1985

DIRECTLY-COUPLED CHROMATOGRAPHY - ANALYTICAL ATOMIC SPECTROSCOPY FOR TRACE METAL SPECIATION

HILL, STEPHEN JOHN

A thesis entitled

DIRECTLY-COUPLED CHROMATOGRAPHY - ANALYTICAL ATOMIC SPECTROSCOPY FOR TRACE METAL SPECIATION

presented by

STEPHEN JOHN HILL, B.Sc.

in part fulfilment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY

of the

COUNCIL FOR NATIONAL ACADEMIC AWARDS

Department of Environmental Sciences

Plymouth Polytechnic

Drake Circus, Plymouth. PL4 8AA

Collaborating establishment

Pye Unicam Ltd.,

York Street, Cambridge.

October 1985

Directly-coupled Chromatography - Analytical Atomic Spectroscopy for trace metal speciation

Stephen John Hill

Abstract

The form of trace metals, so called "speciation", is of vital importance in many fields, e.g., toxicology and environmental monitoring. A promising analytical approach to speciation studies is the coupling of chromatography, for species separation, to the selectivity and sensitivity of atomic spectroscopy for detection. The suitability of such couplings are discussed and the applications of both gas and liquid chromatography reviewed.

The success of coupled gas chromatography (GC) - flame atomic absorption spectroscopy (FAAS) is demonstrated for the unequivocal identification of petrol residues in forensic applications. The advantages and disadvantages of this technique are discussed with reference to both sufficiently volatile and non-volatile compounds.

The advantages of high performance liquid chromatography (HPLC) for many studies are discussed. As coupled HPLC-ETA-AAS (electrothermal atomisation - atomic absorption spectroscopy) suffers from non-continuous detection, coupling is difficult and chromatography constrained. In contrast, a simple HPLC-FAAS coupling utilising pulse nebulisation and a modified atom cell was developed which produced continuous chromatograms in real time. Application of this system to determining tributyltin (TBT^+) compounds in seawater yielded a detection limit of 200 ng ml^{-1}.

A directly coupled system utilising continuous flow hydride generation is described, and for species with non-volatile hydrides, on-line UV photolysis was incorporated. The effects of various parameters on analytical performance are discussed, and applications to real samples given. Detection limits for TBT^+ were improved 100 fold.

A novel sample transport interface using rotating platinum wire spirals controlled by a microprocessor, utilised the attractive features of flame atomisers but sample introduction via the nebuliser was avoided. Applications are reported using both minibore HPLC for alkyllead speciation and fast protein liquid chromatography for speciating zinc in human serum.

Applications of the above techniques for determining organometallic species of Pb, As, Sn, Cu, Zn and Cd are described and possible future work discussed.

Acknowledgements

I wish to express my sincerest gratitude to Dr. L. Ebdon for his invaluable guidance and enthusiasm throughout the duration of this work. I would also like to thank Dr. P. Jones for his active role as second supervisor. I am also grateful to the Science and Engineering Research Council for the provision of a studentship, and Pye Unicam Ltd. for their support in finance and instrumentation.

I am indebted to many people, in particular Dr. A. Brown and Dr. W.J. Price, for helpful discussions on all aspects of this work. I must also acknowledge the help of Dr. R. Ward who initially compiled much of the material used in the tables in the review section, which I was able to include with suitable updating.

I would also like to thank Dr. F. Mantura and the Institute for Marine Environmental Research for the loan of the quartz photolysis equipment and Dr. R. Hanson, Biological Sciences Department, for arranging access to the FPLC instrumentation. I am particularly grateful to Mr. A. Hopkins for his electronic and computing expertise used in constructing the interface units.

Finally, I would like to thank Beverley, for her patience and support, and for the typed presentation of this thesis.

CONTENTS

List of Plates

List of Figures

List of Tables

CHAPTER 1

INTRODUCTION

1.1 The need for trace metal speciation

It is now generally recognised that the physico-chemical form and occurrence of trace metals is a primary factor in controlling their behaviour and fate in the environment. Until fairly recently most environmental research on trace metals was based on an assessment of total metal concentrations. However it has become increasingly evident that the environmental impact of particular metal species may be more important in such studies. The molecular characterisation, or speciation, of a variety of organometallic substances in environmental media have thus received increasing attention (1-2), particularly in view of demonstrations of their biogenic formation (2-4) and widespread commercial use (1,5,6).

Today research studies extend beyond elucidating the formation, pathways, toxicity and fate of organometallics in the environment to incorporate many other fields including clinical, industrial and forensic applications. With this growing awareness however, new techniques have had to be developed which could not only distinguish between the various species present, but also detect them at very low levels (tyically pg or ng per millilitre), in highly polar solvents, such as biotic fluids or natural waters. The problem for the analyst is further complicated by the striking range of chemical types that exist, including comparatively labile neutral molecules such as trimethylarsine, Me_3As and in contrast, involatile solvated species such as $Al(OH)_4^-$ (7). In addition, the various species present in a

sample are not necessarily in equilibrium with each other, and even if they are, any procedure applied to the sample may disturb the equilibria and hence the speciation, although if the equilibrium changes are slow and the separation rapid, the disturbance may be minimal. The act of filtering samples may also affect the equilibria by changing the concentrations of dissolved O_2 and CO_2 and by removing adsorbents in the form of particulate matter. Recognition of the great importance of determining individual species has however led to the development of various techniques for the direct analysis of such metal containing compounds which overcome many of the above problems, and have successfully provided the methodology for many trace metal speciation studies.

1.2 Bioaccumulation, toxicology and transport mechanisms

Although there is now evidence that organometallic compounds are formed in the environment, for example mercury methylation (8), some organometallic products are applied directly to the environment as biocides, in anti-fouling paints or in petrol. Others reach the environment indirectly such as leaching from organotin-based PVC stabilizers. The necessity of considering the direct toxicity of these compounds in fairly obvious, although the toxicity of possible metabolites at points other than that of initial application must also be considered, since often organic derivatives are of greater toxicity than their parent inorganic metals or ions. The need for speciation studies which help elucidate our understanding of cycling and transport mechanisms in the environment are also widely recognised in studies of toxicity of metals on aquatic organisms. The continuous formation of certain organometallic compounds, via environmental methylation (biomethylation), even at low levels, may result in food

chain effects leading to much higher concentrations in food used by man. Probably the best known incident of this nature occurred at Minamata Bay in Japan. Originally inorganic mercury was released in effluent from a chemical factory using mercuric sulphate catalysts in acetaldehyde production. However after assimilation by fish and shellfish the comparatively non-toxic inorganic mercury was converted to methyl mercury. As a result of this 115 people were killed and many more left paralysed for life.

Although many elements are essential to life, often there exists a fairly narrow "concentration window" between the essential and toxic levels (9). Variation in the speciation of trace metals however can dramatically affect their bioavailability or toxicity. Chromium clearly demonstrates this effect - Chromium (III) being essential for life, whereas chromium (VI) is highly toxic. Most of the usable chromium is reported to be provided by the group of chromium amino acid complexes, sometimes known as the glucose tolerance factor (9, 10), and the only assimilable form of cobalt is cobalamin (Vitamin B_{12}). Other elements showing this toxicological effect are arsenic, arsenic (III) being much more toxic than As (V) or its methyl derivatives, and in contrast the alkyl compounds of mercury and lead which are more toxic than the inorganic forms and especially dangerous due to their lipid solubility (11).

The toxicity of a particular dissolved metal species towards aquatic organisms is probably related to its ability to react with a biological membrane (12). The ability of a metal ion to cross the membrane and react with cell components depends on the direct lipid-solubility of the metal species (usually only unchanged organometallic

species), or the extent and rate of reaction of the metal ion with a membrane transport. Metal-protein interactions leading to carrier-mediated transport of the metal across the membrane, will, for bivalent ions, be thermodynamically favoured when the metal is in the simplest chemical form e.g $Cu(H_2O)_4^{2+}$, $CuCl^+$ or $Cu(OH)^+$ (12). For tervalent and trivalent ions such as Fe (III), however, the most bioavailable form may be an organic complex, because hydrolysis and polymerization could render the free ion inactive (13). In some cases, kinetics rather than thermodynamics may dictate the biologically-active chemical species. The toxic form of aluminium appears to be $Al(OH)_2^+$, which has been shown to be the kinetically-favoured species in the reaction between aluminium (III) and a hydroxyazo compound (14). In general, the reaction of metal ions with biological membranes is a particularly complex process and cannot be explained by simple diffusion models (15).

Most fresh waters have a pH which is in the critical range for the adsorption of heavy metals onto particles, a change of as little as 1 pH unit can cause the difference between complete adsorption or desorption (16). There is little doubt that for many heavy metals a major fraction of the dissolved metal in fresh waters exists as species adsorbed on colloidal humic acid and colloidal particles of iron oxide coated with humic acid (17). Even at low pH it has been shown that for river water containing little dissolved organic matter, over 50% of copper present was associated with colloidal organic matter (18). In fresh water with a high organic content, molecular complexes of heavy metals with fulvic and tannic acids may also be formed (19). In the fresh water/sea water mixing zone of estuaries, the precipitation of high molecular-weight humic substances and

hydrous oxides of iron and manganese result in the transfer of much of the dissolved heavy metals from the river water to the estuarine sediment (20,21). The river water is thus effectively a scavenger of trace heavy metals. A fraction of the most stable precipitated forms will stay in solution as colloidal particles and be transported to the oceans, along with their load of adsorbed heavy metals. The most stable colloids are likely to be iron and manganese oxides and clay particles, coated with humic materials. These colloids will then carry an important part of the measured concentration of dissolved copper, lead, cadmium and zinc in seawater (22). Most computer models of speciation in sea water have used silica as a model adsorbent (23). Silica is not however nearly as powerful an adsorbent as a particle coated with humic acid, nor does it have the same adsorption capacity (24). The formation of simple, molecular organic complexes of bivalent heavy metals in sea water is unlikely because of competition by chloride, magnesium and iron (III).

1.3 Analytical approaches to metal speciation

Although the importance of the determination of the chemical speciation of trace elements has been recognised, advances have been slow due to the lack of suitable speciation techniques. Various chemical methods have been used including sequential extraction techniques, using differing strengths of acids and oxidising or reducing agents to leach the various forms of the element into solution. Ion-exchange and solvent extraction have also been employed principally to separate organically bound forms from aqueous solution. More recently various biochemical systems have also been reported, for example enzyme solubilization which exploits the selective nature of biological systems.

In many early attempts at metal speciation however two analytical techniques predominated: anodic stripping voltame try (ASV) and ultrafiltration. The first technique divides the metal species into two categories: electroactive (aquo ions and "labile" complexes) and electroinactive (organic complexes and colloidal species). Ultrafiltration and dialysis techniques divide the metal species into different sized fractions, where the species that pass through the smallest pore size are generally taken to be free metal ions or small complexes (25).

The most popular of these two techniques is ASV, where proponents claim that the method can be used for speciation studies since some forms (e.g. certain organic complexes) are not reducible at the mercury drop whereas other forms are. By changing solution conditions and measuring changes in peak potential and peak current, one should (theoretically) be able to make inferences about metal speciation - in a manner similar to the way one obtains information on complex formation from conventional polarography, except at much lower concentrations. Brezonik (26) gives an example of this where complexation of metals with weak organic acids such as EDTA and amino acids is pH dependent, and the strength of complexation increases with pH. Since most known organic complex formers in natural waters are weak acid salts, the inference is generally made that metal-organic interactions occur <u>in situ</u> under neutral or alkaline conditions but under acidic pH (pH < 3) conditions the metals exist as the free hydrated species. Most metal-organic complexes are thought to be non-reducible at the mercury electrode and hence not measureable by ASV. The difference in peak current under ambient (neutral) conditions and under acidic (pH <3) conditions has then been interpreted (27) as

representative of the amount of metal ion complexed by organic species or more generally as representative of the metal present in "non-labile" complexes of some undetermined nature (27).

There are however at least two circumstances under which the above theory would be invalid. Firstly in the presence of an organic substance that forms metal complexes more strongly under acidic conditions than at neutral or alkaline pH (e.g. chelating acid salts such as amino acids), or forms complexes independent of pH (at least within a broad pH range). Secondly in the presence of surface active substances in solution that coat the mercury electrode and prevent metal ion deposition or metal oxidation. If sorption effects are pH dependent, which seems likely for surface active substances occuring in natural waters, interpretation of ASV results in terms of metal speciation will be in error.

Recently a new voltammetric technique cathodic stripping voltammetry (CSV) has been reported (28) based on the apparent adsorptive behaviour of metal complexes with certain organic complexing ligands. The degree of adsorption is directly related to the dissolved metal concentration and is measured by reduction of the metal complex adsorbed on to the HMDE. The sensitivity of this technique is reported to be superior to that of ASV as the metal is collected in a monomolecular layer on the electrode. A reduction efficiency of 100% is therefore obtained, and the detection limits claimed are typically of the order of 10^{-10} M after two minutes collection. A further advantage of this method for speciation studies is that elements that have a reduction potential within the stability constraints of water can be determined voltammetrically, without the necessity (for ASV) of

reduction to the metallic state. The method has been called CSV because of the cathodic direction of the current, but it has also been called adsorption voltammetry (29).

One of the main problems with techniques such as ASV, solvent extraction, ultrafiltration and dialysis is that they will cause some metal to dissociate from metal complexes or colloidal particles as a result of the electrical potential across the electrode-solution interface in ASV (30) or the concentration gradient across the membrane-solution interface in ultrafiltration and dialysis (31). Under these circumstances, it is pointless to specify that these techniques should apply only at the natural pH of the sample, since the original solution equlibria will be disturbed by the procedure, whatever the analytical pH (32). With CSV this restraint would not apply since collection at the electrode is the result of adsorption of a negligibly small fraction of the surface active metal-organic complexes, at a potential more positive than the reduction potential of the complex. Thus no dissociation of the natural organic complexes can take place during the measurement. However this technique of ligand competition has only been applied to copper and zinc at the present time.

Clearly a more promising analytical approach to speciation studies which overcomes the above problems and which is equally suitable for ionic and molecular species, including organo-metallics incorporated into large biomolecules, is the direct coupling of the capabilities of chromatography, principally gas chromatography (GC) and high performance liquid chromatography (HPLC) for species separation, to the selectivity and sensitivity of atomic spectroscopy for detection -

so called coupled or hybrid techniques.

1.4 The advantage of coupled systems

The essential simplicity of atomic-absorption spectroscopy (AAS) has led to its adoption as the technique of choice for environmental trace-metal monitoring. However, unless preceeded by time consuming sample pre-treatment atomic-spectrochemical techniques do not yield any information as to the species in which the metals are bound. Chromatography, particularly gas chromatography (GC) and high-performance liquid chromatography (HPLC) on the other hand, offer excellent separation of different species, but often the identification of the important organometallic moieties is difficult to achieve unambiguously when working with complex samples. Hence, recently there has been a growth in hybrid chromatographic-atomic spectroscopic instrumentation for trace metal speciation. The use of such specific detection allows less than optimum chromatographic separation to be tolerated with consequent saving in time for sample clean-up and analysis. If two species co-elute and only one contains the metal of interest, the use of metal-specific detection means that only the metal containing species is detected. Thus complete chromatographic separation is not required, only separation of the species containing the metal of interest being necessary.

The choice of which analytical technique to couple is influenced by a consideration of likely sample types, simplicity of coupling and other practical considerations such as economy of operation. Several successful coupled GC-FAAS (33) and coupled inductively coupled plasma (ICP) AES (34) systems have been developed and applied to a variety of practical analytical problems. However, although coupled GC-atomic

spectroscopy has the advantage that the sample supplied to the detector is in a gaseous form, allowing direct delivery to a flame, or plasma, thus avoiding the inefficiency associated with nebulisation, gas chromatography is limited to volatile species whilst the majority of interesting trace metal speciation problems concern involatile species.

Liquid chromatography is the separation technique of choice for a much wider range of species. The aim of this work was therefore to develop sensitive methodologies and instrumental coupling to allow trace metal speciation in a range of samples by directly coupled high-performance liquid chromatography-analytical atomic spectroscopy. Emphasis has been placed upon the use of a continuous detector, which parallels the work done with GC-AAS (34) in which appropriate optimisation of a flame cell led to a continuous detector with superior powers of detection to electrothermal atomisers. The practical problems associated with earlier systems which provided an interim solution in the absence of a sensitive conventional flame AAS coupling, by collecting the HPLC effluent in an auto-sampler, and then making discrete injections of the sample into an electrothermal atomiser, have been overcome.

CHAPTER 2

COUPLED GAS CHROMATOGRAPHY-ATOMIC SPECTROSCOPY

2.1 Separation by gas chromatography

Gas chromatography is basically a separation technique for volatile materials, which achieves separation by utilising the differences in partition coefficient of the materials to be separated, the partition being between gas and liquid or gas and solid. If the stationary phase is a solid, we speak of gas-solid chromatography (GSC), and if the stationary phase is a liquid, gas-liquid chromatography, (GLC). In the latter of these two techniques the liquid is spread as a thin film over an inert solid and the basis for the separation is the partitioning of the sample in and out of this liquid film. The wide range of liquid phases with usable temperatures up to approximately 400 ^{o}C make GLC the most versatile and selective form of gas chromatography, and consequently the form used in this work.

The theory of separation by gas chromatography has been extensively reviewed in a number of publications (35 - 37). However, a brief overview of the main principles involved is given below.

The resolution of chromatographic peaks is related to two factors: column efficiency, and solvent efficiency. Column efficiency is concerned with peak broadening of an initially compact band as it passes through the column. It can be measured by the number of theoretical plates, N, which may easily be measured from a chromatogram. N is given by 16 $(x/y)^2$, where "y" is the length of the baseline cut by the two tangents, and "x" is the distance from

injection to peak maximum (including the dead-volume).

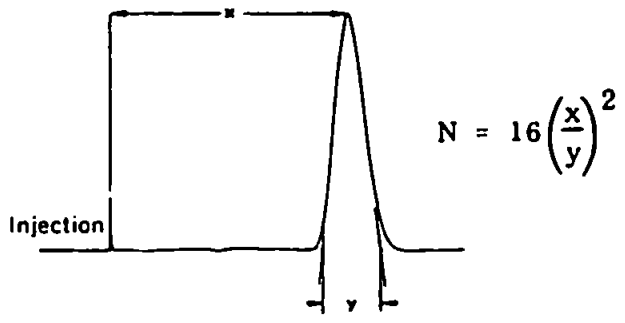


Figure 1 Calculation of theoretical plates

Many factors affect column efficiency and most of these have been evaluated by their effect on N, or the height equivalent to a theoretical plate, HETP. This is related to N by:

$$HEPT = L/N$$

where L is the length of the chromatographic column, usually in centimetres. HEPT calculation allows comparisons between columns of different lengths and is the preferred measure of column efficiency.

The Rate Theory of Van Deemter (38) and its extension by Glueckauf(39) and other workers help account for the slope of elution curves from chromatographic columns. Three principle contributions to band broadening are: (a) the multipath effect or eddy diffusion, (b) molecular diffusion, and (c) resistance to mass transfer. From these a basic equation can be derived (38) for the height equivalent to a theoretical plate in a gas-liquid column:

$$HEPT = A + B/u + C \cdot u$$

where A, B and C are the terms given above, and u the linear gas velocity (or flow rate) through the chromatographic column.

Solvent efficiency, or relative retention (α) results from the solute-solvent interaction and determines the relative position of solute

bands on a chromatogram. It is expressed as the ratio of peak maxima (adjusted retention times), and determined by the respective distribution coefficients of the solutes in the solvent at a given temperature.

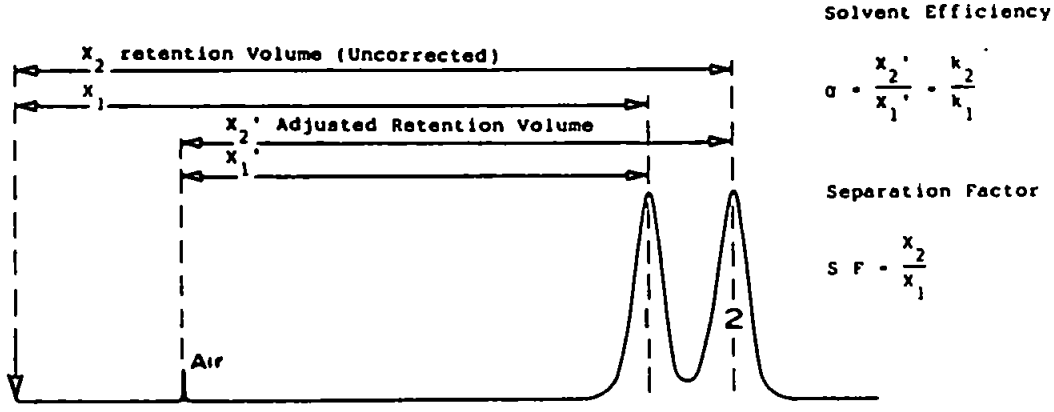


Figure 2 Calculation of solvent efficiency

There are four interaction forces which can aid in the GC separation: (i) orientation or Keesom forces, (ii) induced dipole, or Debye forces, (iii) dispersion, London or non-polar forces, and (iv) specific interaction forces resulting from chemical bonding, and complex formation between solute and solvents (40). These forces of interaction determine the solubility and thereby the separation achieved. Their combined effects are expressed by the partition coefficient k, where

$$k = \frac{\text{amount of solute per unit volume of liquid phase}}{\text{amount of solute unit volume of gas phase}}$$

The value of k is high when most of a substance is retained in the liquid phase. This means that the substance moves slowly down the column because only a small fraction will be in the carrier gas at any given time. Transport is negligible in the liquid phase, and only that fraction in the gas phase is carried through the column. Thus,

separation between two compounds is possible, if their partition coefficients are dissimilar. The greater the difference in their k values, the fewer the plates or the shorter the column length that is required to achieve a separation.

2.2 Available detectors for metallic species

For the determination of metal species by GC, the ideal detector should be sensitive, and may also benefit if it is specific for the analyte metal. In general, electron capture, flame ionization and thermal conductivity detectors do not offer the required sensitivity and selectivity for metal speciation applications. The combination of GC and mass spectrometry provides high sensitivity and selectivity but the systems are complex, high in cost and require highly skilled operators. Flame photometric detectors are specific only for several elements and provide rather poor sensitivity. Emission detectors based on microwave excitation have been described (41) and are commercially available. Overall, the requirement for specificity and sensitivity are well met by the use of atomic spectroscopy for selective GC detection.

2.2.1 Choice of spectroscopic technique as detector

There are a number of advantages in using atomic spectroscopy in conjunction with gas chromatography. These include the ability to speciate various metals, the ability to withstand less than optimal GC conditions, i.e. only the species containing the metal of interest need be separated, and importantly, increased sensitivity to metals compared with conventional GC detectors. Each of the atomic spectrosocpic detectors available has its own advantages and disadvantages, although all have the attributes listed above.

Flame atomic absorption sepctroscopy (FAAS) has the advantage of simplicity and well understood mode of operation. The instrumentation is relatively inexpensive and readily available in most laboratories. Flame atomic absorption is also noted for its excellent selectivity. Absorption techniques do however suffer from short linear ranges, normally 1 - 2 orders of magnitude. The detection limits reported for flame systems are also inferior to those achieved using electrothermal atomisation (ETA-AAS) although such instrumentation is more expensive, and is not designed to take a continuous sample stream. Atomic fluorescence spectroscopic detectors offer an improvement in detection limits and an extended working range compared to AAS, although the detectors are not so readily available.

The alternative to the above techniques is the use of plasma emission. These techniques have extensive linear working ranges, although do not give such low detection limits particularly when compared to ETA-AAS. Although the excitation cells, being comprised of flowing gas streams, are well suited for interfacing with a GC, such instrumentation is expensive both in capital outlay and running costs.

From the above considerations, it was decided to centre most development around interfaces for FAAS, both for GC couplings and HPLC couplings in later chapters. However, applications of all the various atomic spectroscopic detectors are outlined in Section 2.3 and particular detail to the theory and applications of coupled ETA-AAS systems given in Chapter 4.

2.3 Review of existing directly coupled gas chromatography-atomic spectroscopy techniques, and their applications

There are many applications in the literature for the various coupled gas chromatography - atomic spectroscopy techniques outlined above. The review below details many of these with particular emphasis to the design of the interface used in each case.

2.3.1 Coupled gas chromatography - microwave induced plasma

The microwave induced plasma, MIP, has two basic characteristics which may be utilised when coupling to GC. The low gas temperature of the MIP allows small amounts of sample, compatible with that of gas chromatograph solutes, to be introduced without extinguishing the plasma. In addition, sample introduction is easily facilitated since the carrier gas and plasma gas are the same. These advantages have made coupled GC-MIP a popular technique and many applications have been reported - Table 1.

The first use of the MIP as an element selective detector for organic compounds was reported by McCormack et al. in 1965 (41). The effluent from a gas chromatograph was connected directly to the silica tube containing the plasma discharge. Both the more sensitive tapered cavity and coaxial cavity for larger samples were used. Two plasma types were utilised: low pressure helium and atmospheric argon, the latter being favoured due to the complexity of the associated vacuum systems required with low pressure helium plasmas. Later Buche and Lisk used the atmospheric argon plasma to determine pesticides in various samples by selective detection of phosphorus (42) and iodine (43). Using a low pressure argon plasma the same authors lowered the detection limit by a further order of magnitude (44). The more

energetic reduced pressure helium plasma has been used for the determination of halogens, phosphorus and sulphur using atomic lines (45,47). Moye (46) found that using a tapered rectangular cavity with a mixed argon/helium carrier, a lower background emission for chlorine, iodine and phosphorus detection in pesticide residues could be obtained. Dagnall et al. (48,49) used a quarter wave radial cavity with low pressure argon or helium plasmas for the determination of sulphur in various compounds. It was found that the most sensitive and specific emission wavelength was not the same for all the compounds examined. In addition thioglycolic acid was found to be very difficult to fragment (48) although a platinum wire in the base of the detector was found to catalyse the fragmentation process (49). Bache and Lisk (53) were the first to use the low pressure helium plasma for detection of organomercury compounds after extracting the compounds from salmon using the established procedures of Westöö (51,52). A potential use of the MIP detector for obtaining inter-element ratio has been reported by Dagnall et al. (55), using two monochromators, one set at a carbon line and the other set to monitor a heteroatom. Other workers (61,77,83) have also used the MIP detector to determine inter-element ratios in an attempt to estimate empirical formulae. The commercially available MPD850 (Applied Chromatography Systems) low pressure helium plasma system has also been used in this role (76,77). However Dingjan and De Jong (100) found it was necessary to use a reference compound if accurate ratio formulae were to be obtained. An oscillating slit mechanism for the determination of hydrogen isotope ratios has been used by Schwarz et al. (82), but the poor signal to noise ratios obtained gave poor precisions.

The passage of organic compounds through the plasma however may result in the formation of carbon deposits on the walls of the quartz capillary, absorbing part of the radiation and increasing background emission (41). This can be prevented by either initiating the plasma after the solvent has passed through the detector (42), or by adding traces of a scavenging gas. The gas may be either nitrogen (61), oxygen (61,62) or air (41) added to the plasma gas; however as a result the spectral background is considerably increased.

The MIP has proved popular as a detector for various metal chelates (58,60,62,69), and also as a detector for various hydride forming elements (68,75,87,88). Talami and Bostick (68) determined alkylarsenical acid compounds in pesticides by generating their hydrides prior to GC-MIP analysis. The separation and sequential detection of As, Ge, Se, Sn and Sb hydrides has also been demonstrated using a mixed argon/helium plasma (78,87,88). Little difference in detection levels have been found using various cavities for microwave plasmas by Mulligan et al. (85), although the Beenakker TM_{010} cavity was found to be the easiest to operate. This method was used to determine the above hydride forming elements in whole blood and enriched flour (87) and NBS orchard leaves (87,88).

Coupled GC-MIP has also been used for the detection of various metals in volatile organometallic compounds. Lead has been determined as the tetraalkyl species (79,80), in petrol (104,106), in the atmosphere (80), and as trialkylleadchloride in water samples (101). Mercury as the diphenyl (79), dimethyl and diethyl derivatives (98) have been detected using the TM_{010}, cavity. Quimby et al. (79) used the same cavity to determine manganese as the methylcyclopentadienyltricarbonyl

derivative in petrol and as a silicon specific detector for tetravinylsilane. The coupling of capillary columns with the TM_{010} cavity has also been demonstrated with great success for metal specific detection of volatile organometallics (90,101,104). In a study of the pyrolysis of carborane silicone polymers (102) the group at Amherst found that doping of the plasma gas with hydrogen inhibited oxide or silicate formation by promoting borohydride formation, which increased the populations of atomic boron rather than ionic states. Hanie et al. (94) have also used capillary columns for the determination of halides in pesticides using a helium plasma and a surfatron cavity (93).

Recent developments of coupled GC-MIP systems have largely been based on the development of software for both systems control and data handling. One such system described by Eckhoff et al. (107) uses a polychromator/microcomputer system to simultaneously monitor four atomic emission wavelengths throughout an entire chromatographic run. The same system has also been used by Hass and Caruso (114) as an element specific detector for the gas chromatography of halogenated compounds. Delaney and Warren (109) have used a minicomputer to modify the interface described by Estes et al. (101), so that in addition to controlling the switching valves it also controls the monochromator wavelength setting and acquires the analytical data the MFD and FID monitor.

Finally a number of authors (107,109,114) have postulated the use of GC-MIP for determining interelement ratios (and possibly empirical formulas) by measuring the systems response to several elements. The need for careful control over the experimental conditions has been

stressed since it is necessary to quantify the response obtained for each element individually and to ensure the response is independent of chemical form. The imprecision between injections is also cited as a major problem in determining interelement ratios. However it has been suggested (109) that the use of capillary gas chromatographic columns, computerised data acquisition and peak area measurements may improve the precision and accuracy attained. A recent publication by Hass and Caruso (114) has now reported the determination of C/Cl ratios with <1% error in their study of dioxins and other halogenated compounds.

The above and other work in coupled GC-MIP systems are summarized in Table 1.

Table 1 Coupled Gas Chromatography - Microwave Induced Plasma Optical Emission Spectroscopy

Detector	Chromatography	Matrix	Comments	Element (Wavelength/ nm)	Reference
Tapered and co-axial cavities used, the former more sensitive, the latter accepted larger samples. 10 mm. i.d. discharge tube at low pressure.		Solutions of simple and heteroatom containing organic compounds.	At low pressure He was the preferred carrier gas. At atmos. pressure Ar was used since it gave a stable discharge. Dynamic range 4 orders of magnitude. Detection limits ranged between 2×10^{-16} to 2×10^{-7} g/sec	C 388.3 nm F 516.6 nm 251.6 nm Cl 278.8 nm Br 298.5 nm I 206.2 nm S 257.5 nm	41
Atmos. pressure Ar 1 mm i.d. quartz discharge tube in a tapered cavity	2' glass 'U' column, 5 mm i.d. 5% SE 30 on 80/100 Chromosorb W T_c = 160-200 Ar = 20-115 ml/min used.	Organophosphorus insecticide residues in pure form, agricultural + food samples.	Diazinon Dimethoate, Ethion Parathion and Ronnel determined. Detection limits ranged between 1.4 to 9.2 pg s^{-1}.	P 253.565 nm	42
As in ref. 42	See ref. 42	Iodinated herbicide residues and metabolites in wheat, oats and soil.	Detection of Ionynil and metabolites Recoveries from 66-108% achieved. Detection limit 4×10^{-10}g I_2 s^{-1}	I 206.2 nm I_2 band	43
As in ref. 42 except reduced pressure Ar plasma.	See ref. 42	Diazinon in grapes; see ref. 42	See ref. 42 achieved increased sensitivity with low pressure discharge. Detection limit 6×10^{-13} g s^{-1} of P	P 253.565 nm	44

Detector	Chromatography	Matrix	Comments	Element	Reference
Reduced pressure helium plasma using tapered cavity. 5-10 mm. Hg pressure.	6' glass column 10% DC-200 on 80/100 mesh Gas Chrom Q, Isothermal at various T_C = 130°C to 210°C.	Organic compounds and pesticides	Detection limits ranged between 9×10^{-12} to 6×10^{-11} g.s^{-1}	Br 478.55 nm Cl 479.45 nm I 533.82 nm P 253.57 nm S 545.38 nm	45
Ar/He (15 + 85) mixed plasma, tapered cavity as longer life-times and less background emission obtained.	4' x 1/8" i.d. glass, 5% SE 30 on Gas Chrom. Q T_C = 180°C, T_I = 215°C Flow rate = 27 ml min^{-1}.	Pesticide residues of various P, Cl and I containing compounds.	Detection limits ranged between 0.07 ng to 11.5 ng	P 253.57 nm Cl 221.00 nm I 206.20 nm	46
Reduced pressure helium plasma	6' x 1/8" i.d. glass, 10% DC-200 on 100/120 mesh Gas Chrom. Q.	Phenol substituted insecticides in agricultural samples.	Monitored atomic S and Cl lines.	Cl 479.45 nm S 545.38 nm	47
1/4 λ radial line cavity, Ar or He low press. (13-40 mbar) plasma.	2.7 m x 6.5 mm. i.d. Cu tubing packed with dinonyl phthalate 1.0 µl injections.	S compounds CS_2 thiophene, thioglycolic acid, DMSO, SO_2.	Thioglycollic and difficult to fragment. Detection limit 0.2 ng for CS_2 at C = S bandhead.	S S - 190.0 nm S - 191.5 nm C=S - 257.6 nm C_2 - 516 nm common to all compounds	48
See ref. 48	0.6 mm x 6 mm i.d. Cu tubing packed with either Porapack P or Q.	S compounds CS_2, thiophene, dimethyl-sulphide and thioglycolic acid.	Used Pt wire in base of detector to catalyse fragmentation process. Detection limit low ng range.	S C Monitored C=S bandhead at 257.6 nm and atomic C line at 247.9 nm.	49

Detector	Chromatography	Matrix	Comments	Element	Reference
Low pressure (5-10 T) He plasma. See ref. 44.	See ref. 44.	S, halogen and P containing pesticide residues in a wide range of food products.		Br 478.55 nm Cl 479.45 nm I 533.82 nm P 253.57 nm S 545.38 nm	50
Reduced pressure He plasma in a tapered cavity cf. ref. 45.	2' x 5/32" i.d. glass column, 60/80 mesh Chromosorb 101 T_C = 100°C T_I = 140°C He = 80 cm^3/min. 6' x 5/32" i.d. glass column, 20% OV17 and OV1 (1+1 w/w) on 80/100 mesh, Gas Chrom Q T_I = 208°C, T_C = 152°C.	Me_2Hg Methylmercury dicyandiamide, Phenylmercuric acetate, methylmercury dithizonate, MeHgCl in salmon.	Westöö extraction procedure, (51, 52) for MeHgCl in salmon. Linear range: 0.1-100 ng for MgHgCl.	Hg 253.7 nm	53
Atmospheric Ar plasma, 20 cm x 2 mm i.d. quartz tube surrounded by 3/4 λ cavity.	30 and 70 cm x 6 mm i.d. packed with Porapak S.	Range of C, O, N and halogen containing compounds.	Several cavities examined, 3/4 λ preferred because it produced a long (ca. 8 cm) stable discharge with little local overheating. Detection limits 10 - 20 pg.s^{-1}.	C Monitored, atomic C line at 247.9 nm, C_2 bandhead at 516.5 nm and C_2/CN bandhead at 385-9 nm.	54

Detector	Chromatography	Matrix	Comments	Element	Reference
See ref. 54	0.7 m x 1/8" stainless steel, Chromosorb 101.	Range of C, S, and halogen containing compounds.	Use of 2 monochromators - 1 set to atomic C line, other set to hetero-atom line. By monitoring emission from both obtain inter-element ratios. Detection limits ranged between .04 ng s^{-1} to 4.5 ng s^{-1}.	C 247.9 nm I 206.2 nm S 182.0 nm P 253.5 nm Cl 259 nm band Br 292 nm band	55
Ar plasma. Essentially the same as Ref. 41.	10' x 1/4" i.d. stainless steel column, 20% Carbowax 20 M on Chromosorb P 60/80 mesh. Ar = 48 cm^3 min^{-1} T_c = 75°C	Me_2Hg	Found selectivity for Hg over various organic compounds, always > 10^3. Detection limit 0.3 ng	Hg 253.7 nm	56
Low pressure He plasma using MPD 850 system, O_2 and N_2 used as scavengers to prevent C build up.		Various organic compounds.	Detection limits range between 0.03 ng s^{-1} to 3.0 ng s^{-1}.	C 247.8 nm H 486.1 nm D 656.2 nm O 777.2 nm N 746.9 nm F 685.6 nm Cl 479.4 nm Br 470.5 nm I 516.1 nm S 545.4 nm	57

Detector	Chromatography	Matrix	Comments	Element	Reference
Similar system to Ref. 54, except 1/4 λ Evenson cavity 70W forward power Ar plasma, ignited after elution of solvent.	2 columns, both 0.6 m x 4.8 mm i.d., 1. Universal B coated with 10% Apiezon L. 2. 0.5% Apiezon L on glass beads (0.2 mm diam.). Both conditioned for 36 hrs at 200°C.	acac and tfa chelates of Al, Cr, Cu, Ga, Fe, Sc, V.	MIP responded both non-specifically to C or specifically to the metal of interest. Detection limits in the range 2 x 10^{-12} to 2 x 10^{-11} g s^{-1}.	Al 396.2 nm Cr 357.9 nm Cu 324.7 nm Ga 294.4 n Fe 344.1 nm Sc 361.4 nm V 318.4 nm	58
1/4 λ Evenson cavity used, reduced pressure (10 T). He plasma generated in a 6 cm x 8 mm i.d. quartz tube.	1 cm or 2 cm x 1 mm i.d. Cu tubing packed with either Poropak Q or 5A molecular sieve T_C = 125°C 50 µl injections	CO, CO_2, SO_2, N_2 in air.	Gas mixtures were prepared by injecting known amounts of pure gas into an air filled flask fitted with a septum. Detection limits ranged from 20 ppm to 50 ppm	C 247.9 nm N 337.1 nm (N_2 bandhead) S 190.0 nm	59
Ar plasma generated in a quartz capilllary 1.6 mm i.d. x 25 cm placed in a tapered rectangular type cavity.	Stainless steel tubing 72 cm x 4 mm i.d., 0.5% SE-30 on glass beads 60/80 mesh. T_C = 160°C T_I = 200-210°C Ar = 150 cm^3 min^{-1}.	Metal acac chelates dissolved in chloroform.	A CN band was observed for all complexes probably due to N_2 impurity in the Ar. Failed to chromatograph acac chelates of Cu (II), Fe (III) and V(IV). 2 orders of magnitude for Be and Cr. 1 order for Al. Detection limits ranged between 0.01 ng and 100 ng.	Al 396.2 nm Be 234.9 nm Cr 425.4 nm	60

