se) by in atomiser trapping

The characteristic mass and instrument and method detection limits along with the typical calibration data are shown in *Table 4.12*. The instrumental detection limits are based upon the IUPAC recommended definition[209] and the method detection limits are based upon the method described by Miller and Miller.[210] The detection limit for As was dependant upon the purity of the sodium tetrahydroborate. Consequently, the detection limit for As could be decreased further if the source of contamination were to be eliminated.

When the absolute detection limits from this procedure are compared against either single element or simultaneous FI-HG-ETAAS methods already in the literature, as expected the single element detection limits are lower by factor of 2-6 depending upon the element.[92,103,200,211-212] However, when the detection limits are compared with the simultaneous method,[183] the absolute detection limits are approximately equivalent.

The advantage that a simultaneous multi-element analysis has over a comparable single element analysis is that it is quicker with respect to the overall analysis time, and that the time reduction is proportional to the number of elements run together. There is also reduced consumption of reagents per sample. The disadvantage of the approach is shown in the analysis of the lake water samples, whereby the multi-element analysis tends to have higher detection limits than the single element analysis because compromise, rather than optimised conditions for each analyte are used.

	Group A				Group B	
	As^{III}	Bi^{III}	Sb^{III}		Bi^{III}	Se^{IV}
Gradient	0.01242	0.01464	0.01904		0.02410	0.02532
Intercept	0.0024	0.0021	0.0028		0.0003	-0.0017
r^2	0.99736	0.99367	0.99612		0.99984	0.99938
m_0 / pg	175	136	107		91	90
IDL / pg	107	28	27		43	73
MDL / $\mu g\ l^{-1}$	0.82	0.04	0.26		0.12	0.29

Table 4.12 : **Analytical Figures of Merit for the simultaneous determination of Group A (As, Bi and Sb) and Group B (Bi and Se) by "in atomiser" trapping**

However, a decision as to which approach is most appropriate depends upon the demands of the particular type of analysis, especially with respect to the detection limit level required. It should not be forgotten that the instrumentation used in this study also allows single element determination and therefore either multi or single element mode can be selected depending upon the requirement of the analysis. Similarly, while a FI approach with 500 µl volumes was examined in this study, improvements in the detection limit have been obtained by applying continuous flow sampling.[204]

4.5.8 Conclusion

This study has demonstrated that the simultaneous multi-element determination of combinations of hydride forming elements by "in atomiser" trapping electrothermal atomic absorption spectrometry is possible using conditions derived from single element determinations.

The optimised reducing conditions for Group B (Bi and Se) elements are dependant on the fact that Se must be in the tetravalent state for the hydrides to be formed, but for the Group A elements (As and Sb), the hydrides can be formed from either the trivalent or the pentavalent state. This work describes one possible way of determining the hydride forming elements in the lowest oxidation state by using L-cysteine as the reducing agent. The use of L-cysteine also appears to have the advantage of easing the interferences upon the analytes of interest, though with the disadvantage that the stability of the chemical system is limited. The alternative approach as used by Kraus[184] is to oxidise the As and Sb to the higher oxidation state. This entailed using a similar instrumental set up except that a longer reaction coil (1m instead of 60cm) was need to give the hydrides a longer reaction time with the tetrahydroborate. Clearly there are two possible approaches to the simultaneous multi-element determination of hydride forming elements with "in-atomiser" trapping. The hydrides may be formed either from the most sensitive oxidation states, which requires grouping of compatible elements due to the different reductants required, or by determining the elements As and Sb from the pentavalent oxidation state, as examined by Kraus[184] This latter approach does allow elements such as As, Sb, Bi and Se to be determined together.

The "in atomiser" trapping technique can easily be expanded from single element analysis to multi-element analysis providing that an iridium-coated tube is used. The analysis can be set up and left with only occasional re-sloping of the calibration graph to allow for unattended long term analysis for up to five hours with the same precision and accuracy associated with short term analysis. Iridium was the only coating material found to be applicable to this multi-element

procedure, as a universal trapping temperature could not be found for a Zr-coated tube. The conditions developed were shown to produce acceptable results for the analysis of the reference materials. When real samples were analysed, results with acceptable recoveries were obtained, and these results were comparable to values from a dedicated single element hydride generation atomic absorption spectrometry method.

Chapter Five : Multi element determination of metals in seawater

Chapter Five : Multi-element determination of metals in seawater

5.1 Introduction

This chapter describes work performed on developing a simultaneous multi-element method to determine six metals (Cd, Cu, Fe, Mn, Ni and Pb) in seawater with detection by electrothermal atomic absorption spectrometry.

The chapter is split into four sections. The introduction section gives an overview to the methodology of on-line column preconcentration. The experimental section describes the instrumentation used if not already discussed in Chapter Two. This section also details the optimised operating parameters and the reagents used in this study. The following results section is split into three parts. The first part describes the results obtained and highlights the problems encountered in the initial trials and how these difficulties were overcome. The next part details the analysis of various certified reference materials (CRMs) used to validate the method, and the final part looks at a further problem caused by a potential chemical interference and the initial attempts made to solve this problem. Finally, the conclusion section discusses the optimisation and validation of the proposed method. This section will also highlight the problems and the interferences encountered during the method development.

5.1.1 On-line Column Preconcentration

There have been many successful single element methods for determining trace metals in seawater by on-line preconcentration with various atomic spectrometry detection systems, including ETAAS. Review articles by Fang *et al.*[109] Ebdon *et al.*[213] and Carbonell *et al.*[214] highlight the diversity of the many methods as well as the different detection systems used *e.g.* FAAS, ETAAS, ICP-AES / MS.

There are two main types of columns (ion exchange and sorbent extraction) used in the on-line systems as described in *Section 1.3.2.3.* The majority of the preconcentration methods are not element specific, *i.e.* the ion exchange or the chelating resin materials will react with a range of metals. However, only ICP-AES / MS offers a multi element detection and so some of the overall efficiency of the method is lost by using a single element detection technique *e.g.* FAAS or ETAAS.

Materials such as Chelex-100 or Dowex A-1 will react with many transition elements, rare earth elements and some alkali and alkaline earth metals.[215] If the sample contains a large excess of metals, then competition for the chelation sites occurs between the various metals, causing loss of selectivity and sensitivity for the weakly chelated metals, which are removed from the column by the more strongly chelated metals. Better selectivity and a wider scope of applications are achieved by sorbent extraction[54-55] wherein the metal chelates (*e.g.* dithiocarbamates) formed in solution are retained upon a column filled with a non-polar modified silica with C_{18} groups bonded to the silica support, and the subsequent elution with the aid of an organic solvent directly into the spectrometer.

Dithiocarbamate reagents are commonly used as the chelating agent in most published FI sorbent extraction systems, however they are unstable in acidic media and are unselective towards heavy metals.[216] The complexation of dithiocarbamates with large number of metals (*Figure 5.1*) limits their application to the separation of heavy metals from matrixes where coexisting heavy metals are at low concentrations[217] *e.g.* seawater matrices.

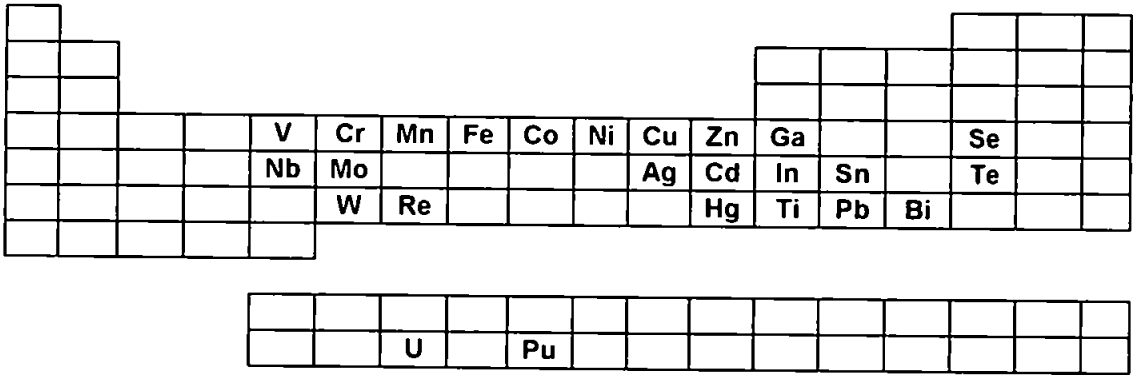


***Figure 5.1* : Periodic table showing elements that can be complexed with diethyldithiocarbamate[218]**

Diethylammonium N, N, diethyldithiocarbamate (DDDC) was chosen as the complexing agent instead of sodium diethyldithiocarbamate (NaDDC) as it is slightly more stable in acidic media.[219] When using DDDC as the complexing agent, the eluting solution would be ethanol rather than methanol due to safety reasons. The complexation reactions and mechanisms of the diethyldithiocarbamates are fully explained by Hulanicki.[220]

5.1.2 Aims of Study

The aim of this study was to see if a simultaneous method for determining trace metals in seawater by on-line column preconcentration with detection by ETAAS could be developed from the successful single element methods already developed. Initially seven elements were identified, Cd, Co, Cu, Fe, Mn, Ni and Pb, although Co was discarded in early work, as it interfere with the Fe and Ni lines.

5.2 Instrumentation

A Perkin Elmer SIMAA 6000 simultaneous multi-element electrothermal atomic absorption spectrometer with a transversely heated graphite atomiser (THGA) and longitudinal Zeeman effect background correction and an AS-72 autosampler were employed for this study. Standard THGA graphite tubes with integrated platforms were used. Multi element hollow cathode lamps and Perkin-Elmer EDL 'System 2' electrodeless discharge lamps were used for all experiments. The final optimised instrumental parameters are shown in *Table 5.1* and THGA program *in Table 5.2.*

Flow injection (FI) on-line column preconcentration was performed with a Perkin-Elmer FIAS 400 flow injection system equipped with an AS-91 autosampler. The optimised FIAS 400 program and flow rates are shown in *Tables 5.3* and *5.4.*

Parameter	Cd	Cu	Fe	Mn	Ni	Pb
Wavelength / nm	228.8	324.8	248.3	279.5	232.0	283.3
Read Time / s	5	5	5	5	5	5
Read Delay / s	0	0	0	0	0	0
Type of Lamp	EDL	HCL	HCL	HCL	HCL	HCL
Signal Measurement	Peak area					
Signal Type	Background correction AA					

Table 5.1 : **Optimised ETAAS instrumental parameters**

Step	Temp / ° C	Ramp Time / s	Hold Time / s	Gas Flow / ml min^{-1}
1	110	1	45	250
2	130	5	75	250
3	280	10	30	250
4	2400	0	5	0
5	2500	1	2	250

Table 5.2 : **Optimised THGA temperature programme for on-line column preconcentration experiments**

	Time / s	Pump 1 / rpm	Pump 2 / rpm	Valve Position
Prefill	40	0	40	Inject
1	60	0	40	Fill
2	10	0	20	Inject
3	60	40	0	Inject
4	20	10	0	Fill
5	60	20	0	Inject

Table 5.3 **: Optimised flow injection programme for on-line column preconcentration (36 μl eluent loop)**

Solution	Tubing	i.d / mm	Flow rate / ml min^{-1}	Flow rate / rpm
Sample	blue / yellow	1.52	3.0	40
DDDC	blue / yellow	1.52	3.0	40
Ethanol	solvent resistant	1.02	0.5	10
Acid wash	red / red	1.14	0.8	20
Air	red / red	1.14	2.4	40
Air	red / red	1.14	1.2	20

Table 5.4 **: Tubing and flow rates used for original flow injection system configuration**

5.2.1 Reagents

All reagents used were of *pro analysi* grade (Merck, Darmstadt, Germany) unless otherwise stated. Dilutions were made using deionised water (Nanopure, Barnstead, USA).

Buffer Solution pH 9.0 : 0.03 mol l^{-1} CH_3COOH and 0.06 mol l^{-1} NH_3

The acetic acid solution was prepared by diluting 1 ml of concentrated acetic acid (Merck, Darmstadt, Germany) to 500 ml with deionised water. The ammonia solution (Merck, Darmstadt, Germany) was prepared by diluting 1 ml of concentrated ammonia solution to 1000 ml with deionised water. These solutions were made when needed.