Detector	Chromatography	Matrix	Comments	Element	Reference
Reduced pressure He plasma, 0.1-1%. O_2 or N_2 added as scavenger.	3 m x 2.5 mm i.d., 10% Apiezon L on 60/80 mesh DCMS treated Chromosorb W. Effluent split 1:1 to FID and MIP.	Wide range of organic solutions.	Multi-nonmetallic element detection used to calculate empirical formula of organic compounds. Linear range: 4 orders of magnitude for F. Detection limits in the range 0.03 ng s^{-1} to 3.0 ng s^{-1}.	C 247.8 nm H 486.1 nm D 656.2 nm F 685.6 nm Cl 479.4 nm Br 470.5 nm I 516.1 nm S 545.4 nm N 746.9 nm O 777.2 nm	61
Reduced pressure He plasma doped with 1% O_2, 1/4 λ Evenson cavity.	'U' tube columns packed with Chromosorb W-HP with 3% OV-101 loading.	Chelates $Cr(tfa)_3$, $Cr(acac)_3$, $Cr(hfa)_3$.	Use of MPD as a specific detector for Cr; and as a non-specific detector, by monitoring the atomic C line. Detection limits ranged between 1.5×10^{-11} to 8.0×10^{-10} gs^{-1} of Cr.	Cr 357.87 nm	62
Tapered cavity system essentially the same as Ref. 41. Ar plasma, 35W forward power	4' x 0.5 mm i.d. glass column packed with 4% SE-30 on 30/60 mesh Chromosorb GHP.	Se cpds in environmental samples, looked at various NBS materials, with good agreement.	Se(IV) complexed with PD to form the volatile piaselenol complex followed by toluene extraction. Detection limit 40 pg Se 0.1 μgl^{-1} for water samples and 15 ppb for solid samples.	Se 204. nm	63

Detector	Chromatography	Matrix	Comments	Element	Reference
Ar plasma (see Ref. 23) atmospheric pressure.	3' column, 4% FFAP on 80/100 mesh Gas Chrom Q. Ar = 90 cm^3 min^{-1}. T_C = 150°C T_I = 200°C.	MeHgX in benzene extracts of biological samples, and air.	X designates Cl, Br, I or OH since all eluted simultaneously; see 64, 65 for explanation of this. Detection limits range between 0.5 pg to 1 ng g^{-1}.	Hg 253.7 nm	
	3' column, 1% FFAP on 80/100 mesh carbon beads. Ar = 95 cm^3 min^{-1} T_C = 135°C T_I = 200°C	CH_3HgX in water and air.			
Reduced pressure 1 - 10 T, He plasma.	2' column, Chromosorb 101 He = 80 cm^3 min^{-1} T_C = 115°C T_I = 135°C.	$(CH_3)_2Hg$ in water and air.			66
Atmospheric pressure Ar plasma, 30W forward power, see 63.	3' column, 4% FFAP on 80/100 mesh. Gas Chrom Q. Ar = 110-120 cm^3 min^{-1}. T_C = 220-240°C T_I = 245-260°C.	As and Sb in environmental samples.	As(III) and Sb(III) converted to Ph_3AsH and Ph_3 SbH, extracted into ether, separated by GC. Detection limits: 20 pg As, 50 pg Sb.	As 228.8 nm Sb 259.8 nm	67
See 63.	6' column, 5% Carbowax 20 M on 80/100 mesh Chromosorb 101 T_C = 175°C T_I = 180°C Ar = 100 cm^3 min^{-1}.	Alkylkarsonic acids in pesticide and environmental samples, MMA and DMA.	As cpds converted to hydrides. Detailed study of hydride generation and trappings of the evolved arsines. Linear range 0.01 - 20 ppm. Detection limits 20 pg as As in water samples.	As 228.8 nm	68

Detector	Chromatography	Matrix	Comments	Element	Reference
1/4 λ Evenson cavity. Atmospheric pressure. Ar plasma, 70W forward power.	0.9 m teflon column 3 mm i.d. 10% SE30 on 70/80 mesh Gas Chrom Z. T_C = 180-190 T_I = 200 Ar = 30-150 ml min^{-1}.	Human blood serum.	Low temp. ashing followed by chelation with H(tfa) to form $Cr(tfa)_3$ which is extracted into benzene. Linear range 1 - 10 pg Cr. Detection limit 9 x 10^{-13} g.	Cr 357.9 nm	69
Low pressure (150 mbar) He plasma cavity type 214L. Interelement selectivity improved by use of wavelength modulation.		Organic compounds Hg(Me)Cl.	Demonstrated that at low pressures fragmentation occurs via collisions with atomic He whereas at high pressures the collisions are with He_2. Linear range 0.02-0.5 ng. Detection limit 5 x 10^{-14} g.	Hg 253.65 nm	70
Atmospheric pressure Ar plasma in quartz capillary 1.6 mm i.d. x 25 cm. Tapered rectangular cavity, 50W forward power.	Column - 45 cm x 3 mm i.d. glass, 0.5% SE30 on 60/80 mesh glass beads. T_C = 140°C. T_I = 180°C. Ar = 80 cm^3 min^{-1}.	Trace levels of Cu and Al in Zn metal.	Cu and Al extracted as tfa chelates in CCl_4. Linear up to 60 ng Cu, 100 ng Al. Detection limits: 0.5 ng Al, 1 ng Cu.	Cu 324.8 nm Al 396.2 nm	71
See 63, 66 Ar plasma, 5-10T pres. 18W forward power.	3' x 5 mm. i.d. glass, 6% FFAP on 80/100 mesh. Gas Chrom Q. T_C = 180-190°C T_I = 200°C Ar = 130-150 cm^3 min^{-1}	MeHgCl in water samples.	MeHgCl extracted as quaternary amine adducts. Detection limit 1 - 2.5 ng l^{-1} for water samples.	Hg 253.7 nm	72

Detector	Chromatography	Matrix	Comments	Element	Reference
Atmospheric pressure, He plasma using TM_{010} cavity.	Used exponential diluter to demonstrate the applicability of MIP for GC detection.	Gas mixtures	Demonstrates advantages of atmospheric pressure He plasma and discusses excitation mechanism. 3-4 orders of magnitude linear ranges. Detection limits ranged between 2×10^{-11} to 2×10^{-9} mol l^{-1}	C 193.1 nm 247.9 nm H 486.1 nm Cl 479.5 nm 481.0 nm Br 470.5 nm 478.5 nm I 516.1 nm 206.2 nm S 545.4 nm	73
Low pressure (3- 5T), Ar plasma; see 63 and 66.	Glass column, 6' x 3.5 mm, 4% OV-101 on Chromosorb G (HP) 80/100 mesh. Ar = 80 ml min^{-1}.	Mixture of n-paraffins and TMS- derivatives of carboxylic acids.	Dual FID/MPD (5:1 split) to demonstrate specificity of response to TMS derivatives. Linear range 0.5-150 ng.	Si 251.6 nm	74
Low pressure (90T) He plasma, observation 8-9 mm downstream from centre of discharge, 75W forward power. 0.25% v/v O_2 as scavenger or 0.4% v/v N_2. 1/4 λ Evenson cavity model 214L and 1/4 λ coaxial cavity model 217L.	Constant sample introduction for optimisation studies.	Various organic compounds.	Optimized plasma conditions for: gas flow rates observation position, microwave power, and gas pressure with the 217L cavity up to 10% of power reflected, with 217L only 1% reflected. 3-4 decades except for H where a non-linear response is found. Detection limit 0.01 ng s^{-1} to 0.5 ng s^{-1}.	Br 470.47 nm C 247.86 nm Cl 479.45 nm F 685.6 nm H 486.13 nm I 516.12 nm N 746.88 nm O 777.19 nm S 545.39 nm	75

Detector	Chromatography	Matrix	Comments	Element	Reference
See ref. 57. Reduced pressure He plasma.		Various organic compounds.	Signals for four elements are monitored simultaneously added by a SYNC signal, stored for latter computer analysis, resulting in interelement ratios: main concern is in data acquisition and processing.	C 247.8 nm H 486.1 nm D 656.2 nm O 777.2 nm N 746.9 nm F 685.6 nm Cl 479.4 nm Br 470.5 nm I 516.1 nm S 545.4 nm	76
See ref. 57. Reduced pressure He plasma.	None given	Trace S in MeOH, yellow P in PCl_3 specific detection of vinylidene and PCB's.	Using MPD 850 to obtain accurate empirical formulae, obtain detection limits comparable to manufacturers' claims.	C 247.8 nm H 486.1 nm D 656.2 nm O 777.2 nm F 685.6 nm Cl 479.4 nm Br 470.5 nm I 516.1 nm P 253.6 nm S 545.4 nm	77
Mixed Ar/He plasma, 110W forward power OW reflected.	Polypenco nylaflow pressure tubing 4.7 mm i.d., 1', 3', and 6' lengths. Packed with Chromosorb 102 60/80 mesh.	Hydrides generated from solutions of As, Ge, Sb, Se and Sn.	Hydride trapped in liq. N_2 then chromatographed. Elements determined sequentially. Linear over 2 orders of magnitude.	Ge 303.9 nm As 193.7 nm Se 196.0 nm Sn 317.5 nm Sb 259.8 nm	78

Detector	Chromatography	Matrix	Comments	Element	Reference
Atmospheric He plasma, TM_{010} cavity, 75-80W, forward power, axial viewing	3' x 1/8", 5% OV 17 on 100/120 mesh Chromosorb 750 He = 70 cm^3 min^{-1}.	Diphenyl mercury.	A design for heating the interface between GC and plasma utilising nichrome resistance wire coupled to a variance given. Detection limits ranged between 0.49 pg s^{-1} to 63 pg s^{-1}.	Hg 253.7 nm	
	3' x 1/8", 3% OV-1 on 100/120 mesh. Varaport 30 He = 50 cm^3 min^{-1}	TBP		P 253.6 nm	
	6' x 1/8", 6% Carbowax 20M on 100/120 mesh Chromosorb P; He = 50 cm^3 min^{-1}	Tetravinylsilane		Si 251.6 nm	
	6' x 1/8" 2.5% Dexsil 300 on 100/120 mesh Chromosorb 750 He = 50 cm^3 min^{-1}.	MMT		Mn 257.6 nm	
		TEL 2-5 dimethylthiophene. Halobenzenes.		Pb 283.3 nm	
				S 545.4 nm	
				Cl 481.0 nm	
				Br 470.5 nm	
				F 685.6 nm	
				I 206.2 nm	79
3/4 λ cylindrical cavity, 125W forward power Ar plasma, background correction by wavelength modulation.	1.8 m x 3.1 mm, 3% OV-1 on 80/100 mesh Chromosorb W. Ar = 22 cm^3 min^{-1} T_C = 80°C T_I = 130°C.	Tetraalkyllead compounds in the atmosphere.	Samples cold trapped on SE50 on Chromosorb P at -80°C. Removed by freeze drying and concentrated in organic solvent. Detection limits range between 6 pg and 40 pg.	Pb 405.78 nm	80
Low pressure (5T) He and Ar plasmas tapered rectangular cavity, 100W forward power. 0.3% O_2 added to plasma gas.	Stainless steel, 3 m x 3 mm i.d., 3% w/w Dexsil 300 on 80/100 mesh Chromosorb W(AW) 6 m x 3 mm i.d. Squalane on 80/100 mesh Chromosorb W(AW).	H in organic cpds.	He plasma twice as sensitive as Ar plasma due to higher energy and therefore more complete fragmentation. Detection limit 10^{-11} g s^{-1}.	H 656.28 nm	81

Detector	Chromatography	Matrix	Comments	Element	Reference
See ref. 81	See ref. 81	H isotope ratios in organic cpds in water samples.	OSM measures alternatively ^{1}H and ^{2}H emissions of hydrocarbons major disadvantage is high S/N ratios .	^{1}H 656.28 nm ^{2}H 656.10 nm	82
Reduced pressure He plasma. See ref. 57.		PCBs in seal blubber, cleaning fluids in water.	Applications of MPD850 in analysis and also empirical formula determinations. Detection limit in the 50 pg s^{-1} range.	C, H, P, N, F, Cl, Br, I, P, Se, As, Hg, Pb.	83
See ref. 57.		Biological tissues, coal tars, pesticides.	Brief resumé of the possible uses of the MPD850 system.	Cl, Br, I, S, P, Hg.	84
Beenakker, 3/4 λ Evenson, 1/4 λ Broida, 3/4 λ cavities were compared with He/Ar or Ar plasmas 100W forward power.	2.5" x 4.7 mm. Packed with Chromosorb 102, Served only to reduce rate of sample through-put to give stable plasma.	Standard solutions	Semi-automated hydride generation from stock solutioncontaining As, Ge, Sb, Sn. Beenakker cavity proved easiest to operate. Detection limit 1 ppb at 3 σ level for all cavities.	As 234.984 nm Ge 303.906 nm Sb 259.806 nm Sn 317.502 nm	85
Beenakker TM_{010} cavity viewed axially He plasma.	10' x 1/8" i.d., stainless steel Tenax G.C.	Haloforms in drinking water.	Compared MIP with HECD. Fund MIP was preferable since it gave uniform molar response and also gave selective detection. Detection limit 1 ppb.	Cl 481.0 nm Br 470.5 nm I 206.2 nm	86

Detector	Chromatography	Matrix	Comments	Element	Reference
Mixed Ar (400 ml min^{-1}) and He (300 cm^3 min^{-1}) plasma, 110 W for forward power. Evenson 1/4 λ cavity.	3' x 4.7 mm i.d. Polypenco Nylaflow tubing packed with Chromosorb 102 60/80 mesh T_c = 23 ±3°C.	Whole blood enriched flour NBS orchard leaves (SRM 1571).	Hydrides trapped on liq. N_2 cooled condensation tube packed with glass helices prior to separation on GC column. Detection limits ranged between 3 ng and 40 ng.	As 193.7 nm Ge 303.9 nm Se 196.0 nm Sb 259.8 nm Sn 317.5 nm	87
See ref. 87.	See ref. 87.	NBS orchard leaves; hydride generation.	Elements except Ge determined both sequentially and simultaneously. The former giving lower detection limits. Detection limits range between 20 ng and 600 ng.	simultaneous As 235.0 nm Se 196.0 nm Sb 259.8 nm (2° order) Sn 317.5 nm (2° order) (for sequential see ref. 87)	88
See ref. 81.	See ref. 81.	H emission from organic compounds.	Characterisation of emission from atomic H in MIP accounts for non-linearity observed.	H 656.28 nm	89
He plasma, TM_{010} cavity viewed axially.	12.5 m fused silica WCOT, SP2100, capillary column 0.2 mm i.d., T_c = 80-116°C at 4°C min^{-1} to 170° 0.1 µl injections. Column passed to within 5 mm of plasma.	Toluene solutions of volatile organo-metallic compounds. $[CpV(CO)_4]$, MMT, $[Cp_2Fe]$, $[Cp_2Ni]$, $[CpCo(NO)(CO)_2]$ $[(CH_3)_5CpCo(CO)_2]$.	The low volume of GC^2 column (approx. 80 ul) is ideally compatible with MIP. Specificity of detection aids identification of the unresolved $[Cp_2Ni]$ and $[CpCr(NO)(CO)_2]$ complexes.	C 247.9 nm Cr 267.7 nm Co 240.7 nm Ni 231.6 nm Mn 257.6 nm	90

Detector	Chromatography	Matrix	Comments	Element	Reference
He plasma TM_{010} cavity viewed axially. He = 450 cm^3 min^{-1}.	OV-225 SCOT, 100 m x 0.25 mm i.d. He = 4 cm^3/min. T_I = 210°C T_C = 40°C then 4° min^{-1} T_{in} = 250°C	Friedel-Crafts catalysed alkyl group redistribution reaction of methyl-ethyl-n-propyl-n-butyl silane.	35 redistribution products are formed, due to requirement to vent the solvent the low MW products which elute with the solvent are not recorded.	Si 251.6 nm	91
He atmospheric plasma, using TM_{010} cavity, 85-90W forward power.	Glass, 1.5 mm x 4 mm i.d. 2% OV-101 on 80/100 mesh Chromosorb WHP. T_C = 238°C. He = 60 cm^3 min^{-1}.	PBB and related compounds.	Not as sensitive as the ECD but offers element selectivity. Detection limit 1 ng.	Br 478.55 nm	92
He plasma in a surfatron cavity (see ref. 93)	30 m capillary column coated with OV-101 methyl silicone He = 5.9 ml min^{-1} T_I = 275°C. T_C = 250 for pesticides.	Pesticides.	The surfatron He plasma gives slightly higher detection limits than those obtained with other cavities. Detection limit 0.5 to 20 ng.	C 247.8 nm Cl 479.5 nm 481.0 nm Br 470.5 nm I 206.2 nm	94
See refs. 79 and 86.	See refs. 79 and 86.	Aqueous chlorination products of humic and fulvic substances.	In addition to trihalomethanes significant number of chlorinated phenolic cpds were found.	Cl 479.5 nm	95
See ref. 96.	See ref. 96.	Selenium biomethylation products from soil and sewage.	$(CH_3)_2Se$, $(CH_3)_2Se_2$, and $(CH_3)_2$-SeO_2 found. Detection limits 20 pg for $(CH_3)_2$-Se.	Se	97

Detector	Chromatography	Matrix	Comments	Element	Reference
TM_{010} cavity, He plasma, 80W forward power O_2 as scavenger. He = 40-70 cm^3 min^{-1}.	15.2 m x 0.508 mm i.d., SCOT column packed with finely ground diatomaceous earth on silica support coated with m-bis (m-phenoxyphenoxy) benzene and Apiezon L, He = 0.5-8 cm^3 min^{-}. T_C = 90°C.	Hydrocarbons, $(CH_3)_2Hg$, $(C_2H_5)_2Hg$.	FID proved 50X more sensitive than MIP for C (at 193.1 nm) Both had the same sensitivity for (C_2H_5)-Hg. The MIP was 2X as sensitive as FID for $(CH_3)_2Hg$ using Hg specific detection. Detection limits 3.8 x 10^{-12} g s^{-1} and 9.1 x 10^{-12} g s^{-1}.	C 193.1 and 247.9 nm Hg 254.3 nm	98
TM_{010} cavity He plasma		Organic compounds; elemental analysis.	Linear ranges of 3 orders of magnitude for all elements. Detection limits range between 2 x 10^{-11} mol l^{-1} to 8 x 10^{-10} mol l^{-1}.	C 193.1 nm 247.9 nm H 486.1 nm Cl 479.5 nm 481.0 nm Br 470.5 nm 487.5 nm I 516.1 nm 206.2 nm S 545.4 nm	99
1/4 λ Evenson low pressure (40 T). TM_{010} atmospheric pressure. Ar and He plasmas. The latter viewed axially.		n-hydrocarbons C_4-C_7.	With the aid of a reference compound it is possible to determine ratio formulae, however results are inadequate for unknown compounds. Detection limits: TM_{010} He Ar; C 0.67 0.2; H 0.13 4.7; 1/4 λ Evenson He Ar; C 0.44 0.35; H 0.16 0.36; in ng s^{-1}	C 247.86 nm H 656.28 nm C_2 576.52 nm CH 431.42 nm	100

Detector	Chromatography	Matrix	Comments	Element	Reference
Atmospheric pressure He plasma in a TM_{010} cavity. Background correction by quartz refractor plate.	SP-2100 WCOT fused silica column 12.5 m x 200 um i.d., and 30 m x 350 m i.d. OV-101 SCOT glass column.	Trialkyllead chlorides in spiked tap water samples.	Gas switches interface illustrated which prevents the solvent extinguishing the plasma linear from 10 ppb to 10 ppm. Detection limits 10 - 30 ppb.	Pb 405.8 nm C 247.9 nm	101
TM_{010} cavity. Atmospheric pressure, He plasma, viewed axially.	12.5 m x 0.2 mm i.d. SP2100 fused silica WCOT capillary column. T_C = 60°C - 104°C at 4°C min^{-1} 0.1 µl injections 100:1 split. For boration studies.	Detection of volatile B compounds from the pyrolysis of Dexsil series carborane silicone polymer, and form boration of diols with n-butylboronic acid.	H_2 doping of the plasma inhibits formation of oxides of silicates, promotes boron hydride formation and the population of B atomic, rather than ionic, states.	B 247.77 nm	102
TM_{010} cavity. Atmospheric pressure; He plasma, 75W forward power; He = 80 cm^3 min^{-1}.	Glass 3 m x 3 mm i.d. columns packed with either: 3% OV17 on 80/100 mesh Shimarate W, 10% Carbowax 6000 on 30/60 mesh Shimarate TPA, or Poropak Q, 80/100 mesh. T_I = 190°C T_{in} = 190°C.	Various organic compounds.	Relative sensitivities for C and H in different compounds were not the same. Attributed to incomplete fragmentation in low power plasma used. Detection limits 1.8 pg s^{-1} to 39.0 pg s^{-1}.	H 656.279 nm C 193.091 nm F 685.602 nm Cl 479.454 nm Br 470.486 nm I 206.238 nm S 545.388 nm	103
Atmospheric pressure, He plasma, TM_{010} cavity. See ref. 101.	12.5 m SP2100 fused silica capillary column. 100:1 split ratio. T_C = 40°C-100°C at 5°C min^{-1} 0.01 µl sample.	Tetraalkyllead compounds in petrol.	Demonstrates advantages of element specific detection by comparison of Pb and C responses.	Pb 283.3 nm C 247.86	104

Detector	Chromatography	Matrix	Comments	Element	Reference
Atmospheric pressure, He plasma, TM_{010} cavity. 75W forward power, 12W reflected.	1 m x 3 mm i.d. glass column, 15% DC-200 on 80/100 mesh Uniport B and 3% OV-17 on 80/100 mesh Uniport HP. He = 80 ml min^{-1}	F in urine.	F extracted with TCMS and converted to TMFS in toluene. Linear over 4 orders of magnitude. Detection limit 7.5 pg s^{-1}.	F 685.6 nm	105
Atmospheric pressure, He plasma, TM_{010} cavity. See ref. 101.	12.5 m x 200 um i.d., SP2100 fused silica WCOT. Terminated within 1-5 mm of cavity wall.	Redistribution reactions for Ge, Sn and Pb alkyls. Pb alkyls in gasolines.	H_2 doping of He enables plasma to withstand 1-2 ng s^{-1} throughputs of Pb, Ge or Sn. Linear over 3 orders of magnitude. Detection Limits ranged between 0.71 pg to 6.1 pg	Ge 265.1 nm Sn 284.0 nm Pb 283.3 nm	106
Beenakker TM_{010} cavity, He as the support gas. 50 W forward and 0-1 W reflected power. Modified Jarrell-Ash 66000 polychromator.	6' stainless steel column (1/8" o.d. x 2 m i.d.) packed with 10% Apiezon L on 80/100 mesh Chromosorb PAW at 110 °C. 3' silinised glass column (1/4" o.d. x 4 mm i.d.) packed with 2% OV 101 on 80/100 Chromosorb HP at 270 °C.	Chlorinated pesticides and brominated flame retardants.	The polychromatogr/ microcomputer system developed to simultaneously monitor four emission wavelengths. Detection limits at nanogram level with precision in order of 5% RSD.	C 247.9 nm Cl 479.5 nm BR 470.5 nm	107
Atmospheric pressure microwave sustained helium plasma with Beenakker TM_{010} resonant cavity. Effluent split by 3-way valve with 20% going to FID.	2' x 1/8" stainless steel column packed with Porapak QS, 80/100 mesh, using 1 ml gas injections. Bentone 34/DC-550 mixed phase on Chromasorb W-HP.	Application to a number of halomethane and monochlorobiphenyl separations.	Notes on design, optimisation and utilisation of interface. Detection limits: 20 pg Cl; 8.8 pg P; 2.5 pg Fe; 10 pg Br; 14.0 pg S	Cl 481.0 nm 479.5 nm P 213.6 nm Fe 259.94 nm Br 478.6 nm S 213.6 nm 545.5 nm	108

Detector	Chromatography	Matrix	Comments	Element	Reference
Copper Beenakker cavity, 2450 MHz microwave generator and McPherson model 270 scanning UV/vis monochromator. Interface similar to Ref. 101.	6' x 0.125" column packed with OV-17 on Chromosorb WHP. Carrier gas He at 28 ml min^{-1}. Column temp. 85 °C (140 °C for derivations).	Technique used in combination with chemical derivitization of selected compounds in complex samples e.g. trichloroacetyl derivatives of aliphatic amines.	Microcomputer used to switch valves, can also be used to control monochromator wavelength settings and acquire analytical data.	C 247.9 nm Cl 479.5 nm Br 470.5 nm	109
System as described in ref. 92. Minor modification by inserting a stainless steel tube from the column into the plasma containment tube in hope of reducing dead volume.	Details not given.	Application to halogenated compounds e.g. Cilex BC-26.	Modification undesirable in quantitative studies since results in degradation of detection limits. Paper recommends interface in ref. 86. Most of paper concerned with hardware and software development for control, data acquisition etc.	Cl 479.45 nm Br 478.55 nm	110
Reduced pressure He plasma in parallel with either an FID or ECD. Plasma viewed transversely by a multichannel spectrometer.	Two capillary columns of 30 m x 0.25 mm i.d. and 1.0 μm film thickness DB-5. Temp. programme 70 °C - 300 °C at 10 °C min^{-1} with a helium carrier at 1 ml min^{-1}.	Characterisation of fluorine containing metabolites in blood plasma.	Inlet splitter to divide effluent between the two columns. Interaction with fluorine species with quartz tubing gives rise to peak tailing.	F 685.6 nm C 495.7 nm	111

Detector	Chromatography	Matrix	Comments	Element	Reference
Spectrospan IIIB Multi-Element Analyser equipped with a three-electrode DCP-Spectrojet III and multielement cassette. Wavelength scan achieved using Spectrametrics DBC-33 system. Series UV monitor at 280 nm.	Gel filtration - 2.6 x 100 cm column packed with Sephacryl S-300. 5 ml sample applied to column.	Speciation of protein-bound Cu, Fe, and Zn in serum and intravenous infusion fluids.	Gel filtration separation requires several hours, therefore spectrometer recalibrated every hour. Detection limits: Cu 3.2 $\mu g\ l^{-1}$ Fe 3.9 $\mu g\ l^{-1}$ Zn 9.3 $\mu g\ l^{-1}$	Cu 324.7 nm Zn 213.8 nm Fe 373.4 nm	112
Atmospheric pressure plasma utilizing a Beenakker type TM_{010} cavity. Low resolution scanning monochromator with approximately 0.1 nm resolution.	Capillary column - 11 m x 0.25 mm i.d. SE30 fused silica. He at 1 ml min^{-1}. Temp. programme at 4 °C min^{-1} after first 6 mins. Pyrolysis using Model 100 Pyroprobe Unit.	Pyrolysis products of novel linear silarylene-siloxanes.	The interface allowed venting of column effluent containing large quantities of solvents which would disrupt helium discharge, while passing labile species without loss.	C 253.6 nm P 247.6 nm	113
System similar to ref. 107 with the internally tuned resonant cavity mounted on the GC oven.	3' x 1/4" column packed with 2% OV 101. Flow rate 25 ml/min. Column temp. 300 °C.	Dioxins and other halogenated compounds.	The H line was monitored with a red-sensitive photomultiplier. Data manipulation as ref. 107, but modified to store chromatographic data.	C 247.9 nm BR 470.5 nm Cl 479.5 nm H 656.3 nm	114

2.3.2 Coupled gas chromatography - inductively coupled plasma

The high capital cost of ICP instrumentation together with the high running costs have resulted in its use mainly as a multi-element excitation source for routine analysis. Consequently use of the ICP as a detector for GC has been limited. However, it does offer the advantage of withstanding organic solvents more readily than the MIP due to the higher gas temperature; and so may possibly be further utilised in this role in the future.

The first couplings of GC-ICP were made by Windsor and Denton (115-117) in Arizona, and Sommer and Ohls (118,119) in Dortmund. The former group showed the capability of ICP OES for the elemental analysis of organic compounds (115) using an all-argon plasma. This capability was then utilised in a GC-ICP coupling (116) for simultaneous multi-elemental analysis of organic and organometallic compounds. A natural extension of this work was the derivation of empirical formula. Windsor and Denton (117) used carbon, hydrogen and halogen ratios to find the empirical formula of various organic compounds; however, while the technique provided the ability to analyse for a large number of elemental constituents, suitable lines for oxygen and nitrogen were not found. Sommer and Ohls (118) used both all-argon and the nitrogen cooled plasmas for the determination of tetraalkyllead compounds in various petrols by monitoring the lead emission. The same authors (119) determined nickel and zinc as diethyldithiocarbamates, using a nitrogen cooled plasma. Fry *et al.* (121) investigated a large number of fluorine atom lines for selective detection of various fluorine-containing organic compounds, using off-line correction to remove interference from the solvent emission. Brown *et al.* (120) monitored near infra-red oxygen emissions to enable

oxygen-specific detection. The determination of volatile hydrides of arsenic, germanium and antimony by GC-ICP, using a sequential slew-scanning monochromator (122) demonstrates how the use of chromatography enables rapid multi-element analysis using a monochromator. Table 2 lists applications of GC-ICP which have appeared to date.

Table 2 Coupled Gas Chromatography - Inductively Coupled Plasma Optical Emission Spectroscopy

Detector	Chromatography	Matrix	Comments	Element (Wavelength/ nm)	Reference
All Ar plasma observations made 9 cm above load coil. Computer controlled data acquisition system. See ref. 115.	6' x 1/8" packed with 8% Carbowax 1540 on 80/100 mesh fire-brick.	Elemental analysis of various organic compounds.	Used single and multi-channel mono-chromators. Using the latter monitored C and H channels for TMT, toluene and p-xylene. Detection limits range between 0.8 ng - 1 mg depending on the element.	Br 700.57 nm C 247.86 nm Cl 725.67 nm F 634.67 nm H 656.28 nm I 206.16 nm Si 251.61 nm Fe 371.99 nm Pb 217.00 nm Sn 284.00 nm	116
All Ar plasma. See refs 115, 116. Power = 0.8 kW Coolant = 12 l min^{-1} Plasma = 0.5 l min^{-1} Sample = 0.9 l min^{-1} Makeup = 0.9"	See ref. 116.	Halogen containing hydrocarbons.	Elemental ration determinations for each peak typically 200 elemental ratio determinations were achieved to yield an average figure.	C H I Cl	117
Uses both high power Ar/N_2 and low power Ar/Ar plasmas.	SP1000. T_c = 140°C (Si) T_c = 150°C (Pb) N_2 = 30 cm^3 min^{-1}.	Looked at lead in petrols using standard addition also TML/TEL ratio and C background at 220.35 nm.		Si 212.4 nm 288.1 nm Pb 220.35 nm	118 119
Ar/Ar plasma 1.75 KW forward power. Used elongated torch, observation zone 5.5 mm above load coil.	10% Carbowax 20M on Chromosorb P 80/100 mesh. Ar = 25 cm^3 min^{-1} T_c = 100°C T_{in} = 100°C.	Monitored near IR oxygen emissions for various gases and organic liquids.	Studied effect of varying various plasma gas flows on signal and background levels. Detection limit 650 ng	O 777.194 nm	120

Detector	Chromatography	Matrix	Comments	Element	Reference
All Ar plasma	6' x 1/8" packed with Amine 200 T_c = 105°C Ar = 25 cm^3 min^{-1} sampling loop used.	Separation of benzenetrifluoride and o-fluorotoluene.	F/C selectivity of 1.0 at 685.602 nm without background correction. By using "off line" correction solvent peak disappears.	F Considered 1 µg 56 lines in the region 350 to 895 nm	121
All Ar plasma with slew scanning monochromator. 1 KW forward power. Observation 15 mm above load coil.	3.5' x 3 mm i.d. Chromosorb 102 at ambient temperature.	Hydrides generated, cold trapped and passed through column into plasma.	Sequentially eluting hydrides monitored. Linear over 2-3 orders of magnitude. Detection limits 4 ng Ge 50 ng As and Sb	Ge 303.9 nm As 278.0 nm Sn 317.5 nm Sb 287.8 nm	122

2.3.3 Coupled gas chromatography - direct current plasma

The DCP is essentially a direct current arc struck between two or more electrodes and stabilized by a flow of inert gas. There are few reported couplings of GC with DCP OES, although the group at Amherst have been particularly active (91,104,123,124). They found it possible to use argon, helium or nitrogen as a carrier gas (124), although in certain spectral regions interference from cyanogen bands can occur with nitrogen. The use of a sheathing gas, heated to prevent sample condensation around the injector nozzle, was found to increase sensitivity (123,124). This coupled technique has been used as an element-selective detector for: manganese as the cyclopentadienyltricarbonyl derivative (123); copper, chromium, nickel, palladium and zinc chelates (124); iron in ferrocene (126), and various group IV metals in an interesting study of Friedel-Crafts catalysed alkyl group redistribution reactions (91). Treybig and Ellebracht (127) utilised a vacuum ultra-violet plasma spectrometer for sulphur-specific detection which compared favourably with MIP detection and has the advantage that solvent venting is not required. Applications of GC-DCP are summarised in Table 3.

Table 3 Coupled Gas Chromatography - Direct Current Plasma Optical Emission Spectroscopy

Detector	Chromatography	Matrix	Comments	Element (Wavelength/ nm)	Reference
Prototype spectraspan III dc plasma echelle spectrometer.	6' x 1/8" i.d. stainless steel 2% Dexsil 300 GC on 100/120 mesh Chromosorb 750. 1:1 split with FID. T_c = 130°C T_I = 160°C T_{in} = 170 He = 25 cm^3 min^{-1}.	MMT in gasoline, standards in iso-octane. Eymantrene as internal standard.	Only sample modification required was addition of the internal standard. 3 min analysis time. Upper limit of linear range was 340 ng. Detection limit 3 ng	Mn	123
See ref. 123. Details of heated interface design given. Dual detection with FID used sheathing gas heated to 230°C to prevent condensation of eluents. T_{in} = 230°C	6' x 1/8" i.d., 3% Dexsil 300 on 100/120 mesh Chromosorb 750. T_c = 170°C He = 60 cm^3 min^{-1} T_c = 220°C 6' x 1/8" i.d. 2.5% Dexsil 300 GC T_c = 230°C T_c = 280°C 6' x 1/8" i.d. 3.2% Dexsil 300 GC on 100/120 mesh Chromosorb 750 T_c = 190°C 6' x 1/8" i.d 10% SE-30 on 60/80 mesh Gas Chrom. S. T_c = 170°C.	$Cr(tfa)_3$ $Cu(en)(tfa)_2$ $Cu(pn(tfa)_2)$ Ni $pn(tfa)_2$ Pd $pn(tfa)_2$ Zn $(dtc)_2$ $CpCr(NO)(CO)_2$ benzenechromium tricarbonyl. C_{10}, C_{12}, C_{14}, and C_{16} hydrocarbons.	Sheathing gas around the issuing g.c. effluent prevented excessive diffusion as the sample travelled into the plasma from the interface tubing. Linear from 2 - 150 ng for Cr. Detection limits ranged between 0.28 pg s^{-1} to 320 pg s^{-1}	Cu 324.7 nm Ni 341.7 nm Pd 340.4 nm C 247.8 nm Cr 267.7 nm C 247.8 nm	124

Detector	Chromatography	Matrix	Comments	Element	Reference
For spectrometer and interface see ref. 125. Except used 3 electrode jet rather than a 2 electrode one. Ar flow rates: Sheathing = 1.42 - 1.65 l min^{-1} Cathode = 2.0 l min^{-1} Anode = 1.3 l min^{-1} Current = 7 A Voltage = 40 60 V	6' x 1/8" stainless steel, 5% OV-101 on 100/120 mesh Chromosorb 750. He = 40 cm^3 min^{-1} T_C = from 80°C to 6 or 8°C min^{-1} T_I = 210°C T_{in} = 220° Nickel tubing 1 m x 1/8", 3% OV-201 on 100/120 mesh ultrabond 20 M. He = 40 cm^3 min^{-1} T_C = from 80°C at 8°C min^{-1} T_I = 210°C T_{in} = 220°C	Friedel-Crafts catalysed alkyl group redistribution reactions.	Redistribution reactions of the following pairs: $^nPr_3Sn$+Et_4Pb Et_4Sn+$^nBu_4Ge$ $^nPr_4Si$+$^nBu^4Ge$ $^nBu_4Ge$+Et_4Pb Vn_4Si+Et_4Sn Vn_3Si+$^nBu_4Ge$ studied. Formation of PbR_3Cl and SnR_3Cl by reactions with $AlCl_3$ studied.	Si 251.6 nm Ge 265.1 nm Sn 286.3 nm Pb 368.3 nm Pb 368.3 nm Sn 286.3 nm	91
See refs. 91 and 124.	100' x 0.03" i.d. stainless steel PLOT OV-101 T_C = 170°C.	Ferrocene and halo-derivatives.	Paper contains many other organometallic separations, however the detector used is the FID.	Fe 372.0 nm	126
Vacuum UV spectrometer with spectrametrics dc plasma.	122 cm x 2 mm i.d., Poropak super Q. 183 cm x 2 mm i.d., 3% OV-101 on Chromosorb W HP, 80-100 mesh. N_2 = 80 cm^3 min^{-1}.	CS_2, Thiophene 3-methylthiophene. hexanethiol benzenethiol methylsulphoxide. Detection Limit 0.3 ng S s^{-1}.		S 180.7 nm	127

Form CNAA 10 Decl

COUNCIL FOR NATIONAL ACADEMIC AWARDS

Candidate's declaration form

Note: This form must be submitted to the Council with the candidate's thesis and the Examiners' Recommendation Forms (Appendix 2, paragraph 7 of the Regulations refers)

Name of candidate: Stephen John Hill

Sponsoring establishment: Plymouth Polytechnic

Degree for which thesis is submitted: Doctor of Philosophy

1 Statement of advanced studies undertaken in connection with the programme of research
(Regulations 3.8–3.10 refer)

A series of post-graduate lectures in atomic spectroscopy, attendance at relevant lectures and meetings of the Royal Society of Chemistry, Analytical Division. Departmental colloquia and weekly meetings of the Chemistry of Natural and Polluted Environments research group at the Polytechnic.

2 Concurrent registration for two or more academic awards (Regulation 2.5 refers)

either *I declare that while registered as a candidate for the Council's research degree, I have not been a registered candidate or enrolled student for another award of the CNAA or other academic or professional institution

or ~~I declare that while registered as a candidate for the Council's research degree, I have been a registered candidate or enrolled student for the following award~~

3 Material submitted for another award

either *I declare that no material contained in the thesis has been used in any other submission for an academic award

or ~~I declare that the following material contained in the thesis formed part of a submission on the basis of which the award of~~

.....*(state award and awarding body and list the material below):*

Signature of candidate..... Date 24/11/85

*delete as appropriate

2.3.4 Coupled gas chromatography - atomic fluorescence spectroscopy

Van Loon (205) was the first to suggest the possible use of non-dispersive AFS as a detector for chromatography, noting its multi-element capability, ability for low level detection and simplicity of usage. Although this latter point is debatable, the most likely reason for the dearth of published work using GC-AFS is probably the lack of sufficiently intense, stable and simple light sources. To date only line sources have been utilised in chromatographic applications, Van Loon's group in Toronto having published the only GC-AFS work (157). A nitrogen separated circular air/acetylene flame was used with an inert gas shielded electrothermally heated quartz tube and a modified graphite cup atomiser. In the lead specific detection of tetraalkyllead compounds flame AFS proved a factor of three more sensitive than FAAS; however, no increase in detectability was found using AFS over AAS when the graphite cup or quartz tube atomisers were used. The availability of a commerical AFS instrument should increase the usage of the technique since the advantages of multi-element analysis and sensitive detection make AFS an excellent method for the determination of metals.

Table 4 Coupled Gas Chromatography - Atomic Fluorescence Spectroscopy

Detector	Chromatography	Matrix	Comments	Element (Wavelength/ nm)	Reference
Circular N_2 shielded circular Air/C_2H_2 flame. Electrothermally heated quartz tube furnace. Graphite cup furnace at 1000°C.	See Table 4 ref. 109.	Tetraalkyllead compounds.	FAFS 3 x more sensitive than FAAS, however, Electrothermal AFS was no better than electrothermal AAS.	Pb	109

2.3.5 Coupled gas chromatography - atomic absorption spectroscopy

Coupled GC-AAS can be split into flame (FAAS) and electrothermal (ETA) atomisation systems - Table 5. Flame atomisation offers the advantages of continuous operation, simplicity and low cost instrumentation. Although it would appear that the low nebulisation efficiency of about 10% for solutions, would be a disadvantage compared with ETA where the whole sample is atomised, this is unimportant in coupled GC-AAS since the analyte is in the gas phase prior to entry into the atom cell. However FAAS does suffer the disadvantage of higher detection limits due to the shorter atomic residence times in the flame. In addition to the increased sensitivity, it is also claimed that ETA is safer and lends itself to the possibility of unattended operation.

The simplest way of interfacing a gas chromatograph with an atomic absorption spectrometer is to pass the column effluent via an interface tube into the nebulisation chamber, to be swept by oxidant and fuel gases into the flame. The first reported GC-FAAS coupling by Kolb *et al.* (128) used this method to determine tetraalkyllead compounds in petrol with an air/acetylene flame. This interfacing method has been utilised by various authors (129,136,140). Morrow *et al.* (129) used the nitrous oxide/acetylene flame for the silicon specific detection of silylated alcohols and an air/acetylene flame for atomic emission detection of the same species. A similar coupling was used to determine lead in petrol (136,140,153), and in the atmosphere (140). Hahn *et al.* (162) used such an arrangement to determine As, Ge, Se and Sn, after hydride generation, using a hydrogen diffusion flame. Coker (125) realised that dilution of the sample and excessive peak broadening caused by passage through the

nebulisation chamber could be avoided, and so passed the chromatographic effluent into a manifold just below the burner slot, thus achieving lower detection limits for tetraalkyllead compounds in petrol than with previous couplings. Wolf (146,152) used a similar coupling to specifically determine chromium in standard orchard leaves after chelation with trifluoroacetylacetone, as did Chan (167) when investigating tetraalkyllead ratios in petrols from varying sources. The work of Ward (34) has emphasised that in order to enable true trace level determinations by GC-FAAS, the residence times of atoms in the flame must be increased. This was achieved using a ceramic tube suspended over a flame in various configurations (33,206). In the most successful arrangement, described by Ebdon et al. (33), the effluent from the gas chromatograph was taken to a 'T' piece where a flow of hydrogen is introduced to enable a small hydrogen diffusion flame to burn at the end of the interface tube. The atoms produced in the hydrogen flame are then swept into the ceramic tube. This approach has given detection limits of 17 pg for lead in tetramethyllead and tetraethyllead, 80 and 95 pg for mercury as Me_2Hg and Et_2Hg respectively, and 0.12 ng for selenium in organoselenium compounds. The system has now been adopted for routine use in a number of laboratories (33,175,177), particularly for the speciation of alkyllead compounds.

The electrothermal devices used in coupled GC-AAS, fall into three main categories:

(i) home made electrothermally heated quartz or ceramic tubes;

(ii) commercial graphite furnaces;

(iii) commercial cold vapour mercury analysers.

This latter atom cell has been used for mercury specific detection of organomercurials in various samples. Hey (130) passed the effluent from the chromatograph into a continuous wet chemical reduction cell, the reduced Hg(0) being swept into the cold vapour absorption cell of a commercial system (MAS 50, Coleman Instruments). Other authors (132-135) used a flame ionisation detector flame to atomise the organomercury species which were then passed into the same cell. Dressman (132) used this method to speciate dialkylmercury compounds in spiked river waters. Blair et al. (135) also used this method in a study of mercury transformations in aquatic environments. Gonzalez and Ross (131) used a quartz combustion furnace prior to the detector to determine methyl- and ethyl- mercury chlorides in fish tissues, and found better selectivity towards mercury than that exhibited by electron capture detectors towards the organomercury chloride.

The use of an electrothermally heated silica tube as an atom cell for coupled GC-AAS was pioneered by Chau et al. (138). The furnace, heated to around 1000 ^{o}C with a through flow of air and hydrogen, was used with a selenium specific detector for the separation of dimethyldiselenium and dimethylselenium (138). Chan, with a number of co-workers, then used this coupled technique for numerous environmental applications (138,139,142,168,169). This group also developed the technique for metal specific detection of organolead in the atmosphere (140,141), the aquatic environment (155,159) and for methylation studies of lead (139,203), tin (168) arsenic, mercury and selenium (169). Thompson (171) utilised a similar atom cell to study methylation pathways in coastal sediments, whilst Brueggemeyer and Caruso (172) used the same system for the determination of inorganic lead in aquatic samples after methylation of the extracted

dithiocarbamate lead complex. Van Loon and Radziuk (143-145) developed a silica 'T' tube for coupled GC-AAS. This low cost arrangement had the chromatographic column contained in the long arm of the 'T', the effluent then passed into the cross piece atomiser purged with flows of hydrogen and nitrogen. The system was used as a metal specific detector for organoselenium compounds (143) and in the study of organoselenium transpiration by Astragalus racemosus (144,145). Bye and Paus (154) used an electrothermally heated quartz furnace to atomise organomercurial compounds prior to their detection in an unheated silica cuvette. In a comprehensive study of various tetraalkyl, methyl- and ethyl-tin chlorides (165) Burns et al. used an electrothermally heated quartz tube as atomiser. They found that detection limits could be lowered substantially if the hydrides were generated prior to atomisation. In a comparison of various atom cells for coupled GC-AAS by Radziuk et al. (157) the graphite furnace proved the most sensitive for lead, and gave a factor of fifty increase in response when compared to the early simple Kolb type flame coupling.

The first gas chromatograph coupling to a commercial graphite furnace was rather crudely achieved by Segar (137). The end of a tungsten transfer line was passed through an enlarged hole in the graphite tube so that the effluent impinged on the hot tube wall. Parris et al. (150) considered the effect of using pyrolytically coated, alumina lined and standard graphite tubes at various atomisation temperatures with and without hydrogen (10%) added to the chromatographic effluent. The best detection levels were achieved for As, Se and Sn, using standard graphite tubes with hydrogen added to the effluent flow and an atomisation temperature of 1800 ^{o}C. Robinson et al. (151) passed the chromatographic effluent through a graphite electrode into the

optical path of a home made atomiser which was kept at 2000 °C throughout the chromatographic run. This atomiser was used for lead specific detection of tetraalkyllead compounds in petrol (151) and in a study of the degradation of TEL in sea water (158). Bye and Paus (154) found graphite furnace atomisation was 100-fold more sensitive than flame atomisation for the determination of TML in petrol. The determination of tetraalkyllead compounds in various matrices has again been well researched; for example, Cruz et al. (204) in fish, water, sediment and vegetation samples. The group in Antwerp developed the most sensitive GC-GFAAS coupling for tetraalkyllead compounds (163) and used it to determine these compounds in petrol (163,166), the atmosphere (164,166) and in a preliminary study of their degradation in river water (166). Determination of another "anti-knock" petrol additive, methyl-cyclopentadienylmanganese tricarbonyl, in the atmosphere, was achieved by Coe et al. (160) down to levels of 0.05 ng m^{-3}. Winefordner and co-workers (174) have demonstrated a novel method of avoiding matrix interference by selective volatilisation using coupled high temperature (ca. 2093 K) GC-AAS. They used a molybdenum column/atomiser for the separation of sodium, copper, manganese and magnesium ions with excellent correlation of analytical signals for each metal in pure and mixed solution. This work opens a new area of application for GC-AAS, since prior to this only elements which form volatile hydrides or chelates in inorganic matrices could be separated. The technique thus offers a possible method for separating interfering concomitants from the analyte prior to atomic spectroscopic analysis.

Table 5 Coupled Gas Chromatography - Atomic Absorption Spectroscopy

Detector	Chromatography	Matrix	Comments	Element (Wavelength/ nm)	Reference
Flame AAS, GC effluent passed via a heated tube into the nebulisation chamber.	2 m x 2 mm i.d., 10% Apiezon M on Chromosorb R. N_2 = 40 ml min^{-1} T_c = 150°.	Pb alkyls in petrol, TML and TEL	First paper to describe GC-AAS coupling for element specific detection. Linear range 50-700 ppm.	Pb 217.0 nm	128
Flame AAS, N_2O/C_2H_2 FAES air/C_2H_2 flame. Coupling was through the nebulisation chamber.	6' x 0.25" i.d. steel column, 20% SE30 on 30/60 mesh chromosorb W. He = 100 ml min^{-1}. T_c = 130°C.	Silylated pyridine solutions of n-alcohols C_1 - C_7.	Interface tube, stainless steel (0.0345" i.d.) heated in excess of T_c. AAS 4-20 µg AES 3-100 µg Linear range. Detection limits AAS 0.11 µg AES 0.72 µg	Si 251.6 nm	129
Using cold vapour analyser.		Organomercury compounds.	Passed GC effluent into a continuous wet chemical reduction vessel; Hg then flushed into cold vapour cell. Linear up to 10 µg. Detection limit 50 ng	Hg 253.7 nm	130
As ref. 130.	Glass 6' x 0.25" column, 5% HIEFF-2AP on Chromosorb WHP, 80/100 mesh. N_2 = 120 ml min^{-1} T_I = 200°C T_c = 170°C T_{in} = 200°C.	Alkyl mercury compounds in fish tissue MeHgCl and EtHgCl.	GC effluent passed into a quartz tube combustion furnace (780°C) prior to passing into the cold vapour cell. Linear up to 45 ng. Detection limit - 2.5 x 10^{-11} g of MeHgCl gives 1% absorption.	Hg 253.7 nm	131

Detector	Chromatography	Matrix	Comments	Element	Reference
See ref. 130.	6' x 2 mm i.d. glass column, 5% DC-200 + 3% QFI on 80/100 mesh Chromosorb Q, T_c = 70°C hold 2 min then 20°C min^{-1} to 180°C.	Dialkyl mercury compounds in spiked river waters.	The effluent was passed through the FID to combust the mercury compounds prior to entry into the cold vapour analyser. Detection limit 0.1 ng	Hg 253.7 nm	132
see ref. 130	See ref. 132	Dialkyl mercury compounds, Me_2Hg, Et_2Hg, $^nPr_2Hg$, $^nBu_2Hg$.	See ref. 84. Linear from 0.05 ng to 100 ng. Detection limit 0.02 ng for Me_2Hg.	Hg 253.7 nm	133
See ref. 130.	See ref. 132.	Dialkyl mercury compounds.	See ref. 84. Linear from 0.05 to 100 ng for Me_2Hg and Et_2Hg. Detection limit 0.02 ng for Me_2Hg.	Hg 253.7 nm	134
See ref. 130.	6' x 0.125" glass column, 5% SP2100 + 3% SP2401 on 80/100 mesh Supelcon AW-DCMS. N_2 = 20 ml min^{-1}. T_c = 60°C hold 2 min then 32°C min^{-1} to 180°C.	Mercury compounds involved in transformations of microorganisms, in soils and sediments.	Study of methylation pathways in microorganisms.	Hg 253.7 nm	135
Air/acetylene flame.	3 m x 3 mm Teflon tube. N_2 = 40 ml min^{-1} T_c = 110°C.	Pb alkyls in gasoline samples.	Effluent passed from GC into spray chamber. 5 cm burner. Linear from 0.2 to 40 µg.	Pb 217.0 nm	136
Graphite furnace kept at 2700°C with background correction.	6' x 5/16" i.d. on glass column, 4% SE-30 + 6% OV210 on Gas Chrom Q. Ar = 50 ml min^{-1} T_c = 150°C 2.0 µl injections.	Pb alkyls in gasoline.	10 cm W transfer line connected into an enlarged hole in graphite tube. Detection limit 10 ng Pb.	Pb 217.0 nm	137

Detector	Chromatography	Matrix	Comments	Element	Reference
Electrothermally heated silica tube (60 mm x 7 mm i.d., T = 1000°C) Furnace gases: air = 120 ml min^{-1} H_2 = 120 ml min^{-1}.	1.8 m x 6 mm glass column, 3% OV-1 on Chromosorb W 80/100 mesh. T_C = 40°C hold 2 min then 15°C min^{-1} to 120°C T_I = 225°C.	Me_2Se and Me_2Se_2 in synthetic air samples.	Air samples trapped at -80°C on 3% OV-1 on Chromosorb W and desorbed into the GC at 80°C. The trap being heated in a commercial 'toaster'. Linear up to 50 ng. Detection limit 0.1 ng Se	Se 196.0 nm	138
Air/C_2H_2 flame.	3' x 3/16" i.d. steel column, 10% Carbowax 20M on 100/120 mesh Porasil C. H_2 = 120 ml min^{-1} T_C = 130°C Home made column heating system. 5 µl injections.	Pb alkyls in gasoline.	The effluent from the GC passes into a manifold just below the burner slot which evenly distributes the effluent along the flame. Linear up to 200 ppm for TML and 1000 ppm for TEl. Detection limit 0.2 ppm.	Pb 283.3 nm	127
AAS using an electrothermally heated silica furnace. See ref. 138.	See ref. 138.	TML from methylation of Me_3Pb^+ salts.	Reported that Me_3Pb^+ salts were readily converted to TML by microorganisms in lake water or nutrient medium.	Pb	139
Air/C_2H_2 flame. All-glass lining for nebulisation chamber used to prevent absorption of organo-lead on chamber walls.	1.8 m x 6 mm glass column, 3% OV-1 on 80/100 Chromosorb W. N_2 = 65 ml min^{-1}. T_C = 40°C for 2 min then 5° C min^{-1} to 90°C.	Tetraalkyllead compounds in the atmosphere and gasolines.	The air sample was trapped (see ref. 90); passed through nebulisation chamber into flame. Detection limit 80 ng.	Pb 217.0 nm	140

Detector	Chromatography	Matrix	Comments	Element	Reference
Electrothermally heated silica tube. See ref. 138.	Column (see ref. 92). N_2 = 70 ml min^{-1} T_c = 50°C for 2 min then 15°C min^{-1} to 150°C T_I = 150°C.	Tetraalkyllead compounds in the atmosphere.	For sample trap and chromatographic interface see ref. 138. Linear up to 200 ng. Detection limit 0.1 ng.	Pb 217.0 nm	141
Electrothermally heated silica tube. See ref. 138.	1.8 m x 6 mm, 3% OV-1 on Chromosorb W 80/100 mesh. Lead see 92. Selenium N_2 = 70 ml min^{-1} T_c = 40°C for 2 min then 15°C min^{-1} up to 120°C . T_I = 225°C Arsenic 10% OV-1 on chromosorb W. N_2 = 30 ml min^{-1} T_c = 25°C = T_I T_{in} = 100°C Mercury 5% DEGS on Chromosorb W N_2 = 80 ml min^{-1} T_c = 145°C T_I = 150°C T_{in} = 150°C Cadmium N_2 = 70 ml min^{-1} T_c = 70°C T_I = T_{in} = 80°C	Organometallic compounds in liquid or gaseous samples. For gaseous sample trapping method see ref. 138.	Compounds determined were: tetraalkylleads methylseleniums methylarsines, alkylmercury chlorides, and dimethylcadmium. Detection limits 0.1 ng for each element.	Hg 253.6 nm Pb 217.0 nm Cd 228.5 nm As 193.7 nm Se 196.0 nm	142
'T' Furnace atomiser (900-1000°C; dimensions, 100 mm x 20 mm i.d.) flows into atomiser. H_2 = 1 l min^{-1}; N_2 = 6 l min^{-1}. Quartz 'T' furnace,	122 cm x 3 mm i.d. Al tube, 20% polymetaphenylether on 60/80 mesh Chromosrob W. N_2 = 23 ml min^{-1} T_c = 82°C T_I = 180°C	Dialkylselenium compounds	The homemade chromatographic system was contained in the quartz 'T' arrangement.	Se 196.0 nm	143

Detector	Chromatography	Matrix	Comments	Element	Reference
see ref. 143.	See ref. 143.	Organoselenium compounds transpired by Astragalus racemosus.	The transpired compounds were trapped on DC-550 on Chromosorb W in a dry ice bath and desorbed at 175°C into the chromatographic column. Detection Limits Me_2Se = 10 ng Me_2Se_2 = 20 ng Et_2Se_2 = 20 ng	Se 196.0 nm	144,145
Flame, with chromatographic effluent being delivered directly to the burner cavity.	2' x 3 mm i.d. Teflon tubing, 10% SE30 on Chromosorb WHP 80/100 mesh. T_c = 180°C N_2 = 65 ml min^{-1} 20 ul injection.	Inorganic Cr in NBS SRM 1571 Orchard leaves as $Cr(tfa)_3$ chelates.	After a H_2SO_4:O_2 digestion, Cr chelated with Htfa (0.1 ml) extracted with hexane (0.5 ml) prior to injection. Linear from 0.5 ppm to 5 ppm Cr. Detection limit 1 ng	Cr	146
Electrothermally heated silica furnace, see ref. 138, or directly coupled through the nebulisation chamber to an air/C_2H_2 flame, see ref. 140.	See ref. 141.	Tetraalkyllead compounds in petrol and air samples.	For atmospheric sampling see ref. 138. Linear up to 200 ng for furnace. Detection limit 0.1 ng for furnace system.	Pb 217.0 nm	147
H_2 diffusion flame burning in quartz cuvette. H_2 = 250 ml min^{-1}. Air = 150 ml min^{-1}.	6 m stainless steel column, 16.5% DC-550 on 80/100 mesh Chromosorb W AW DMCS; He = 80 ml min^{-1}.	Reducible As species in natural waters.	The hydrides of the As compounds isolated by cold trapping, passed down a column and into a furnace. Linear up to 50 ng. Detection limit 0.05 ng for AsH_3.	As 193.7 nm	148

Detector	Chromatography	Matrix	Comments	Element	Reference
See ref. 132.	80 cm x 6 mm i.d. glass column, 10% Carbowax 20M on Chromosorb W AW. 5 to 100 µl injections; T_I = 200°C T_c = 60° for Me_2Hg; 200°C for MeHgCl N_2 15 ml min^{-1} for Me_2Hg. N_2 = 200 ml min^{-1} for MeHgCl.	Me_2Hg, MeHgCl.	Detection limit 10 ppb Hg.	Hg 253.7 nm	149
Graphite furnace with pyrolytic or alumina lining or standard graphite tubes, at various temperatures with and without Ar/H_2 (90 + 10) flow (20 ml min^{-1}).	6' x 1/8" i.d. glass column, 5% SP2100 and 3% SP2401 on 80/100 mesh Supelcon AWDMCS T_c = 40°C Ar = 30 ml min^{-1} T_{in} = 100°C.	Me_3As, Me_4Sn and Me_2Se in N_2. To stimulate an atmosphere over a lake system.	Best detection levels achieved using standard graphite tubes with an Ar/H_2 flow at 1800°C. Linear up to 320 ng As, 313 ng Se, 363 ng Sn. Detection limits between 5 and 12 ng.	As Se Sn	150
Graphite furnace 2000°C. The furnace kept at this temperature throughout chromatographic run.	Teflon column, 8' x 1/8", 20% TCP on Chromosorb W, Ar = 30 ml min^{-1} T_c = 100°C T_I = 125°C T_{in} = 100°C.	Tetraalkyllead compounds in gasoline and the atmosphere.	TEL undetecteed in all 10 air samples. Detection limit 0.1 ng.	Pb 283.3 nm	151
Air/C_2H_2 flame; see ref. 146.	18" x 3 mm i.d. PTFE tubing, 5% SE-30 on Chromosorb P AWDMCS, 80/100 mesh. N_2 = 120 ml min^{-1} T_c = 160°C. T_I = 150°C.	Inorganic Cr in NBS SRM 1571 orchard leaves and SRM 1569 brewers yeast as chelates, also Co, Fe and Cu chelates.	The chelates determined were: $Co(fod)_3$ $Fe(fod)_3$ $Fe(tfa)_3$ $Cu(ofhd)_3$. Linear from 0.5 to 8.0 µg. Detection limits ranged between 1.0 ng to 500 ng.	Cr Co Fe Cu	152

Detector	Chromatography	Matrix	Comments	Element	Reference
Both a flame, air/C_2H_2, the effluent introduced through the nebuliser, and a graphite furnace at 1300°C.	20% SE-52 on Chromosorb W, Ar = 90 ml min^{-1} $T_C = T_I = 125°C$ $T_{in} = 130°C$.	Tetraalkyllead compounds in gasoline samples.	The furnace technique was 100X and 75X more sensitive than the flame coupling for TML and TEL respectively. Detection limits <u>Flame</u>: TML = 17 ng TEL = 81 ng <u>Furnace</u>: TML = 0.12 ng TEL = 1.1 ng.	Pb 283.3 nm	153
Hg compounds atomised in electrically heated quartz furnace at 620°C.	10% SP 2300 on Chromosorb W. N_2 = 90 ml min^{-1}. T_C = 145°C T_I = 200°C	Alkylmercury compounds in fish.	A rapid method for quantitative extraction of organomercury compounds from fish given. Linear up to 120 ng. Detection limit 3.5 ng.	Hg 254.0 nm	154
Electrothermally heated silica tube. See refs. 138 and 141.	See ref. 138.	Tetraalkyllead compounds in water, sediment and fish.	Extraction procedures for three sample types given. Detection limits water (200 ml) = 0.5 µg l^{-1}. Sediment (5 g) = 0.01 µg g^{-1}. Fish (2 g) = 0.025 µg g^{-1}.	Pb 217.0 nm	155
Graphite furnace atomisation at 1700°C.	150 cm x 6 mm i.d., glass column, 3% OV-101 on Chromosorb W, 80/100 mesh. T_{in} = 80°C T_C = 90°C then 40°C min^{-1} to 200°C. Or isothermal at 150°C.	Tetraalkyllead compound in air.	The Pb compounds from 70 l air samples were trapped at -72°C on the chromatographic packing. Detection limit 40 pg Pb.	Pb 283.3 nm	156

Detector	Chromatography	Matrix	Comments	Element	Reference
Various atom cells; air/C_2H_2 flame; flame and electrothermally heated quartz tubes, graphite cup and furnaces.	150 cm x 6 mm i.d. glass column, 3% OV-101 on Chromosorb W, 80/100 mesh N_2 = 140 ml min^{-1} T_c = 50°C then 40°C min^{-1} up to 200°C.	Tetraalkyllead compounds.	If T_{in} > 300°C decomposition of lead compounds occurred and interference from remobilization by the solvent resulted. Detection limit 30 pg with HGA2100 furnace.	Pb 183.3 nm	157
Graphite furnace atomisation (see ref. 151) at 1500°C.	18" x 1/8" i.d. Teflon column, 20% Ucon Non-Polar on Chromosorb P. Ar = 60 ml min^{-1}. T_c = 140°C T_I = 150°C T_{in} = 140°C.	TEL in sea water.	Some TEL migrates to surface and evaporates. The majority forms the soluble Et_3PbCl. Evidence of further degradation was found. Detection limit 1 µg ml^{-1}.	Pb 283.3 nm	158
Electrothermally heated silica furnace; see ref. 138.	See ref. 138.	TML in methylation of Pb (II) salts in aqueous solution.	Found a chemical methylation pathway for converting Pb(II) salts into methyl derivatives.	Pb	203
Electrothermally heated silica tube furnace; see refs. 155 and 141.	See refs, 155 and 141.	Tetraalkyllead compounds in fish, sediment vegetation and water samples.	Samples were analysed for total Pb, volatile Pb tetraalkyllead and hexane extractable Pb.	Pb 283.3 nm	159
Graphite furnace atomiser.	2.3 m x 6 mm i.d., 3% OV-101 on Chromosorb WHP, 80/100 mesh. T_c = 115°C T_I = 150°C T_{in} = 150°C N_2 = 80 ml min^{-1}.	MMT in air samples.	The air samples were collected (see ref. 108) at 70 ml min^{-1} for 8 hours. Detection limit 0.05 ng m^{-3}.	Mn 279.5 nm	160

Detector	Chromatography	Matrix	Comments	Element	Reference
Graphite furnace atomisation.	Same as ref. 159.	Determination of total, hexane extractable volatile and tetraalkyllead in fish, water sediment and vegetation samples. See ref. 159.	Coupling of chromatograph transfer line to the furnace was via friction fitted Ta connector (157). Detection limits 2 ppb hexane extractable, 0.5-1.5 ppb volatile, and 0.5 ppb tetraalkyllead.	Pb 283.3 nm	161
H_2 diffusion flame, samples introduced through nebuliser.	3' x 4.7 mm i.d. Polypenco Nylaflow tubing, Chromosorb 102, T_C = 23°C.	Determination of As, Ge, Se and Sn after hydride generation and cold trapping of hydrides.	Chromatographic separation allowed manual lamp change and monochromator change between peaks. The overlap of SeH_2 and S_4 required their separate detection. Detection limits ranged between 60 ng and 260 ng.	As 193.7 nm Ge 265.2 nm Se 196.0 nm Sn 224.6 nm	162
Graphite furnace atomisation at 2000°C. External gas flow of 0.9 l min^{-1}.	Glass column 180 cm x 2 mm i.d., 3% OV-101 on Gaschrom Q, 100/120 mesh Ar = 30 ml min^{-1} T_C = 50°C the 20°C min^{-1} up to 150°C T_{in} = 200°C.	TML and TEL in petrol.	Coupling via 1 m x 0.5 mm i.d. glass tube. Linear up to 50 ng. Detection limits 40 pg TML 90 pg TEL	Pb 283.3 nm	163

Detector	Chromatography	Matrix	Comments	Element	Reference
Graphite furnace atomisation; see ref. 115.	Same as ref. 163; samples desorbed from short glass column of chromatographic material at 90°C into chromatograph.	Tetraalkyllead compounds in air sampled for 1 hr at 6 l min^{-1}.	Pb compounds sampled onto glass beads at -130°C. Then transferred to a short column of chromatographic packing at -196°C. Detection limits TML = 0.1 ng m^{-3} TEL = 0.3 ng m^{-3}.	Pb 283.3 nm	164
Electrothermally heated quartz tube.	2 m x 6 mm i.d. glass column, 3% SE30 on Chromosorb GAW DMCS. For R = Me T_c = 120°C N_2 = 16 ml min^{-1} For R = Et T_c = 180°C N_2 = 50 ml min^{-1}.	Tetraalkyltin and alkyltinchlorides (R_nSnCl_{4-n}) R = Me and Et.	Owing to column rearrangements all four methyltin compounds cannot be examined. Passed column effluent directly to atomiser and also generated hydrides prior to atomisation. Linear up to 400 ng. Detection limits 1.0 ng for Me_4Sn 2.0 pg for Me_4Sn if hydride is atomised.	Sn 286.3 nm	165
Graphite furnace atomisation; see ref. 115.	Same as ref. 163.	Tetraalkyllead compounds in air (cf. 116), petrol (cf 115), river and rain water.	Degradation of TML and TEL in river water investigated. Detection limits TML = 0.2 µg l^{-1} TEL = 0.5 µg l^{-1}.	Pb 283.3 nm	166
Air/C_2H_2 flame; effluent from chromatograph introduced just below burner slot.	10' x 1/8" steel column, 20% Carbowax 20M on Chromosorb P N_2 = 120 ml min^{-1} T_c = 120°C T_I = 140°C T_{in} = 110°C 2 µl injected.	Tetraalkyllead compounds in petrol from a variety of sources.	Interface line was 4' x 0.02" i.d. stainless steel. Linear up to 400 ng for TML up to 1400 ng for TEL.	Pb 217.0 nm	167

Detector	Chromatography	Matrix	Comments	Element	Reference
Electrothermally heated silica furnace (see refs 138 and 141) at 850°C H_2 = 150 ml min^{-1}.	4' glass column 20% OV-3 on Chromosorb W, 80/100 mesh. N_2 = 80 ml min^{-1} T_C = 30°C for 3 min then 20°C min^{-1} up to 110°C. T_I = 85°C T_{in} = 65°C.	Methyltin compounds sampled from the headspace above sediment samples in a methylating environment.	Headspace sampling (see ref. 138). Experiments indicated Sn(II) was methylated by CH_3I but Sn(IV) was not. Detection limit 0.1 ng Sn.	Sn 224.6 nm	168
Electrothermally heated silica furnace; see refs. 138 and 141).	For chromatographic conditions see refs. 140, 141 and 142.	Methylated derivatives of As, Hg, Pb and Se.	Study of the effect of pH on methylation in the aquatic environment. Detection limits 0.1 ng of each element.	As 193.7 nm Hg 253.6 nm Pb 217.0 nm Se 196.0 nm	169
Graphite furnace atomisation; see refs. 163 and 164).	See refs. 163 and 164.	Tetraalkyllead compounds in the atmosphere. Samples taken from rural, urban and gasoline station environs.	Elevated levels of tetraalkyllead compounds were found around gasoline stations and in areas with heavy traffic. Linear up to 50 ng. Detection limits 40 pg TML 90 pg TEL.	Pb 283.3 nm	170
Electrothermally heated silica tube, see ref. 138.	180 cm x 6.4 mm, 3% OV-1 on Chromosorb HP 80/100 mesh. N_2 = 25 ml min^{-1} T_C = 70°C T_I = 150°C.	Tetraalkyllead compounds formed in study of methylation pathways in coastal sediments.	Reported that bioconversion of Pb(II) to TML unlikely in marine environments.	Pb 217.3 nm	171

Detector	Chromatography	Matrix	Comments	Element	Reference
Electrothermally heated quartz tube (cf. 141) at 980°C.	8 cm x 3.2 mm i.d. stainless steel column, Porapak Q 80/100 mesh. TML was trapped on column and flushed off with N_2 (150 ml min^{-1}) by placing the column in a toaster (cf. 141) at T = 235°C.	Determination of inorganic Pb in aqueous samples as tetramethyl derivative formed by methylation of the extracted dithiocarbamate complex.	Methylation was affected by methyl lithium and only a 50% conversion was achieved. Linear up to 200 ng. Detection limit 5 ng.	Pb 283.3 nm	172
Electrothermally heated quartz tube (see ref. 141).	1.8 m x 6 mm glass column, 3% OV-1 on Chromosorb W, 80/100 mesh. N_2 = 65 ml min^{-1} T_I = 180°C T_C = 90°C then 20°C min^{-1} up to 190°C T_{in} = 165°C.	Organotin compounds, $Me_nSn\text{-}Bu_{4-n}$ in water.	Tin compounds were extracted with a 0.1% tropolone in benzene solution from spiked water samples. Linear up to 33 ng. Detection limit 0.1 ng.	Sn 224.6 nm	173
Flame and a flame heated ceramic tube.	1.5 m x 4 mm glass column, 5% Carbowax 20M on Chromosorb 750, 80/100 mesh, $T_C = T_I = T_{in}$ = 159-175°C.	Tetraalkyllead compounds.	Various atom cells developed, and simplex optimised. Detection limit 17 pg Pb for most sensitive atom cell.	Pb 283.3 nm	33
Mo furnace surrounded by an alumina sleeve, heated at 250 K s^{-1} to 2473 K.	247 mm x 1.22 mm i.d. Mo column with a wall thickness of 0.81 mm. Carrier gas of either: Ar at 44.7 ± 2.1 µl s^{-1} or Ar + H_2 at 35 l ± 0.8 µl s^{-1} and 13.5 ± 0.4 µl s^{-1} respectively. T_C = 2093 K.	Na, Cu, Mn, Mg in inorganic salts.	Ar (3.8 l min^{-1}) and H_2 (1.2 l min^{-1}) used to provide an air free atmosphere around tube.	Na Cu Mn Mg	174

Detector	Chromatography	Matrix	Comments	Element	Reference
Modified form of flame AAS system used in ref. 33 to enable the use of Perkin Elmer burners requiring high gas flow rates.	1.5 m x 6 mm o.d. x 2 mm i.d. column packed with 10% OV-101 on Chromosorb W (80/100 mesh). Temp. programme 50-250 °C at 10 °C min^{-1}.	Ionic alkyllead compounds in water.	Problem of sample introduction into the atom cell overcome using commercially available open silica cell normally employed with the P.E. MHS-10 mercury/hydride system. Detection limits ng l^{-1}.	Pb 283.3 nm	175
Silica furnace consisting of an electrically heated quartz T-tube encased in a shaped firebrick. Assembly mounted in an aluminium cardle positioned within the optical beam of the spectrometer.	1.8 m x 6 mm glass column packed with 10% OV-101 on 80/100 mesh Supelcoport He flow rate 35 ml min l^{-1}. Temp. programme up to 250 °C.	Alkyllead compounds in environmental samples.	Furnace operating conditions - 900 °C and hydrogen makeup gas at 50 ml min^{-1}. Detection limits of about 30 pg, with claims of possible improvement by improving the chromatographic efficiency.	Pb	176
Flame AAS system based on ref. 33.	1 m x 6 mm o.d. x 2 mm i.d. glass column containing 3% OV-101 on Gaschrom Q (100/120 mesh).	Tetraalkyllead compounds in air.	Samples collected using cryogenic trapping at -196 °C then flash-heating.	Pb	177

2.3.6 Summary

Historically, the MIP has proved the most popular excitation source coupled with gas chromatography. This is probably a reflection of the MIP's ability to monitor certain non-metallic elements in addition to metals, and particular mention should be made of the ability of the helium MIP to monitor halogens. The only commercially available GC-MIP system unfortunately uses a low pressure plasma and thus has the attendant problems of vacuum lines and gas transfer from atmospheric pressure in the chromatograph to low pressure in the detector. The availability of the Beenakker TM_{010} cavity, which allows an atmospheric He plasma to be sustained may yield a more satisfactory GC-MIP coupling.

All the plasma emission detectors offer a multi-element facility and long linear ranges which make them attractive as GC detectors. Unfortunately the ICP, and to a lesser extent the DCP, involve high capital investment and high operating costs, so that coupling of these detectors to GC may not prove cost effective for all but the largest laboratories.

Atomic absorption detectors, whilst having relatively short working ranges, offer adequate sensitivity for trace metal speciation work. It is often quoted that flame atomisers do not offer such low detection limits as electrothermal atomisation. however, flame atomisers have been shown to give superior detection limits if the atom cell is carefully optimised. In addition, flame systems offer simplicity of design and have the advantage that the instrumentation is readily available in the majority of laboratories concerned with the analysis of metals. Directly coupled GC-FAAS techniques are now

used routinely in a number of laboratories.

CHAPTER 3

THE EVALUATION OF DIRECTLY COUPLED GAS CHROMATOGRAPHY-FLAME ATOMIC ABSORPTION SPECTROSCOPY

It can be seen from the review in Chapter 2 that a number of different interface designs have been published for directly coupled GC-atomic spectroscopy. This chapter evaluates the most successful of these couplings, utilising flame atomic absorption spectroscopy, for two very different applications. The first case describes the use of directly coupled GC-FAAS for the determination of alkyllead compounds. Although this particular application has been described in the literature, it has been extended here to form the basis of a forensic study looking at petrol residues on hand swabs. The second case described is the determination of tributyltin compounds in seawater. This application demonstrates the limitations of coupled GC-FAAS techniques for speciation studies involving compounds with unfavourable gas-solution partition coefficients.

3.1 Use of flame atomic absorption spectroscopy

The use and characteristics of flames used for absorption spectroscopy have been widely reported (178-180). Premixed flames are in the most common usage since they offer high temperature with relatively low background emission, have well documented chemical composition and are easily reproduced (181). For many elements the air/acetylene flame produces sufficient atomisation to enable good sensitivity with freedom from inter-element interferences. It is not just the enthalpy of the flame which decides the degree of atomisation but also the flame chemistry. This is demonstrated by the fact that the elements,

Bi, Cu, Cr, Ga, Sn and Sr, are atomised substantially more in the hot reducing nitrous oxide/acetylene flame and the cool hydrogen/air flame than in either the hot nitrous oxide/hydrogen flame or the cooler air/acetylene flame (182).

The theory of atomic absorption spectroscopy is detailed widely in the literature (183-185). Briefly, atomic absorption follows an exponential relationship between the intensity of transmitted light, I, and absorption path length, l , similar to Lambert's law in molecular spectroscopy

$$I = I_o \exp(-k_v\, l)$$

where I_o is the intensity of the incident light and k_v is the absorption coefficient at the frequency Y. For quantitative spectrosocpy the absorbance, A, is defined by:

$$A = \log(I_o/I)$$

thus we obtain the linear relationship:

$$A = k_v\, l \log e$$

$$= 0.4343\, k_v\, l.$$

It is possible to demonstrate (183) from classical dispersion theory that in practical terms k_v is proportional to the number of atoms per cubic metre in the flame, i.e. absorbance is proportional to analyte concentration.