The buffer solution was prepared by adding the ammonia solution to the acetic acid solution, and balancing the pH with appropriate volumes of either solution until the desired pH value of 9.0 was obtained. This solution was prepared in 300 ml volumes and stored in a Nalgene bottle until used.

Complexing Reagent : 0.05 % (m/v) DDDC in pH 9.0 buffer

This reagent was prepared by dissolving 0.05 grams of diethylammonium N, N, diethyldithiocarbamate (DDDC) (Merck, Darmstadt, Germany) in 100 ml of pH 9.0 buffer. This solution was prepared fresh daily.

Washing Solution : 0.02% HNO_3

The concentrated nitric acid (Suprapur, Merck, Darmstadt, Germany) was first distilled in a sub boiling quartz distillation apparatus as described by Mitchell.[221] The final solution was prepared by diluting 20 µl of the distilled nitric acid to 100 ml with deionised water. This solution was prepared daily.

Eluting Solution : Ethanol

The ethanol (Merck, Darmstadt, Germany) was first distilled in a sub boiling quartz distillation apparatus as described by Mitchell.[221] Once purified, the ethanol was used as stated.

Analytical Columns :

The column materials used were :-

i) polygosil silica bonded reversed phase sorbent with octadecyl functional groups (RP-C_{18}) 40-63 μm and

ii) octadecyl functional groups (RP-C_{18}) 40-63 μm bonded onto a polymeric support material.

Two different column sizes were used. The smallest column was the analytical preconcentration column supplied by Perkin Elmer, in which the C_{18} material was pre-packed. The column was connected between two ports on the FIAS valve by small lengths of 0.35 mm i.d. PTFE tubing.

The larger column was the on-line purification column for the complexing agent. This column was a 4.3 mm i.d. x 10 mm C270 cartridge guard column (Upchurch Scientific, USA), which was manually packed with the required C_{18} material. The column was placed in-line between the eight port valve and the convergence point for the sample and complexing reagent. Each day, the columns were flushed in both directions with ethanol to rejuvenated the columns before use.[222]

Metal Standards :

The first set of working metal calibration standards were in the range 0-2.0 $\mu g\ l^{-1}$ and were made by stepwise dilutions from the original single element stock standards (1000 mg l^{-1} of the metal as nitrate in 0.5 mol l^{-1} HNO_3). The standards were made up in 0.02 % HNO_3 solution.

The second set of working metal calibration standards were made from the stock GFAAS mixed element standard in 5% HNO_3 (Perkin Elmer). The working metal calibration standards were in the range as stated in *Table 5.5.* The calibration standards were made by stepwise dilutions from the original stock standard and made up to volume with 0.02% HNO_3.

Metal	Conc in stock std mg l^{-1}	Standard range $\mu g\ l^{-1}$
Cd	5	0.01 - 0.1
Cu	50	0.1 - 1.0
Fe	20	0.04 - 0.4
Mn	20	0.04 - 0.4
Ni	50	0.1 - 1.0
Pb	100	0.2 - 2.0

***Table 5.5* : Concentration range of working standards and original stock solution concentration of the multi element standard**

5.3 Initial results and problems encountered during analysis

Calibration was first attempted with the instrument set up using the default conditions as stated in *Section 2.4.3.3* and using the first mixed standard in the

range 0-2 μg l^{-1}. A 36 μl eluent loop was chosen as this was the maximum amount of ethanol that could be injected into the furnace by the FIAS without the ethanol "creeping" off the tube platform. The initial results were encouraging in that the preconcentration method was working for all six metals, but the blank results were very high. Large peaks were observed for all the standards, but those for Fe and Cu showed signs of roll-over.

The conclusion from this study was that a serious source(s) of contamination was present and which would have to be eliminated or reduced to a minimum before the method development could proceed.

5.3.1 Sources of Contamination

Various potential sources of contamination exist originating from the glassware used, the tubing in the FIAS and the reagents used in all the reactions.

All the glassware used was washed with 10% HNO_3 and rinsed three times with deionised water. When not in use the volumetric flasks and PFA bottles were filled with deionised water and sealed to prevent atmospheric contamination.[223] Before use, the bottles were rinsed three times with deionised water. All new FIAS tubing was flushed with 10% HNO_3 and then finally with deionised water before use

The reagents however, remained a potential source of contamination. A large column (*Section 5.2.1*) containing C_{18} material and placed in-line was used to clean up the complexing reagent. This remedy immediately reduced the blank levels by half. The nitric acid originally used was *pro analysi* grade, but when this was replaced by purified nitric acid (sub boiled) grade, the blank levels again

were reduced, but only slightly. This left the ethanol as the last identified contamination source.

To prove that the ethanol was indeed the source of contamination, a sub boiling still was set up to purify the ethanol. This procedure was partly successful as shown by the results in *Table 5.6*. The blank values were reduced for five out of the six elements, but Fe still showed elevated levels. The experiment was performed by directly injecting 40 µl of the ethanol into the furnace and running the default THGA program. Five replicates were run for both ethanol batches. It has been suggested[224] that the Fe is organically bound to the ethanol, hence why it is transferred from the contaminated to the clean ethanol in the distillation process. Thus since the contamination problem with the Fe was proving problematic, it was removed from the list of metals to be determined.

Ethanol	Contaminated		Cleaned	
	Mean / Int Abs	SD	Mean / Int Abs	SD
Cd	0.0062	3.4×10^{-3}	0.0006	3.0×10^{-4}
Cu	0.0255	1.7×10^{-2}	0.0133	9.6×10^{-3}
Fe	1.3152	0.4013	0.1200	8.3×10^{-2}
Mn	0.0649	5.5×10^{-3}	0.0052	1.0×10^{-3}
Ni	0.0753	6.2×10^{-3}	0.0043	2.4×10^{-3}
Pb	0.0030	1.0×10^{-3}	0.0018	7.0×10^{-4}

(n = 5) for both experiments

***Table 5.6* : Experimental results for both contaminated and clean ethanol (after sub boiling purification)**

Another possible contamination source was atmospheric contamination. It was assumed that since all of the reactions took place in the closed system of the FIAS tubing, atmospheric contamination should not affect the system.

5.3.2 Multi-element calibration graphs

Once the sources of contamination had been reduced to a minimum, a new set of calibration graphs were prepared based on measurement of multi-element standard prepared from the single stock solutions. During the initial experiment stages, all standards were analysed with five replicates, to provide sufficient data for statistical analysis. However, the resulting graphs were of poor quality with inferior correlation coefficients, with no calibration possible for Ni due to uneven peak signals. The statistical data for these first calibration graphs are shown in *Table 5.7.* The reason for these poor results was determined to be co-contaminants present in the single element standards which had been used to make the multi element standard.[225] The contamination present in the single element standards are at low levels but due to the sensitivity of the instrument and the preconcentration factor applied by the method, these low concentrations were multiplied and caused the errors noted in the calibration graphs.

The next set of calibrations used the GFAAS mixed standard. This original stock standard contained Pb at 1000 $\mu g\ ml^{-1}$, Cu and Ni at 50 $\mu g\ ml^{-1}$, Mn and Fe at 20 $\mu g\ ml^{-1}$ and Cd at 5 $\mu g\ l^{-1}$. These values reflect the relative sensitivities of the metals in the ETAAS. With these new working standards (calibration range stated in *Table 5.5*) made by dilution of the original stock standard, very much

better calibration graphs were obtained, and the statistical data are shown in *Table 5.8.*

	Cd	Cu	Mn	Ni	Pb
Slope	0.2937	0.0069	0.0913	-	0.0211
Intercept	-0.0079	-0.0004	-0.0033	-	-0.0010
r^2	0.9739	0.8922	0.9539	-	0.9297
Blank / A	0.0033	0.0033	0.0044	0.0045	0.0016
SD	0.0010	0.0020	0.0014	0.0031	0.0006
IDL / pg	1.4	26.7	9.55	-	4.05
MDL / ng l^{-1}	50	1410	130	-	210
Conc range	0.2 -0.6 µg l^{-1}				

Table 5.7 : **Calibration data obtained from using multi-element standards prepared from single element stock solutions**

	Cd	Cu	Mn	Ni	Pb
Slope	3.3678	0.0170	0.0755	0.0165	0.0116
Intercept	0.0019	0.0008	-0.0003	-0.0002	0.0001
r^2	0.9925	0.9954	0.9971	0.9982	0.9986
Blank / A	0.0035	0.0043	0.0014	0.0019	0.0014
SD	0.0026	0.0050	0.0011	0.0026	0.0011
IDL / pg	6.7	25.6	1.1	14.2	8.5
MDL / ng l^{-1}	3	1086	66	60	40
Conc range	See *Table 5.6*				

Table 5.8 : **Calibration data with GFAAS mixed multi element standard**

5.3.3 Optimisation of the furnace parameters

When consistent atomisation signals were being attained from the metal standards solutions, the peak shapes were observed more closely. The default furnace parameters were determined by choosing the lowest pyrolysis temperature (Cd at 400°C) and the highest atomisation temperature (Ni at 2300°C), and these conditions potentially needed further optimisation.

Two problems regarding the peak shapes were observed. The nickel peak showed signs of tailing and did not return to the baseline within the read time period of five seconds. The cadmium peak, showed a negative baseline before the start of the peak. For nickel, the problem was eventually removed, by raising the atomisation temperature. At 2350°C there was a slight improvement, but a much better peak shape with baseline resolution was attained at 2400°C. The clean-out temperature was 2500°C. The increase in atomisation temperature also had two other effects on the analysis, in that the other peaks shapes became slightly sharper and with the increased clean-out temperature, the tube lifetime decreased.

Solving the problem of a negative baseline for cadmium was slightly more difficult. The pyrolysis temperature was decreased in steps of 50°C to a final temperature of 200°C when poor peak shapes were seen for the other elements. The final solution was achieved by decreasing the pyrolysis temperature slightly to 280°C and increasing the hold time from 20 to 30 seconds for the pyrolysis step.

5.3.4 Optimisation of the flow injection system parameters

The next stage for the optimisation was the flow injection parameters to be investigated *i.e.* length of each step, flow rates, tube lengths, and in the case of the delivery tube, the length and the internal diameter of the tube.

The two most important steps to optimise were the prefill step and the sample introduction step. The FIAS prefill step had to be long enough to ensure that fresh sample was reaching the valve before the new preconcentration step was started. The sample introduction step was optimised by watching the eluent slug reach the furnace.

5.3.4.1 Pulsation in the flow injection system

During the analysis of the standards, pulsation was observed in the sample and reagent line around the convergence point. In one extreme case, the complexing reagent flow was observed to be flowing backwards towards the reagent reservoir. It was assumed that this pulsation was the cause of the imprecision observed for the standard replicates (*i.e.* RSD values greater than 10%). The original FIAS configuration used 1.52 mm i.d. pump tubing for both sample and complexing reagent lines (*Table 5.4*). Various combinations of this and 1.14 mm i.d. tubing were tried to reduce the pulsation. In order to help observe the pulsation, an air bubble was introduced into the line and its passage was noted, *i.e.* if the pulsation did not occur then the air bubble would have a smooth passage through the tubing.

Fang[226] has suggested three ways to minimise pump pulsation :-

i) use sufficiently high rotation speeds, with the rollers pressing the pump tubing no less than 200 times per minute,

ii) use short pump tubes on the downstream to minimise the "bellows effect",

iii) lubricate pump tubes with silicon oil.

In order to make the optimisation process easier, the flow rates were kept as close as possible to the original stated flows of 3.0 ml min^{-1}, as higher flows resulted in too much back pressure and caused the tubes to rupture (at approx. 5 ml min^{-1}). In addition, if the flow rates were reduced too much, longer preconcentration times would be required to produce the same response, hence increasing the overall time for the analysis.