Atomic absorption corresponds to transitions from low to higher energy levels, hence the degree of absorption will be dependent on the low level population. The proportion of excited to ground state atoms in a population at a given temperature can be considered with the aid of the well known Boltzmann relation:

$$\frac{N_m}{N_n} = \frac{g_m}{g_n} \exp - \frac{(E_m - E_n)}{kT}$$

where N is the number of atoms in an energy state E_n or E_m, g is the statistical weight for a particular state and k is the Boltzmann constant. The population of the ground state is generally much greater than that of higher energy levels and as a result absorption is greatest for transitions from the ground state. (i.e. resonance lines)

Since the width of atomic lines is typically about 0.002 nm, the amount of radiation isolated by a conventional monochromator is not significantly reduced by the narrow absorption signal if a continuum source is used. The considerable contribution made by Walsh (186) to atomic absorption spectroscopy was to use a line source. Since absorption and emission lines have the same wavelength the narrowness of the absorption line is a positive advantage. Thus overlap of an absorption line of one element with an emission line of another is negligible and, hence, only resolution from other lines in the lamp, e.g. filler gas lines, is required. This 'lock and key' mechanism is responsible for the greater selectivity of AAS over AES.

3.1.1 The coupled gas chromatography - flame atomic absorption spectroscopy system

The coupled GC-FAAS system used in the following applications is based on the technique described by Ebdon et al. (33). In essence the technique employs a conventional gas chromatograph which is coupled to a conventional atomic absorption spectrometer by a readily demountable interface. Effluent from the chromatograph is led via a heated glass-lined metal-tube to a small 'T' piece. Here an auxillary flow of

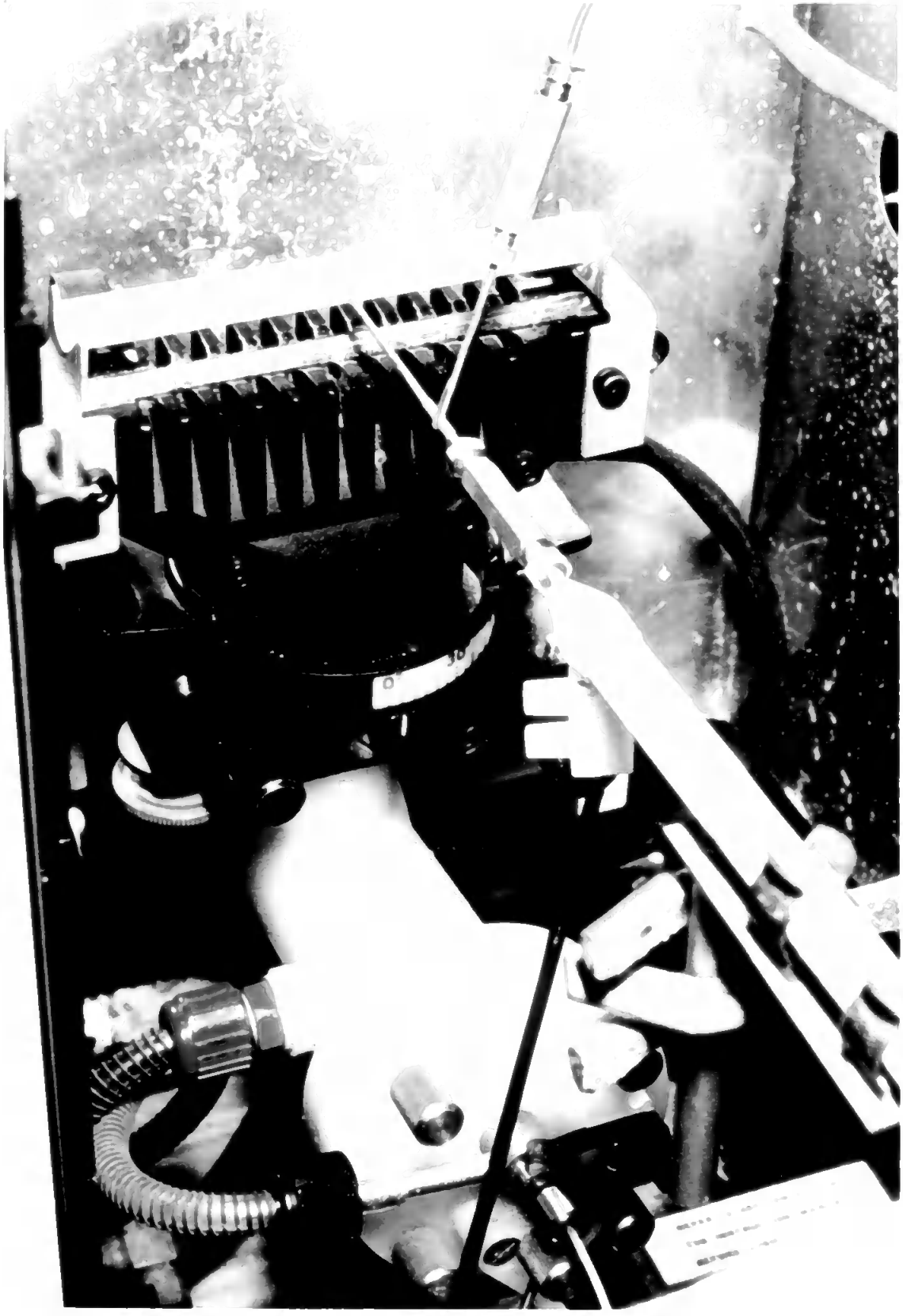
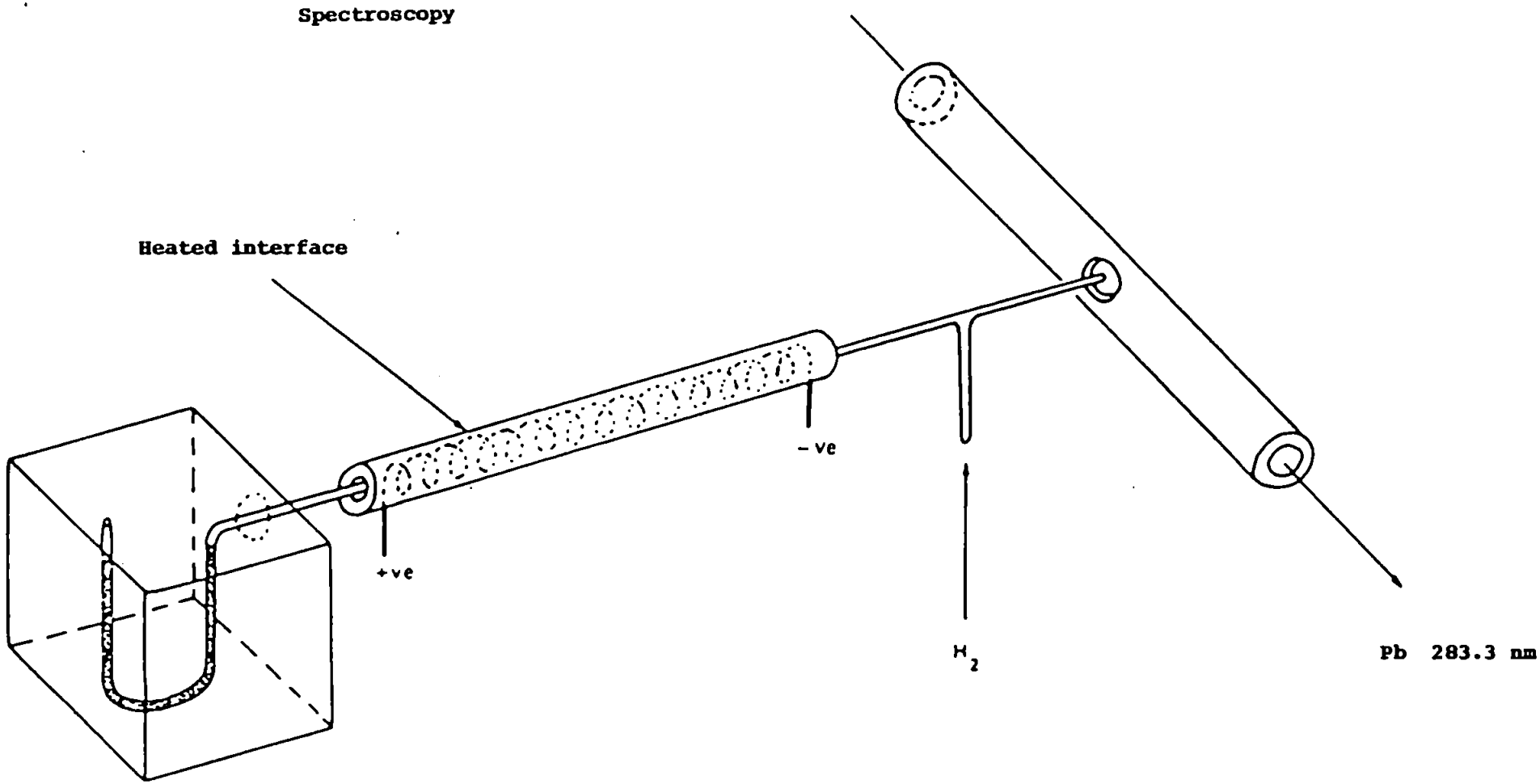


Plate 1

Interface for directly coupled gas chromatography - flame atomic absorption spectroscopy

Figure 3

Interface for Coupled Gas Chromatography - Atomic Absorption Spectroscopy



hydrogen is introduced to produce a miniature hydrogen diffusion flame which burns at the end of the interface tube and atomises the effluent. The atoms are immediately swept into a ceramic tube suspended above and heated by a conventional air/acetylene flame see Plate 1 and Figure 3. Using this arrangement the air/acetylene flame serves the dual purpose of keeping the ceramic tube hot and thus preventing condensation in the tube, and keeping the hydrogen flame alight. It does not however serve to atomise the sample. The axis of the tube is aligned in the light path of the spectrometer and the resultant atomic absorption signals monitored.

Since organometallic species can be selectively and specifically detected using this technique, the system was evalauted for two very different applications - (i) the identification of lead alkyl compounds extracted from hand swabs of individuals with previous contact with petrol (Section 3.2) and (ii) the determination of organotin species in seawater (Section 3.3)

3.2 Application to the determination of tetraalkyllead compounds on hands after contact with petrol

Additives to petrol can be classified into two major categories. Possibly the most important of these are the tetraalkyl compounds of lead which are added to petrol to improve its octane rating. The actual mixtures used vary widely depending on the individual batch of crude oil and the requirements of the refined product. The tetraalkyl leads are usually adjusted to give an even octane rating across the petrol boiling range, although the maximum level permitted by legislation varies from country to country. Of the five tetraalkyllead compounds, tetramethyllead (TML), ethyltrimethyllead

(ETML), dimethyldiethyllead (DEDML), triethyldimethyllead (MTEL), and tetraethyllead (TEL), only TML and TEL are usually found in British petrols. Since these compounds are virtually unique to petrol, their presence can be utilised for the unequivocal determination of petrol residues. The application described here is for the determination of petrol residues collected on hand swabs. Obviously such an application has direct forensic applications, although a suitable analytical technique capable of measuring the lead alkyl distribution in petrol is also of value in helping to identify spillages and leakages, predicting petrol properties by compositional analysis, calculating their effect on sensitivity (difference between the motor and research octane ratings), and quality control in blending (125). The second group of additives, the so-called 'scavengers' - organohalides which act as engine cleaners by preventing the build up of PbO_2 deposits are not of interest in this investigation.

Until the introduction of coupled GC-AAS techniques the satisfactory determination of individual lead alkyls was a lengthy process. The one standard method that exists (IP 188/66) works on separating the sample into two fractions by distillation after dilution with a xylene-toluene mixture (187). The distillate which distils below 133 ^{o}C, contains the tetramethyl lead and the residue contains the tetraethyl lead.

Coupled GC-AAS involves no lengthy preparation prior to analysis, and has in addition many other advantages, such as increased sensitivity, specificity for lead, complete resolution of TML and TEL, speed of analysis and freedom from interferences.

3.2.1 Experimental

Instrumentation

Atomic absorption spectrometer (SP9, Pye Unicam, Cambridge) fitted with background correction and lead hollow cathode lamp. The 283.3 nm lead line was used. Gas chromatograph (series 104, Pye Unicam, Cambridge) fitted with glass column packed with 5% carbowax 20 M on chromosorb 750 (80-100 mesh), column temperature 160°C. The interface 0.76 mm i.d. glass lined tubing (Phase Separation, Queens Ferry, Clwyd), was resistance heated.

Method

Before beginning work on the detection of tetraalkyllead compounds in the hand swab extracts, a number of preliminary experiments were made to investigate a range of possible solvents and the efficiency of the extraction technique.

Choice of solvent

Four solvents were selected and used to extract standard solutions of 1 and 2 ppm TML/TEL solutions (1 ml) from defatted cotton wool swabs. The solvents used were:

1. isooctane;
2. pentane;
3. hexane;
4. chloroform.

Extractions were made using 2 x 5 ml of solvent and then rotary evaporated down to 0.5 ml before injection into the instrumentation. Each extraction was repeated three times and the results obtained compared with those of standard injections.

Extraction efficiency

The solvent selected as offering the best recovery of TML/TEL was used

for all further work. However to determine the least amount of solvent required to extract the maximum amount of lead, five different extraction methods were used.

A. 5 ml + 5 ml extraction - 1 ppm TML/TEL spike

B. 5 ml + 3 ml extraction - 1 ppm TML/TEL spike

C. 3 ml + 2 ml + 2 ml extraction - 1 ppm TML/TEL spike

D. 3 ml + 2 ml extraction - 1 ppm TML/TEL spike

E. 2 ml + 2 ml + 2 ml extraction - 1 ppm TML/TEL spike

Again each method was repeated three times and the efficiency of each extraction calculated by comparison with a 3 ppm standard injection.

Hand swabs

Before beginning the work on petrol a series of tests were made to invesigate the possibility of interference due to the solvent removing other compounds from the hands. The solvents used were:

a. isooctane; b. pentane; c. acetone.

Each swab was prepared by taking a wad of defatted cotton wool (1 cm^3) moistened with solvent and extracting from the palm of the hand.

The technique was further developed by spiking the hands with 1 ml of TML/TEL solution (10 ppm). Initially swabs were taken 10 minutes after application but this period was extended to several hours in later analysis.

Finally the hands were spiked with two star petrol allowing periods of up to seven hours before extraction. During these experiments investigations were also made to examine the effects of hand washing on the detection levels. Quantification of the results obtained in these tests were facilitated by the use of CR50 (a catalytically

reacted equimolar mixture of TML and TEL containing all 5 tetraalkyllead compounds mentioned above supplied by Associated Octel, Bletchley) as an internal standard.

Since deterioration of the tetraalkyllead compounds after extraction may also be an important factor - both (a) on the swabs before solvent extraction and (b) during storage in the vial before injection into the GC-AAS, these factors were examined. A series of swabs were prepared by extraction 3 hours after spiking the hands with petrol (1 ml). These were then left in sealed containers for 3 days before extracting the TEL with solvent for analysis. The results obtained were then compared with those of a similar experiment in which the solvent extraction of TEL was made immediately after preparing the swabs. To examine the effects of storage on the final extracts, samples were kept for a number of days after the initial analysis and then reinjected so that the results obtained in both sets of injections could be compared.

3.2.2 Results and discussion

It was found from the investigations on various solvents that maximum extraction of the tetraalkyllead from the swabs was achieved using iso octane. Although the extraction efficiency of each solvent was not quantified rigorously, a comparison of the areas obtained for similar injections (1 μl) of each sample was made with that obtained with a direct injection of TML/TEL standard solution (1 ppm). The results indicated almost 100% extraction of TEL with isooctane compared to ~ 60% extraction for hexane and less for pentane and chloroform. The extraction of TML was found to be lower in all cases possibly due to its volatility. The second experiment on extraction technique

suggested that by using a 3 ml + 2 ml + 2 ml extraction instead of the initial 5 ml + 5 ml extraction, evaporation time could be reduced and less solvent used, although the extraction efficiency was reduced by some 18%. Although evaporating the solvent down to 0.5 ml from 7 ml should increase the concentration this is to some extent affected by evaporation of the analyte. Using CR50 as an internal standard this latter technique indicated increases in concentration of 79% for TEL and 19% for DMDEL. TML however showed a 39% decrease indicating the greater volatility of the lower molecular weight components.

During the initial studies the hands were not washed in the period prior to taking the hand swabs. The first attempts to quantify the TEL after introducing hand washing however, resulted in poor results compared with those of unwashed samples. This was overcome by further concentrating the sample using nitrogen blowdown. The total volume of extract being reduced from 500 to 100 µl. This refinement allowed easy definition of the TEL peak (retention time 0.53 minutes) and reduced baseline noise - Figs 4 and 5. CR50 was again used to quantify the results and showed increases in concentration of 41% TML, 57% DMDEL and 110% TEL, i.e. some evaporation losses were obtained but there was still a net gain in concentration. Thus the complete extraction procedure gave a concentration factor of 18.5 for TEL allowing increased sensitivity - Fig. 6.

Investigations of hand extracts using iso-octane, pentane and acetone as solvents showed little evidence of interference on the AA signal at 283.3 nm. Later tests using 1 ml spikes of TEL/TML solution (10 ppm) gave good detection for TEL in all samples for up to seven hours (the longest period examined), and so were followed by more quantitative

Figure 4

Chromatograms after rotary evporation to 0.5 ml

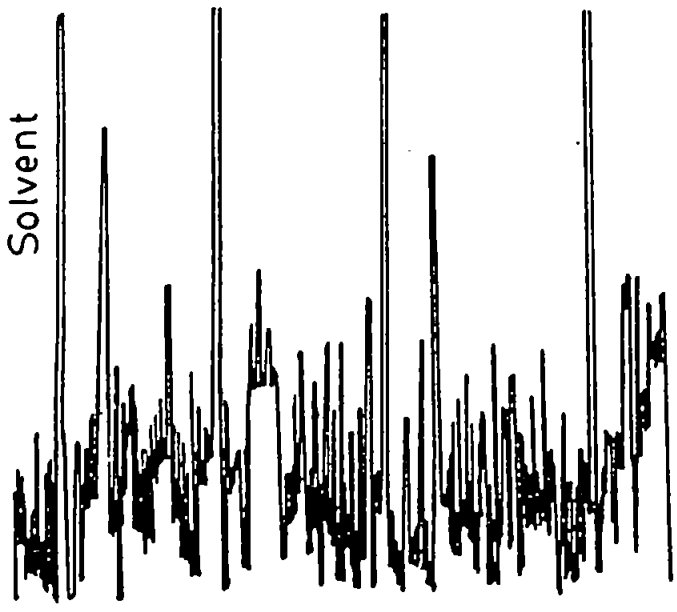


Figure 5

Same extract after N_2 blowdown to 100 µl

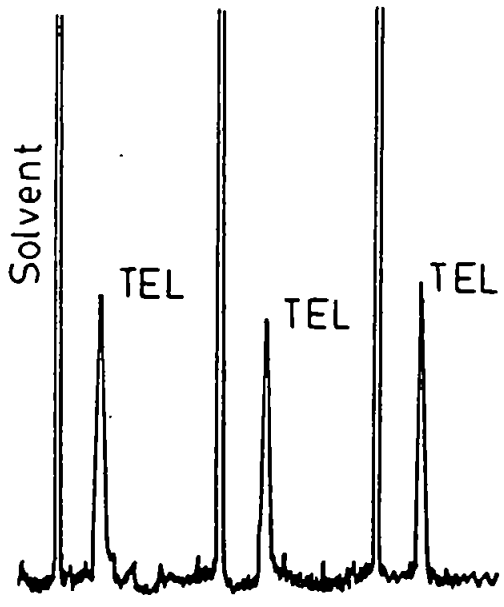
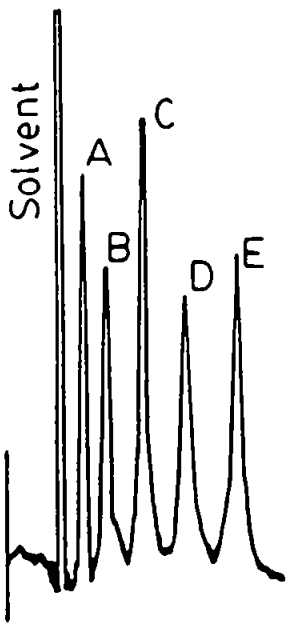
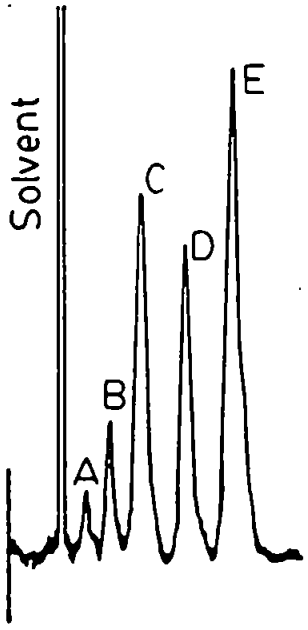


Figure 6

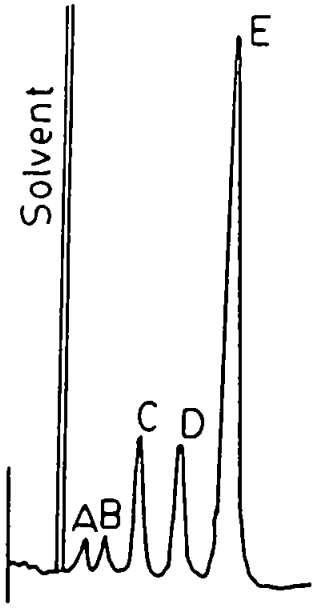
Gas chromatograms showing the concentration of tetraalkyllead compounds in CR50 during the extraction procedure



1 ppm CR50



Extract after rotary evaporation



Extract after N_2 blowdown

A Tetramethyllead

B Trimethylethyllead

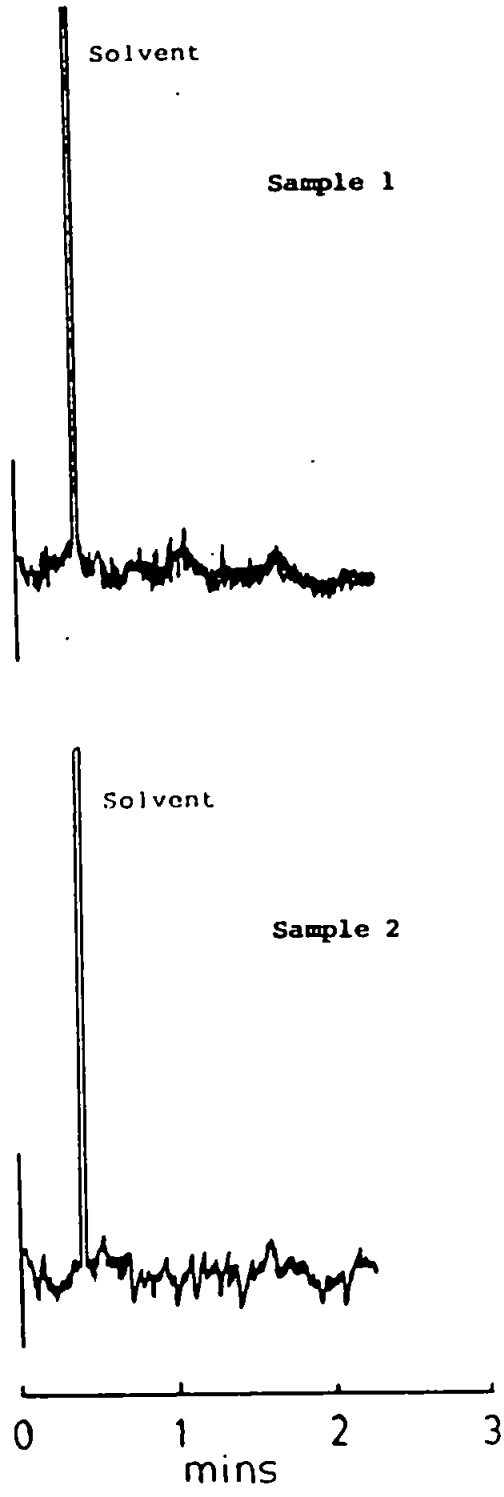
C Dimethyldiethyllead

D Triethylmethyllead

E Tetraethyllead

Figure 7

Gas chromatograms from swab extracts taken after filling a car petrol tank



work using two star petrol. Here again good detection was possible for periods of up to seven hours, the results indicating between 0.3 and 1.8 ng of TEL in eight samples after six hour period (limit of detection 17 pg). TML was not detected although this was expected from earlier work which had indicated TML is lost rapidly by volatisation or even possibly through the skin.

The deterioration of samples left for a period of 2-3 days before extraction was not found to be a problem (longer periods may also be possible). Swabs taken 3 days prior to extraction gave similar levels of TEL to those with immediate extraction. Samples once extracted also kept well on storage for up to five days (longer periods may again be possible but were not investigated). Amounts detected by GC-AAS in these tests were consistent with those of freshly prepared extracts.

The final experiment investigated possible contamination of the hands with tetraalkyllead due to contact with petrol via filling up a car petrol tank. Swabs taken 2 hours after contact gave no detection of TEL - Fig. 7.

This study was later extended to include a 'blind test' of swabs - (spiked with known amounts of petrol) supplied by the Central Research Establishment Home Office Forensic Science Service. All swabs spiked with petrol were correctly identified, the levels determined by about 60% of the original spike values which was considered satisfactory considering the losses due to volatisation and extraction. The technique thus proved superior to conventional gas chromatographic analysis with flame ionisation detection which also responds to

various other hydrocarbons on the hands when used for this particular forensic application.

3.3 Application to the determination of organotin compounds

During the last ten years there has been an increasing demand for analytical techniques capable of speciating organotin compounds. More is now known about the marked differences in toxicity of the various organotin compounds according to the variation of the organic moiety in the molecules and this has stressed the importance of speciation studies. The presence of n-butyltin (188, 189) and methyltin (189-191) species at concentrations in the ng/l to µg/l range have been reported in a variety of natural waters as well as rain. This is probably due to the increased use of organotin compounds as stabilizers for polyvinylchloride, catalysts, and in pesticide preparations. (192) In addition the recent controversy concerning the use of tributyltin compounds in antifouling paints, which may lead to legislation on its use, has stimulated interest in developing new techniques capable of detecting such species at environmental levels. A more detailed discussion of the chemistry of organotin compounds and the particular problems associated with their determination is given in Chapter 5. This section however evaluates the use of coupled GC-AAS for the direct determination of organotin compounds, with particular emphasis on the determination of tributyltin compounds in sea water.

Several methods have been reported to separate and detect methyltin species. One technique commonly used is the conversion of such species to volatile hydrides (see Chapter 5) that are then separated by their boiling points (188,190,191). In another method used by Chau

(193) the methylated tin species is first extracted with benzene using tropolone as the complexing agent, butylated, and finally separated by GC. In further work, Burns et al.. (194) reported that mono- and dimethyltin chlorides could not be separated by GC. They also observed on-column rearrangement of the methyltin species present in a mixture, and found that monomethyltin trichloride or tetramethyltin should be absent in order to avoid redistribution. Maguire et al.. (189) determined n-butyltin species by GC of the volatile n-pentyl derivatives (Bu_n Pe_{4-n} Sn) with detection by a modified flame photometric detector. This technique gave detection limits of about 100 pg with good reproducibility of peak area with multiple injections. However, it has since been shown that the detector response is severely diminished by injections of large amounts of (i) organotin compounds, i.e. more than 100 ng, (ii) tropolone, which is used in extracting butyltin and Sn^{4+} species from water (195), and (iii) organic co-extractives from natural waters and sediments. These difficulties can usually be avoided though a judicious choice of concentration of butyltin species to inject for GC, or the use of a silica gel column to remove tropolone and organic co-extractives from water and sediment; however the inadvertant "poisoning" of the flame photometric detector necessitates a time consuming disassembly of the detector and removal of a white powder (presumably SnO_2) by mechanical and chemical means from accessible metal and optical surfaces followed by repeated injections of chlorofluorocarbons (191,193) at 250°C to restore the sensitivity of the detector. The whole process has taken as long as 3 weeks (196).

3.3.1 Determination of tributyltin chloride in seawater

The techniques described above all require some form of sample

pretreatment prior to analysis. However a more ideal system would enable direct injection of sample without such lengthy preparation stages, and so the coupled GC-AAS system previously described (Section 3.1.1) for organolead compounds was evaluated for the direct analysis of tributyltin chloride (TBTC). A number of different GC packing materials were examined, Table 6; however it was found that the use of coupled GC-AAS was not really compatible with such compounds as tributyltin chloride due to its low volatility and tendency to be retained by the column. This tendency to 'plate out' in the column was also observed in the results obtained using a microwave plasma detector coupled to a gas chromatograph (Applied Chromatography Systems Model 850 Helium Microwave Plasma Detector, Luton England, coupled to a Pye Unicam Series 104 gas chromatograph, Cambridge, England). Here it was found that when using Dexsil 300 (3%) on Chromosorb W HP and monitoring the Cl and Sn channels, detection of the TBTC was masked by the solvent front when using chloroform to prepare the samples. Hexane proved more suitable, although the detection limits obtained were poor, and gave little linearity with variations in injection size. A double peak was also observed for tin at higher concentrations indicating redistribution on the column. At low temperatures the TBTC was totally retained by the column. The published chemistry of organotin supports these findings and augmented the idea of changing the technique to coupled HPLC-AAS.

The above work was however utilised forming the basis of a chromatographic analysis using GC with a flame ionisation detector (FID) to detect TBTC in local harbour water. The method used a glass column packed with 3% SE30 on Chromosorb G (AW.DMCS) for the separation, and gave evidence of 0.75 $\mu g\ l^{-3}$ TBTC in the samples

Table 6 **Gas chromatographic column packings examined for use in the determination of tributyl tin compounds**

	Packing material	Chromatographic conditions	Remarks
1.	3% OV101 on Chromosorb 750	Isothermal runs tried between 130-170°C Carrier gas - N_2 Flow rate varied between 30-60 ml/min 1 µl sample.	Peaks very broad with long retention times (over 1.5 hours at 170°C.)
2.	2.5% XE60 on Chromosorb G	Temperature programme 150-250°C at 10°C/min. Carrier gas N_2 Flow rate 30 ml/min 1 µl sample.	Good separation from solvent front in 3-4 mins at 200°C. With higher temperatures peaks merge with solvent front. Severe peak tailing with lower temps. Column very difficult to condition.
3.	3% Dexsil 300 on Chromosorb G	Temperature programme 150-300°C at 10°C/min. Carrier gas N_2 Flow rate 30ml/min. Also isothermal run at 300°C	Offered best resolution of the columns tried although columns tend to be difficult to condition leading to broad peaks and severe tailing
4.	3% SE-30 on Chromosorb G	Isothermal runs at 120°C Carrier gas N_2 Flow rate 30 ml/min 1 µl injections	Good resolution from solvent front and good reproducibility Selected for use with the GC-FID analysis of TBTC in seawater

[All columns glass 1.5 m x 3 mm]

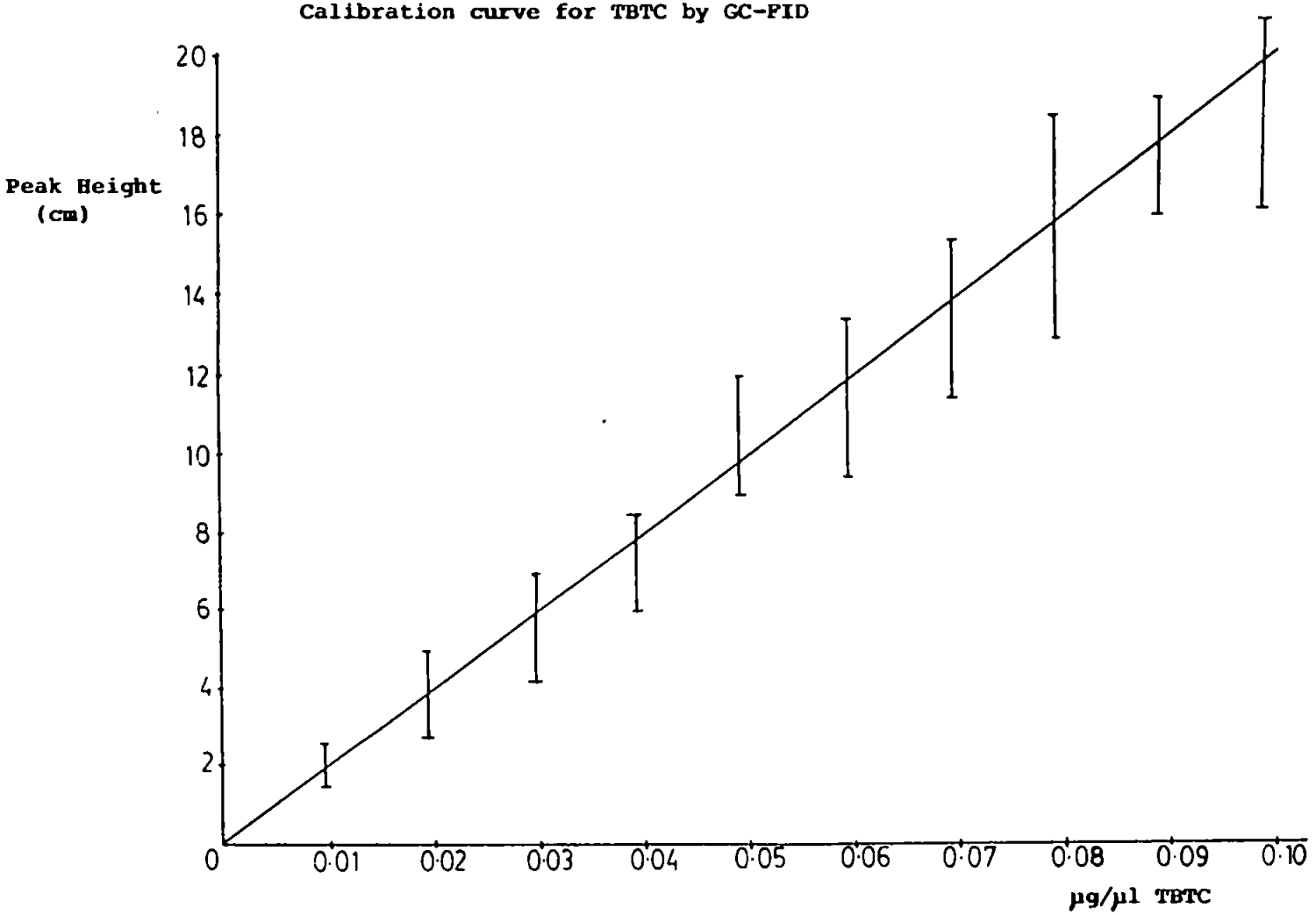
taken, although problems were encountered due to fouling of the FID by tin oxide as recently reported by Maguire and Tkacz (196) and poor reproducibility from repeat injections. The calibration curve obtained using this method is shown in Fig. 8. Such a system also lacks the element specific detection and sensitivy shown to be possible with coupled chromatographic - AAS techniques for some species of organotin (193,194), although identification of tributyltin species have not been reported using these techniques.

3.4 Limitations of coupled gas chromatography - atomic absorption spectroscopy

Although the use of GC coupled with highly sensitive element specific detectors has proved effective where thermal stability and favorable gas-solution partition coefficients exist, often this is not the case. In many circumstances, the true identity of metal containing metabolites or anthropogenic materials cannot be reliably inferred, nor can they be adequately quantified by GC-AAS, owing to their strong solvation in aqueous, lipid or tissue phases. Several isolation procedures have been developed in which, usually either by chelation (197), or reductive cleavage (198,199) of small organometallic moieties from retentive donor sites, "characteristic" analytes of sufficient volatility are derived for GC-AAS determination. Recoveries may be variable, but more importantly in the methods mentioned, original oxidation states or co-ordination numbers of metals can be significantly altered. Moreover, evidence exists (2 , 3, 4) suggesting that for some metals specific biotransformation, will occur stepwise to produce ionic intermediates. Consequently, future assessment of the primary fate or kinetically important forms of certain metals in environmental media require means for their direct

Figure 8

Calibration curve for TBTC by GC-FID



speciation as trace reactive intermediates, probably occuring in their most polar (ionic) forms as solvates in liquid samples. Such analysis is potentially possible using coupled high pressure liquid chromatography - atomic-absorption spectrometry (HPLC-AAS).

One of the significant advantages of HPLC over gas chromatography is that the columns are operated at ambient temperatures, thus permitting the elution of thermally unstable substances such as biologically active materials, high molecular weight organic compounds, polymers, and metal-organic compounds. Another important advantage of HPLC is the availability of a variety of separation modes such as liquid-liquid partition, liquid-solid adsorption, reversed-phase partition, ion exchange, and size exclusion. Another fundamental difference between the two techniques is that in gas chromatography the mobile phase is always inert whereas in HPLC the eluent may range from being relatively inert to being highly selective in effecting column separations. The interchange of solvents in HPLC therefore often provides added experimental resolving capabilities which are not available in gas chromatography. Also, recent column technology advances have made it possible to use very small support particles (tyically in the 5-10 μm diameter range) for achieving high efficiencies comparable to gas chromatographic columns.

CHAPTER 4

COUPLED HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

4.1 Separation of organometallics by high performance liquid chromatography

The rapid development of efficient high-pressure liquid chromatographic (HPLC) separation techniques over the past decade has provided a desirable method for application to the speciation of trace organometallics. The basic theory behind the separations obtained using this technique has been well documented in the literature (203, 204). The following summary however outlines the key practical considerations to be borne in mind when using this technique.

The retention of a sample component, or peak, can be expressed in terms of volume (or time), with respect to a non-retained component. If the elution volume of the non-retained component is V_0 (or time T_0) and that of the sample component is V_r (or T_r), then the capacity factor (k'), may be expressed as

$$k' = \frac{V_r - V_0}{V_0}$$

At constant flow, this is equivalent to

$$k' = \frac{T_r - T_0}{T_0}$$

This value, k', represents the ratio of the amount of a constituent in the stationary phase to the amount of the constituent in the mobile phase, thereby quantifying retention. Capacity factor (k') for a given substance is a complex function of the relative affinities of that

substance for the stationary and mobile phases and the capacities of the stationary and mobile phases in the column. Capacities may depend on a number of characteristics of the column and the stationary phase, including the fraction of the column volume occupied by the mobile phase and the surface area of the stationary phase.

The selectivity of a column for two different solutes is given by a. Selectivity is a physico-chemical factor, and reflects the composition and nature of both the mobile phase and the stationary phase.

$$a = \frac{k'_2}{k'_1}$$

Two components must have differing capacity factors, or retention times, in order to be separated. Perhaps the most important factor in determining column performance is its efficiency. Efficiency is the ability of a chromatographic system to maintain sharp peaks. Sharp peaks result when there is minimal dilution of sample constituent zones (bands) as they pass through the column, relative to the time spent in the column. An efficient system produces minimal zone dilution (or band-broadening) and therefore, sharp peaks. Broad peaks result if there is excessive dilution of sample constituent zones as they pass through the column. A system which produces broad peaks has poor efficiency. Efficiency is measured as the number of theoretical plates (N) of the system (see Section 2.1). For a given column length a system which produces sharp peaks (good efficiency) has a large number of theoretical plates. A system which produces broad, diffuse peaks (poor efficiency) has a small number of theoretical plates. In measuring the efficiency of a chromatographic system, it is normally assumed that the volume of sample injected at the column inlet is a small fraction of the volume of a constituent peak as measured by the

detector after passing through the column. In practice, this condition is usually met. However, it should be remembered that injecting too large a sample volume will result in broader peaks than predicted by the number of theoretical plates. Also, in expressing the efficiency of a chromatographic column, it is usually assumed that in the system used to measure efficiency of the column, only the column itself produces band-broadening. In fact, this is almost never true. The injector, system plumbing, and detector always make finite contributions to the width of a constituent peak. With care, these extracolumn effects can be minimized so the measured efficiency approximates the efficiency of the column itself. However, the measured number of theoretical plates will always be lower than the true number of theoretical plates for the column. Extracolumn effects also have a greater effect on measured theoretical plate numbers for low k' peaks than for high k' peaks.

Band-broadening in a chromatographic column results from a variety of causes including: diffusion, non-uniform flow patterns within the packed adsorbent bed, and limitations in mass transfer rates of molecules entering and leaving the stationary phase. Assuming that retention of a substance on a column occurs by a single mechanism, and that events leading to band-broadening occur randomly, statistical theory predicts that constituent peaks emerging from the column will have a symmetrical Gaussian shape. Peaks obtained from high quality HPLC columns will normally approximate this Gaussian shape, and for such peaks the number of theoretical plates can be calculated using the expression

$$N = \frac{5.54\ t_R^2}{w_{1/2}^{\ 2}}$$

where N = number of theoretical plates for the column

t_R = retention time

$w_{1/2}$ = width of peak at half height (in same units as time)

Thus, when comparing columns of the same length, N is a measure of the ability of the column to make sharp peaks since, for any given value of t_R, a narrower peak will have a smaller $w_{1/2}$ and therefore a higher calculated value for N, Fig. 9. Efficiency is sometimes expressed as theoretical plates per unit length. This is simply the value of N divided by the length of the column usually expressed per centimetre. (However care should be taken to express efficiencies in the same way when comparing different HPLC columns). When theoretical plates per metre are given, this value must be multiplied by the length of the column in metres to obtain the number of theoretical plates for the column.

The resolution of a column R_s, is the amount of separation between two adjacent peaks. It is a function of column efficiency, selectivity, and capacity factors for the compounds. Expressed as

$$R_s = (1/4)(a - 1)(N)^{1/2} \frac{k'}{1 + k'}$$

where

k' is the average value for the two peaks.

Peaks with R_s values greater than 1.25 are generally well resolved, while smaller values indicate overlapping peaks. Resolution requires differences in retention, therefore, if two components have the same retention, they cannot be separated, no matter what the efficiency of the column. A more efficient column will simply give one very sharp peak containing two unresolved substances, whereas a less efficient

Figure 9

Determination of the number of theoretical plates for the column

$$N = \frac{5.54\ t_R^2}{W_{1/2}^2}$$

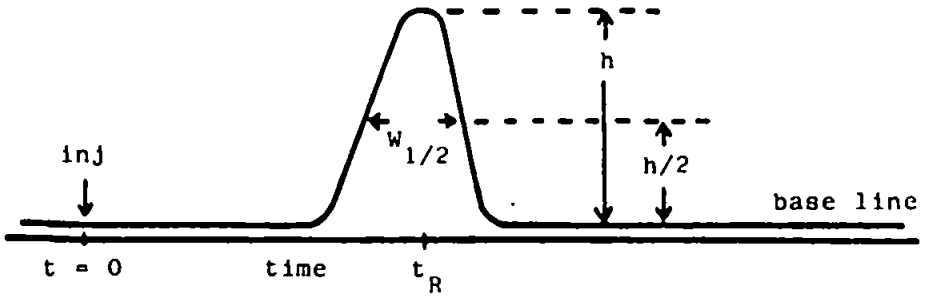
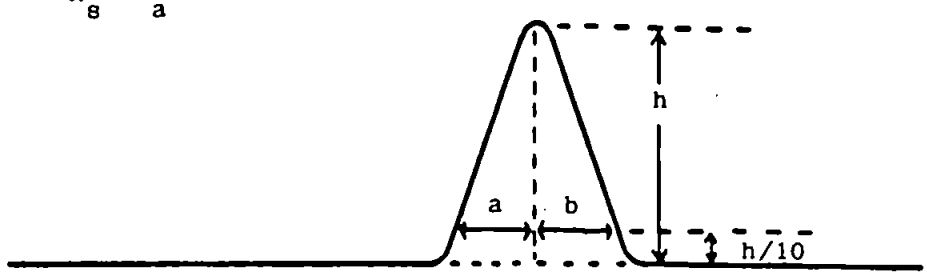


Figure 10

Determination of Asymmetry Factor

$$A_s = \frac{b}{a}$$



column would give one rather broad peak. However, if two constituents are only partially resolved on a column with lower efficiency, they may be completely resolved on a column with higher efficiency (assuming there is no difference in selectivity between the two columns). Also, where a short column is desired in order to increase the speed of separations, the highest efficiency short column will give the best chance of resolving the constituents of interest. Selectivity is also an important parameter in determining resolution, and may be easily altered by changing mobile phase constituents or column packings, since resolution increases linearly with a, but increases only as the square root of the efficiency. Thus, a solvent or pH change may be the best way to improve the resolution of peaks that are only partially separated.

The asymmetry factor, A_s, is a measure of peak shape defined in Fig. 10. For a symmetrical peak, $A_s = 1.0$. A tailing peak will have a value of A_s greater than 1.0, while a fronting peak will have a value less than 1.0. Assymetrical peaks indicate that the behaviour of the column is deviating from a set of linear sorption isotherms. The most frequent cause of this is mixed-mode behaviour of the adsorbent. Asymmetrical peak shape will decrease the resolving power of the column since the tail of the peak may interfere and overlap with other peaks.

Although many publications have now appeared devoted to the particular application of HPLC to the separation and analysis of organometallic compounds (205, 206), until the late 1970's HPLC had been applied almost exclusively to the separation of organic and biochemically active compounds. However, since HPLC columns are usually constructed

of stainless steel, (sometimes lined with PTFE in trace analysis to overcome problems from leaching), the compounds of interest are isolated from the atmosphere and light, and can be separated with degassed, inert mobile phases at ambient temperatures. The use of HPLC for determining inorganic metal complexes which are often unstable is therefore obvious, and HPLC has now been shown to be an effective means for the separation and determination of many different types of coordination complexes and organometallic compounds (207).

4.2 The need for element specific detectors

In general HPLC offers acceptable recoveries of nanogram amounts of eluents, provided an appropriately sensitive detector is available. Many types of detectors have been coupled to HPLC which offers prospects for trace detection of metal containing eluents. These include UV absorption (208), fluorescence (209), electrochemical (210), chemiluminescence (211), mass spectrometric (212), flame ionisation (213), electron capture (214) and electron spin resonance (215) schemes. However, all suffer somewhat in adequately meeting several of the criteria cited for the GC element specific detector case above, and notably, all fail to satisfy the need for unambiguous element selectivity.

A number of criteria may be used for the characterisation of a good HPLC detector. Firstly, the noise level governs the detection limit available with a particular detector. A chromatographic peak can only be recognised as such if its height is at least twice that of the highest noise peak. In addition to purely electrical sources, air bubbles and impurities in the eluent may also cause noise. A drift in the baseline is also undesirable. The primary causes here are slow

changes in the ambient temperature, the flow rate, or stripping of the stationary phase from the column.

In considering the sensitivity, distinction must be made between the absolute and the relative sensitivity of a detector. The former is a function of the instrument design, the measuring technique employed, and the noise level; the latter depends on the amount of a certain substance that is just detectable under a definite set of chromatographic conditions (216). The sensitivity is one of the most important characteristics of a detector. However, there are other factors to consider, such as band spreading in the detector, dependence of the response to external parameters, and the convenience of servicing. For quantitative analysis, the linearity of the response plays an important role.

As with the coupled GC-AAS system described in Chapter 3, the use of atomic spectroscopy for detection in HPLC meets many of the above criteria, offers element specific detection and reduces the constraints placed on the chromatography. However interfacing the two techniques presents a number of problems of sample introduction since the eluent is not so readily introduced into the atom cell. Various methods for overcoming these difficulties have been reported and these are reviewed in the next section.

4.3 Review of existing techniques for directly coupled high performance liquid chromatography - atomic spectroscopy and their applications

While gas chromatography has been coupled with analytical atomic spectroscopy for trace metal speciation it is limited to volatile and thermally stable organometallic species or metal chelates. The use of liquid chromatography considerably expands the type of chemical and physical species which may be studied. The separation of ions, and involatile high molar mass organometallic species, in addition to volatile species, is possible using one or other of the popular LC configurations. Adsorption, ion-exchange, gel-permeation, normal and reverse phase chromatography have all been used in conjunction with atomic spectroscopy.

The coupling of LC with atomic spectroscopy has been reviewed in a number of publications (217 - 219), and frequently the complication of atomising large volumes of a liquid mobile phase is noted. Thus the atom cells used, e.g. flame, furnace or plasma, must be capable of handling solvent flows, typically 0.1 - 4.0 ml min^{-1}, which may be aqueous or organic in nature. The following review looks at the existing techniques for directly coupled HPLC - atomic spectroscopy and the utilisation of these techniques for various applications.

4.3.1 Review of coupled high performance liquid chromatography - flame atomic absorption spectroscopy techniques

In addition to offering excellent inter-metal selectivity, flame atomic absorption has the advantage that it readily accepts liquid samples. Coupled HPLC-FAAS systems also offer on-line, real time analysis and produce a continuous chromatogram. The various LC-FAAS

couplings reported in the literature are summarised in Table 7.

Several workers have utilised FAAS in conjunction with simple ion chromatography. Manahan and Jones (220) noted the potential of FAAS as a metal specific detector for the determination of (ethylenedinitrilo) tetraacetic acid (EDTA) and nitrilotriacetic acid (NTA) chelating agents separated as copper complexes by ion exchange chromatography. The column eluent was passed into the nebuliser of the spectrometer in mixed solutions and also in spiked sewage effluents (221). The same group also expanded the range of amino-carboxylic acid-copper chelates which could be monitored (222) to include ethylenebis(oxyethylene-nitrilo) (EBTA) and (1,2-cyclohexylenedinitrilo) tetraacetic acid (CDTA), and demonstrated that organic mobile phases, toluene/pyridine, could be used for the separation of various chromium chelates (223). Pankow and Janauer (224) also employed an ion exchange system to separate and preconcentrate chromate in natural waters and reported detection limits as low as 0.1 ppb.

The potential of FAAS as a detector for gel chromatography was first noted by Yoza and Ohashi (225). In a study to monitor Mg and K in chloride solutions after separation, they used a 'T' piece with one end placed in a water reservoir to balance LC and aspiration flow rates. Later, Yoza et al. (226) coupled a gel chromatography column directly to a FAAS instrument for the determination of condensed phosphate anions (diphosphate, triphosphate etc) measured as magnesium complexes.

A number of other groups (227, 228) have used the separation of

tetraalkyllead compounds in petrol to demonstrate LC-FAAS couplings using a H_2O-methanol mobile phase. Cassidy et al. (229) used molecular-sieve and reversed-phase chromatography for the separation of various organosilicon compounds. The organosilicons were first preconcentrated on porous polymer columns prior to HPLC separation. After eluting the adsorbed organosilicons with (MIBK), the HPLC effluent was fed directly to the nebuliser and into a nitrous oxide-acetylene flame. Detection limits reported for the various organosilicons ranged from 0.5 to 5 μg.

Van Loon et al. (230) used direct coupling of the column eluent to the nebuliser to monitor copper aminoacid complexes, used in the treatment of metal poisoning, and also to study zinc aryl and alkyl compounds in lubricating oils. Kahn and Van Loon (231) used a similar coupling to preconcentrate and speciate Au and Pt complexes from aqueous solutions.

Slavin and Schmidt (232) in their LC-FAAS coupling, operated the nebuliser in a starved mode by using an injection cup (233) for the determination of amino-acids after metal labelling. The concept of metal labelling of species to enable determination by atomic spectroscopy has great potential, however, the low sensitivity of flame AAS would be problematic for the analysis of amino acids in body fluids at the level they occur. A flow injection sample manipulator (FISM) was used by Renoe et al. (234) as an interface between the chromatograph and spectrometer. This FISM allowed the addition of matrix modifiers, in this case acidified lanthanum chloride, to the HPLC eluent prior to introduction to the nebuliser of the spectrometer.

In a study of the effect of various mobile phases on nebulisation efficiency Jones et al. (235) found that with methanol, ethanol, chloroform and benzene, 100% nebulisation efficiencies could be achieved at flow rates of 1 ml min^{-1}, whereas for water at the same flow rate only 32% was nebulised into the flame. The nebuliser in this case was "starved" of liquid, i.e. the column flow rate was less than the nebuliser aspiration rate, which was adjusted to maximize sensitivity for the aspiration of standard solutions, as is normal in AAS. However, starving the burner results in a reduced pressure region post column; this condition can lead to gas bubble formation which would be particularly deleterious if a flow cell detector is also operated post column. Yoza and Ohashi (225) preferred to operate their nebulzier at the same flow rate as the chromatographic pump, but found balancing of the two flow rates to be difficult. Their solution was to incorporate an additional solvent reservoir at the end of the column from which any additional solvent required by the nebuliser could be drawn. This, however led to further sample dilution which is obviously undesirable for trace analysis.

An alternative to the above approaches would be to operate the nebuliser at a flow rate less than the column flow rate, i.e. flood the burner. Since LC flow rates are generally between 0.5 and 3 ml min^{-1}, a very small aspiration rate would be necessary to encompass the entire range while still maintaining aspiration. Koropchak and Coleman (236) found that operating a nebuliser at slight backpressure not only negated the use of a post column diluter to match LC flow rates with nebuliser uptake rate, but also gave improved signal to noise ratios with a standard nebuliser arrangement.

Several of the more recent publications on coupled LC-FAAS have stressed relatively simple interface systems, and have reported increased sensitivity by attention to the atom cell. One such approach has been reported by Haswell et al. (237) in a study of arsenic speciation in soil pore waters. The arsenic species are first preconcentrated by control of the mobile phase and then passed into a continuous flow hydride generator, the atomisation taking place in a heated quartz tube. It is this approach of paying particular attention to the design and optimisation of the atom cell that has been paramount in the work presented in this thesis.

4.3.2 Review of coupled high performance liquid chromatography - electrothermal atomisation - atomic absorption spectroscopy techniques

Electrothermal atomisation, mainly using graphite furnaces, offers the advantage of high sensitivity for a small aliquot of sample, however, the necessity to dry and ash a sample prior to atomisation makes it practically impossible to directly couple the eluent from a chromatograph to a furnace. Thus various indirect couplings have been used to overcome this problem, see Table 7.

Brinckman's group, at the National Bureau of Standards, developed two such indirect couplings (238). The first utilised a PTFE flow through cell from which the eluent was periodically sampled and injected into a graphite furnace, so called pulsed-mode operation. In the second, termed survey-mode, the eluent was colleted by an auto-sampler and each collected fraction analysed by GF-AAS. These two sampling modes were demonstrated for the speciation of various Sn, Hg, As and Pb compounds (238); the detection limits quoted were not evaluated by any

conventional method and should therefore be treated with care. The survey-mode of operation was also used for the speciation of organometallic polymers and organo-tin and silicates by the same group (239).

Koizumi et al. (240) used HPLC-Zeeman GFAAS for the speciation of tetraalkyllead compounds in gasoline. The eluent was sampled every 250 ul whilst the flow was stopped and the sample vaporised in a high temperature furnace. The interference caused by background absorption was avoided by using Zeeman effect background correction. Vickrey's group also used Zeeman effect background correction in their couplings (241-243). They described an interface device which consisted of a sampling valve, timing circuit and automatic coanalyte addition, in this case nickel ions (241) for selenium speciation. This interface was later microprocessor controlled (243) and 37 µl samples injected into the furnace from each 100 or 220 µl of eluent. They also used stream splitting of chromatographic peaks (242) prior to atomisation for the speciation of tetraphenyllead and pulsed mode operation for the speciation of Cr (III) and Cr (VI) (242), where the eluent was sampled every 30 or 120 seconds. The coupling was also used for tetraalkyllead (244) and organo-tin speciation (244). With the former, the addition of iodine prior to atomisation was found to enhance both the signal and precision. A similar effect was found by using zirconium coated cuvettes in the speciation of organo-tin compounds (244). Irgolic's group at the same institution used a similar automated interface for the speciation of arsenobetaine, arsenocholine and inorganic arsenic at the micro-gram level (245). In a joint study, Brinckman's and Irgolic's groups (246) demonstrated various chromatographic separations for the speciation of arsenic

compounds in soil and water samples. The extremely high background molecular absorption levels encountered when ion pair reagents, such as THAN, were used, were reported to require Zeeman effect background correction, since normal deuterium arc correction proved insufficient.

Workers at the U.S. Department of Agriculture (247,248) utilised a flow-through PTFE sampling cup as an interface between a low-capacity anion exchange column and graphite furnace. They speciated organic and inorganic reducible forms of arsenic in pesticide residues, and gave full details of a clean up procedure for use in the analysis of soil arsenical residues by the same procedure (248). This flow through PTFE sampling cup was made commercially available and a data sheet available on its application to arsenic speciation studies (249).

Another indirect form of coupling was utilised by Burns and co-workers (194) and Ricci et al. (251), namely hydride generation prior to atomisation. In their comprehensive study of organotin compounds, the former group (194) found a thousand-fold increase in response to tin by using hydride generation followed by ETA as opposed to coupling the eluent directly to the nebuliser for flame atomisation. The speciation of reducible forms of arsenic was achieved by Ricci et al. (251), using hydride generation prior to atomisation by heated quartz tube. The use of hydride generation circumvents the problems of low nebulisation efficiency normally encountered with FAAS, thus enabling sensitive detection along with "real time" detection.

Recent reports of coupled LC-graphite furnace systems have either reported dual detector systems or emphasised the use of micro-

processors. Fish et al. (252, 253) have used GF-AAS in conjunction with a rapid-scan UV-visible detector to investigate vanadyl and nickel compounds in heavy crude petroleum and asphaltenes, although here the sample was loaded into the furnace in an auto-sampler. The coupled LC-graphite furnace system used in our laboratories is based on a previous design (245) although the injector sequence, valve operation, and activation of pneumatic injector are all microprocessor controlled. This allowed automatic sampling of the eluent stream without the need for conventional fraction collection. Such a system emphasises the complexity of coupling an LC to a graphite furnace which cannot continually monitor the eluent from the column directly, in contrast to the simpler but less sensitive flame AAS and hydride generation AAS and ICP couplings.

Table 7 Coupled Liquid Chromatography - Atomic Absorption Spectroscopy

Detector	Chromatography	Matrix	Comments	Element (Wavelength/ nm)	Reference
Flame AAS. The column is connected to the nebuliser and the venturi effect pulls the eluent through the column.	Chelex ion exchange resin, 100/120 mesh, packed into a 1 ml. syringe, resin volume 0.5 ml. 0.1-0.5 ml samples. Resin washed with 0.1M $CuCl_2$ to generate Cu form.	Solutions of EDTA and NTA; pH 4 - 9.	Chelates strip Cu from the column which is monitored by AAS, Cu signal then related to chelate concentration. Linear up to 50 x 10^{-6} M EDTA or NTA	Cu	220
Flame AAS with column connected directly to nebuliser.	60 cm x 1.0 cm i.d. Sephadex G-15 column. Eluent: 0.1M NaCl or 0.1M NaCl + 0.001M EDTA. 1 ml sample volume.	Detection of Mg and K in $MgCl_2$/KCl solution.	To balance column flow with aspiration rate, a 'T' piece used with third arm placed in water reservoir. Linear from 10^{-5}-1.7 x 10^{-4} M.	Mg K	225
Flame AAS directly coupled through the nebulizer by PTFE tubing (0.023" i.d.)	60 cm x 2 mm i.d. Porasil A column, Eluent 0.5% (v/v) pyridine in toluene. Sample volume = 10 µl.	Cr as the $Cr(acac)_3$, $Cr(HAP)_3$ and $Cr(HFAA)_3$ chelates.	Adjustment of oxidant and fuel flows were made to accommodate the solvent in the flame. Detection limit of 40 ng.	Cr	223
Flame AAS see ref. 225.	94.5 cm x 1.5 cm Sephadex G-25 column. $NH_4.H_2O/NH_4Cl$ (pH 10) eluent 0.02M, at 1.83 ml min^{-1}. Column preequilibrated with $MgCl_2$ solution.	Determination of various condensed phosphates by on column complexation with Mg.	Phosphates elute in order tetra, tri, di, mono, with free magnesium eluting last after 73 min. W1/2 = 5 min. Linear up to 20 µg phosphate as triphosphate.	Mg (285.2 nm)	226
Flame AAS, direct coupling through nebuliser. Flow spoiler removed from chamber, ca. 80% of eluent reaching flame	50 cm x 2.6 i.d. ODS column. T_c = 50°C Eluent, 3:2 v/v H_2O/ MeOH at 1.0 ml min^{-1}, at 1200 psi, 1 µl sample.	Tetraalkyllead compounds in petrol.	No background problems found, possibly due to the large and constant amount of MeOH in eluent. Linear from 0.25 to 50 µg.	Pb	227

Detector	Chromatography	Matrix	Comments	Element	Reference
Flame AAS, direct coupling, c.f. ref. 223 Air/C_2H_2 flame.	5 cm x 2.1 cm i.d. Aminex A-14 resin, 4% cross linked with SDVB, 20÷3 um. 0.05M $(NH_4)_2SO_4$ eluent at 2.0 ml min^{-1}	Separation of Cu_2(EGTA), Cu)NTA0$^-$, Cu(EDTA)$^{2-}$ and Cu(CDTA)$^{2-}$.	pH of sample affects formation of Cu_2(EGTA) but not of other complexes. Detection limits/ng Cu of EGTA = 13.5 NTA = 16.2 EDTA = 29.4 CDTA = 450 in order of elution.	Cu	222
Flame AAS, direct coupling through nebuliser see ref. 222.	Identical to ref. 222	Copper chelates of aminocarboxylate ions in spiked sewage effluents.	Assumptions made as to detection limits and hence feasibility of method. Detection limits/ng Cu NTA = 10.7 EDTA = 23.6	Cu	221
GFAAS, HPLC eluent passed into a sample well then sampled, 10-50 µl, into a standard furnace.	250 x 4.6 cm columns		Coupling operated in either a pulsed mode, where the eluent was passed into a PTFE flow through cup periodically sampled, or in a survey mode where the eluent was collected by an autosampler and each fraction analysed.		
Program					
Dry - 80°C 15s	Lichrosorb C^{18} RP on 10 um silica, Eluent MeOH at 0.12 ml min^{-1}	Triphenylarsine.		As (193.3 nm)	238
Atomise - 2700°C 5s					
Dry - 100°C 10s	Lichrosorb C_2 RP 1.5 ml min^{-1} of MeOH	Ph_3SnCl, Pr_3SnCl, Bu_3SnCl.		Sn (253.7 nm)	
Char - 100°C 10s					
Atomise - 2500°C 15s					
Dry - 80°C 25s	Lichrosorb C_8 RP Eluent (a) 0.01M NH_4OAC (b) 25 ppm mercaptoethanol in MeOH. Flow a + b (96 + 4) for 25 min then gradient, 10% min^{-1}, to 100% b at 0.30 ml min^{-1}.	MeHgCl, EtHgCl, PhHgCl, ^nPrHgCl, in 1 + 1 H_20/-MeOH.		Hg (253.7 nm)	
Atomise - 750°C 12s					

Detector	Chromatography	Matrix	Comments	Element	Reference
Dry - 25°C 20s Char - 80°C 10s Atomise - 2000°C 10s	Lichrosorb Si-100 10 µm silica, eluent - hexane/CH_2Cl_2 (95 + 5) 0.33 ml min^{-1}.	Ph_6Pb_2		Pb (283.3 nm)	238
Flame AAS, column coupled directly to nebuliser. Air/C_2H_2 flame.	Partisil-10 SCX cation exchange column T_c = 55°C 1M NH_4NO_3 at 4.0 ml min^{-1} as eluent 25 µl sample size	Separation of Cu EDTA. Cu-trien, Cu-glycene. Complexes	Use of UV/Vis detection enabled only Cu-trien to be monitored, with reduced sensitivity compared to AAS.	Cu	
	25 cm ODS-SILXI column. Eluent: 50- 100% methanol/water gradient in 10 min.	Alkyl and aryl Zn additives in lubricating oils samples diluted in CH_2Cl_2.	AA detector shown superior to UV/Vis detection	Zn (213.9 nm)	230
Flame AAS. Use of column directly coupled to nebuliser. Aspiration rate controls flow of eluent through column.	Basic anion exchange Dowex 2X-8 column soaked overnight in 3M HCl followed by water rinsing. Pt and Au complexes eluted with NH_4OH (75%)	Pt and Au in aqueous solutions.	The Pt and Au solutions (pH 6) passed through the column, the metals retained and then eluted with NH_4OH into nebuliser. Linear from 2 to 10 µg for Au and from 35-175 µg for Pt.	Au Pt	231
GFAAS using Zeeman background correction. Dry - 100°C 25s Ash - 1000° 1s Atomise - 3000° fs using $NiNO_3$ as co- analyte and Ar shield gas (4 l min^{-1}) 37 µl injections.	10 cm Partisil-PXS-ODS column. Eluent: MeOH/H_2O (2 + 1) at 0.3 ml min^{-1} 20 µl injection.	Se specific detection of $Me_2NC(Se)NH_2$ and $(C_6H_5CH_2)_2Se$.	Design and operation of interfacing device consisting of sampling valve, timing circuit and coanalyte addition described. Linear from 10 to 100 ppb for a single atomisation.	Se (196 nm)	241

Detector	Chromatography	Matrix	Comments	Element	Reference
Flame AAS: see refs 225, 226 for interface.	97.5 x 1.5 i.d., Sephadex G25 column	Monitoring of Kurrol's Salt, $(KPO_3)_n$, di-, tri-, and ortho-phosphate as Mg complexes	Kurrol's salt used as useful marker for void volume of column. Estimation of stability constants also made.	Mg (285.2 nm)	254
GFAAS, Dry - 100°C 20s Char - 700°C 30s Atomise - 2500°C 10s	8 cm Bio-Rex 70, weak acidic cation exchange resin.	Cu-amino acid complexes in human serum	The eluent from the column collected by an autosampler and then automatically injected into furnace.	Cu (324.7 nm)	255
	24 cm, silica gel (100/120 mesh, ASTM D1314 - 61T, grade 923) Flow rate = 0.40 ml min^{-1} acetone/water (60÷40), pH 7-8.	Naturally occurring Cu-amino acids, Cu-histidine and Cu-glutamine from an aqueous mixture.			
GFAAS using Zeeman effect background correction.	500 x 2.5 cm column, Hitachi Gel No. 3010. Eluent: MeOH at 0.67 ml min^{-1}.	Tetraalkyllead compounds in petrol.	10 µl samples from each 250 µl of eluent injected into furnace.	Pb (283.3 nm)	240
GFAAS using auto-sampler as interface, see ref. 238 T_{at} = 2700°C	300 x 7.8 mm column, SDVB copolymer (10 µm) Eluent: THF at 1 ml-min^{-1} or THF/CH_3CN (19 + 1).	SEC used for organo-metallic polymers, OMP-1, OMP-2, OMP-4.	A 50s interval exists between injections, thus at 1 ml min^{-1} only 2.4% of eluent sampled.	Sn (286.3 nm)	
	Lichrosorb C_{18} (10 µm) 250 x 3.2 mm column, Eluent: ethanol at 0.25 ml min^{-1}.	RPC used for organo-tin and silicates.	Linear up to 20 ng Sn or Si for a 20 µl injection	Si (251.6 nm)	239

Detector	Chromatography	Matrix	Comments	Element	Reference
Flame AAS using standard flame conditions eluent passed into nebuliser	25 x 0.46 cm Partisil-10 SCX column. Eluent, NH_4NO_3 at various molarity and pH, 1 to 2 ml min^{-1}.	Use of metal labelling to determine amino-acids, in this case histidine as copper complex.	Nebuliser operated in starved mode by use of injection cup (see ref. 233). 100 µl drops from column into cup. Detection limit of 48.5 ng.	Cu	232
GFAAS using Zeeman effect background correction. Automated interface which controls eluent sampling, coanalyte addition, injection and furnace operation.	µ - Bondapak (C_{18}) RPC column. Eluent: H_2O/acetonitrile/acetic acid and 0.005M heptane-sulphonic acid (95/5/6).	Separation of arseno-betaine, arsenocholine and inorganic arsenic.	Chromatograms illustrating separation of arsenic compounds at 1 µg level given.	As	245
GFAAS with microprocessor controlled interface, details of interface and computer control program given.	Partisil SCX cation exchange column. Eluent: 0.1M acetate buffer (pH 4.3).	Separation of Cr(III) and Cr(VI).	Pulsed mode operation, eluent sampled for GFAAS only every 30 to 120s.	Cr	
Dry - 60°C 20s Ash - 250°C 12s Atomise - 2400°C 5s	Lichrosorb C_{18} (10 µm) Eluent: MeOH/H_2O (90/10) at 0.5 ml min^{-1} 20 µl injection.	Tetraphenyllead	Total consumption mode, peak containing eluent stream is stored prior to GFAAS analysis.	Pb (283.3 nm)	242
GFAAS using Zeeman effect background correction. Dry - 60°C 25s Ash - 500°C 12s Atomisation - 2400°C 5s	25 cm Lichrosphere (10 µm) RPC column. Eluent: MeOH/H_2O (90/10)	Tetraphenyllead.	Eluent stream containing lead compound is stored, after separation, in tubing (10' x 0.05 cm) prior to injection into furnace.	Pb (283.3 nm)	

Detector	Chromatography	Matrix	Comments	Element	Reference
Microprocessor controlled interface, see ref. 242. 37 µl injection from each 100 µl or 220 µl sample of eluent.			Detection limit of 480 pg		243
GFAAS, 20 µl injections every 45s. Dry - 150°C 15s and 200°C 5s Atomise - 2700° 10s No background correction.	25 cm x 3.2 mm i.d. Lichrosorb SAX (10µm) column. Eluent: 0.05M NaH_2PO_4 at 0.5 ml min^{-1}.	Speciation of DMA, MMA and arsenilic acid.	The HPLC separation schemes were employed for As speciation work with several soil and drinking water samples. Linear from 0.1 to 10 ng As.	As (193.7 nm)	
	Same column but with 0.03M ammonium acetate/0.045M acetic acid Eluent at 0.25 ml min^{-1}	Speciation of MMA, DMA and As(III).			246
No background correction.	25 cm x 3.2 mm i.d. Altex SCX column (10 µm) with 0.0375M ammonium acetate/ acetic acid.	Speciation of As(III) and As(V)			246
Zeeman effect background correction.	Eluent at 0.15 ml min^{-1} 30 cm x 4 mm i.d., µ-Bondapak C_{18} RPC (10 µm) column, H_2O/-MeOH (95/5) 0.005M w.r.t. TBA, at pH 7.3 adjusted with phosphoric acid.	Speciation of As(III) and As(V)	The use of the ion-pair reagents THAN or TBAP requries the superior background correction afforded by the Zeeman effect. Linear up to 500 ng As.		246

Detector	Chromatography	Matrix	Comments	Element	Reference
Zeeman effect background correction.	25 cm x 4.6 mm i.d., Altex Chromosorb RP-18 column (10 µm). H_2O/-MeOH saturated with THAN for 23 min then MeOH, at 1.0 ml min^{-1}.	Speciation of As(III), DMA, MMA and As(V).			246
GFAAS, using PTFE flow through sampling cup as interface 20 µl injections at 43s intervals. Dry - 110°C 8s Char - 1200°C 7s Atomise - 2500°C 8s 20s furnace cooling period.	25 cm x 3 mm i.d., low capacity anion exchange column (Dionex) gradient elution from H_2O/MeOH (80 + 20) to 0.02M $(NH_4)_2CO_3$-MeOH (85 + 15) at 1.2 ml min^{-1}. 5-25 µl injections. 8-12 min equilibration time.	Separation of DMA, MMA, As(III), and As(V).	The column packing prepared by passing a suspension of a high capacity strong anion exchange latex over a cation exchange resin Linear from 5 to 200 ng As.	As (193.7 nm)	247
GFAAS, see ref. 247	HPLC column and conditions same as ref. 247.	Arsenical residues, DMA, MMA, As(III) and As(V) in soils.	Extraction and extensive clean up procedure is given.	As (193.7 nm)	248
GFAAS using Zeeman effect background correction. Dry - 80°C 20s Ash - 370°C 10s Atomise - 2300°C 5s	25 cm Lichrosorb 10 µm C-18 ODS column Eluent 0.5 ml min^{-1} 80:20 MeOH/H_2O for 28 min followed by a step gradient to 100% MeOH.	Tetraalkyllead compounds.	Addition of Iodine found to enhance signal and precision.	Pb	244
Dry - 80°C 20s Ash - 400°C 10s Atomise - 2300°C 5s	Same column, eluent: MeOH/H_2O (97.5 + 2.5) isocratic at 0.1 ml min^{-1}.	Organotin compounds.	Increased signal and precision found when Zr coated graphite cuvettes were used.	Sn (224.6 nm)	244

Detector	Chromatography	Matrix	Comments	Element	Reference
Flame AAS using N_2O/C_2H_2. Directly coupled through nebuliser or hydride generation followed by electrothermal, quartz furnace, AAS.	250 x 3.0 mm i.d., ODS Spherisorb S5W T_c = 23 ± 0.1°C, Eluent: acetone/pentane (3 + 2) at 1.0 ml min^{-1} for methyltin compounds; acetone/pentane (7 + 3) at 1.2 ml min^{-1} for ethyl tin compounds.	Methyl and ethyl tin compounds both SnR_4 and $SnR_{4-n}Cl_n$.	The design of a miniature, continuous flow hydride generation system given. Linear up to 50 µg using flame and up to 100 ng for hydride generation.	Sn (286.3 nm)	250
GFAAS, 10-100 µl injections.	35 x 1 cm i.d. column 9 cm AG50 W-X8 (100/120 mesh) cation exchange resin, 26 cm AG1-X8 (100/120 mesh) anion exchange resin.	As(III), As(V), MMA, and DMA in arsenic contaminated, sediment interstitial water, up to 2 ml injected.	The separated As species were collected in fractions from which injections were made into furnace.	As	256
	Column conditioned with 50 µg of each arsenic species.		Detection limit 10 ppb in original sample.		256
AAS, using air/C_2H_2 flame directly coupled through nebuliser.	300 mm x 3.9 mm i.d., µ-Bondapak C_{18} column, Eluent: acetonitrile/ water (70 + 30) at 3.0 ml min^{-1}. 20 µl injections.	Tetraalkyllead compounds in petrol.	The relative merits of UV and AAS detection discussed with latter proving more suitable for this application. Linear from 1.1 - 11 µg.	Pb (283.3 nm)	228
Flame AAS using flow injection sample manipulator (FISM) interface with fuel rich air/C_2H_2 flame.	100 mm x 7.5 mm i.d. Spheregel TSK 2000SW (10 µm). Eluent: 130 mmol NaCl, 6.8 mmol NaOH, 3 mmol NaN_3, 4 mmol KCl and 10 mmol TES at 0.4 ml min^{-1}, pH = 7.43 at 37°C.	Study of metal ligand binding in clinical samples.	FISM interface decribed enabled La/HCl to be mixed with eluent prior to introduction through nebuliser. Linear up to 3.75 mmol Ca.	Ca (422.7 nm) Mg (285.2 nm)	248

Detector	Chromatography	Matrix	Comments	Element	Reference
AAS using hydride generation and electro-thermal, quartz tube, atomisation T = 800°C.	3 x 500 mm standard Dionex anion column, Eluent: 2.6 ml min^{-1}. 0.0024M $NaHCO_3$/ 0.0019M Na_2CO_3/ 0.001M $Na_2B_4O_7$.	Speciation of As(V), MMA, p-APA.	Miniature hydride generation system, see ref. 257. 1 hour reequilibration time between eluent systems. Detection limit of 10 ng ml^{-1}.	As (193.7 nm)	
	0.005M $Na_2B_4O_7$.	Speciation of As(III) and DMA.			251
GFAAS using a fraction collector as interface Dry - 100°C 30s Char - 1300°C 30s Atomise - 2700°C 10s 20 µl injections.	25 cm x 2.6 mm, ODS-HC Sil-X-1. Eluent: either gradient from 50% MeOH to 100% MeOH in 25 min or 20% MeOH for 10 min then gradient to 100% in 30 min.	Organophosphorus compounds in lubricating oil.	The chromatographic analysis time = 25 to 40 min, whereas GFAAS analysis time = 100-120 min. Detection limit of 0.3 mg l^{-1}.	P (213.6 nm)	
GFAAS, see refs. 238, 245, 246.	See ref. 247.	Inorganic and organo-arsenic compounds in oil shale retort and process waters.	Compounds found were: arsenite, arsenate, methylarsonic acid, phenylarsonic acid, along with one unidentified compound.	As (193.7 nm)	258
GFAAS using fraction collector as interface with manual injections.	Anion exchange resin Dowex 1-X4, 200/400 mesh in acetate form, 115 cm x 10 mm, Eluent: 0.1% acetic acid 65 min, 5% acetic acid 130 min then 1M HCl for 65 min. Flow rate = 20 drops min^{-1}.	Separation of DMA, MMA and As(III)/As(V) As(III) levels found separately in soil polluted with As.	Extraction procedure given for soils. The chromatographic separation does not speciate As(III) and As(V).	As (193.7 nm)	259

Detector	Chromatography	Matrix	Comments	Element	Reference
AAS using fraction collector as interface.	33 x 1.0 cm Sephadex G15 column. Eluent: 0.2M $NaClO_4$ (pH 2) at 40 ml hr^{-1}. 46 x 10 cm Sephadex G-10, eluent 0.1M $HClO_4$ at 19 ml hr^{-1}.	Separation of successive Cr(III) isothiocyanato complexes with SCN/Cr ratio of 1 - 6.		Chromium	260
GFAAS rapid-scan uv-vis detector connected to furnace via auto-sampler	Reverse-phase HPLC/GFAA ODS C-18 column (Altex 6 mm i.d. x 250 mm length) with guard column (Waters 3.2 mm i.d. x 40 mm length). Size exclusion chromatography using a series combination of 50/100/1000 A μ-spherogel column (Altex, 8.0 mm i.d. x 300 mm length) with swelled divinylbenzene as the packing.	Vanadyl and Nickel compounds in heavy crude petroleums and asphaltenes	Spectra from the rapid-scanning detector were stored by micro computer. GFAAS histogrammic data were recorded by both strip chart recorder and digital integrator	Vanadium (318.4 nm) Nickel (232.0 nm)	252, 253
GFAAS with micro-processor controlled interface based on previous design - see ref. 245.	Either single column, Hypersil 5 μm ODS, (250 mm x 5 mm i.d.) or 5 μm ODS and one Hamilton 10 μm PRP 1 (250 mm x 5 mm i.d.) in series in a constant temperature (28.5°C) enclosure.	Determination of organo-copper species in soil pore waters.	Injector consists of a pneumatic slider injection valve and a solenoid-controlled stainless steel syringe needle. N_2 used to deliver the sample via sample loop, through syringe needle into the cuvette. Injection sequence valve operation, and activation of solenoid controlled by micro computer.	Copper	261

Detector	Chromatography	Matrix	Comments	Element	Reference
AAS using continuous flow hydride generation and heated quartz tube atomisation.	Zipax ion-exchange precolumn attached in series to strong-base anion exchange BAX 10 resin (5 μm, 250 x 5 mm column). Eluent 10^{-4}% sulphuric acid (flow rate 4.0 ml min^{-1} switched to 0.01M ammonium carbonate (flow rate 4.0 ml min^{-1}).	Arsenic speciation in soil-pore waters.	Precolumn acted as a guard column and enabled preconcentration step to be incorporated in the analysis. Arsenate, arsenite and monomethylarsonic acid characterized in soil-pore water.	Arsenic (193.6 nm)	237
UV detector or atomic-absorption spectrophotometer connected directly via the nebuliser. Various types of tubing used for the interface.	Column, HS-3 C-18 (10 cm x 0.46 cm i.d.); solvent A, MeOH; solvent B, 0.01M Na_3PO_4 in water; gradient, linear from 0% MeOH to 70% MeOH for 10 min; and flow rate, 2 ml min^{-1}.	Determination of iron in blood.	Main aim of paper is an investigation of peak dispersion in a coupled LC-AAS system. Three types of tubing examined:- (i) serpentine tube of 0.25 mm i.d. (ii) straight tube 1.27 mm i.d. (iii) polyethylene tube 0.55 mm i.d. All tubes 49 cm long.	iron (248.3 nm)	262
	P.E. 3 x 3 column (0.3 cm x 4.6 mm i.d.) packed with octadecyl-bonded silica gel (3 μm). Mobile phase MeOH flow rate 2 ml min^{-1}. Solute $Mg(NO_3)_2$.		System used for peak dispersion measurements.	Mg	262

Detector	Chromatography	Matrix	Comments	Element	Reference
GFAAS using Zeeman effect background correction. Conductivity detector in series with automated interface based on Ref. 245.	Dionex system consisting of 50 x 3 mm anion precolumn (Dionex 30008), 150 x 3 mm anion separator column (Dionex 30589) and a 250 x 3 mm anion suppressor column (Dionex 30066), in series. Mobile phase 0.0080M Na_2CO_3 at 0.46 ml min^{-1}.	Distilled water, synthetic river water and Texas river water spiked with selenite and selenate.	Dionex model 16 ion chromatography used. Detection limit 20 ng Se for each selenium compound. Preconcentration from a max. of 4 ml of an anion-rich water sample extends detection limit to 5 ng Se.	Se	263
Directly coupled flame atomic absorption utilising pulse nebulisation and a slotted tube atom trap.	Partisil-10-SCX (250 x 4.6 mm i.d.) column. Mobile phase of 80:20 methanol:water in 0.1M NH_4 OAC.	Tributyltin determination in coastal waters.	Organotin compounds quantitatively extracted from seawater into chloroform and then into methanol to facilitate injection onto the HPLC column. Total analysis within 8 minutes.	Sn (224.6 nm)	264

4.3.3 Review of coupled high performance liquid chromatography - atomic fluorescence spectroscopy techniques

The advantages of AFS as a chromatographic detector have been extolled by Van Loon (265) as a simultaneous multi-element detector with greater sensitivity than AAS. Such detectors have been utilised a little more with LC, Table 8, than with GC. Van Loon's own group have used non-dispersive simultaneous multi-element FAFS for the speciation of Cr(III), Ag(I), Mn(II) and Mn(VII) in synthetic sea water (266). Excellent resolution was demonstrated for a mixed solution (10 mg l^{-1} of each species, 10 ml injection); however, the high sensitivity of AFS was not tested. This group also demonstrated the multi-element capability of coupled LC-FAFS for the speciation of Cu, Ni and Zn amino-acid and amino-carboxylic acids (267). Unfortunately no mention of the metal concentrations was made.