Taking into account the suggestions of Fang[226] and recognising the flow rate limitations, the answer to the problem was to use a smaller internal diameter tubing (1.14 mm i.d.) rather than the previous larger diameter (1.52 mm i.d.) tubing for both reagent and sample lines. No pulsation was observed as the air bubble had a smooth passage through the tubing. A calibration graph was attempted with this new tubing configuration, but unfortunately the calibration failed since no signals were recorded. It was concluded that for complexation to occur between the metal and the DDDC, some mixing in the tubes was required. The original calibration graphs were re-produced when the original (1.52 mm i.d.) tubing was re-installed and the old flow rates used, but of course, the poor precision remained.

5.3.4.2 Minimisation of Tube Lengths

The most important tube length to be kept to a minimum is the tube between the convergence point and the column, because as soon as the DDDC mixes with the sample, the metal-DDC complexes are formed. If the tube is not kept to a minimum, some complex could be retained and not eluted with the ethanol as it does not flow through this piece of tubing.[58]

When the first experiments were run, the FIAS unit was placed on a table about 50 cm below the level of the furnace. This meant that the sample in the delivery tube, had to flow uphill from the valve to the autosampler arm and then to the furnace. This operation required a substantial pressure in the air flow line. The original delivery tube was PTFE tubing with a 0.7 mm internal diameter and a length of approx. 70 cms. It was observed that the ethanol when flowing up the tube would start off in one single slug but by the time it reached the furnace head, it had become fragmented and it seemed as though some liquid was flowing back down the tube under gravity rather than being pumped out of the tube. Hence not all the ethanol plus desorbed analyte were being transported to the furnace for the measurement step.

The problem was solved in two stages. The first stage was to raise the FIAS unit to a level where the top of the valve was just below the furnace head so the ethanol then only had a slight incline to flow up under air flow. If the FIAS was raised above the furnace head, there was less control on the eluent delivery step due to gravity forcing the ethanol down the tube.

The second stage involved using a smaller internal diameter tube; eventually 0.3 mm was chosen and used for all future experiments. The greatly

reduced internal volume of the delivery tube allowed the front end of the sample slug to be entering the furnace while the tail would still be in the column. In this way, the slug tended to remain in one piece and not become fragmented, which helped to improve precision.

5.3.5 First injection problem

It was hoped that after optimising the furnace / flow injection parameters and solving the three previous problems, (contamination, calibration and pulsation) that the method could be validated.

However, it was noted that there was a problem with the first sample run in any batch, *i.e.* the first run was always significantly higher than replicate injections. This was thought to be due to the fact that before the first injection, the column was washed with acid during the prefill step.

In order to investigate this further, an experiment was conducted, where an extra step (identical to the prefill step) was added to the end of the FIAS program. Thus after every measurement step, the column was washed with the acid washing solution before the next preconcentration step. The results obtained showed a high result for the first injection, and then poor and imprecise results for the replicates.

Different configurations of flow rates and pump speeds were evaluated to solve this problem, including halving the flow rate of the acid washing solution and reducing the time for the step. A detrimental effect was seen on the precision of the standards and samples, as a result of these different configurations. This

effect was due to the same pump controlling all three reagent lines, *i.e.* sample, acid washing solution and complexing reagent.

A potential solution to this problem would, therefore, have been to remove the prefill step or stop the acidic washing solution from flowing through the column during this stage. However, this was not possible since :-

i) the prefill step was required to ensure that only the new sample is present during the preconcentration step and

ii) due to the way the FIAS is configured, the acid is always being pumped at the same time as the reagent / sample.

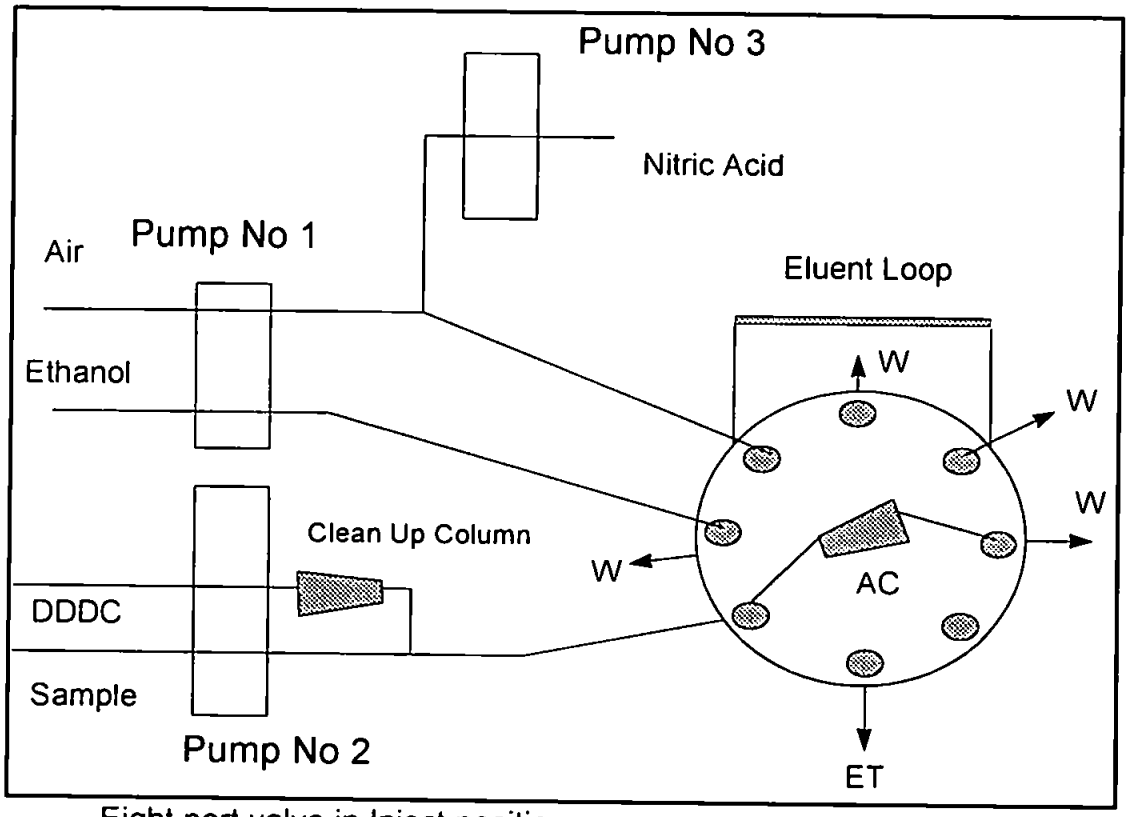
5.4 Reconfigured flow injection system

As suggested in *Section 5.3.5*, a potential way to remove the first injection problem was to re-configure the flow injection system so that the acid sample was not being pumped during the prefill step prior to the next analysis, since no alternative could be provided for flushing the old sample from the tubing and replacing it with new sample.

To achieve the new configuration (*Figure 5.2*), the addition of a third pump to control the flow of the acid washing solution was required. This arrangement also had the advantage of allowing for more control over the manipulation of solutions during the preconcentration operation (*Table 5.9*).

The new configuration, contained the eight port value and the two original pumps. However, now Pump No 2 only controlled the pumping of the sample solution and the DDDC reagent. Pump No 1 controlled the air and the ethanol

desorbing solution. The third auxiliary pump controlled only the acidic wash solution and was under control of the FIAS system.



Eight port valve in Inject position
W = Waste, AC = Analytical Column, ET = To ETAAS

Figure 5.2 : **Reconfigured flow injection system on-line multi-element preconcentration by electrothermal atomic absorption spectrometry**

In the original system the acid wash solution was controlled by Pump No 2, so when the prefill operation was initiated, the sample and DDDC solution filled the tubing and then flowed to waste. At the same time, the acid wash solution flowed through the silica column. It is this acid washing step that was believed to

have caused the activation of complexation sites upon the silica support material which contributed to the poor precision problem with the first injection.

	Time / s	Pump 1 / rpm	Pump 2 / rpm	Pump 3 / rpm	Valve Position
Prefill	40	0	100	0	Inject
1	60	0	100	0	Fill
2	10	0	0	10	Inject
3	60	40	0	0	Inject
4	20	10	0	0	Fill
5	60	20	0	0	Inject

Table 5.9 : **Optimised flow injection program for re-configured on-line column preconcentration (36 μl eluent loop)**

By removing the acid solution from this pump, this solution only flowed through the column during the acid washing step, and for a shorter length of time. This step only occurred after the preconcentration operation, when the previously activated silica sites had been complexed with excess DDDC reagent.

A number of other advantages were also been noticed when using the new configuration. These included less reagent consumption per sample, and more control over the flow rates. The pump tubing (*Table 5.10*) for the sample and DDDC reagent lines (0.76 mm i.d.) required high pump rates to achieve the previous flow rates (approx. 3 ml min^{-1}). These high pump rates would have caused the acid line to rupture using the old configuration. They also improved the precision of the method. During this re-configuration, a fume extraction hood

was fitted over the FIAS 400 and the furnace to remove any possible contamination from the atmosphere.

Solution	Tubing	i.d / mm	Flow rate / ml min^{-1}	Flow rate / rpm
Sample	black / black	0.76	2.4	100
DDDC	black / black	0.76	2.4	100
Ethanol	Solvent resistant	1.02	0.75	10
Acid wash	red / red	1.14	5	50
Air	red / red	1.14	2	40
Air	red / red	1.14	1	20

Table 5.10 : **Tubing and flow rates used for re-configured flow injection system**

5.4.1 Comparison of two different C_{18} support materials.

Two different support materials (the original silica support and a new polymeric support material) were compared using the new instrumental configuration set up described in *Section 5.4.* The silica support is a rough irregular shaped support to which the C_{18} is bonded, while the polymeric material is a regular shaped spherical support, smaller in size than the silica support material. At present this is all that is known about these two materials.[227] *Tables 5.11* and *5.12* show the analytical figures of merit used to compare and contrast the two support materials.

Both columns show similar figures of merit for most of the metals, with the exception of Mn on the polymeric support column, which exhibits very much lower absorbance values for the top standard than were obtained with the silica column. The polymeric material shows better blank levels for all but Cd.

	Cd	Cu	Mn	Ni	Pb
Slope	0.5260	0.0527	0.0807	0.0406	0.0325
Intercept	0.0001	-0.0059	0.0004	0.001	-0.0009
r^2	0.9988	0.8994	0.9967	0.9903	0.9980
Blank / Int Abs	0.0054	0.0215	0.0010	-0.0013	0.0025
Std Devn (n = 3)	0.0008	0.003	0.0006	0.0011	0.0005
Top Std / Int Abs	0.0394	0.0191	0.0245	0.0349	0.0465
Conc of Std / $\mu g\ l^{-1}$	0.075	0.75	0.3	0.75	1.5
Method DL / $ng\ l^{-1}$	15	690	30	25	151
Instrumental DL / pg	1.6	14.1	0.8	3.0	1.6

Table 5.11 : **Analytical figures of merit for silica support material**

	Cd	Cu	Mn	Ni	Pb
Slope	0.5139	0.0269	0.0142	0.0290	0.0312
Intercept	0.0011	-0.0047	-0.0007	-0.0011	-0.0002
r^2	0.9980	0.9994	0.9993	0.9981	0.9998
Blank / Int Abs	0.0065	0.0066	0.0000	-0.0002	0.0017
Std Devn (n = 3)	0.0004	0.0004	0.0002	0.0014	0.0002
Top Std / Int Abs	0.0383	0.0200	0.0042	0.0213	0.0464
Conc of Std / $\mu g\ l^{-1}$	0.075	0.075	0.3	0.75	1.5
Method DL / $ng\ l^{-1}$	13	464	92	175	79
Instrumental DL / pg	0.1	2.2	1.5	5.9	0.7

Table 5.12 : **Analytical figures of merit for polymeric support material**

In this work, there appeared to be a contamination problem for Cu with the silica material. Efforts were made to reduce this value but these were unsuccessful. The polymeric material gave better precision for all the standards than the silica material, and also gave better correlation data but this is probably due to the precision for this material being better than the silica.

The polymeric material gave lower detection limits for three of the five metals (Cd, Cu and Ni) and a high result for Mn (157 ng l^{-1} c.f. 25 ng l^{-1} for Mn with the silica material). Both materials showed high Cu results (due to the Cu contamination in the silica column material), which were reflected in the corresponding poor detection limits for both columns, Polymeric 464 ng l^{-1} and 690 ng l^{-1} for Cu on the silica material.

5.4.2 Analysis of Certified Reference Materials NASS-1, NASS-4 and CASS-3.

Using the reconfigured flow injection system, it was decided to validate the proposed method by the direct analysis of three appropriate CRMs. The CRMs chosen were two open ocean waters NASS-1 and NASS-4 and one coastal water CASS-3. The concentrations of the five elements in the CRMs are shown in *Table 5.13*. With the exception of Mn in the CASS-3 CRM, all the analytes fall within the calibration range but Pb is at an extremely low level in all three reference materials.

	NASS-1 $\mu g\ l^{-1}$	NASS-4 $\mu g\ l^{-1}$	CASS-3 $\mu g\ l^{-1}$
Cd	0.029 ± 0.004	0.016 ± 0.003	0.030 ± 0.005
Cu	0.099 ± 0.010	0.228 ± 0.011	0.517 ± 0.062
Mn	0.022 ± 0.007	0.380 ± 0.023	2.510 ± 0.360
Ni	0.257 ± 0.027	0.228 ± 0.009	0.386 ± 0.062
Pb	0.039 ± 0.006	0.013 ± 0.005	0.012 ± 0.004

Table 5.13 : **Concentration of the five selected elements in three Certified Reference Materials, NASS-1 and NASS-4 and CASS-3.**

	NASS-1 $\mu g\ l^{-1}$	NASS-4 $\mu g\ l^{-1}$	CASS-3 $\mu g\ l^{-1}$
Cd	0.047 ± 0.006	0.009 ± 0.003	0.030 ± 0.013
Cu	0.072 ± 0.062	-0.169 ± 0.012	-0.022 ± 0.035
Mn	0.025 ± 0.006	0.113 ± 0.018	0.974 ± 0.118
Ni	0.353 ± 0.095	0.008 ± 0.003	0.127 ± 0.051
Pb	3.178 ± 0.494	0.369 ± 0.098	0.564 ± 0.280

Table 5.14 : **Experimental results for all three reference materials using a silica support column**

	NASS-1 $\mu g\ l^{-1}$	NASS-4 $\mu g\ l^{-1}$	CASS-3 $\mu g\ l^{-1}$
Cd	0.083 ± 0.032	0.047 ± 0.015	0.051 ± 0.005
Cu	0.175 ± 0.026	0.123 ± 0.003	0.374 ± 0.053
Mn	0.065 ± 0.017	0.211 ± 0.012	1.195 ± 0.037
Ni	1.126 ± 0.564	0.464 ± 0.125	0.610 ± 0.051
Pb	4.650 ± 0.801	1.125 ± 0.049	2.108 ± 0.119

Table 5.15 : **Experimental results for all three reference materials using a polymeric support column**

Table 5.14 and *5.15* shows the results for each of the three RMs with the silica and polymeric column support materials respectively. Before analysis, the reference materials were adjusted to pH 2.5 with concentrated ammonia solution, since when analysed without buffering, no peak signals were observed.

5.4.2.1 Summary of work potential with Certified Reference Materials

Overall, the results obtained for the analysis of the CRMs with both columns were not good and with all results outside the certified ranges. Clearly, other factors such as matrix interference and standard stability were affecting the analysis of these materials.

Further trials were therefore conducted to investigate these factors and in particular to investigate the effect of the matrix (NaCl) upon the analysis of the five metals and the stability of the calibration standards.

5.4.3 Matrix interference experiment

In this experiment, with a silica column, the top metal standard (Cd 0.1, Cu, Ni 1.0, Mn 0.4 and Pb 2.0 $\mu g\ l^{-1}$) was analysed in increasing amount of simulated sea water *i.e.* sodium chloride solution to determine the effect of the matrix on the analysis. In theory, this should not have been a problem since the matrix should have been washed straight through the column so that only the metals were retained upon the column. The results for each metal in this experiment are shown graphically in *Figure 5.3a-e.*

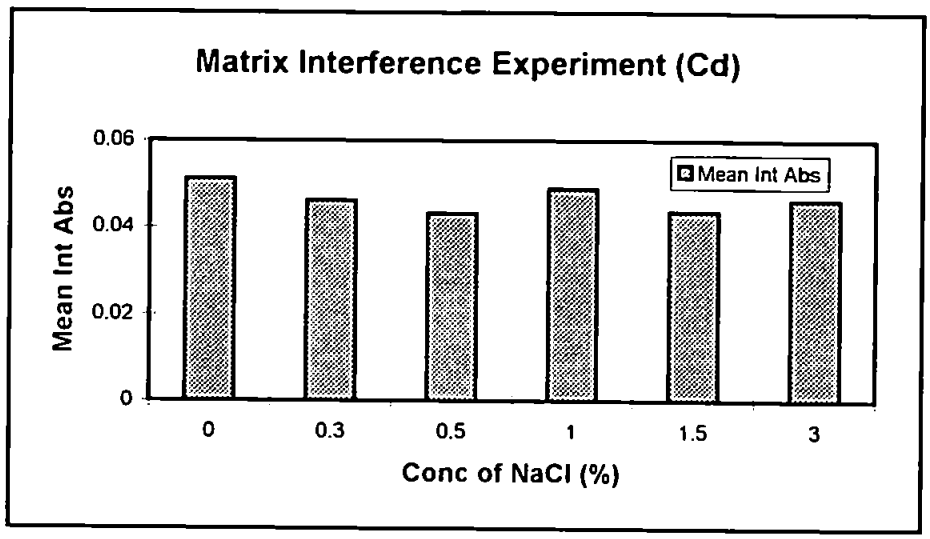


Figure 5.3a : **Results for matrix interference experiment for cadmium**

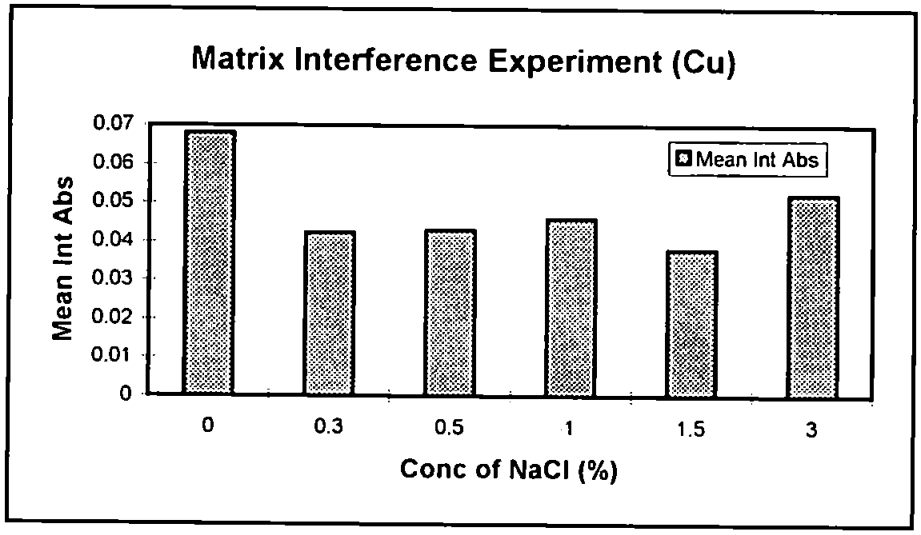


Figure 5.3b : **Results for matrix interference experiment for copper**

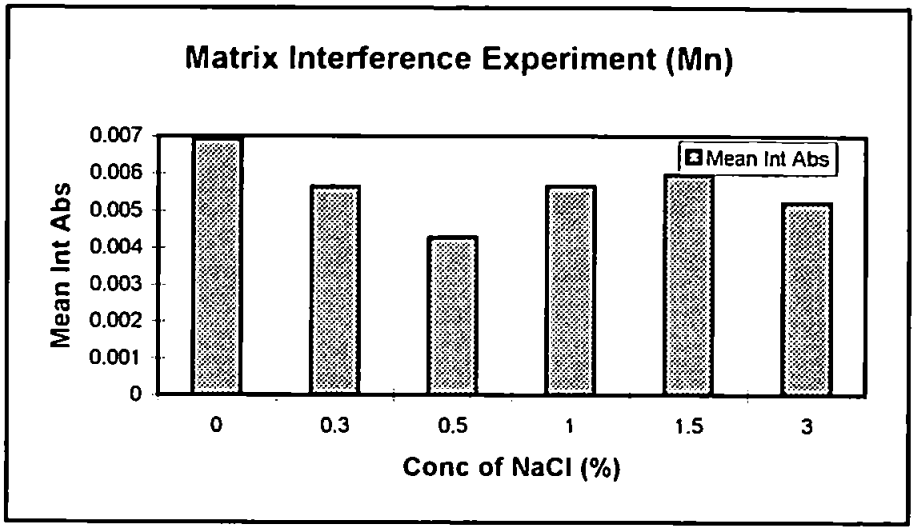


Figure 5.3c : **Results for matrix interference experiment for manganese**

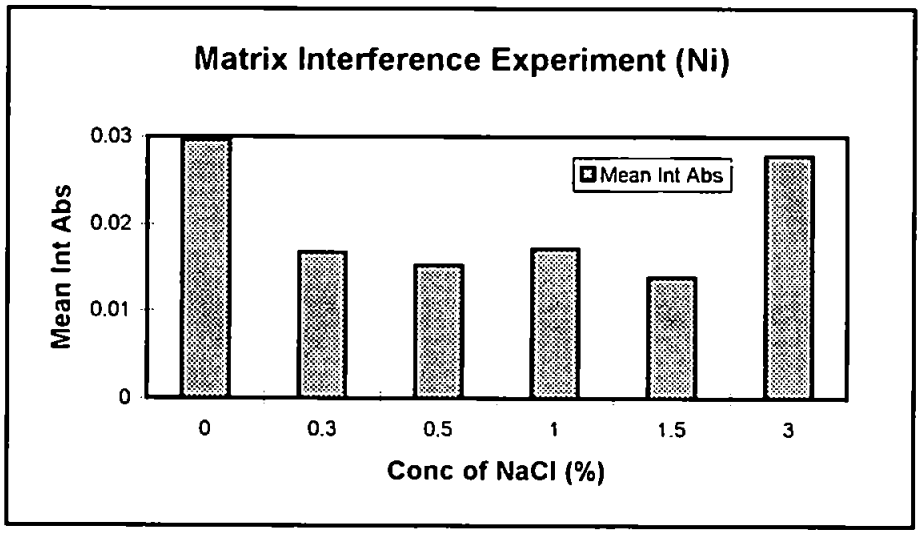


Figure 5.3d : **Results for matrix interference experiment for nickel**

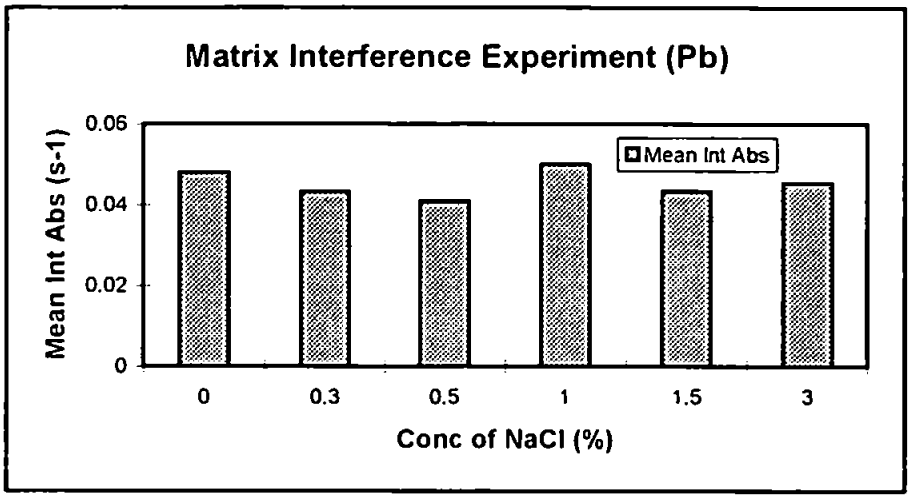


Figure 5.3 e : **Results for matrix interference experiment for lead**

5.4.3.1 Summary of results from matrix experiments

All five elements showed an interference *i.e.* a suppression of the standard signal in the presence of 0.3-3.0% sodium chloride. This was not expected since the matrix should have been washed straight through the column and not retained.