Siemer and co-workers (268) reported the use of continuum source FAFS in the study of the acetylation reaction of ferrocene by acetic anhydride. They found it much easier to follow the reaction by Fe specific detection than by conventional UV detection. Mackey (269) in a study of the interactions of simple cations, Cu, Fe and Zn, with macroreticular resins used multichannel FAFS but quantified the results by batch measurements using graphite furnace AAS. The LC-FAFS system was found to be linear up to 1.0, 1.6 and 0.6 mg l^{-1} for Cu, Fe and Zn respectively, deviation from linearity was said to occur at 20 times the detection limit as defined by Larkins (270).

Table 8 Coupled Liquid Chromatography - Flame Atomic Fluorescence Spectroscopy

Detector	Chromatography	Matrix	Comments	Element (Wavelength/ nm)	Reference
FAFS using a N_2 shielded circular air/C_2H_2 flame, eluent passed directly into nebuliser.	10 cm x 1 cm column, 80 mesh Chelex 100 washed with HCl (40 ml) and water (40 ml) at 1 ml min^{-1}. Eluent: H_2O (pH 6) for 4 min then 2M HNO_3.	Speciation of Cr(III), Cr(VI), Ag(I), Mn(II), and Mn(VII) in standards and synthetic sea water.	In the sea water a scattering peak, due to NaCl, appears well before Cr(III), Mn(II) or Ag(I) elutes.	Cr Mn Ag	266
As above	Partisil-10 SCX column at 55°C. Eluent: water until first peak eluted then a 5 min convex gradient to 100% 1M NH_4NO_3 at 4.0 ml min^{-1}.	Separation of Cu, Ni, and Zn EDTA, Trien and glycine complexes.	The glycine and EDTA complexes have almost identical retention times, however multielement AFS gives excellent resolution.	Cu Ni Zn	267
FAFS using air/C_2H_2 capillary tube burner, Ar shielded, Xe continuum lamp sources, direct coupling to nebuliser.	50 cm x 2 mm chromosep S column packed with pellicular 10 µm silica gel. Eluent: diethylether/methanol (40/1) at 0.5-2.0 ml min^{-1}	Investigation of acetylation reaction of ferrocene by acetic anhydride.	Progress of reaction more specifically followed using AFS than normal UV detection.	Fe (283.3 and 252.2 nm)	268
FAFS; see ref. 266.	6 cm column of XAD-2 resin. Various elution systems used.	Study of absorption of trace metals on Amberlite resins	Metals are not desorbed by MeOH but by methanolic HCl, methanolic NH_3 and Na_2H_2EDTA. Linear up to: 1 mg l^{-1} Cu, 1.6 mg l^{-1} Fe and 0.6 mg l^{-1} Zn.	Cu Fe Mg Zn	269

4.3.4 Review of coupled plasma techniques

The relatively low excitation temperature of the various atomic spectroscopic flames limits their usefulness as atom cells for coupled LC-AES applications. Flames have been used in various configurations as molecular emission detectors; for example, McGuffin and Novotny (271) monitored HPO bands for phosphorus selective detection of various compounds eluting from a microbore LC column. Similarly Cope and Townshend (272) have used a phosphorus sensitive MECA detector as a detector for HPLC.

The low neutral gas temperature of the MIP makes it very sensitive to large solvent flow-rates. Although several workers have devised continuous nebulisation systems (273-275) for the plasma, it has proved singularly unpopular in LC applications. In contrast, both the direct current and inductively coupled plasmas with their ability to withstand both organic and aqueous solvent flows have found various applications as LC detectors (276).

Direct Current Plasma

The group at Amherst, Massachusetts, have been one of the main exponents of coupled LC-DCP OES, Table 9, using both two and three electrode plasmas (276-279). They found (278) that the standard nebulisation arrangement was sufficient for eluents used in ion exchange and reverse phase chromatography but when used in conjunction with the organic solvents used for adsorption chromatography a rapid build-up of carbon resulted. Thus they designed a novel nebuliser which had an efficiency of 20 - 25% and could be run continuously for up to ten hours with no carbon deposits forming. The couplings were used in the speciation of diethyldithiocarbamate complexes of Co, Cu,

Ni (277), Hg and Cr (278). The study of mixed ligand complexes of the type $Cr(HFA)_n$-$(TFA)_{3-n}$, for n = 0, 1, 2, 3 (279), was aided by the metal specific detection afforded by the coupled system.

Koropchak and Coleman (280) used a conventional cross-flow nebuliser in their LC-DCP coupling. They studied nebulisation parameters to optimise the plasma detection capabilities when interfaced to a liquid chromatograph. They demonstrated its capability in the speciation of three cadmium salts; however, the hope that the DCP could also provide sensitive specific detection for the halogens was not realised.

The group at the University of Massachusetts (281) have also studied the characteristics of the nebuliser/spray chamber interface. They again used a cross-flow nebuliser but fitted a modified spray chamber and located the entire interface directly below the excitation region with the solvent-analyte spray in the same vertical plane as the plasma. The performance of this arrangement was investigated using a range of aqueous and organic solvents and copper hexafluoracetyl acetonate.

Other recent workers using coupled LC-DCP have paid more attention to the chromatography using direct connection of the column to the nebuliser. Krull et al. (282) have studied the speciation of chromium in various water, biological and tannery samples, reporting detection limits of 5 - 10 ppb with at least 3 - 4 orders of magnitude linearity in the calibration plots. Chromatographic details were given for a number of columns used in the investigations.

Table 9 Coupled Liquid Chromatography - Direct Current Plasma Optical Emission Spectroscopy

Detector	Chromatography	Matrix	Comments	Element (Wavelength/ nm)	Reference
DCP, using Spectraspan III instrument	250 x 4 mm i.d., 8 µm Spherisorb SEP. Eluent: 5:15:80 acetonitrile, diethylether and skelly B at 2.2 ml min^{-1}. Column washed prior to use with 0.5% pyridine in skelly B.	Separation of metal diethyldithiocarbamates.	DCP detector in series with UV detector used to confirm metal content of eluted peaks. Linear from 5 to 500 ng Co and from 10 to 500 ng Cu.	Co Ni Cu	277
DCP (Spectraspan III) For reverse phase and ion exchange chromatogrpahy, eluent passed directly into standard nebuliser system.	250 mm Partisil ODS column. Eluent: H_2O/acetronitrile (60:40) at 0.65 ml min^{-1}.	Speciation of: $Cu(enAA_2)$. $Cu(enTFA_2)$ and the Ni analogues. $Cr(HFA)_3$ and various mixed ligand chelates formed by reaction of Cr with TFA and HFA.	Nebulisation of eluents used for adsorption chromatography caused rapid C build up and thus required a new design of nebuliser. Eluent was directed at chamber wall in a fine jet and resulting mist swept into plasma. Nebulisation efficiency of 20-25% was attained with no C build up over 10 hr period. Linear from 30 to 4000 ng Cu and from 60 ng to 2.5 µg Cr.	Cu (324.7 nm) Ni (341.5 nm) Cr (267.7 nm)	278
	250 mm, 8 µm Spherisorb, eluent: 5:20:75 acetonitrile/diethyl-ether/skellysolve B.	$Hg(DEDTC)_2$; $Cr(DEDTC)_2$.		Hg (253.7 nm) Cr (267.7 nm)	
DCP plasma, same interface for hydrocarbon eluents as ref. 278.	300 x 4 mm i.d., 10 µm Partisil silica, eluent: 6% acetonitrile in CH_2Cl_2 1.5 ml min^{-1}.	Speciation of: mer and fac isomers of $Co(BAA)_3$ and $Co(PAM)_3$.	Detection limit of 100 ng for Cr.	Cr (267.7 nm)	

Detector	Chromatography	Matrix	Comments	Element	Reference
	8% CH_2Cl_2 in hexane	Mixed ligand complexes of $Cr(HFA)_n$-$(TFA)_{3-n}$ and the mer/fac isomers of Cr-$(TFA)_3$.			
	Concave gradient of 3-20% CH_2Cl_2 in hexane.	As for above only better peak shape and shorter analysis time achieved.			279
DCP using crossflow nebuliser with direct introduction of eluent.	500 mm x 5 mm, Sephadex 10 column. Eluent: H_2O at 2.0 ml min^{-1}.	Separation of Cd; sulphate, bromide and acetate.	Examination of nebulisation parameters concerned with coupling reported.	Cd (228.8 nm)	280
DCP (Spectraspan IIIb). The end of the analytical column was attached to the DCP via a short length of stainless-steel tubing connected to a section of flexible plastic inlet tubing.	The HPLC separations of the two Cr ions was achieved using paired ion, reversed-phase conditions, with either a tetrabutylammonium counter-ion or a camphor sulphonate counter-ion in solution.	Speciation of Cr(VI) and Cr(III) in various water samples, biological samples and tannery samples.	The order of elution of the two Cr ions is completely reversed going from one counter ion to the other in the mobile phase. Det. limits for both Cr species in the range 5-10 ppb with at least 2-4 orders of magnitude linearity in the calibration plots.	Chromium (425.4 nm)	
	The analytical columns used were as follows: 1. 5 µm, 15 cm x 4.6 mm i.d. C_{18} Resolv column (Waters). 2. 5 µm, 15 cm x 4.6 mm i.d., C_{18} Altex column (Altex/Beckman). 3. 10 µm, 25 cm x 4.6 mm i.d., C_{18} column (Altex).				282

Detector	Chromatography	Matrix	Comments	Element	Reference
	4. 10 µm, 25 cm x 4.6 mm i.d. C_8 column (Altex)				282
On-line detection with SpectraSpan IIIB DCP. Outlet of column connected directly to the cross-flow nebuliser.	Separation by gradient elution HPLC, using either a Du Pont Zorbax ODS (4.6 mm x 250 mm) column or a Hamilton PRP-1 (4.1 mm x 150 mm) column previously equilibrated with the tetraalkylammonium ion selected for use.	Polyphosphate oligomers.	Linear oligomers ranging from P_1 (orthophosphate) to P_{12} can be observed in neutralised polyphosphate samples. A detection limit of 0.2 ug of P is observed with the 214.9 nm emission line. Precision for each of each of the major oligomers (P_1-P_{10}) is in the 1-5% RSD range.	Phosphorus (214.9 213.6 nm) nm	283
SpectraSpan IV three electrode direct current argon plasma equipped with an echelle grating spectrometer, and ceramic cross-flow nebuliser fitted with a PTFE collar to a modified 10 ml pyrex round bottom flask spray chamber.	Silica 5.0 µm column (250 mm x 4.5 mm i.d.) Mobile phase 100% CH_2Cl_2 at 1.0 ml min^{-1}.	Various aqueous and organic solvent systems. Copper hexafluoroacetylacetonate.	Paper based on the design and characterisation of a nebuliser/spray chamber interface. The entire interface was situated in the SpectraSpan IV so that the chimney tip was 15 mm below the plasma excitation region, placing the solvent-analyte spray in the same vertical plane as the plasma.	Copper (324.7 nm)	281

Inductively Coupled Plasma

The coupling of LC with ICP-OES (Table 10) is normally directly through standard nebuliser arrangements. Browner and co-workers (284) considered the effect of nebulisation chamber position using both Meinhard (285) and fixed cross-flow (286) nebulisers for LC-ICP couplings. Although they only studied aqueous eluents, they found peak broadening and distortion occurred when the chamber was placed inside the ICP gas box, due to extended liquid transport. If, however, the chamber is sited outside the gas box, then a loss in signal commensurate with aerosol transport over an equivalent distance occurred.

Fraley et al. (287, 288) built on their experience with hybrid techniques and compared FAAS and ICP-OES as HPLC detectors for the speciation of copper amino-carboxylic acid chelates (287). Both techniques were found to yield a similar response; however, the multi-element facility of ICP-OES was demonstrated using a dummy column to simulate chromatographic conditions. The simultaneous detection of Ca, Cu, Mg and Zn amino-carboxylic acid chelates with linearity up to 1 ug (288) illustrates another advantage of OES over AAS, i.e. long linear calibrations.

Gast et al. (289) demonstrated a coupling using a fixed cross-flow nebuliser for the speciation of carbonyl complexes of Fe and Mo, various forms of As, dialkyl-mercury compounds, tetraalkyllead compounds and various ferrocene derivatives. The ICP-OES detector was evaluated by injecting small samples into the nebuliser. They studied the effect of solvent composition and determined both linear ranges and detection limits by this method. Morita et al. (290) used direct

sampling of eluent to the nebuliser for the estimation of Co/P/C ratios in vitamin B_{12} and also in the simultaneous multi-element detection of various proteins. Kurosawa et al. (291) used the same coupling to unequivocally identify the presence of arsenobetaine in shark liver and muscle.

Hausler and Taylor (292, 293) used ICP-OES in conjunction with size exclusion chromatography and evaluated a number of spray chamber designs. Using toluene as eluent (292) it was found that cooling the chamber to $0^{o}C$ resulted in better sensitivity being obtained. This evaluation, along with the determination of detection limits, was performed in the absence of the chromatographic column. When pyridine was used as eluent (293), best sensitivity was achieved with the chamber thermostated at $20^{o}C$. Detection limits, found by the same procedure as above, were slightly worse than those obtained with toluene. Gardner et al. (294) used ICP-OES in series with UV detection to monitor the speciation of Ca and Mg in natural water filtrates. If both detectors gave a response, the tenuous implication that the metal was organically bound was made. This example illustrates one of the main advantage of hybrid chromatographic techniques, i.e. they provide unambiguous identification of metal. The most definite conclusion from the chromatographic data obtained (294) was that a species contained Ca, Mg, or neither, the nature of the organic moiety remained speculation.

Recent papers on coupled LC-ICP have shown a tendency to move towards the use of microbore-HPLC. The group at Toyohashi University of Technology have used micro columns in their studies on various interface couplings (295-297) utilised for the determination of Cu,

Zn, Fe and Co in organometallic compounds and the analysis of carbon containing materials such as saccharides. Fassel's group (298) have also used microbore columns although they have in addition reported the first departure from using conventional cross-flow, concentric or Babington-type pneumatic nebulisers and have developed a microconcentric nebuliser which is inserted directly into the tip of a conventional sample introduction tube of an ICP torch. However at the present time problems with low residence times of the analyte species in the plasma and possible solvent interaction in the excitation process have been reported to impair detection limits.

Table 10 Coupled Liquid Chromatography - Inductively Coupled Plasma Optical Emission Spectroscopy

Detector	Chromatography	Matrix	Comments	Element (Wavelength/ nm)	Reference
ICP, eluent from column passed directly into nebuliser. All Ar plasma. For FAAS work air/C_2H_2 flame used.	Aminex A-14 column; see ref. 222.	Separation of EDTA and NTA chelates.	Compared with FAAS detection for Cu chelates and found both gave similar response. Also used dummy column to simulate chromatography for various metals.	Cu (324.7 nm)	287
ICP all-Ar plasma, outlet of column connected by capillary PTFE tubing to nebuliser of cross-flow design.	250 x 4.6 mm i.d. Zorbax-C8 column, eluent: 70% (v/v) Eluent: 5 min. linear gradient of 50-55% ethanol then 5 min. linear gradient up to 80% EtOH, 1 ml min^{-1} 20 µl injection.	Separation of iron carbonyl complexes. Separation of various molybdenum carbonyl complexes.	ICP was tested as a HPLC detector by injecting small samples through an injector into the nebuliser, to evaluate effect of various solvents; sensitivity; linearity and detection limits.	Fe (259.94 nm) nm Mo (281.615 nm)	289
	Hypersil (6 um), 100 x 4.6 mm i.d., eluent: 30% MeOH, 1% (w/w) n-hexadecyltrimethyl-ammonium bromide, 0.08M, pH 5, at 1.2 ml min^{-1}.	Separation of DMA, MMA, p-APA, As(V), phenylarsonic acid.		As (278.022 nm)	
	Eluent: EtOH - 0.05M NaBr (1:2) pH 3, 1.2 ml min^{-1}	Separation of Hg(II) methylmercury, ethyl-mercury and propyl-mercury.		Hg (253.652 nm)	
	Eluent: 75% EtoH 1.4 ml min^{-1}. 30 µl injection	Tetraalkyllead compounds in petrol.		Pb (283.306 nm)	289

Detector	Chromatography	Matrix	Comments	Element	Reference
	250 x 3 mm i.d., silica Gel Si60 (8 µm), eluent: toluene 1.4 ml min^{-1}	Separation of various ferrocene compounds.		Fe (259.94 nm)	289
All-Ar ICP, eluent passed directly into nebuliser.	600 x 2 mm, TSK GEL 3000 SW eluent: 0.9% NaCl, 1.0 ml min^{-1}.	Separation of vitamin B_{12}. Separation of various proteins; ferritin, catalase, aldolase, albumin, cytochrome C, chymotrypsinogen A.	Multi-element detection used to calculate Co/P/C ratio. Simultaneous multi-element detection of Cu, Fe, Mn, P, Zn.	C (246.7 nm) Co (228.6 nm) Cu (324.9 nm) Fe (259.9 nm) Mn (257.6 nm) P (241.9 nm) Zn (213.8 nm)	290
All-Ar ICP, eluent passed directly into nebuliser.	Either: Nagel-Nucleosil 10-SA cation or 10-SB anion exchange resin. Eluent: 0.25M phosphate buffer, pH 7.4.	Identification of arsenobetaine in shark muscle and liver by comparison with standard chromatogram of arsenobetaine, DMA, MMA, As(III) and As(V).	Arsenobetaine matched, on both resins, the main As compound found in the shark tissues.	As (193.7 nm)	
ICP, all-Ar plasma, eluent passed directly to nebuliser; varius spray chamber designs evaluated with and without cooling to 0°C.	100-A -Styragel waters column at a flow rate of 1.0 or 0.5 ml min^{-1} of toluene. Bio-Beads SX-2 size exclusion column; Eluent: toluene at same flow rates. 200 µl injected.	Separation of variuos Si, Pb, Sn and Ge organometallic compounds. Separation of a 21-element standard, metal salts of dialkylbenzene sulphonates, in an organic matrix.	The various spray chambers, and detection limits were evaluated without the chromatographic column being used. Detection levels are comparable to those found for aqueous solutions.	Al, Ag, Ba, Cd, Cu, Fe, Mg, Mn, Ni, Pb, Si, Sn, Ti, V, Zn.	292

Detector	Chromatography	Matrix	Comments	Element	Reference
Ar ICP, see ref. 292. Spray chamber thermostated to 20°C.	100-A μ-Styragel waters column, eluent: pyridine at 0.5 or 1.0 ml min^{-1} or toluene at same flow rate.	Separation of a 21-element standard (see ref. 292), ferrocene and derivatives, copper and cobalt complexes, and organically bound metals in solvent refined coal.	Detection limits in pyridine, determined by same method as 292, and are generally slightly worse than those found using toluene.	See ref. 292	293
Ar ICP, eluent taken from UV detector directly to crossflow nebuliser. 32-element polychromator used for simultaneous detection, or monochromator for single channel operation.	250 x 1.6 mm i.d., AGI x 4 (< 400 mesh) anion exchange resin Eluent: 0.05M $(NH_4)_2SO_4$.	Separation of NTA and EDTA chelates of Cu, Zn, Ca and Mg.	The data acquisition storage and output is microprocessor controlled. Linear up to 1 μg for all elements.	Cu Ca Mg Zn	288
Ar plasma; see ref. 287.	600 x 7.5 i.d., TSK 3000 SW size exclusion column, or a 500 x 7.5 mm i.d., TSK 2000 SW column. Eluent: H_2O at 1.0 or 1.5 ml min^{-1}	Speciation of dissolved Ca and Mg in natural water filtrates.	By using UV detection as well as ICPOES, inference as to the organic binding made.	Ca Mg	294
ICP, the eluent from the column being fed to a cross-flow nebuliser via PTFE tubing. (0.5 mm i.d. x 300 mm long)	Strong cation exchange resin (UIPX-210 SC from Toyo Soda Co. Japan). Step gradient elution from 0.2M NaH_2PO_4, pH 0.2 to 0.2M NaH_2PO_4, pH 4.3 column temp. t0°C.	Amino acids.	In the detection of sulphur, a simple Ar purge system was used to reduce light absorption by oxygen. Detection limits of 30-50 μg/ml and 1-3 μg/ml as amino acids were obtained for carbon and sulphur respectively.	Carbon (193.09 nm) Sulphur (180.73 nm)	299

Detector	Chromatography	Matrix	Comments	Element	Reference
ICP, the HPLC column being connected to a Meinhard concentric nebuliser rated at 3 ml min^{-1} by 2' of 1/16th" O.D., 3/64" I.D. PTFE tubing.	Hamilton PRP-1 resin-based, reverse phase column.	Various arsenic, selenium, and phosphorus compounds.	The standard software was modified to allow the chromatogram to be displayed graphically on-line. Det. limit for As 130 µg l^{-1} at 100 µl injection volumes.	Arsenic (189.0 nm) Carbon (247.8 nm) Phosphorus (214.9 nm) Sulphur (180.7 nm) Selenium (203.9 nm)	300
ICP, the eluent from the micro HPLC is carried into the nebuliser using either water or methylisobutylketone as carrier, via a simple or modified stainless T-type connector. Series UV detection also used.	Micro-HPLC. The columns were Teflon tubing of 0.5 mm i.d. by 12 cm length packed with Jasco SC-01 (ODS-silica, 5 µm) for reversed phase mode and Teflon tubing 0.5 mm i.d. by 15 cm length packed with Jasco Fine Sil-5 (silica, 5 µm) for normal phase mode. Mobile phases were MeOH or MeOH-H_2O mixture for reversed phase mode and toluene for normal phase mode.	Cu, Zn, Fe and Co organometallic compounds.	Application of micro HPLC with simple interface for LC-ICP previously reported - Ref. 296.	Copper (324.7 nm) Zinc (213.8 nm) Cobalt (228.6 nm) Iron (259.9 nm)	295

Detector	Chromatography	Matrix	Comments	Element	Reference
ICP, eluent from the column fed to a cross-flow nebuliser using directly coupled PTFE tubing (0.5 mm i.d. x 300 mm long).	Strong cation exchange resin (IEX-210 SC - Toyo Soda Co. Japan) 250 mm x 4 mm i.d. at 50°C. Mobile phase 0.4 - 1.0M ammonium lactate (pH 4.22).	Rare earth elements in geological samples.	Similar interface to Ref. 299. Det. limits for system 0.001-0.3 µg/ml with 100 µl sample injection.	Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu.	301
ICP using a microconcentric nebuliser which is inserted directly into the tip of a conventional sample introduction tube of an ICP torch.	Ion-pairing, reversed phase separation. Microbore 1 mm i.d. x 50 cm C_{18} column (HRSM-50-C_{18}, C-M Laboratories, Nutley, NJ). Mobile phase. Cr - 5 mM sodium pentane-sulphonate in MeOH/H_2O (20/80), pH 3 at 120 µl/min As - 5 mM tetrabutyl-ammonium phosphate in MeOH /H_2O (5/95), pH 7.2 at 140 µl/min	Inorganic and organometallic compounds in various solvents.	First departure from using conventional cross-flow, concentric or Babington-type pneumatic nebulisers. At present some problems with low residence times of analyte species in the plasma and possible solvent interactions in the excitation process thus impairing detection limits.	Chromium (205.6 nm) Arsenic (193.7 nm)	298
ICP, with cross flow nebuliser attached directly to the microcolumn by PTFE tubing. The sample gas carries the eluent as a fog into the plasma torch.	Microcolumn gel permeation chromatography. Column made of PTFE tubing 1 mm i.d. x 20 cm packed with Fine GEL SC-220 (11.7 µm, Jasco, Japan). Mobile phase-distilled water.	Analysis of carbon containing materials - example of sacharides.	Comparison made with previously reported system using various T-type connectors in the interface - Ref. 302.	Carbon	297

4.3.5 Conclusions

Atomic absorption, whilst being the most inherently metal specific of the atomic spectroscopic techniques, introduces restrictions to the potential couplings available with liquid chromatography. In LC-FAAS using reverse phase systems, i.e. mainly aqueous eluents, low nebulisation efficiency may limit the sensitivity of the technique. However, operation of the nebuliser in a starved mode, for example by using modified injection cup devices, has been shown to alleviate much of this problem. When normal phase, i.e. organic, eluents are used, then higher nebulisation efficiencies are possible; however, transport of large amounts of organic solvents to the flame can have adverse effects on its properties, e.g. increased background levels from carbon particles and band spectra. Therefore sample transport systems are being developed in our laboratory which do not use conventional nebulisation. Since such systems allow the sample to be desolvated prior to reaching the flame for atomisation, they offer much promise for the future. The advantages of directly coupled LC-FAAS systems which offer on-line, real-time analysis with simple, cheap, readily demountable interface systems largely offset the lower detection limits obtained compared with electrothermal atomisation.

The use of electrothermal atomisation should circumvent the problem of low nebulisation efficiency; however, the time required to run through an atomiser dry-ash-atomise-cool cycle results in only infrequent samples being analysed out of the flowing chromatographic stream. To minimize the possibility of missing a species, very low flow-rates are normally used although much of the eluent is still not monitored. To overcome this problem in direct interfaces such as autosampler systems are often required and "real time" chromatographic interpretation is

not possible. Thus the advantage of high sensitivity detection obtained when using electrothermal atomisation is only achieved at the expense of real time analysis, and often involves expensive and complicated interface systems. The advent of microbore HPLC may provide some solution to problems of interfacing with electrothermal atomisers. The low flow-rates, microlitres per minute, encountered in microbore HPLC mean that the volume containing a species is very small and providing the peak resolution is good, injection into the furnace of the whole chromatographic peak may be feasible. Other possible, though expensive, ways of making coupled HPLC-ETA-AAS a real time method would be the use of a dual furnace, or the development of a continuous furnace system.

The same problems beset coupled LC-AFS as afflict any AFS method, namely: lack of suitably stable and intense line sources. However, the advent of atomic fluorescence instruments using an ICP as the atom cell will perhaps signal a renewed interest in this technique and enable the advantages of multi-element low level detection afforded by AFS to be utilised.

The plasma emission techniques offer the possibility of multi-element detection and long linear ranges. With reverse phase eluents, both DCP- and ICP-OES, like FAAS, suffer from low nebulisation efficiencies and to increase detectability, then this efficiency must be increased. The use of normal phase eluents affords high nebulisation efficiencies but, as a result, a higher background emission level, and hence an increase in detection limits. This may be offset by the increased analyte flow into the plasma. The ability of plasmas to monitor not only metal emission lines, but also carbon lines, could, so long as

non-carbon containing eluents are used, offer a universal LC detector. Although emphasis has been placed on the advantages of specific or at least selective detection, the value of a universal detector should not be underestimated, as the wide usage of FID in GC shows. By using a simultaneous multi-element facility, DCP- or ICP-OES could offer such universality.

Recent emphasis on studies into characterising the processes which take place within an interface i.e. within the nebuliser/spray chamber, connectors or even connecting tubing itself, should lead to a better understanding of the ideal sample transport system and hence the development of interface techniques which fully realise the potential of coupled techniques. The trend towards micro-bore columns, thus decreasing the eluent flow, also allows experimentation with low flow but high efficiency nebulisers such as the frit nebuliser.

The arrival of commercial ICP-MS systems which provide detection limits significantly lower than ICP-OES, and approaching those obtained by GF-AAS, offers an exciting new detection mode for coupled HPLC systems. Inductively coupled plasma-MS systems operate in a real-time mode operating on flows of liquid similar to elution rates from mini-bore HPLC systems. Thus several groups of workers are already experimenting with coupled HPLC-ICP-MS which may offer the sensitivity in a multi-element mode necessary for trace metal speciation in real samples with the on-line detection capability necessary to enable optimisation of the separation and routine operation.

CHAPTER 5

THE EVALUATION OF COUPLED HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-ELECTROTHERMAL ATOMISATION - ATOMIC ABSORPTION SPECTROSCOPY

5.1 An automatic interface for coupled high performance liquid chromatography - electrothermal atomisation - atomic absorption spectroscopy

Although the coupled HPLC-ETA-AAS systems described in Chapter 4 have found many applications, they all have certain disadvantages intrinsic to their design. The need to transfer a sample from the HPLC effluent to the furnace, often requiring the flow to be stopped, and the necessity to use very low HPLC flow rates thus severely limiting the chromatography, means that such techniques are often slow, give poor resolution and do not produce continuous chromatograms. However to investigate the practicability of such a system, an interface based on the design of Brinckman and Irgolic (246) and modified by Haswell (303) was evaluated for the speciation of arsenic.

5.2 Application to the speciation of arsenic

Current interest in the speciation of arsenic compounds can be attributed to two main areas. Firstly, the use of arsenic compounds as pesticides and herbicides, both as inorganic arsenic salts and as organoarsenicals such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). As with many other metals the toxicity, biological activity and environmental fate depends on the molecular form of the arsenic species. Secondly, it has been shown that different arsenic compounds interconvert in the environment by both chemical and biological pathways. The assimilation of arsenate by

marine phytoplankton which is finally released into solution after reduction and methylation is one such example. As the methylated forms of arsenic are apparently much less toxic than their inorganic parent compounds, speciation of arsenic compounds yields more useful information than total arsenic levels.

Various HPLC-ETA-AA couplings have been developed for the speciation of arsenic, often based on the two couplings developed by Brinckman's group at the National Bureau of Standards (238). The first of these utilises a PTFE flow through cell from which the eluent is periodically sampled and injected into a graphite furnace, so called pulsed mode operation. In the second, termed survey mode, the eluent was collected by an autosampler and each fraction collected analysed by ETA-AAS. Using these two techniques the speciation of As, Sn, Hg, and Pb compounds was demonstrated (238), the survey mode later being extended to the speciation of organometallics, polymers and organotin silicates by the same group (239). The flow-through PTFE sampling cup has also been used as an interface between a low capacity anion exchange column and graphite furnace by workers in the U.S. Dept. of Agriculture (247, 248) when speciating organic and inorganic reducible forms of arsenic in pesticide residues.

Irgolic and co-workers used a similar automated system for the speciation of arsenobetain, arsenocholine and inorganic arsenic at the micro-gram level (245). In a joint study with Brinckman's group (246) they demonstrated various chromatographic separations for the speciation of arsenic compounds in soil and water samples. The extremely high background molecular absorption levels encountered with ion pair reagents, such as tetraheptylammonium nitrate (THAN), were

reported to require Zeeman effect background correction, since normal deuterium arc correction proved insufficient.

5.2.1 Experimental

The HPLC system used in this investigation consisted of a Waters 6000A solvent delivery system (Waters Associates Inc., Massachusetts, USA), Rheodyne 7125 injection valve fitted with a 1000 µl sample loop and Whatman Zipax and SAX HPLC columns in series. Full details of the chromatography are given below and summarised in Table 12. An Instrumentation Laboratories IL 555 furnace (Instrumentation Laboratories Inc., Massachusetts, USA) was modified so that an injector could be fixed to the face plate and aligned with the cuvette sample injection opening - Figure 11. (The modified furnace was also used in conjunction with an IL Video 12 Spectrometer). In addition the vertical access port was replaced by a borosilicate glass tube which allowed nitrogen to be blown into the chamber via a stainless steel lance to accelerate cooling. The increased gas flow reduced the cooling time to about 20 seconds.

The interface consisted of two Altex (4 way) slide injection valves with pneumatic actuators. The sample (76.6 µl) and co-analyte (5 µl) loops were of 0.8 mm I.D. PTFE cut to appropriate lengths. All other inter-connecting tubing was of 0.33 mm I.D. PTFE. Delivery of the co-analyte and sample was by nitrogen pressure through a 1/16th inch OD 316 stainless steel tube activated by a solenoid. The co-analyte followed the sample through the system into the cuvette, thus reducing the possibility of inter-sample contamination.

The analysis sequence of the entire system was controlled by a

Figure 11

IL 555 flameless atomiser with auto-injector

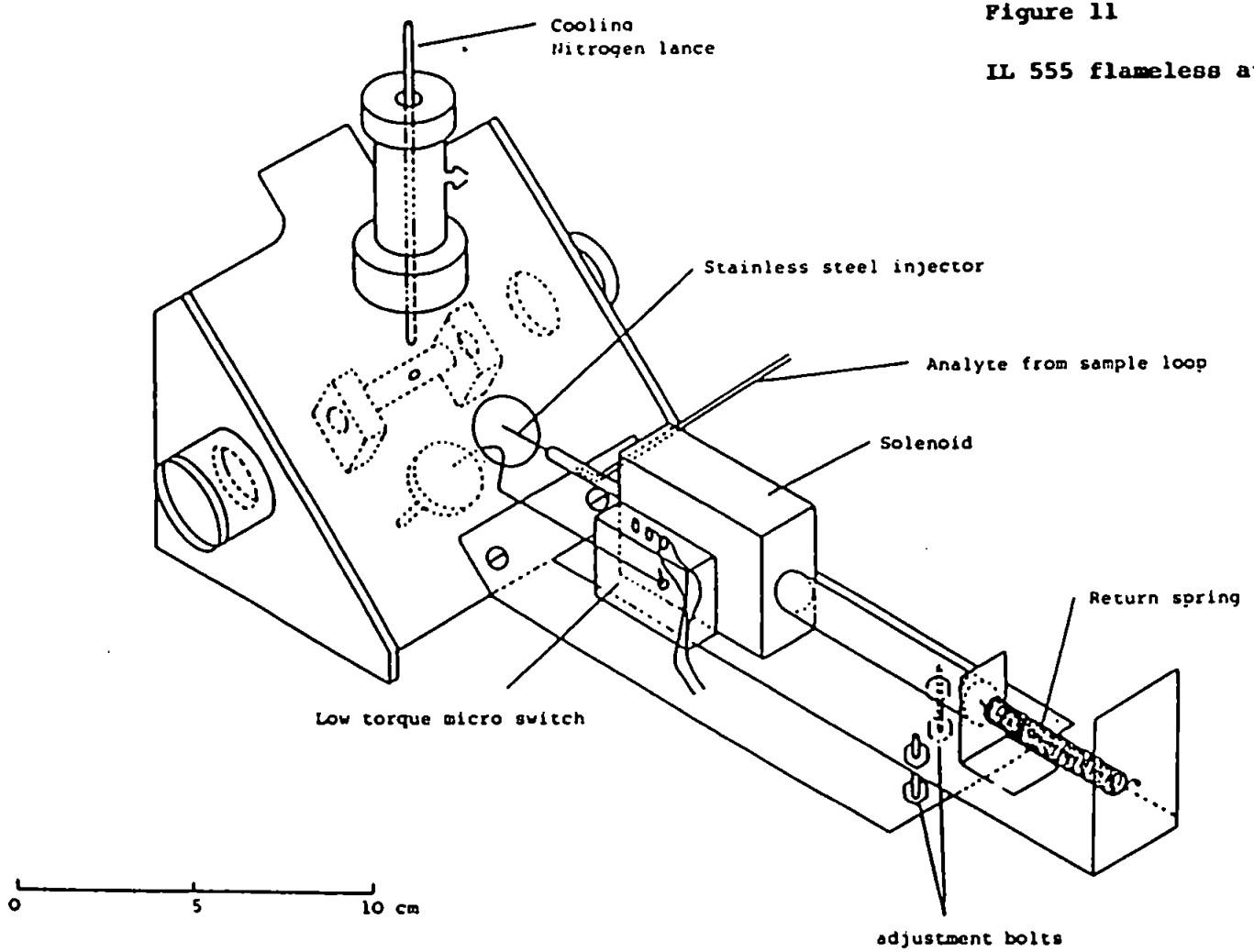


Table 11

Graphite furnace atomic absorption spectrometer conditions for the determination of arsenic

Spectrometer	IL 151 or IL Video 12
Wavelength	193.6 nm
Bandpass	1 nm
Lamp current	6.0 mA

Temperature programme:

Temperature ^{o}C	175	270	1900
Time seconds	10	5	5

Injection temperature:

125 $\pm$ 10^{o}C

Injection:

Sample volume	76.6 µl + 5 µl 0.5% nickel nitrate
N_2 injection pressure	16 psi

Table 12

Chromatographic conditions for the speciation of arsenic

Columns:	Zipax ion-exchange precolumn (100 mm x 5 mm i.d.) in series with a SAX-10 strong anion-exchange column (200 mm x 5 mm i.d.)
Eluent:	Sulphuric acid (0.1 M) switched to ammonium carbonate (0.1M) after the first peak has appeared.
Flow rate:	1.0 ml
Sample injection:	20 µl - 1 ml.

microprocessor control system. The use of A/D convertors allowed the determination of peak area from the atomic absorption signal and the determination of furnace temperature. Status lines were also used to inform the computer of a) whether the furnace door is open and ready for the next injection cycle, and b) when the atomisation of the sample is about to occur so that data acquisition may begin. The results obtained were recorded on a standard chart recorder and peak height measurements taken.

Blowing the sample into the hot cuvette has a number of advantages:

(a) a larger volume of sample can be accommodated by the cuvette as vapourisation of solvent occurs almost immediately, thus increasing the effective sensitivity;

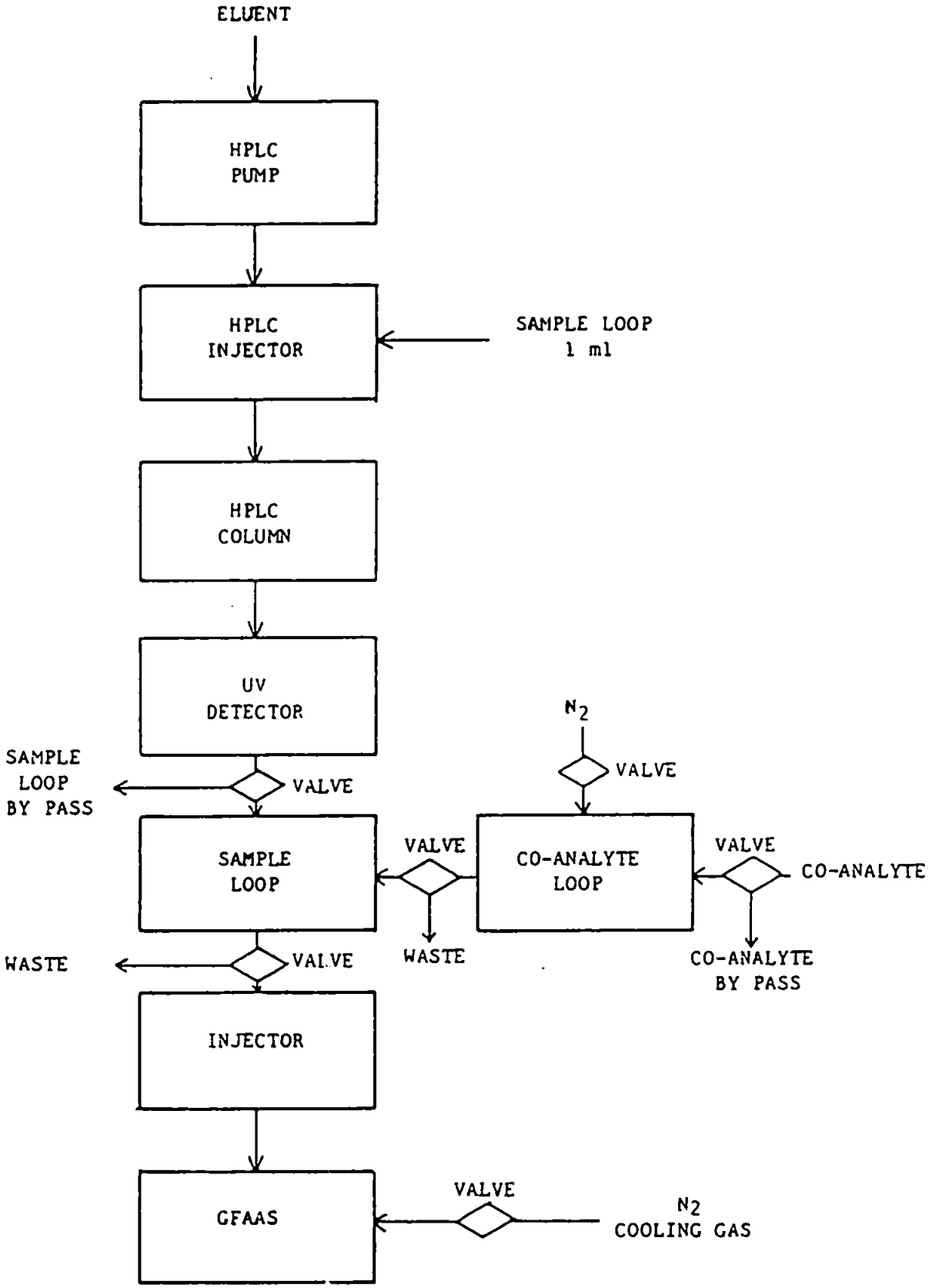
(b) the analysis sequence time for any one determination is reduced both by shortening the drying time and decreasing the cooling range. These effects plus the increased rate of cooling achieved by introduction of extra nitrogen coolant gas reduces the total cycle time from over three minutes to approximately 50 seconds.

A schematic representation of the complete system as used for the determination of arsenic is shown in Figure 12.

All chemicals and solvents used in this work were supplied by BDH Chemicals Ltd., Poole, England. The organoarsenic standards were provided by Dr. K.J. Irgolic, Texas A and M University, USA. The urine samples were supplied as part of a joint study with the Ministry of Agriculture Fisheries and Food. These samples were collected from individuals over a five day period following ingestion of a fish meal

Figure 12

Schematic diagram of HPLC-GFAAS interface for the determination of arsenic



spiked with inorganic arsenic. The object of this work was to speciate the arsenic excreted from the body.

This complete study required the use of coupled HPLC-hydride AAS (see Chapter 7) for the speciation of reducible species - As^{3+}, As^{5+}, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), and the use of coupled HPLC-ETA-AAS for the speciation of any non-reducible forms present such as arsenobetaine $[(CH_3)_3\ AsCH_2\ COOH]^+$ and arsenocholine $[(CH_3)_3\ As\ CH_2\ CH_2OH]^+$ which is probably the precursor from which arsenobetaine is formed.

In many studies, the presence of non-reducible compounds is calculated from the difference between the sum of the reducible forms and the total As level as determined by graphite furnace-atomic absorption. This evaluation was made here as a first step in identifying the species present. The interface was connected for this analysis although a peristaltic pump was used in place of the HPLC to facilitate supplying the sample. The graphite furnace atomic absorption spectrometer conditions are given in Table 11.

Once the total As levels had been determined the HPLC system was connected. To separate the arsenic species, a twin column system was used, consisting of a silica based anion exchange precolumn in series with a SAX resin based strong anion exchange column. The elution system involved switching from sulphuric acid (1.8×10^{-4} M) to ammonium carbonate (0.1 M) which enables a preconcentration on the Zipax column. The success of this system for arsenic speciation has been reported by Tye et al. (304), and is detailed further in Chapter 7. However, in this instance the optimum flow rate of 3 ml min^{-1} was

reduced to 1 ml min^{-1} to meet the requirements of the interface. The chromatographic conditions used here are summarised in Table 12.

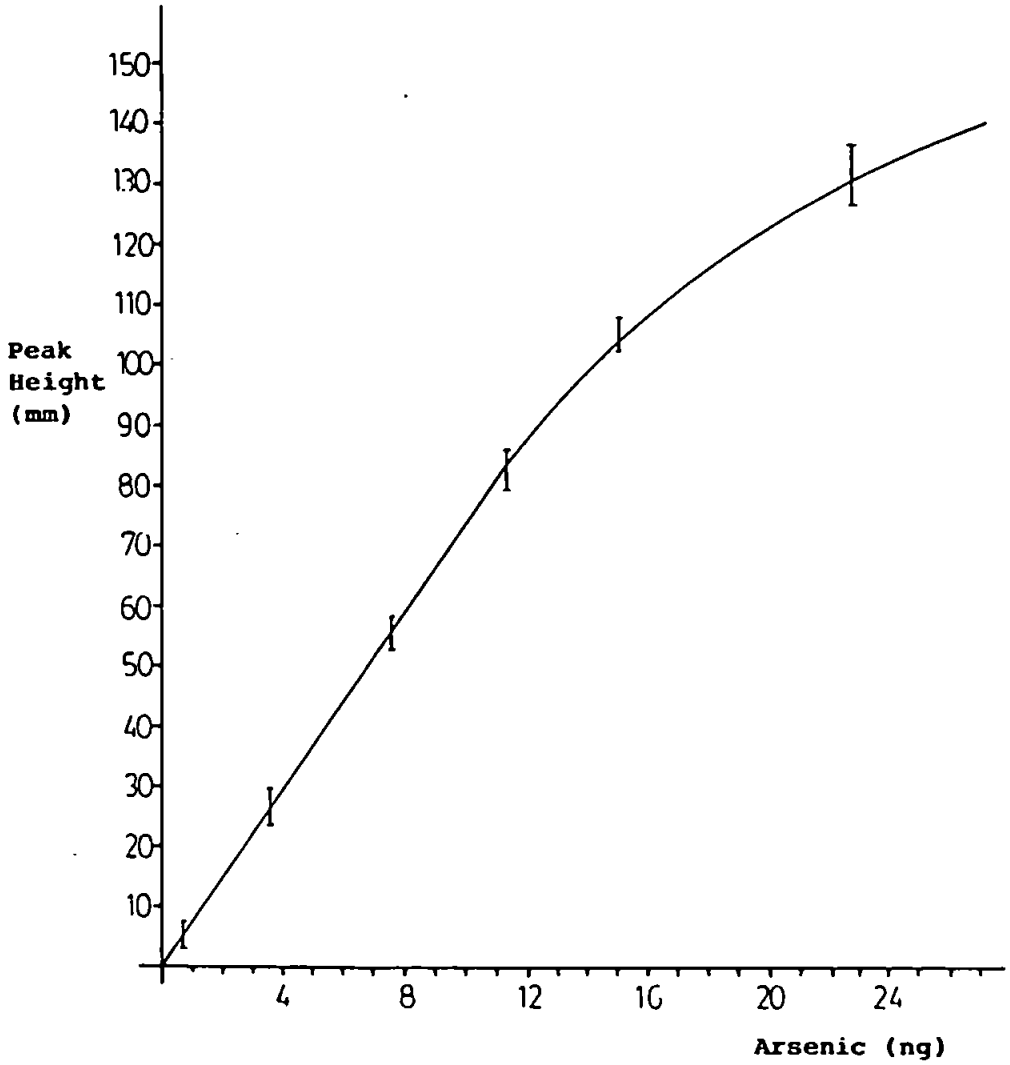
5.2.2 Results and Discussion

The calibration data obtained from a series of arsenic standards (sodium arsenate) using the interface and a peristaltic pump to fill the sample loop is shown in Figure 13. A linear working range of 10 ng with a detection limit of 0.5 $\mu g\ l^{-1}$ was obtained.

When the HPLC was connected difficulty was experienced in obtaining any response from a mixed standard containing 50 ng of As^{3+}, As^{5+}, MMA, and DMA, even when leaving the system running for over an hour. This was attributed to the greatly reduced flow rate which would adversely affect the chromatogrpahy, but which was necessary to avoid the possibility of the species once separated moving through the interface between injections into the graphite furnace and thus not being detected. The chromatographic system was therefore changed, the Zipax/SAX system being replaced with a Hypersil ODS column (3-5 µm 250 mm x 4 mm) and a sulphuric acid isocratic elution system (1.8 x 10^{-5} M) as used by Haswell (303). Using this system and a single standard solution of sodium arsenate, it was found possible to obtain a simple chromatogram via the interface although the peak had severe tailing and took some twelve minutes to elute - Figure 14. It was obvious from these findings that such a system was ill suited to speciation studies and so an attempt was made to reproduce the work of Stockton and Irgolic (245) for the separation of arsenobetaine, arsenocholine and arsenite/arsenate. Since extremely high background molecular absorption levels are encountered with this technique, due to the use of ion pair reagents such as tetraheptylammonium nitrate,

Figure 13

Calibration curve of peak height against weight of arsenic with 0.5% nickel nitrate co-analyte



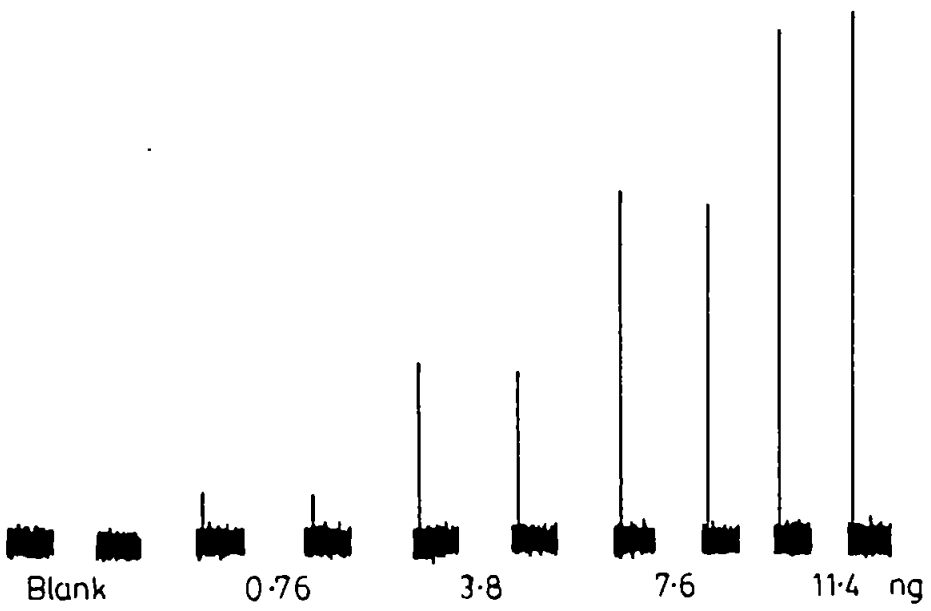
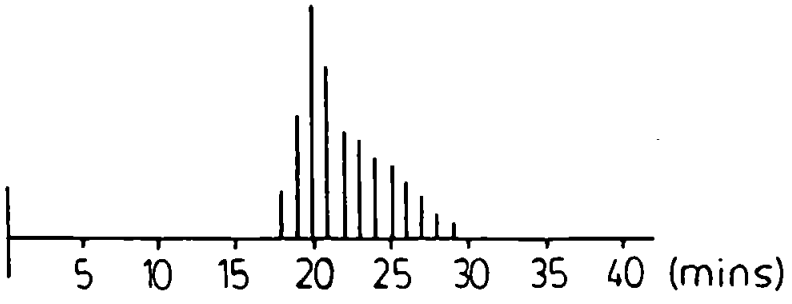


Figure 14

Chromatogram obtained using the HPLC-GFAAS interface

50 ng sodium arsenate



(- overcome by the use of Zeeman effect background correction in the work by Brinckman et al. (246), the modified furnace was fitted to an IL Video 12 instrument fitted with Smith-Heiftje background correction. However, once again problems were encountered due to the difficulty in operating the interface for more than a few minutes without malfunction, and so this work was stopped in favour of developing a less complicated interface for use with flame atomic absorption instruments.

5.3 Limitations of coupled high performance liquid chromatography-electrothermal atomisation - atomic absorption spectroscopy

Although collection of the HPLC effluent in some form of auto-sampler followed by subsequent discrete injection of the collected fraction into an electrothermal atomiser provides an interim solution to the need to improve upon reported couplings to conventional FAAS detectors, there are as explained above several practical problems associated with this approach. In addition the results are not obtained in real-time and the non-continuous nature of the detector is both tedious and likely to lead to peak broadening. Hence in the following work emphasis is placed on the design and construction of a continuous detector which parallels the former work with coupled G.C.-AAS, in which appropriate optimisation of a flame atom cell has led to a continuous detector with superior powers of detection to electrothermal atomisation for typical genuine samples, yet remaining simple, reliable, and giving real-time analysis.

CHAPTER 6

THE EVALUATION OF DIRECTLY COUPLED HIGH PERFORMANCE LIQUID CHROMATOGRAPHY - FLAME ATOMIC ABSORPTION SPECTROSCOPY

In addition to offering excellent inter-metal selectivity, flame atomic absorption has the advantage that it readily accepts liquid samples. Coupled HPLC-FAAS systems also overcome many of the problems associated with coupled HPLC-ETA-AAS since they offer on-line, real time analysis and produce a continuous chromatogram. The various LC-FAAS couplings reported in the literature have been summarised in Chapter 4.

6.1 Development of a simple directly coupled high performance liquid chromatography - flame atomic absorption spectroscopy system for the speciation of organotin compounds

Several of the most recent publications on coupled LC-FAAS have stressed relatively simple interface systems, and have reported increased sensitivity by attention to the atom cell. The system reported below consists of a modified version of Slavin and Schmidt's direct coupling (232) utilising pulse nebulisation via a directly coupled vented tube from the column to the nebuliser, althought the sensitivity has been improved by incorporating a slotted tube atom trap to increase the residence time of atoms in the flame. This system has been evaluated for the determination of tributyltin species in natural waters, detection of which has proved problematical by other techniques.

6.1.1 Instrumental

Atomic absorption spectrometer (SP9, Pye Unicam, Cambridge) fitted with background correction and tin hollow cathode lamp. The 224.3 nm tin line was used. A Pye Slotted Tube Atom Trap (STAT) was fitted to the 5 cm burner head. Waters 6000A solvent delivery system (Waters Associated Inc., Massachusetts) equipped with a Waters U6K injector with 1 ml sample loop and Partisil-10SCX analytical column (10 µm particle size, 25 cm x 4.6 mm i.d.) (Whatman, New Jersey). 10 µl and 100 µl HPLC syringes (Scientific Glass Engineering, Melbourne). Perkin-Elmer 023 chart recorder (Norwalk, Connecticut).

6.1.2 Reagents

The tributyltin chloride and tributyl tin fluoride were supplied by Aldrich Chemical Co. Ltd., Gillingham. All other analytical reagents used were obtained from BDH Chemicals Ltd., Poole, Dorset.

The tin(IV) stock solution was prepared by dissolving 0.1000 g of analytical reagent grade granulated tin in 20 ml of hot conc. H_2SO_4. This was cooled and added cautiously to about 200 ml of water contained in a 1 l calibrated flask. The solution was then cooled and a further 60 ml of conc. H_2SO_4 added. The solution was allowed to cool and the mixture diluted to 1 l with water. Any globules of sulphur formed were ignored.

6.1.3 Results and discussion

The ideal system envisaged at this stage was one enabling direct injection of aqueous samples onto the HPLC column for separation of the organotin species, without sample pre-treatment and then detection by FAAS keeping the interface as simple as possible. Particular

emphasis was placed on the separation of tributyltin species although development of the chromatography was found to be difficult in the absence of a suitable detector; conventional u.v. and fluorescence detectors being unsuitable due to the absence of any active chromophore or fluorophore for the butyltin species. (This last fact is common for all nonaromatic derivatives of organotin (238), thereby giving impetus to general metal-specific HPLC detection schemes for trace alkyl metals or alkyl metalloids). Emphasis was therefore placed on development of the interface to the atomic absorption spectrometer which was to be used as the detector.

The first method of sample introduction considered was that of discrete volume nebulisation (also known as 'direct injection', 'aliquot', or 'pulse nebulisation'). This technique overcomes the problem of small sample uptake, e.g. less than 0.5 - 1.0 ml, which may not give the conventional system time to attain equilibrium and produce a steady reading on the meter or digital display. Using the discrete volume technique sensible readings may be obtained using volumes of solution as small as 10 - 100 µl (305).

The sample uptake capillary from the nebuliser was constructed so that it terminated in the point of a small plastic conical funnel as shown in Figure 15. A standard micropipette tip is ideal for this purpose although the capillary tubing from the nebuliser should only be pushed in about 0.5 mm to avoid forming a liquid trap which would cause cross-contamination (183).

The tip was then mounted vertically in front of the spectrometer. The instrument was set up in the normal way for flame analysis except that

Figure 15

Accessory for discrete volume nebulisation

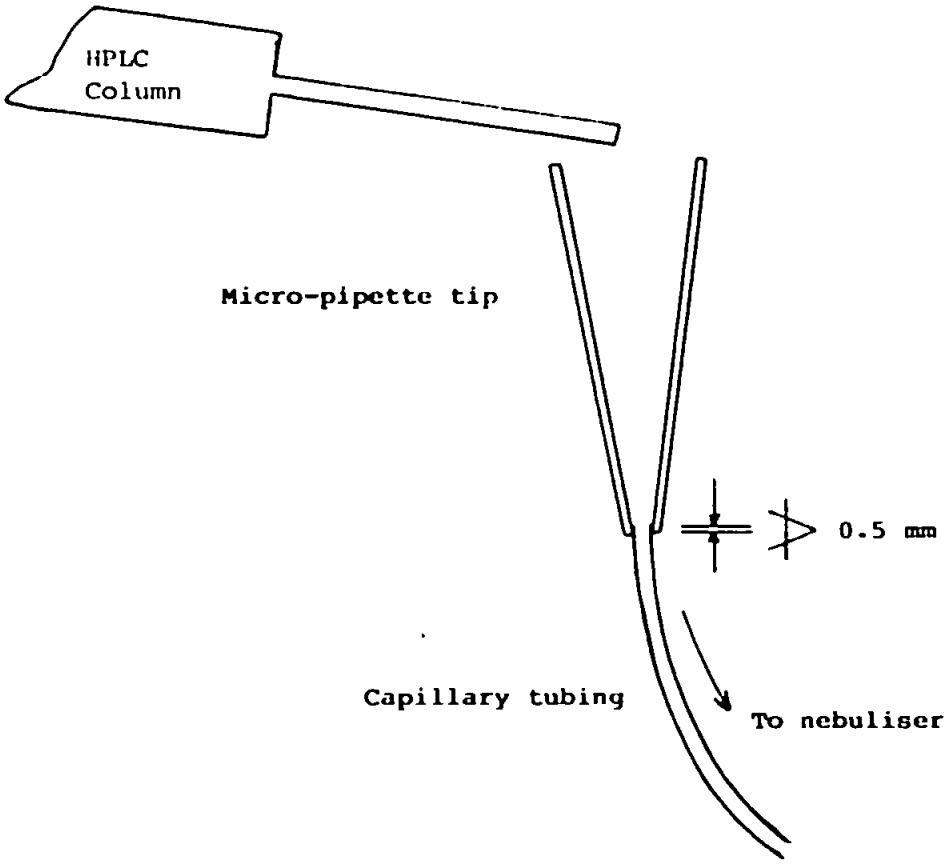


Figure 16

Comparison of discrete volume nebulisation with direct uptake

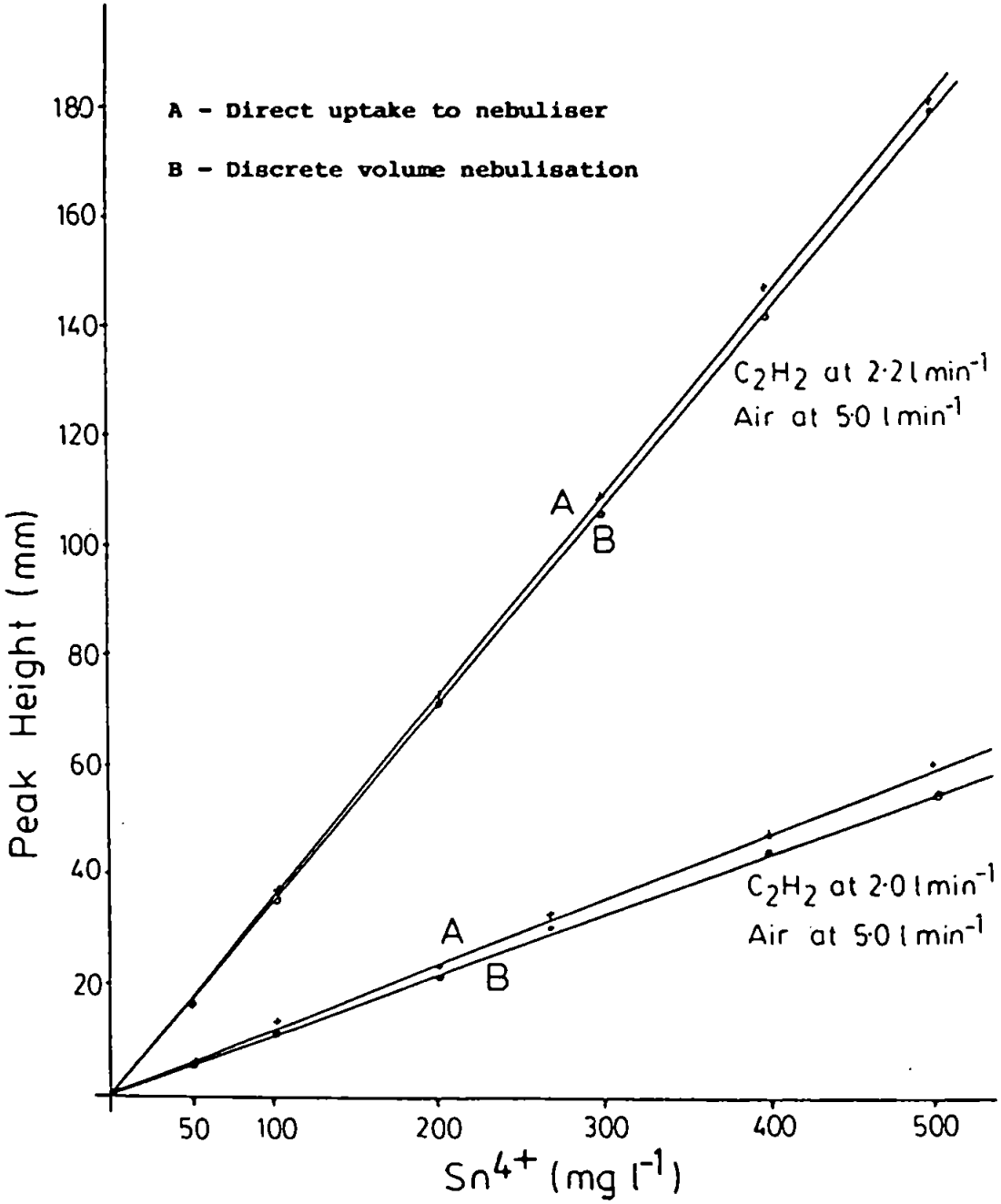


Figure 17

Effect of sample size on the response obtained with discrete volume nebulisation

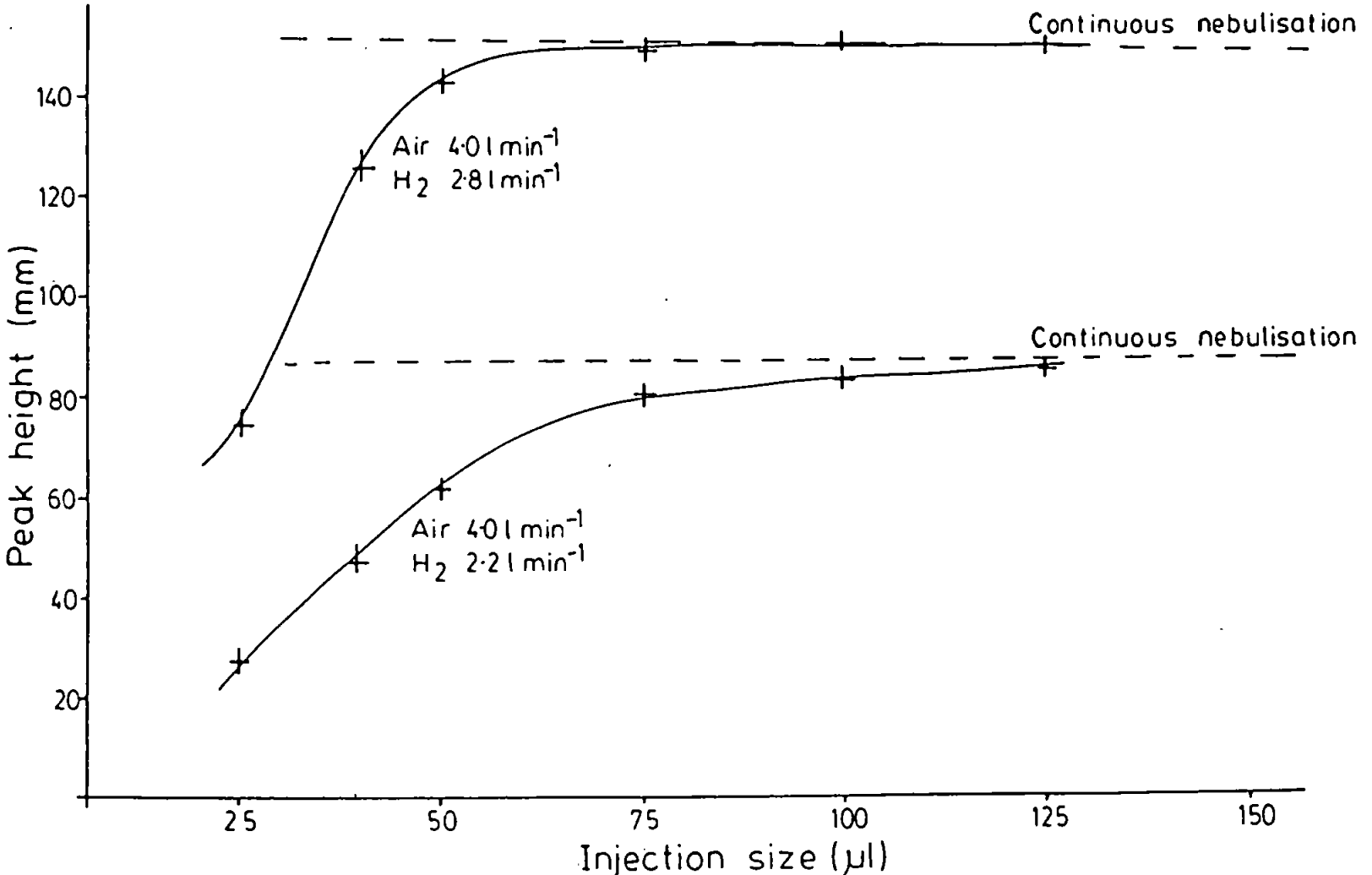


Figure 18

Modified arrangement for discrete volume nebulisation

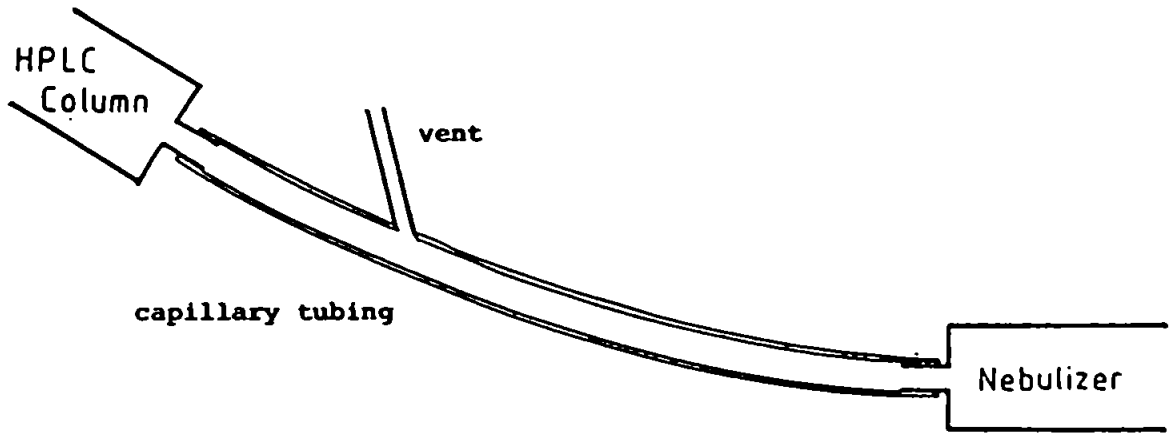
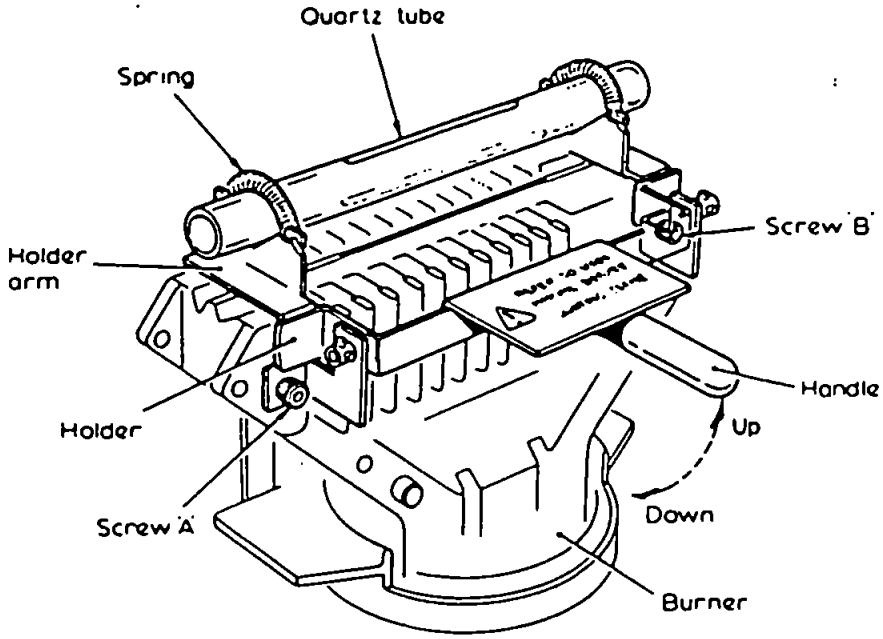


Figure 19

STAT accessory mounted on a burner



the spray chamber and burner were allowed to run dry when no sample was being passed.

Usually analysis would be carried out by transferring 50 or 100 µl aliquots of the sample into the tip by means of a precision micropipette. The sensitivity obtained using this method was found to be only marginally lower than for continuous aspiration, Figure 16. In the analysis of seawater for TBTC, however, the sample input was facilitated by allowing the HPLC column eluent to drip directly into the cup, although the arrangement was later modified by replacing the 'cup' with an open ended tube - Figure 18.

An examination of the effect of eluent drop size on the response was also undertaken under optimal conditions in later work, Figure 17, and revealed that a possible increase in sensitivity could be obtained by using 'gulp' nebulisation, i.e. allowing several drips to fall into the cup before being released to the nebuliser and thus increasing the sample size.

Although discrete volume nebulisation allowed a simple yet effective way of getting the sample into the nebuliser, the detection limit for Sn using conventional FAAS with an Air/H_2 flame was in the order of 0.06 mg l^{-1}. A means of increasing the sensitivity yet keeping the system simple was thus sought, and to this end a slotted tube atom trap (STAT) was used, Figure 19. The use of such a double slotted quartz tube as an atom trap was first described by Watling (306). The tube was supported above the flame from a conventional burner with one of the slots aligned directly above the flame. In the original design the slots were machined laterally so that they were parallel to

each other at 120°. In the commercial tube however the slots are at 180° since this angle was found to give significantly improved detection limits for some elements, although because the slots are superimposed and directly in line with the burner slot the atomic residence time in the tube is probably reduced resulting in a loss in sensitivity. The improvement in detection limits can probably be attributed to the decreased turbulence of the hot gases in the 180° slot configuration.

The improvement in sensitivity when using the STAT is generally confined to those elements readily dissociated to their ground state atoms in the flame. This implies relatively low M-O dissociation energies. Elements with relatively high M-O dissociation energies such as higher molecular weight transition metals and the refractory elements, which are normally best determined in the nitrous oxide/acetylene flame are precluded because of the excessive thermal shock this hotter flame would impose on the quartz tube. The determination of tin using the STAT is however easily faciliated using the cooler air/H_2 flame giving a detection limit of 0.015 mg l^{-1} i.e. a four fold increase on the conventional H_2/air flame mode.