Two possible explanations are that :-

i) the acid wash step was not flushing the column of remaining matrix and

ii) the silica sites which are activated by the acidic solution are retaining some matrix which is being eluted to the furnace along with the metal-DDC complex.

5.4.4 Stability of standard solutions

The stability of the standard solutions was tested by running a calibration graph (blank and four standards) after new stock standards had been made. A time delay of 90 mins was then introduced before the next calibration graph was produced. This procedure was then repeated to obtain a total of four calibration graphs. The results for the low standard and a higher standard are shown in *Tables 5.16* and *5.17* respectively.

Conc / $\mu g\ l^{-1}$	Cd 0.01 / Int Abs	Cu 0.1 / Int Abs	Mn 0.04 / Int Abs	Ni 0.1 / Int Abs	Pb 0.2 / Int Abs
0 mins	0.0260	0.0145	0.0080	0.0122	0.0287
90 mins	0.0256	0.0144	0.0080	0.0121	0.0287
180 mins	0.0250	0.0096	0.0071	0.0102	0.0259
270 mins	0.0031	0.0108	0.0325	0.0006	0.0086

Table 5.16 : **Stability test on the low concentration multi element standard**

Conc /$\mu g\ l^{-1}$	Cd 0.05 / Int Abs	Cu 0.5 / Int Abs	Mn 0.2 / Int Abs	Ni 0.5 / Int Abs	Pb 1.0 / Int Abs
0 mins	0.1061	0.0479	0.0323	0.0645	0.1199
90 mins	0.1056	0.0475	0.0318	0.0637	0.1182
180 mins	0.0889	0.0371	0.0261	0.0442	0.1078
270 mins	0.0053	0.0303	0.1218	0.0030	0.0234

Table 5.17 : **Stability test on the high concentration multi element standard**

5.4.4.1 Summary of results obtained for the stability tests

Both the high and low standards are stable for up to 90 mins, with significant loss of absorbance being reported after 180 mins *i.e.* Cu (0.1 $\mu g\ l^{-1}$) standard dropping to 2/3 of original absorbance. This would indicate that fresh standards have to be made every two to three hours to ensure good reliability in the analytical results. As each sample takes 20 mins for three replicates, only 9 samples can be analysed before re-calibration with fresh standards is required.

5.5 Conclusion

Once the initial contamination problems been minimised, and the system parameters optimised to produce reproducible signals, there was every expectation that the method would work on real samples and successfully validated. There would appear to be no problem using the compromise furnace conditions as good peak shapes and reasonable calibration graphs were obtained. There has been one reported paper[113] in the literature, of the use of the eight port valve for an on-line sorbent preconcentration experiment using C_{18} on a silica support. Welz and co-workers observed different sensitivities depending upon the wash solution used. They attributed the change in sensitivities to the dual retention behaviour of the C_{18} material.

Clearly the most frustrating problem is the poor results obtained for the CRMs. The matrix interference experiment indicated some interference by the matrix. The stability of the standards has been shown to be reasonable as long as fresh standards are made approximately every three hours.

Chapter Six : Conclusion, Discussion and Future Work

Chapter Six : Conclusion, Discussion and Future Work

6.1 Conclusion

As stated in *Section 1.7*, the fundamental aim of this study, was to investigate the possibilities for the determination of trace elements in environmental samples following separation / preconcentration of the sample on-line where possible prior to detection by ETAAS.

A major study was undertaken on the determination of mercury. This work used the technique of cold vapour generation to remove the analyte (mercury) from the environmental matrix (tuna fish and sediment samples). Since the mercury was bound to the matrix, an off line digestion method was employed to release the mercury from the matrix and present it in a form suitable for analysis. The use a microwave assisted digestion method was evaluated due to its ease of operation and short reaction time *i.e.* five mins per digestion.

The method resulting from this study was able to achieve a sample throughput of 20-30 samples per hour, and offered a detection limit of 0.2 ng g^{-1} (3s) with a precision of less than 10% at the 0.1 $\mu g\ g^{-1}$ level for sediment samples. An interference study was conducted and although not totally exhaustive indicated that seven important elements (As^{V}, Cd^{II}, Cu^{II}, Ni^{II}, Pb^{II},

Sb^{V} and Se^{VI}) gave a less than 5% interference when the interferent concentration was at least 2 orders of magnitude greater than the Hg. In addition, Ag^{I} showed 9% interference at just one order of magnitude greater than Hg. Thus the determination of Hg in environmental and biological samples has been successfully achieved.

The hydride forming elements were also studied with a view to developing a method for the simultaneous determination of four hydride forming elements in water samples. Initial experiments showed the potential for simultaneous trapping Bi and Se on an Ir coated graphite tube after hydride generation in a flow injection system. This approach allowed for the high sample throughput (30-40 per hour) and high precision (less than 2% at 3.0 $\mu g\ l^{-1}$ level) with little operator assistance. Further experiments characterised the limitations of the method, and revealed that only certain elements in particular oxidation states could be analysed together. Arsenic and Sb can be determined in either oxidation state but are more sensitive in oxidation state III, while Se and Te must be in oxidation state IV. Careful selection of elements and oxidation states was therefore required along with careful choice of reducing or oxidising agents to facilitate maximum sensitivity. This study also revealed that Hg severely influenced the optimal instrumental parameters (pyrolysis and atomisation temperature) and should therefore be determined by an alternative method *e.g.* CVAAS.

In the final stage of this investigation two groups of elements Group A (As, Bi and Sb) and Group B (Bi and Se) were identified and used

successfully in the analysis of lake water samples. Key to defining these groups was the nature of the reducing agent used to reduce all the elements to the lowest oxidation state, *i.e.* Group A used L-cysteine while Group B used concentrated HCl and an elevated temperature. The method gave detection limits of 0.82, 0.04, 0.26 and 0.29 $\mu g\ l^{-1}$ (500 µl sample loop) for As, Bi, Sb and Se respectively. The characteristic masses recorded were 177 pg for As, 91 pg for Bi, 107 pg for Sb and 90 pg for Se upon an Ir coated tube. During validation of the method good agreement was obtained with certified and standard reference materials and the method was successfully applied to the determination of As, Bi , Sb and Se in lake water samples.

A comparison of Ir- and Zr- tube coatings was also undertaken and clearly showed that Ir was the material better suited for simultaneous multi-element hydride trapping.

A number of areas can be identified from this study for future work. Extending the range of hydride forming elements which can be determined simultaneously would clearly be advantageous. This would require a more in-depth study of appropriate oxidation states. The identification of a "magic bullet" reducing agent which will reduce all the elements to the most sensitive states, but without reducing them to the ground state *e.g.* L-cysteine will reduce Se^{VI} to Se^{IV} and then to Se^{0} would obviously be the ultimate aim. The present range of elements which can be analysed together is shown in *Table 6.1.*

A variety of alternative environmental matrices could also be examined to evaluate the method, either directly or in a slightly modified form for other applications. The results from this current work are only the first of what could be a very interesting and promising area of analysis, allowing multi element determinations after separation from the matrix and a preconcentration of the sample in the graphite tube.

	$As^{(III)}$	$As^{(V)}$	$Bi^{(III)}$	$Sb^{(III)}$	$Sb^{(V)}$	$Se^{(IV)}$	$Te^{(IV)}$
$As^{(III)}$							
$As^{(V)}$							
$Bi^{(III)}$							
$Sb^{(III)}$							
$Sb^{(V)}$							
$Se^{(IV)}$							
$Te^{(IV)}$							

Key :

	Can be analysed together
	Cannot be analysed together

Figure 6.1 : **Diagram showing whether various different elements can be or cannot be analysed together by simultaneous multi element in atomizer trapping**

In latter stages of this work, the simultaneous multi element determination of trace metals in seawater was evaluated, but was not found to work well. The majority of the contamination problems from the reagents, tubing, containers and from the atmosphere were successfully eliminated or reduced to a minimum. However, the largest contamination problem, caused by Fe present in the ethanol, (even after sub boiling distillation) prevented sensitive calibration. This is due to the Fe being organically bound in the ethanol and hence transported with the ethanol. For this reason, Fe was removed from the study. Once the contamination problems had been resolved, an interference problem was noted during the analysis of reference materials. There are two potential causes for this :-

i) competition between the various metal DDC complexes for binding sites on the column, *i.e.* quantitative retention was not occurring or

ii) an interference was being retained upon the column which eluted with the complexes into the furnace causing poor precision and reproducibility.

Further work must therefore address this interference problem to aid validation of the proposed method. Lancaster *et al.*[228] have suggested that when using a silica column material there is competition between the chelating agent and the silanol groups for binding the metal. The metal that binds to the silanol group can be removed by a pH change across the column, while the metal-chelate can only be removed by a change in polarity,

therefore no one solution can remove all the metals in one pass through the column. This interaction could be responsible for the poor results obtained in this study.

This may prove a useful starting point in characterising this problem, which when better understood may be resolved by using a different sorbent material and possibly a polymeric support instead of a silica column material (*e.g.* fullerene[229]). The use of a more selective complexing agent may also be used to retain only selected metals allowing the rest to flow to waste with the matrix.

Overall, this study has shown that novel methods employing flow injection methodologies for separation and preconcentration are a viable way to prepare environmental samples for analysis by ETAAS. Further it has demonstrated that multi-element analysis is possible for low levels of analyte despite the presence of troublesome matrices, although further work is required to achieve the ultimate goal of a universal method suitable for all analytes irrespective of the sample type.

Chapter Seven : References

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7.1 References

1) Pietrzyk, D.J., and Frank, C.W., *"Analytical Chemistry"*, Academic Press, New York, Second Edition, 1979, ch. 1, pp. 1-9.

2) Fifield, F.W., and Haines, P.J., *"Environmental Analytical Chemistry"*, Blackie, London, 1995, ch. 15, pp. 320-351.

3) Dean, J.R., *"Atomic Absorption and Plasma Spectroscopy"*, ACOL, Wiley, Chichester, Second Edition, 1997, ch. 1, pp. 1-20.

4) Howard, A., and Statham, P., *"Inorganic Trace Analysis Philosophy and Practice"*, Wiley, Chichester, 1993, ch. 1, pp. 1-12.

5) Vandecasteele, C., and Block, C.B., *"Modern Methods for Trace Element Determination"*, Wiley, Chichester, 1993, ch. 1, pp. 1-8.

6) Fifield, F.W., and Kealey, D., *"Principles and Practice of Analytical Chemistry"*, Blackie, London, Fourth Edition, 1995, ch. 1, pp. 1-13.

7) Dean, J.R., *"Atomic Absorption and Plasma Spectroscopy"*, ACOL, Wiley, Chichester, Second Edition, 1997, ch. 2, pp. 21-31.

8) Browner, R.F., and Boorn, A.W., *Anal. Chem.*, 1984, **57(7)**, 786A.

9) Fifield, F.W., and Kealey, D., *"Principles and Practice of Analytical Chemistry"*, Blackie, London, Fourth Edition, 1995, ch. 4, pp. 55-183.

10) Vandecasteele, C., and Block, C.B., *"Modern Methods for Trace Element Determination"*, Wiley, Chichester, 1993, ch. 2, pp. 10-53.

11) Howard, A.G., and Statham, P.J., *"Inorganic Trace Analysis Philosophy and Practice"*, Wiley, Chichester, 1993, ch. 7, pp. 113-160.

12) Tolg, G., *Talanta*, 1972, **19**, 1489.

13) Tsai, W.C., and Shiau, L.J., *Anal. Chem.*, 1977, **49**, 1641.

14) Leyden, D.E., and Wegscheider, W., *Anal. Chem.*, 1981, **53**, 1059A.

15) Torre, M., and Marina, M.L., *Critical Reviews in Analytical Chemistry*, 1994, **24(5&6)**, 327.

16) Volynsky, A.B., Spivakov, B.Y., and Zolotov, Y.A., *Talanta*, 1984, **31(6)**, 449.

17) Shabani, M.B., Akagi, T., Shimizu, H., and Masuda, A., *Anal. Chem.*, 1990, **62**, 2709.

18) Filippelli, M., *Analyst*, 1984, **109**, 515.

19) Danielsson, L-G., Magnusson, B., Westerlund, S., and Zhang, K., *Anal. Chim. Acta*, 1982, **144**, 183.

20) Batterham, G.J., and Parry, D.L., *Marine Chemistry*, 1996, **55**, 381.

21) Kinrade, J.D., and Van Loon, J.C., *Anal. Chem.*, 1974, **46(13),** 1894.

22) Johnson, C.A., *Anal. Chim. Acta*, 1990, **238**, 273.

23) Baffi, F., and Cardinale, A., *Intern. J. Environ. Anal. Chem.*, 1990, **41**, 15.

24) Pyrzynska, K., *Analyst*, 1995, **120**, 1933.

25) Ion Exchange Resins, Sixth Edition, Product No 57020 4G, BDH Limited, Poole, UK.

26) Kingston, H.M., Barnes, I.L., Brady, T.J., Rains, T.C., and Champ, M.A., *Anal. Chem.*, 1978, **50(14),** 2064.

27) Pai, S-C., *Anal. Chim. Acta*, 1988, **211**, 271.

28) Sturgeon, R.E., Berman, S.S., Willie, S.N., and Desaulniers, J.A.H., *Anal. Chem.*, 1981, **53**, 2337.

29) Thompson, M., Ramsey, M.H., and Pahlavanpour, B., *Analyst*, 1982, **107**, 1330.

30) Dedina, J., and Tsalev, D.L., *"Hydride Generation Atomic Absorption Spectrometry"*, Wiley, Chichester, 1995.

31) Matusiewicz, H., and Sturgeon, R.E., *Spectrochim. Acta*, 1996, **51B**, 377.

32) Nakamura, T., Oka, H., Ishii, M., and Sato, J., *Analyst*, 1994, **119**, 1397.

33) Fang, Z., *"Flow Injection Separation and Preconcentration"*, VCH, Germany, 1993, ch.1, pp.1-19.

34) Browner, R.F., and Boorn, A.W., *Anal. Chem.*, 1984, **57(7)**, 875A.

35) Jacintho, A.O., Zagatto, E.A.G., Bergamin, F.H., Krug, F.J., Reis, B.F., Bruns, R.E., and Kowalski, B.R., *Anal. Chim. Acta*, 1981, **130**, 243.

36) Fang, Z., *"Flow Injection Separation and Preconcentration"*, VCH, Germany, 1993.

37) Fang, Z, *"Flow Injection Atomic Absorption Spectrometry"*, Wiley, Chichester, 1995.

38) Gallego, M., Luque de Castro, M.D., and Valcarcel, M., *At. Spectrosc.*, 1985, **6(1)**, 16.

39) Tyson, J.F., *Spectrochim. Acta. Rev.*, 1991, **14(3)**, 169.

40) Burguera, J.L., and Burguera, M., *J. Anal. At. Spectrom.*, 1997, **12**, 643.

41) Skeggs, L., *Am. J. Clin. Path.*, 1957, **28**, 311.

42) Ruzicka, J. and Hansen, E.H., *"Flow Injection Analysis"*, Wiley, New York, First Edition, 1981.

43) Ruzicka, J. and Hansen, E.H., *"Flow Injection Analysis"*, Wiley, New York, Second Edition, 1988.

44) Fang, Z, *"Flow Injection Atomic Absorption Spectrometry"*, Wiley, Chichester, 1995, ch. 11, pp. 218-228.

45) Tsalev, D.L., Sperling, M. and Welz, B., *Analyst*, 1992, **117**, 1729.

46) Burguera, M., and Burguera, J.L., *Anal. Chim. Acta*, 1986, **179**, 351.

47) Burguera, J.L., and Burguera, M., *J. Anal. At. Spectrom.*, 1993, **8**, 235.

48) Fang, Z., *"Flow Injection Separation and Preconcentration"*, VCH, Germany, 1993, ch. 3, pp. 47-83.

49) Luque de Castro, M.D., *J. Auto. Chem.*, 1986, **8(2)**, 56.

50) Backstrom, K., Danielsson, L-G., and Nord. L., *Analyst*, 1984, **109**, 323.

51) Backstrom, K., and Danielsson, L-G., *Anal. Chim. Acta,* 1990, **232**, 301.

52) Fang, Z., *"Flow Injection Separation and Preconcentration"*, VCH, Germany, 1993, ch. 4, pp. 85-128.

53) Olsen, S., Pessenda, L.C.R., Ruzicka, J., and Hansen, E.H., *Analyst*, 1983, **108**, 905.

54) Ruzicka, J., and Arndal, A., *Anal. Chim. Acta*, 1989, **216**, 243.

55) Fang, Z., Sperling, M., and Welz, B., *J. Anal. At. Spectrom.*, 1990, **5**, 639.

56) Fang, Z, Ruzicka, J., and Hansen, E.H., *Anal. Chim. Acta*, 1984, **164**, 23.

57) Mohammad, B., Ure, A.M., and Littlejohn, D., *J. Anal. At. Spectrom.*, 1993, **8**, 325.

58) Sperling, M., Yin, X., and Welz, B., *J. Anal. At. Spectrom.*, 1991, **6**, 295.

59) Sperling, M., Yin, X., and Welz, B., *Spectrochim. Acta*, 1991, **46B(14),** 1789.

60) Porta, V., Abollino, O., Mentasti, E., and Sarzanini, C., *J. Anal. At. Spectrom.*, 1991, **6**, 119.

61) Liu, Z-S., and Huang, S-D., *Anal. Chim. Acta*, 1992, **267**, 31.

62) Fang, Z., *"Flow Injection Separation and Preconcentration"*, VCH, Germany, 1993, ch. 7, pp. 169-195.

63) Fang, Z., Sperling, M., and Welz, B., *J. Anal. At. Spectrom.*, 1991, **6**, 301.

64) Fang, Z., and Dong, L., *J. Anal. At. Spectrom.*, 1992, **7**, 439.

65) Fang, Z., *"Flow Injection Separation and Preconcentration"*, VCH, Germany, 1993, ch. 5, pp. 129-157.

66) Dedina, J., and Welz, B., *Spectrochim. Acta*, 1993, **48B(3)**, 301.

67) Sturgeon, R.E., Willie, S.N., and Berman, S.S., *J. Anal. At. Spectrom.*, 1986, **1**, 115.

68) Nielsen, S., Sloth, J.J., and Hansen, E.H., *Talanta*, 1996, **43**, 867.

69) Carrero, P.E., and Tyson, J.F., *Analyst*, 1997, **122**, 915.

70) Price, W.J., *"Spectrochemical Analysis by Atomic Absorption"*, Heyden, London, 1979, ch. 3, pp. 27-69.

71) Welz, B., *"Atomic Absorption Spectrometry"*, Second Edition, VCH, Germany, 1985, ch. 3, pp. 31-82.

72) Hatch, W.R., and Ott, W.L., *Anal. Chem.*, 1968, **40(14)**, 2085.

73) Ebdon, L., *"An Introduction to Atomic Absorption Spectroscopy"*, Heyden, London, 1982, ch. 9, pp. 86-89.

74) Rooney, R.C., *Analyst*, 1976, **101**, 678.

75) Colina de Vargas, M., and Romero, R.A., *At. Spectrosc.*, 1989, **10(5)**, 160.

76) Zhu, L.Z., Lu, J., and Le, X., *Mikrochim. Acta*, 1993, **111**, 207.

77) Kaiser, G., Gotz, D., Schoch, P., and Tolg, G., *Talanta*, 1975, **22**, 889.

78) Matsunaga, K., and Takahashi, S., *Anal. Chim. Acta*, 1976, **87**, 487.

79) Welz, B., Melcher, M., Sinemus, H.W., and Maier, D., *At. Spectrosc.*, 1984, **5(2)**, 37.

80) Price, W.J., *"Spectrochemical Analysis by Atomic Absorption"*, Heyden, London, 1979, ch. 8, pp. 191-286.

81) Thompson, K.C., and Godden, R.G., *Analyst*, 1975, **100**, 544.

82) Guo, T., and Bassner, J., *Anal. Chim. Acta*, 1993, **278**, 189.

83) Welz, B., Tsalev, D.L., and Sperling, M., *Anal. Chim. Acta*, 1992, **261**, 91.

84) Hanna, C.P., Tyson, J.F., and McIntosh, S., *Anal. Chem.*, 1993, **65**, 653.

85) Vandecasteele, C., and Block, C.B., *"Modern Methods of Trace Element Determination"*, Wiley, Chichester, 1993, ch. 5, pp. 93-137.

86) Holak, W., *Anal. Chem.*, 1969, **41**, 1712.

87) McDaniel, M., Shendrikar, A.D., Reiszner, K.D., and West, P.W., *Anal. Chem.*, 1976, **48**, 2240.

88) Siemer, D.D., Koteel, P., and Jariwala, V., *Anal. Chem.*, 1976, **48**, 836.

89) Smith, R.G., Van Loon, J.C., Knechtel, J.R., Fraser, J.L., Pitts, A.E., and Hodges, A.E., *Anal. Chim. Acta*, 1977, **93**, 61.

90) Dedina, J., and Rubeska, I., *Spectrochim. Acta*, 1980, **35B**, 119.

91) Siemer, D.D., and Koteel, *Anal. Chem.*, 1977, **49(8)**, 1096.

92) Haug, H.O., and Liao, Y-P., *Fresenius J. Anal. Chem.*, 1996, **356**, 435.

93) Xiu-ping, Y., and Zhe-ming, N., *J. Anal. At. Spectrom.*, 1991, **6**, 483.

94) Shuttler, I.L., Feuerstein, M., and Schlemmer, G., *J. Anal. At. Spectrom.*, 1992, **7**, 1299.

95) Bulska, E., and Jedral, W., *J. Anal. At. Spectrom.*, 1995, **10**, 49.

96) Tsalev, D.L., D'Ulivo, A., Lampugnani, L. Di Marco, M, and Zamboni, R., *J. Anal. At. Spectrom.*, 1996, **11**, 979.

97) Sturgeon, R.E., Willie, S.N., Irwin Sproule, G., and Berman, S.S., *J. Anal. At. Spectrom.*, 1987, **2**, 719.

98) Schelmmer, G., and Feuerstein, M., *"Verminderung von Gasphaseninterferenzen in der Hydrid-AAS durich FIA-Graphitrofenkopplung.*, Colloquium Analytische Atomspectroscopie", 1993.

99) Dedina, J., and Tsalev, D.L., *"Hydride generation atomic absorption spectrometry"*, Wiley, Chichester, 1995, ch. 4, pp. 91-117.

100) Tsalev, D.L., D'Ulivo, A., Lampugnani, L., Di Marco, M., and Zamboni, R., *J. Anal. At. Spectrom.*, 1995, **10**, 1003.

101) Zhang, L., Ni, Z., and Shan, X., *Spectrochim. Acta*, 1989, **44B**, 339.

102) Barth, B., Krivan, V., and Hausbeck, H., *Anal. Chim. Acta*, 1992, **263**, 111.

103) An, Y., Willie, S.N., and Sturgeon, R.E., *Spectrochim. Acta*, 1992, **47B(12)**, 1403.

104) Fang, Z, *Spectrochim. Acta Rev.*, 1991, **14(3)**, 235.

105) Ruzicka, J., and Hansen, E.H., *"Flow Injection Analysis"*, Second Edition, Wiley, New York, 1988, ch. 4, pp. 139-256.

106) Malamas, F., Bengtsson, M., and Johansson, G., *Anal. Chim. Acta*, 1984, **160**, 1.

107) Fang, Z., Xu, S-K., and Zhang, S-C, *Anal. Chim. Acta*, 1984, **164**, 41.

108) Hartenstein, S.D., Ruzicka, J., and Christian, G.D., *Anal. Chem.*, 1985, **57**, 21.

109) Fang, Z., Xu, S-K., and Tao, G., *J. Anal. At. Spectrom.*, 1996, **11**, 1.

110) Beinrohr, E., Cakrt, M., Rapta, M, and Tarapci, P., *Fresenius J. Anal. Chem.*, 1989, **335**, 1005.

111) Fang, Z., Tao, G., *Fresenius J. Anal. Chem.*, 1996, **355**, 576.

112) Fang, Z., *"Flow Injection Atomic Absorption Spectrometry"*, Wiley, Chichester, 1995, ch. 9, pp. 175-202.

113) Welz, B., Sperling, M., and Sun, S-J., *Fresenius J. Anal. Chem.*, 1993, **346**, 550.

114) Berndt, H., and Schaldach, G., J. *Anal. At. Spectrom.*, 1994, **9**, 39.

115) Ma, R., van Mol, W., and Adams, F., *At. Spectrosc.*, 1996, **17(4)**, 176.

116) Fang, Z., Xu, S-K., and Zhang, S-C., *Anal. Chim. Acta*, 1987, **200**, 35.

117) Fang, Z., Xu, S-K., and Zhang, S-C., *Anal. Chim. Acta*, 1984, **164**, 41.

118) Fang, Z., *"Flow Injection Atomic Absorption Spectrometry"*, Wiley, Chichester, 1995, ch. 8, pp. 143-174.

119) Willard, H.H., Merritt, Jr., L.L., Dean, J.A., and Settle, Jr., F.A., *"Instrumental Methods of Analysis"*, Wadsworth Publishing Company, USA, Seventh Edition, 1988, ch. 9, pp. 224-259.

120) Skoog, D.A., West, D.M., and Holler, F.J., *"Fundamentals of Analytical Chemistry"*, Saunders College Publishing, USA, International Sixth Edition, 1992, ch. 24, pp. 615-651.

121) Welz, B., *"Atomic Absorption Spectrometry"*, VCH, Germany, Second Edition, 1985, ch. 1, pp. 1-17.

122) Willard, H.H., Merritt, Jr., L.L., Dean, J.A., and Settle, Jr., F.A., *Instrumental Methods of Analysis*, Wadsworth Publishing Company, USA, Seventh Edition, 1988, ch. 5, pp. 97-117.

123) Tyson, J.F., *Atomic Absorption Spectrometry ; Theory, Design and Applications*, Editor Haswell, S.J., Elsevier, Netherlands, Volume 5, 1991, ch. 1, pp. 1-20.

124) Fifield, F.W., and Kealy, D., *Principles and Practice of Analytical Chemistry*, Blackie, London, 1995, Fourth Edition, 1995, ch. 7, pp. 267-281.

125) Skoog, D.A., and Leary, J.J., *Principles of Instrumental Analysis*, Saunders College Publishing, USA, Fourth Edition, 1992, ch. 10, pp. 196-232.

126) Ebdon, L., *"An Introduction to Atomic Absorption Spectroscopy"*, Heyden, London, 1982, ch. 1, pp. 1-10.

127) Skoog, D.A., West, D.M., and Holler, F.J., *"Analytical Chemistry : An Introduction"*, Saunders College Publishing, USA, International Sixth Edition, 1990, ch. 24, pp. 453-480.

128) Fifield, F.W., and Kealy, D., *"Principles and Practice of Analytical Chemistry"*, Blackie, London, Fourth Edition, 1995, ch. 8, pp. 282-350.

129) Vandecasteele, C., and Block, C.B., *"Modern Methods for Trace Element Determination"*, Wiley, Chichester, 1993, ch. 7, pp. 159-167.

130) Ebdon, L., *"An Introduction to Atomic Absorption Spectroscopy"*, Heyden, London, 1982, ch. 5, pp. 58-62.

131) Dean, J.R., *"Atomic Absorption and Plasma Spectroscopy"*, Wiley, Chichester, 1997, ch. 3, pp. 33-45.

132) Shuttler, I.L., and Schulze, H., *Analysis Europa*, 1994, **1**, 44.

133) Radziuk, B., Rodel, G., Stenz, H., Becker-Ross, H., and Florek, S., *J. Anal. At. Spectrom.*, 1995, **10**, 127.

134) Harnly, J., *Fresenius J. Anal. Chem.*, 1996, **355**, 501.

135) Ebdon, L., *"An Introduction to Atomic Absorption Spectroscopy"*, Heyden, London, 1982, ch. 4, pp. 42-57.

136) Slavin, W., Manning, D.C., and Carnrick, G.R., *At. Spectrosc*, 1981, **2(5)**, 137.

137) Carnrick, G.R., and Slavin, W., *Am. Lab.*, October 1988, **21(2)**, 90.

138) Carnrick, G.R., and Slavin, W., *Am. Lab.*, February 1989, **20(10)**, 88.

139) Simultaneous Multi element Atomic Absorption 6000 Spectrometer, *Installation, Maintenance and System description*, B3119.10, Release 1.3, Bodenseewerk, Perkin-Elmer GmbH, Uberlingen, 1995.

140) Blankley, M., Henson, A., and Thompson, K.C., *"Atomic Absorption Spectrometry : Theory, Design and Applications"*, Editor Haswell, S.J., Elsevier, Netherlands, Volume 5, 1991, ch. 4, pp. 79-123.

141) Welz, B., *"Atomic Absorption Spectrometry"*, VCH, Germany, Second Edition, 1985, ch. 7, pp. 129-164.

142) Clyburn, S.A., Kantor, T., and Veillon, C., *Anal. Chem.*, 1974, **46**, 2214.

143) Massmann, H., *Spectrochim. Acta*, 1968, **23B**, 215.

144) Model 4100ZL Atomic Absorption Spectrometer, *Installation, Maintenance and System Description*, B3121.10, Release 1.0, Bodenseewerk, Perkin Elmer GmbH, Uberlingen, 1995.

145) Frech, W., Baxter, D.C., and Hutsch, B., *Anal. Chem.*, 1986, **58**, 1973.

146) Price, W.J., *"Spectrochemical Analysis by Atomic Absorption"*, Heyden, London, 1979, ch. 4, pp. 70-94.

147) Dean, J.R., *"Atomic Absorption and Plasma Spectroscopy"*, Wiley, Chichester, 1997, Second Edition, ch. 4, pp. 47-77.

148) Perkampus, H-H., *"Encyclopaedia of Spectroscopy"*, VCH, Germany, 1995, pp. 368-369.

149) Skoog, D.A., West, D.M., and Holler, F.J., *"Fundamentals of Analytical Chemistry"*, Saunders College Publishing, USA, International Sixth Edition, 1992, ch. 21, pp. 535-560.

150) Willard, H.H., Merritt, Jr., L.L., Dean, J.A., and Settle, Jr., F.A., Instrumental Methods of Analysis, Wadsworth Publishing Company, USA, Seventh Edition, 1988, ch. 6, pp. 118-158.

151) Perkampus, H-H., *"Encyclopaedia of Spectroscopy"*, VCH, Germany, 1995, pp. 479-480.

152) Welz, B., "Atomic Absorption Spectrometry", VCH, Germany, Second Edition, 1985, ch. 5, pp. 95-106.

153) Perkampus, H-H., *"Encyclopaedia of Spectroscopy"*, VCH, Germany, 1995, pp. 455-456.

154) Perkampus, H-H., *"Encyclopaedia of Spectroscopy"*, VCH, Germany, 1995, pp. 445.

155) Radziuk, B., Rodel, G., Zeiher, M., Mizuno, S., and Yamamoto, K., *J. Anal. At. Spectrom.*, 1995, **10**, 415.

156) Haswell, S.J., *"Atomic Absorption Spectrometry : Theory, Design and Applications"*, Editor Haswell, S.J., Elsevier, Netherlands, Volume 5, 1991, ch. 2, pp. 21-49.

157) Dulude, G., *"Advances in Atomic Spectroscopy"*, Editor Sneddon, J., Jai Press, London, 1992, Volume 1, ch. 4, pp. 125-161.

158) Morales-Rubio, A., Mena, M.L., and McLeod, C.W., *Anal. Chim. Acta*, 1995, **308**, 364.

159) Lajunen, L.H.J., *"Spectrochemical Analysis by Atomic Absorption and Emission"*, RSC, Cambridge, 1992, ch. 6, pp. 117-131.

160) Tahan, J., Granadillo, V., Sanchez, J., Cubillan, H., and Romero,R., *J. At. Anal. Spectrom.*, 1993, **8**, 1005.

161) Colina de Vargas, M., and Romero, R., *Analyst*, 1992, **117**, 645.

162) Colina de Vargas, M., Ledo de Medina, H., and Araujo, K., *Analyst*, 1994, **119**, 623.

163) Guo, T., and Baasner, J., *Talanta*, 1993, **40(12)**, 1927.

164) Baxter. D.C., and Frech, W., *Anal. Chim. Acta,* 1990, **236**, 377.

165) Adeloju, S.B., Dhindsa, H.S., and Tandon, R.T., *Anal. Chim. Acta,* 1994, **285**, 359.

166) Adeloju, S.B., *Analyst,* 1989, **114**, 455.

167) Quevauviller, P., Imbert, J-L., and Olle, M., *Mikrochim. Acta*, 1993, **112**, 147.

168) Nieuwenhuize, J., and Poley-Vos, C.H., *At. Spectrosc.*, 1989, **5(10)**, 148.

169) Vermeir, G., Vandercasteele, C., and Dams, R., *Anal. Chim. Acta*, 1989, **220**, 257.

170) Navarro, M., Lopez, M.C., Lopes, H., and Sanchez, M., *Anal. Chim. Acta*, 1992, **257**, 155.

171) Schnitzer, M.G., Pellerin, C., and Clouet, C., Appendix 1., Pittsburgh Conference 1988, Ministry of Agriculture Direction of the Quality., CNEVA-Paris.

172) Lan, W.G., Wong, M.K., and Sin, Y.M., *Talanta*, 1994, **41(2)**, 195.

173) Flow Injection Mercury/Hydride Analyses., Recommended Analytical Conditions and General Information Documentation, B050-1820 (B3505), Release 3.0 / Feb 94, Bodenseewerk Perkin-Elmer, Uberlingen, 1994.

174) Rokkjaer, I., Hoyer, B., and Jensen, N., *Talanta,* 1993, **40(5)**, 729.

175) Personnel Communication, I.L. Shuttler, September 1995.

176) Madrid, Y., Gutierrez, J.M., and Camara, C., *Spectrochim. Acta*, 1994, **49B**,163.

177) Bernth, N., and Vendelbo, K., *Analyst,* 1984, **109**, 309.

178) Uggerud, H., and Lund, W., *J. Anal. At. Spectrom.*, 1995, **10,** 405.

179) Morrow, A., Wiltshire, G., and Hursthouse, A., *At. Spectrosc.*, 1997, **18**, 23.

180) Bowman, J., Fairman, B., and Catterick, T., *J. Anal. At. Spectrom.*, 1997, **12**, 313.

181) Stroh, A., and Vollkopf, U., *J. Anal. At. Spectrom.*, 1993, **8**, 35.

182) Sturgeon, R.E., and Gregoire, D.C., *Spectrochim. Acta,* 1994, **49B(12)**, 1335.

183) Garbos, S., Walcerz, M., Bulska, E., and Hulanicki, A., *Spectrochim. Acta*, 1995, **50B**, 1669.

184) Kraus, B., Diplomarbeit *Utersuchungen zur simultanen Multielementbestimmung hydridbildender Elemente und von Quecksilber mit der Graphitrohr-Atomabsorptionsspektrometrie., Fachhochschule Aalen*, Germany, 1996.

185) The FIAS Furnace Technique, *Setting up and Performing Analysis*, B050-9907, **Release 1.0 July 1993**, Bodenseewerk Perkin-Elmer GmbH, Uberlingen, 1993.

186) Bye, R., and Lund, W., *Fresenius Z. Anal. Chem.*, 1988, **332**, 242.

187) Tyson, J.F., Offley, S.G., Seare, N.J., Kibble, H.A.B., and Fellows, C., *J. Anal. At. Spectrom.*, 1992, **7**, 315.

188) Dedina, J., and Welz, B., *J. Anal. At. Spectrom.*, 1992, **7**, 307.

189) Welz, B., and Sucmanova, M., *Analyst*, 1993,**118**, 1417.

190) D'Ulivo, A., Lampugnani, L., Pellegrini, G., and Zamboni, R., *J. Anal. At. Spectrom.,* 1995, **10**, 969.

191) Tsalev, D.L., D'Ulivo, A., Lampugnani, L., Pellegrini, G., and Zamboni, R., *J. Anal. At. Spectrom.,* 1996, **11**, 989.

192) Welz, B., and Sucmanova, M., *Analyst*, 1993,**118**, 1425.

193) Bye, R., *Talanta*,1990, **37(10)**, 1029.

194) Bye, R., *Talanta*, 1983, **30(12)**, 993.

195) The FIAS Furnace technique, *Recommended Analytical Conditions and General Information,* Publication B3212.20, Part No 0993-5204 Bodenseewerk Perkin Elmer Uberlingen 1993.

196) Lide, D.R., Editor in Chief, *"Handbook of Chemistry and Physics"*, 75th Edition, CRC Press, USA, 1995, ch. 8, pp. 21-31.

197) Sperling, M., Xu, S., and Welz, B., *Anal. Chem.*, 1992, **64**, 3101.

198) Pitts, L., Worsfold, P.J., and Hill, S.J., *Analyst*, 1994, **119**, 2785.

199) Hill, S.J., Pitts, L., and Worsfold, P.J., *J. Anal. At. Spectrom.*, 1995, **10**, 409.

200) Xiu-ping, Y., and Zhe-ming, N., *J. Anal. At. Spectrom.*, 1991, **6,** 483.

201) Tsalev, D.L., and Slaveykova, V.I., *Spectroscopy Letters* , 1992, **25(2)**, 221.

202) Personal Communication, M. Feuerstein, June 1997.

203) Welz, B., and Strauss, P., *Spectrochim. Acta*, 1993, **48B(8)**, 951.

204) Schlemmer, G., and Feuerstein, M., CANAS '93, Colloquium Analytische Atomspektroskopie, Ed Welz, B., Bodenseewerk Perkin-Elmer GmbH, Uberlingen, Germany, 1994, 431-441.

205) Wickstrom, T., and Lund, W., and Bye, R., *Analyst*, 1996, **121**, 201.

206) Welz, B., and Schubert-Jacobs, M., *J. Anal. At. Spectrom.*, 1986, **1**, 23.

207) Sinemus, H-W., Kleiner, J., Stabel, H-H., and Radziuk, B., *J. Anal. At. Spectrom.*,1992, **7**, 433.

208) Personal Communication, Bodensee Water Works, July 1997.

209) IUPAC Analytical Chemistry Division, Commission on Spectrochemical and other optical procedures for analysis, *Spectrochim. Acta.*, 1978, **33**, 241.

210) Miller, J.C., and Miller, J.N., *"Statistics for Analytical Chemistry"*, Second Edition, Wiley, Chichester, 1988, ch. 5, pp.115-117.

211) Zhe-ming, N., Bin, H., and Heng-bin, H., *J. Anal. At. Spectrom.*, 1993, **8**, 995.

212) Sturgeon, R.E., Willie, S.N., and Berman, S.S., *Fresenius J. Anal. Chem.*, 1986, **323**, 788.

213) Ebdon, L., Fisher, A.S., Hill, S.J., and Worsfold, P.J., *J. Auto. Chem.*, 1991, **13(6)**, 281.

214) Carbonell, V., Salvador, A., and de la Guardia, M., *Fresenius J. Anal. Chem.*, 1992, **324**, 529.

215) Myasoedova, G.V., and Savvin, S.B., *Critical Reviews in Analytical Chemistry*, 7(1), 1.

216) Ma, R., Van Mol, W., and Adams, F., *Anal. Chim. Acta*, 1993, **285**, 33.

217) Ma, R., and Adams, F., *Spectrochim. Acta*, 1996, **51(14)**, 1917.

218) Freiser, H., and Morrison, G., *Annual Review of Nuclear Science*, 1959, 9, 221.

219) De, A.K., Khopkar, S.M., and Chalmers, R.A., *"Solvent extraction of metals"*, Van Nostrand Reinhold, London, 1970, ch. 8, pp. 141-157.

220) Hulanicki, A., *Talanta*, 1967, **14**,1371.

221) Mitchell, J.W., *Talanta*, 1982, **29**, 993.

222) Ma, R., Van Mol, W., and Adams, F., *Anal. Chim. Acta*, 1994, **293**, 251.

223) Personal Communication, D. Bradshaw July 1997.

224) Personal Communication, M. Sperling, July 1997.

225) Personal Communication, I.L. Shuttler, July 1997.

226) Fang, Z., *"Flow Injection Atomic Absorption Spectrometry"*, Wiley, Chichester, 1995, ch. 2, pp. 14-44.

227) Personal Communication, M. Sperling, September 1997.

228) Lancaster, H.L., Marshall, G.D., Gonzalo, E.R., Ruzicka, J., and Christian, G.D., *Analyst*, 1994, **119**, 1459.

229) Gallego, M., Petit de Pena, Y., and Varcarcel, M., *Anal. Chem.*, 1994, **66**, 4074.

Epilogue

Abbreviations

8-HOQ	8 hydroxyquinoline
A or abs	Absorbance
AAS	Atomic Absorption Spectrometry
AES	Atomic Emission Spectrometry
AFS	Atomic Fluorescence Spectrometry
APDC	Ammonium pyrrolidinedithiocarbamate
BOC	Baseline Offset Correction
CPG	Controlled Pore Glass
CRMs	Certified References Materials
CVAAS	Cold Vapour Atomic Absorption Spectrometry
DDC	Diethyldithiocarbamate
DDDC	Diethylammonium diethyldithiocarbamate
EDL	Electrodeless Discharge Lamp
EDTA	Ethylenediaminetetraacetic acid
ETA	Electrothermal Atomiser
ETAAS	Electrothermal Atomic Absorption Spectrometry
FAAS	Flame Atomic Absorption Spectrometry
FI	Flow Injection
FIA	Flow Injection Analysis
FIAS	Flow Injection Analysis System
FIAS 400	Flow Injection Analysis System 400
FIAS-MHS	Flow Injection Analysis System Mercury / Hydride system
FI-CVAAS	Flow Injection Cold Vapour Atomic Absorption Spectrometry
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
GLS	Gas-Liquid Separator
HCL	Hollow Cathode Lamp
HFE	Hydride Forming Element
HGA	Heated Graphite Atomiser
HG-AAS	Hydride Generation Atomic Absorption Spectrometry
HMA-HMDTC	Hexamethyleneammonium hexamethylenedithiocarbamate
IBMK	Iso-butyl methyl ketone

ICP-AES	Inductively Coupled Plasma-Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
IDL	Instrument Detection Limit
m_o	characteristic mass
mA	milliamp
MDL	Method Detection Limit
MHz	Megahertz
NaDDC	Sodium Diethyldithiocarbamate
ng g^{-1}	nanograms per gram
ng ml^{-1}	nanograms per millilitre
nm	nanometre
PA	Peak Area
PFA	Perfluoralkoxyvinyl ether
PH	Peak Height
PMT	Photomultiplier Tube
PTFE	Polytetrafluoroethene
QF-AAS	Quartz Furnace Atomic Absorption Spectrometry
QF-HG-AAS	Quartz Furnace Hydride Generation Atomic Absorption Spectrometry
r^2	Correlation Co-efficient Squared
RSD	Relative Standard Deviation
SRM	Standard Reference Material
Std Devn	Standard Deviation
STPF	Stabilised Temperature Platform Furnace
THGA	Transversely Heated Graphite Atomiser
µg g^{-1}	micrograms per gram
µg l^{-1}	micrograms per litre
UV	Ultra-Violet

Meetings attended

Application of Atomic Spectroscopy in Trace metal speciation.

Atomic Spectrometry Updates / Atomic Spectroscopy Group.

Royal Society of Chemistry, Analytical Division.

30 March 1995, Bristol University.

Research and Developments Topics in Analytical Chemistry Meetings.

Royal Society of Chemistry, Analytical Division.

10-11 July 1995, University of Hull.

SAC 95 : An International Symposium on Analytical Chemistry.

Royal Society of Chemistry, Analytical Division.

11-15 July 1995, University of Hull.

Young Environmental Chemists Meeting.

Royal Society of Chemistry, Industrial Division.

5 March 1996, De Montfort University.

Spectroscopic Techniques for in situ Water Monitoring.

Western Region, Analytical division and Molecular Spectroscopy Group.

11 July 1996, University of Plymouth.

Eighth Biennial National Atomic Spectroscopy Symposium.

Royal Society of Chemistry, Analytical Division.

17-19 July 1996, University of East Anglia.

Poster Presented :

"Determination of Total Mercury in Environmental and Biological Samples by Flow Injection Cold Vapour Atomic Absorption Spectroscopy."

Research and Developments Topics in Analytical Chemistry Meetings.

Royal Society of Chemistry, Analytical Division.

22-23 July 1996, Nottingham Trent University.

Poster Presented :

"The Application of Flow Injection with Cold Vapour Atomic Absorption Spectroscopy for the Determination of Total Mercury in Environmental and Biological Samples."

Second Young Environmental Chemists Meeting.

Royal Society of Chemistry, Industrial Division.

18 March 1997, De Montfort University.

Speech Given :

"The determination of total mercury in environmental and biological samples by flow injection cold vapour atomic absorption spectrometry".

Towards 2000 : Challenges for Atomic Spectrometry.

Atomic Spectrometry Updates / Atomic Spectroscopy Group.

Royal Society of Chemistry, Analytical Division.

25 March 1997, University of Edinburgh.

Research and Developments Topics in Analytical Chemistry Meetings.

Royal Society of Chemistry, Analytical Division.

2-3 July 1997, University of Northumbria in Newcastle

Poster Presented :

"Investigations into the Simultaneous Determination of Bismuth And Selenium By 'In Atomizer Trapping' Electrothermal Atomic Absorption Spectrometry."

XXX Colloquium Spectroscopicum Internationale

21-25 September 1997, Melbourne World Congress Centre, Australia

Posters Presented

"Investigations into the Simultaneous Determination of Bismuth And Selenium By 'In Atomizer Trapping' Electrothermal Atomic Absorption Spectrometry."

and

"The possibilities for Simultaneous Determination of Hydride Forming Elements by "In Atomizer Trapping" Electrothermal Atomic Absorption Spectrometry."

Speech given by M. Sperling

"Simultaneous multi element determination of ultra trace in complex matrices by electrothermal atomic absorption with on-line preconcentration using flow injection."

Research Meeting, Perkin Elmer Bodenseewerk GmbH

14 November 1997, Überlingen, Germany

Speech given

"On-line sample pretreatment of environmental samples for use with Atomic Spectroscopy."

Lectures and associated courses attended

Erasmus Eurocourse, "Frontiers in Analytical Chemistry :

Trace Environmental Analysis"

10-14 September 1995, University of Plymouth 1995.

MSc credited short course in Analytical Atomic Spectrometry,

9-13 September 1996, University of Plymouth 1996.

Papers published

"The Determination of Total Mercury in Environmental and Biological samples by Flow Injection Cold Vapour Atomic Absorption Spectrometry".

J. Murphy, P. Jones, S.J. Hill.

Spectrochimica Acta Part B Special Flow Injection issue Vol. **51** No **14** 1867-1873

"Investigations into the Simultaneous Determination Of Bismuth And Selenium by 'In Atomizer Trapping' Electrothermal Atomic Absorption Spectrometry".

J. Murphy, G. Schlemmer, I.L. Shuttler, P. Jones, S.J. Hill.

Analytical Communications, November 1997, Vol **34**, 359-362.

"Simultaneous Multi Element Determination of Hydride Forming Elements by 'In Atomizer Trapping' Electrothermal Atomic Absorption Spectrometry on an Iridium - coated graphite tube."

J. Murphy, G. Schlemmer, I.L. Shuttler, P. Jones, S.J. Hill.

Journal of Analytical Atomic Spectrometry, Submitted for publication.