Before using the STAT in conjunction with the simple HPLC interface above, its performance was optimised and comparison made with operation in the conventional flame mode. Obviously to do this the optimal burner height for conventional flame mode must first be determined so that the changes in response obtained when using other parameters can be directly compared with those obtained using the STAT which is fixed such that the burner height, rotation, and lateral alignment ensure maximum radiation passes through the tube. The

results obtained are shown in Figure 20. In all further work using conventional flame mode a burner height of 10 mm was selected.

The next parameters to be optimised were the gas flows to the burner. Using the STAT the hydrogen flow rate was varied whilst keeping the air flow constant and aspirating solutions of both Sn^{4+} and TBTC. The results obtained are shown in Figure 21. Changing the air flow rate did not greatly effect the response, although at lower flow rates severe noise problems was observed. To overcome this problem the air was preset at 4 l min^{-1} so that the interference was eliminated. The final spectrometer conditions selected are summarised in Table 13, and the instrument arrangement is shown in Plate 2.

Having optimised the detector it was now possible to optimise the chromatography. The column selected was a Partisil reverse bonded phase (RBP) SCX column, which may be considered as having a mixture of three distinct properties. These include cation-exchange character which is dictated by the sulphonate group, RBP character due to the hydrocarbon part of the stationary phase, and adsorption character arising from incomplete coverage of Si-OH sites. In most cases, cation-exchange equilibria dominate the chromatographic process. For an ideal RBP-SCX, exemplified by the Partisil SCX siloxane-bonded benzenesulphonic acid function, the individual organotin ion can be regarded as a classical cation. The comparable relation between the basic (anionic) species, a major complexing ion (or buffer) forming the supporting electrolyte, and active RBP anionic exchange sites have been treated fully in principle by Horvath et al. (307). A variant of that approach which appears suited to qualitatively assessing organotin species and column properties found in the present work has

Figure 20

Optimisation of burner height

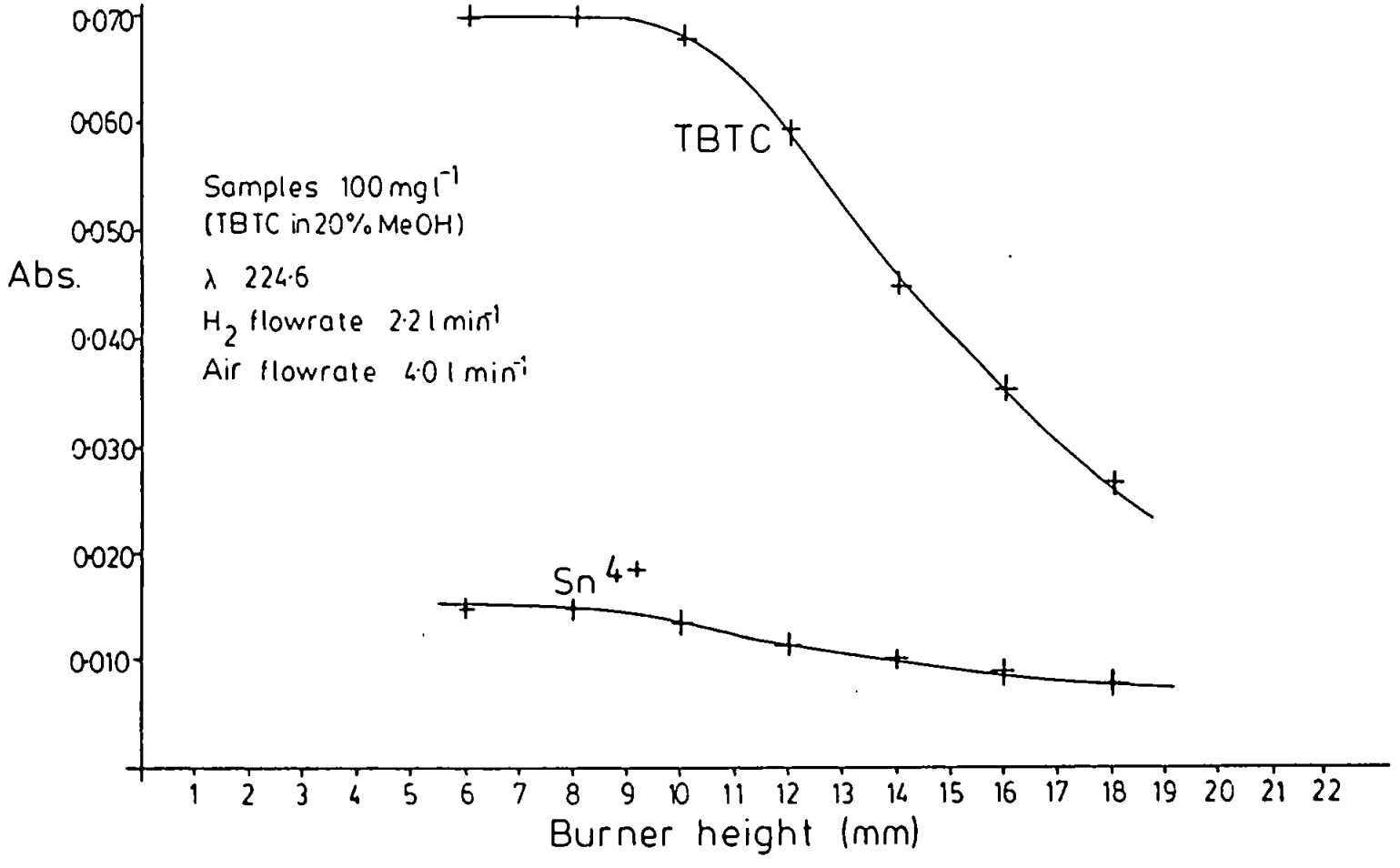


Figure 21

Effect of changing the H_2 flowrate when using the STAT

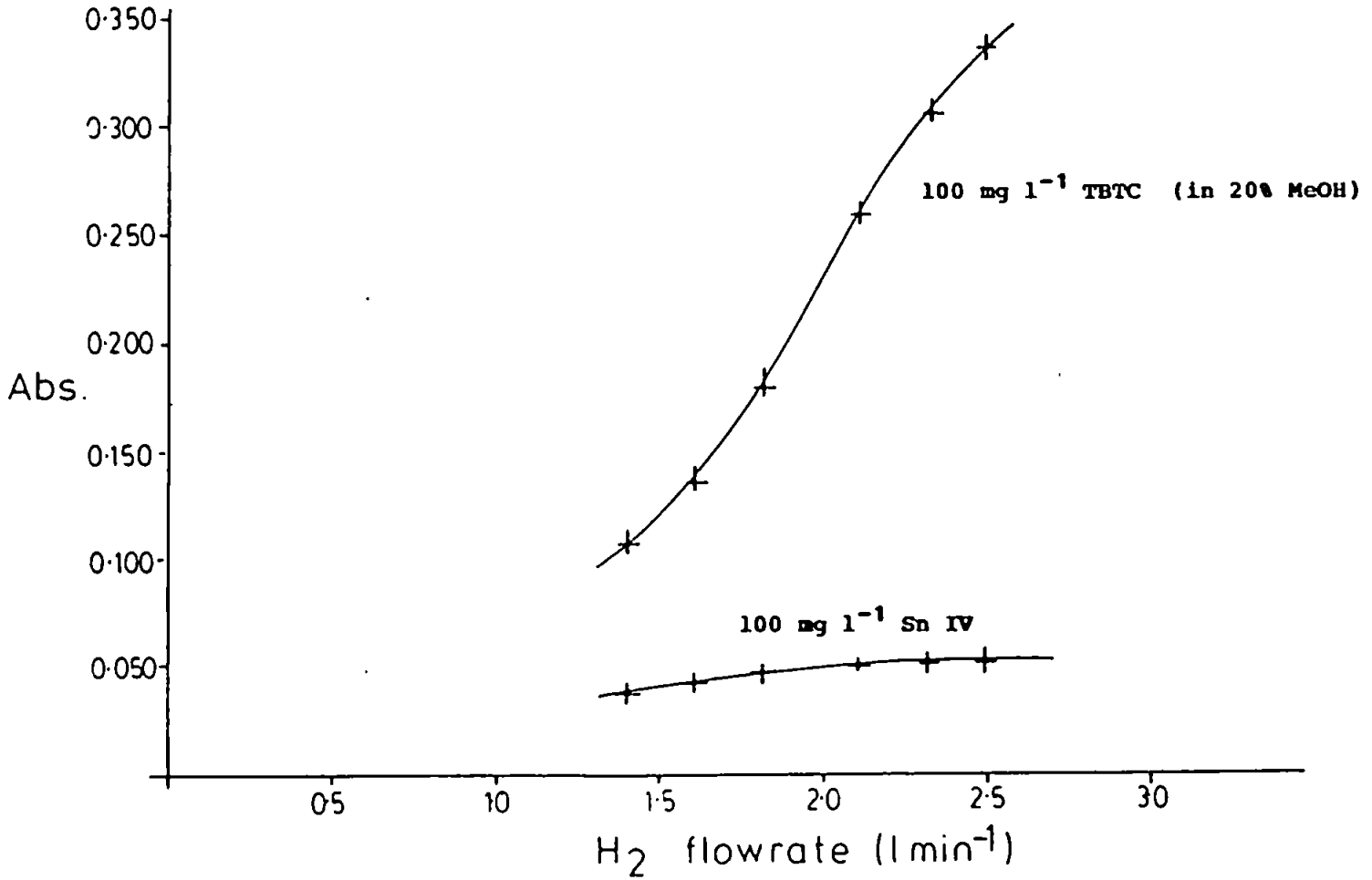


Table 13

Spectrometer conditions used for the speciation of tin

	With STAT	Without STAT
Air	4.0 l min^{-1}	4.0 l min^{-1}
Hydrogen	2.6 l min^{-1}	2.2 l min^{-1}
Burner Height	16 mm (fixed)	10 mm
Lamp Current	6.0 mA	6.0 mA
Wavelength	224.3 nm	224.3 nm
Slit Width	0.1 mm	0.1 mm

Table 14

High pressure liquid chromatograph conditions for the speciation of tin

Column:	Whatman Partisil-10 SCX (10 µm particle size, 25 cm x 4.6 mm i.d.)
Mobile phase:	80:20 Methanol:Water
Buffer:	0.1 M ammonium acetate solution
Flow rate:	3 ml min^{-1}
Injection size:	Up to 2 ml facilitated

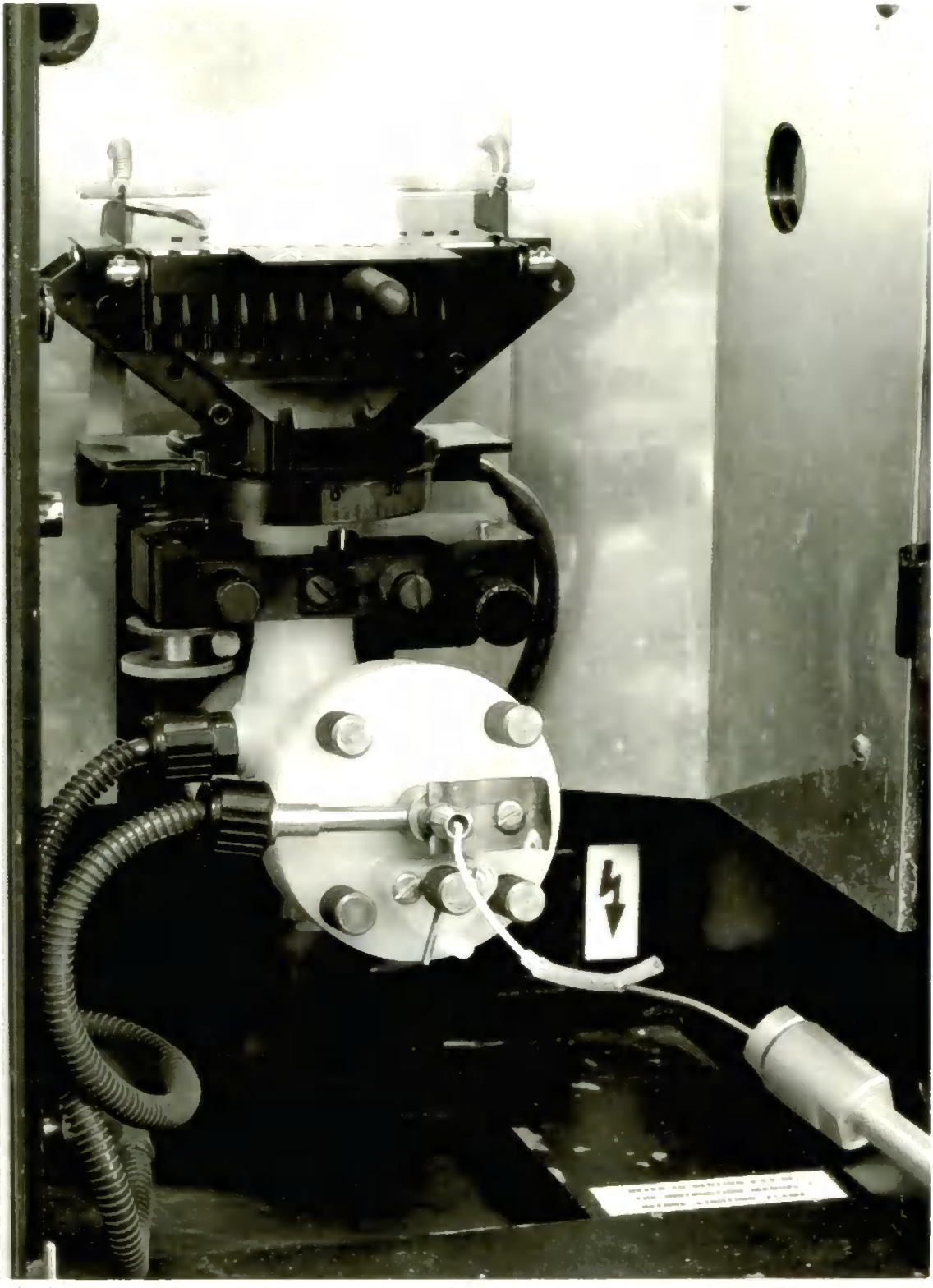


Plate 2

Interface for directly coupled high performance liquid chromatography - flame atomic absorption spectroscopy

been published by Jewett et al. (308) - Figure 22.

The simplest example conceptualized in Figure 22 involves a monoacid, R_3Sn^+, and a singly charged anion, L^-, interacting mutually or competitively with the substrate. Whereas k_o is a measure of the exchange process normally associated with cation-exchange resins, k_1 and k_2 are measures of the less important processes of partition with the organic link between support material and sulphonate groups or adsorption with unreacted silanol sites. Organotin salts are considerably more (10^2) soluble in lower alcohols than water (309, 310). Therefore, under the experimental constraints imposed by optimising their solubility in methanol/water mobile phases, in concert with improved column efficiencies or capacities and necessary ionic strengths to achieve reasonable separation times and sensitivities, the individual effects of pH or ligand L^- selectivity were not measured. Nonetheless, it will be seen that these factors also variably affect k_o, k_1, or k_2 to some extent.

With an SCX column, partial nonionic separation can therefore be qualitatively evaluated by varying solvent properties. Thus, the involvement of a "free" tributyltin cation separation on the SCX column (308) indicates that by varying the mobile phase composition and ionic strength, optimised conditions for speciation of mixtures of organotins can be attained. The effect of changing the percentage of methanol in the mobile phase and the reduction in retention time of TBTC with increasing buffer strength are shown in Figures 23 and 24 respectively. In addition to reducing the retention time, an increase in the percentage of methanol in the eluent improves the resolution and hence increases the sensitivity. The effect can be seen in

Figure 22

Proposed interaction of organotin ion with column substrate

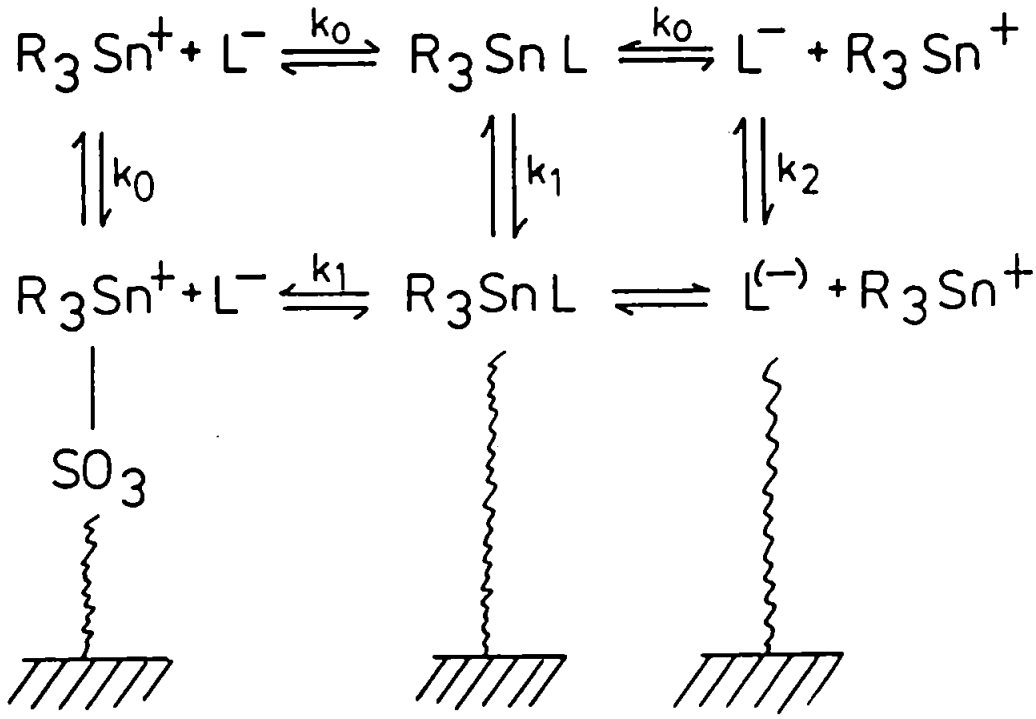


Figure 23

Effect of eluent composition (MeOH : H_2O) on response for TBTC

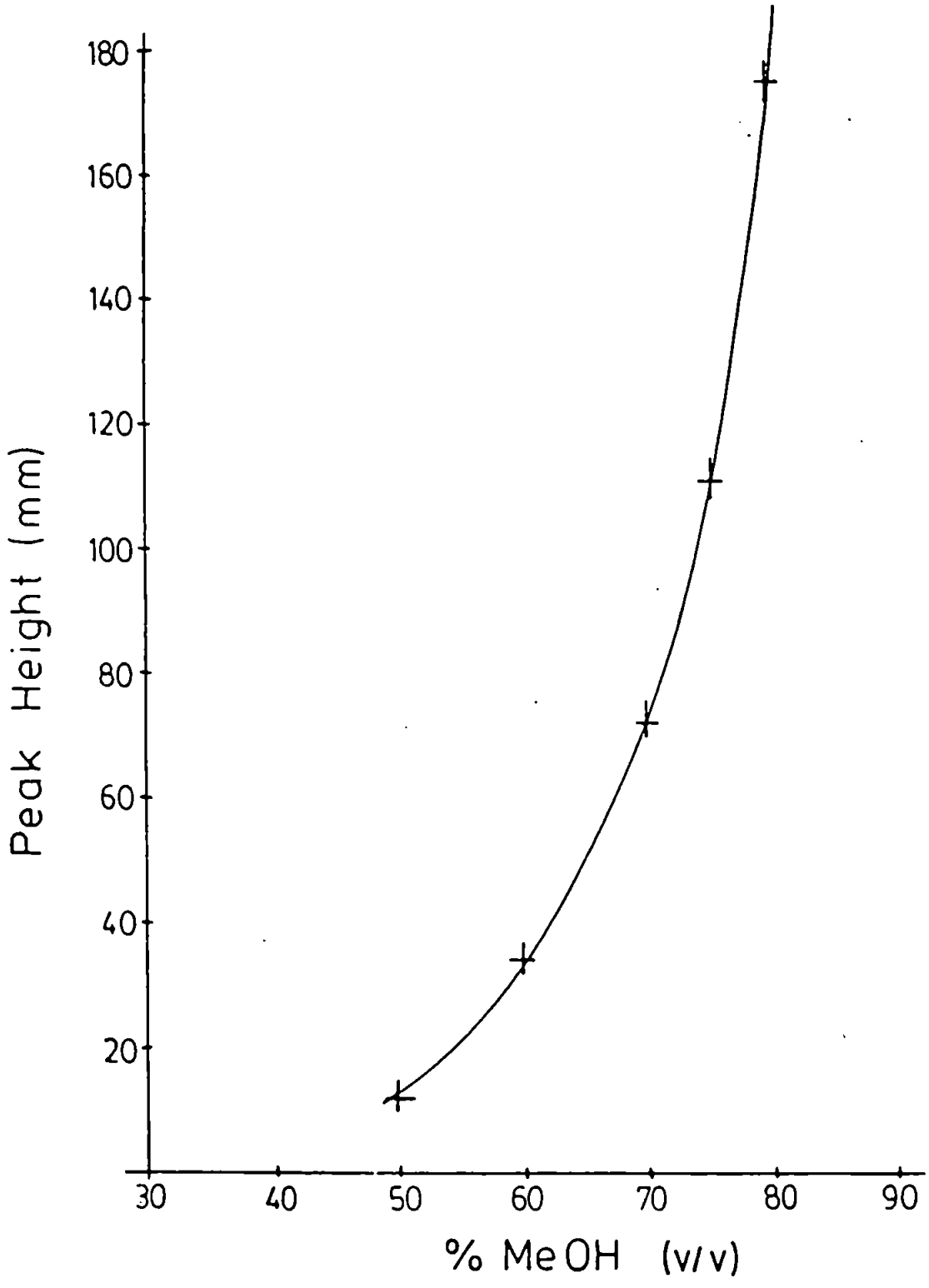


Figure 24

Effect of NH_4O Ac concentration on the retention time of TBTC

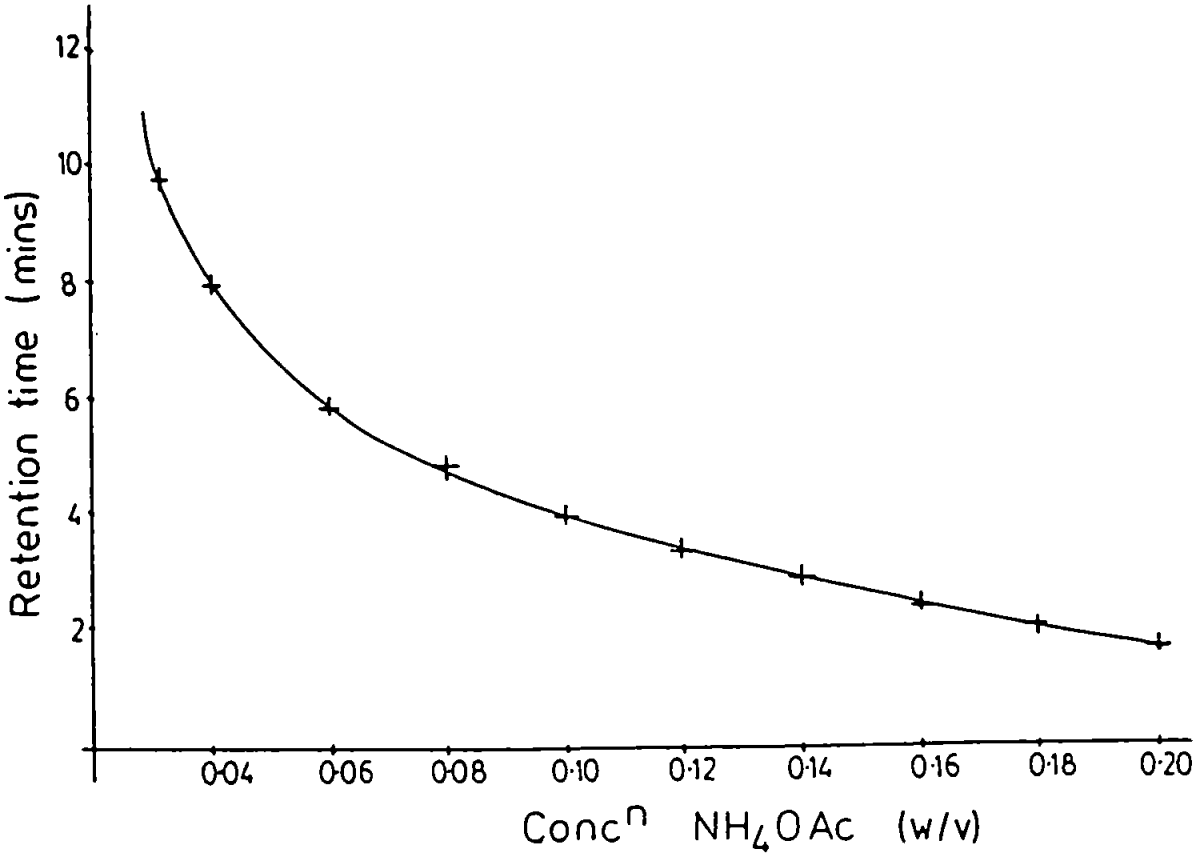


Figure 25

Effect of NH_4OAc concentration on analytical signal for TBTC

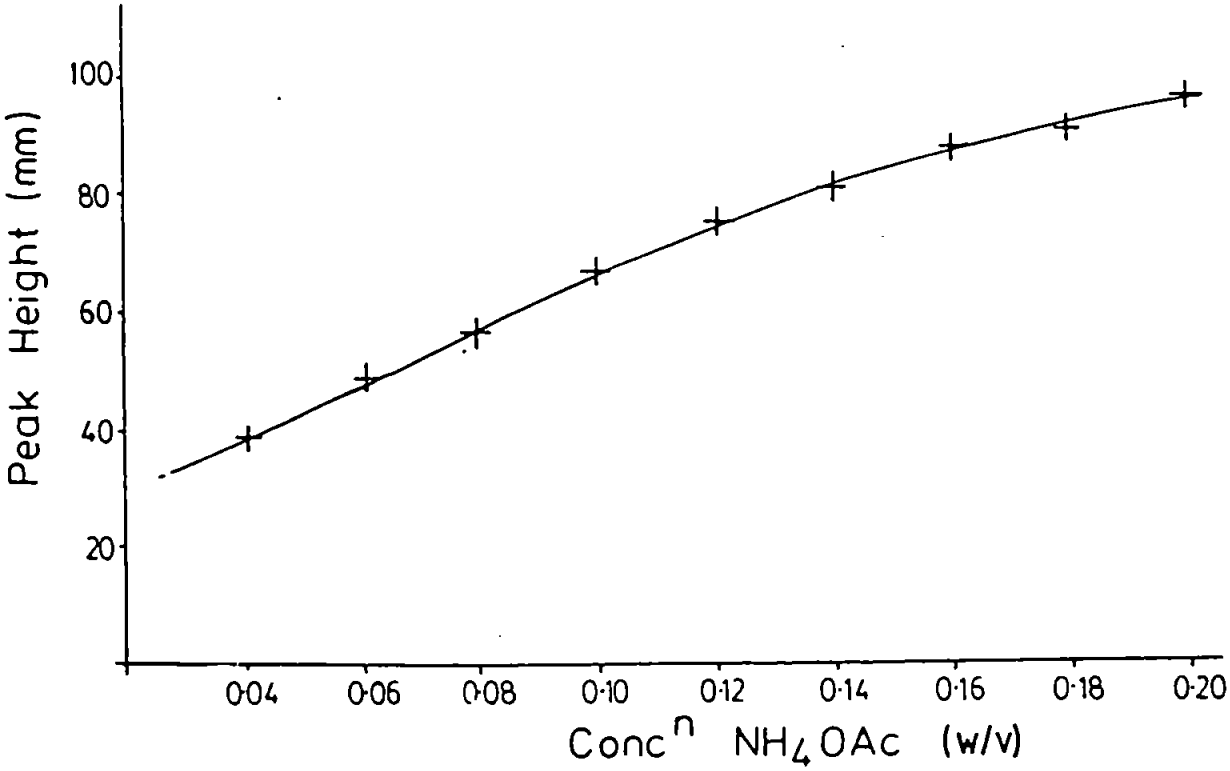
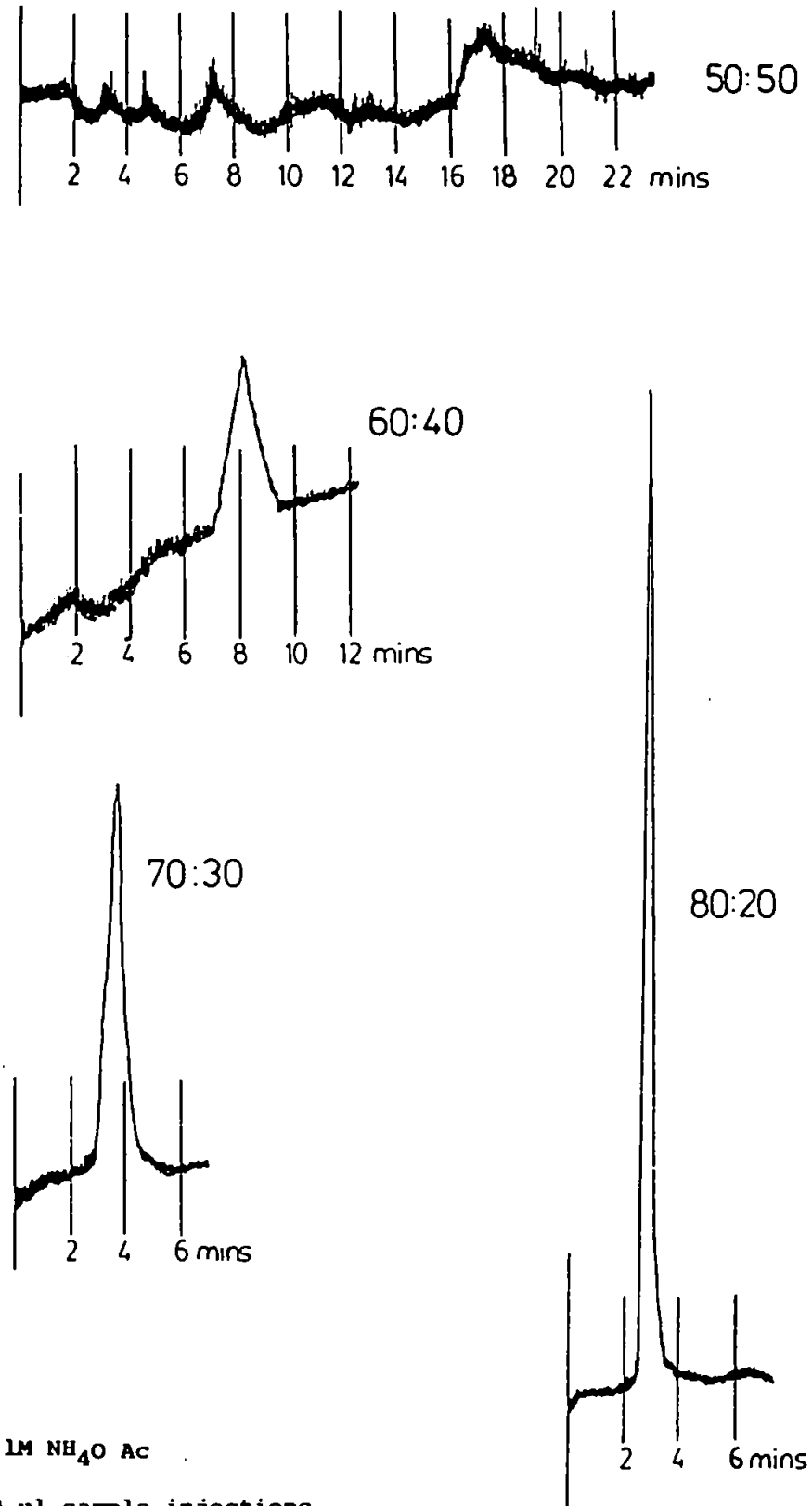


Figure 26

Effect of elute strength ($MeOH:H_2O$) on resolution and retention time of TBTC

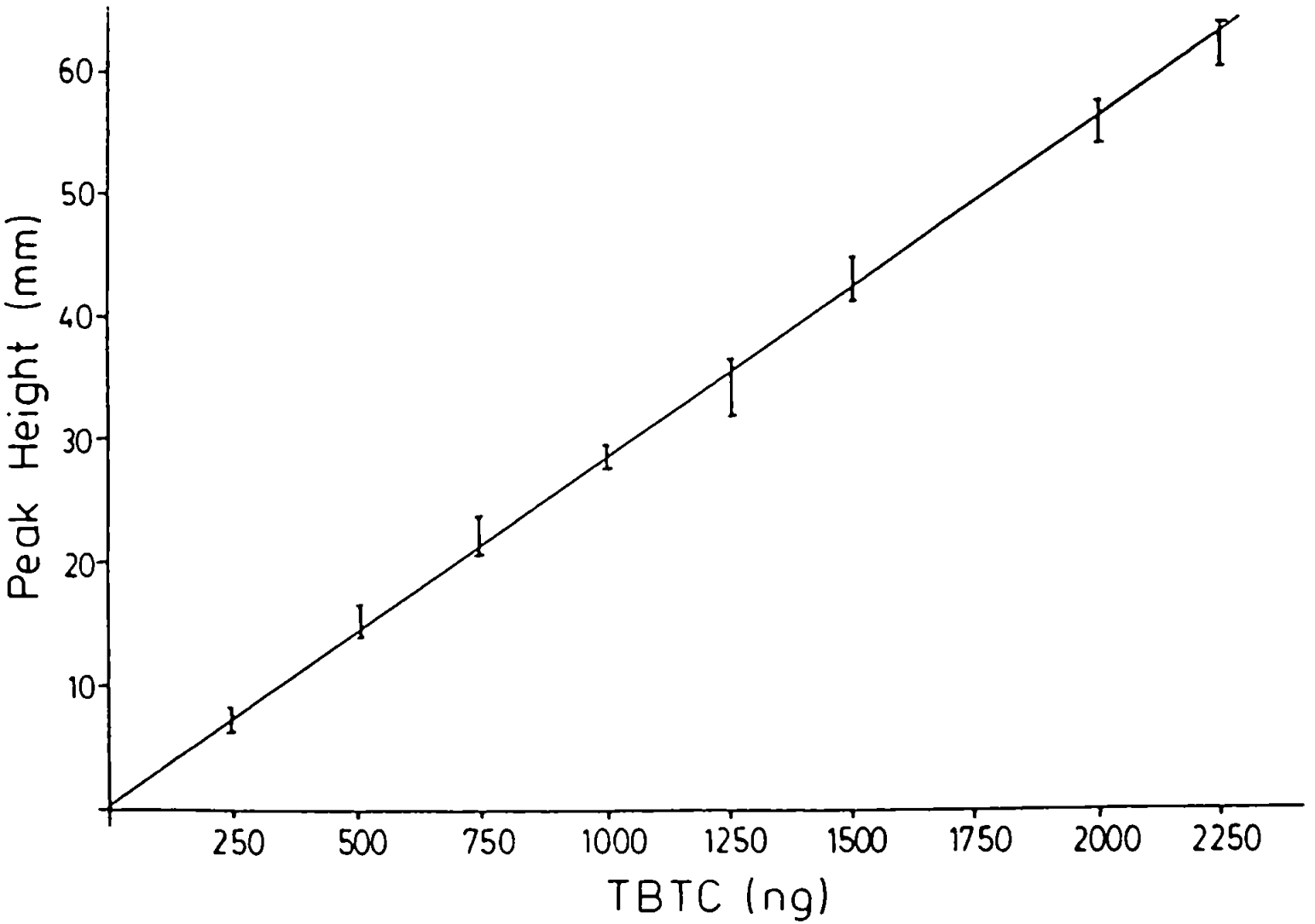


0.1M NH_4O Ac

50 µl sample injections

Figure 27

Calibration curve for TBTC using coupled HPLC-FAAS



Figures 25 and 26. The optimal chromatographic conditions for TBTC are summarised in Table 14.

Using the conditions in Tables 13 and 14 a detection limit of 200 ng TBTC can be obtained. The calibration curve obtained is shown in Figure 27.

6.2 Determination of tributyltin compounds in seawater

Disturbances in calcification mechanisms in shell fish have been reported by several groups of shellfish growers (311). Organotin compounds used in the formulation of antifouling paints (e.g. bis (tributyltin) oxide and tributyltin fluoride and chloride (312)) have been implicated in causing the extreme shell thickening and the formation of an intralamina gel in certain species (311). As a result of this, a series of bans on the use of organotin antifoulings on vessels of less than 25 m have been instigated by the French government. However, in the past analysis of TBTC in seawater has utilised gas chromatography/mass spectroscopy (GC/MS) (313). The technique developed above using coupled HPLC-FAAS was therefore evaluated as a simple yet fast and sensitive means of determining organotins in sea water collected at various sites around Plymouth.

Although optimal conditions for TBTC were determined in section 4.2.1, these were not employed here since it was hoped that by increasing the retention time of TBTC other species could be identified by reducing the chance of co-elution. The conditions selected for the analysis consisted of a mobile phase of 0.03 M ammonium acetate in methanol:water (70:30) and isocratic flow at 3 ml min^{-1}. The separation of Sn^{2+}, Sn^{4+}, and TBTC under these conditions is shown in

Figure 28. Tributyltin chloride and fluoride can not be separated using this system as expected from the "free" tributyltin cation separation mechanism outlined in section 6.1.3. Both compounds have a retention time of six minutes i.e. the retention time of the tributyltin cation.

The organotin compounds were preconcentrated prior to analysis by coupled HPLC-FAAS using quantitative extraction into chloroform. Each spiked sample was placed on a mechanical shaker prior to extraction into two 5 ml aliquots of chloroform. The extraction efficiency using this technique was found to be 92% with a standard deviation of 6.2%. To facilitate injection onto the HPLC column the chloroform was then evaporated and the sample redissolved in methanol. The extraction efficiency of this second stage was 90% with a standard deviation of 5.1%.

The results obtained from the analysis of seawater collected from various sites both on the surface and at depth at Sutton Harbour, Barbican Steps and the Naval Dockyard are shown in Figure 29. A second sample from the Barbican Steps was also used in a quantitative analysis of the tributyltin, identification being confirmed by co-injection - Figure 30. The level of tributyltin in the original sample was 0.47 $\mu g\ l^{-1}$.

6.3 Conclusions

The technique described above shows that by careful attention to the atom cell, an increase in sensitivity may be obtained, even when using an extremely simple interface. The use of the STAT tube above the flame can be used for all elements readily dissociated to their ground

Figure 28

Separation of tin species by coupled HPLC-FAAS

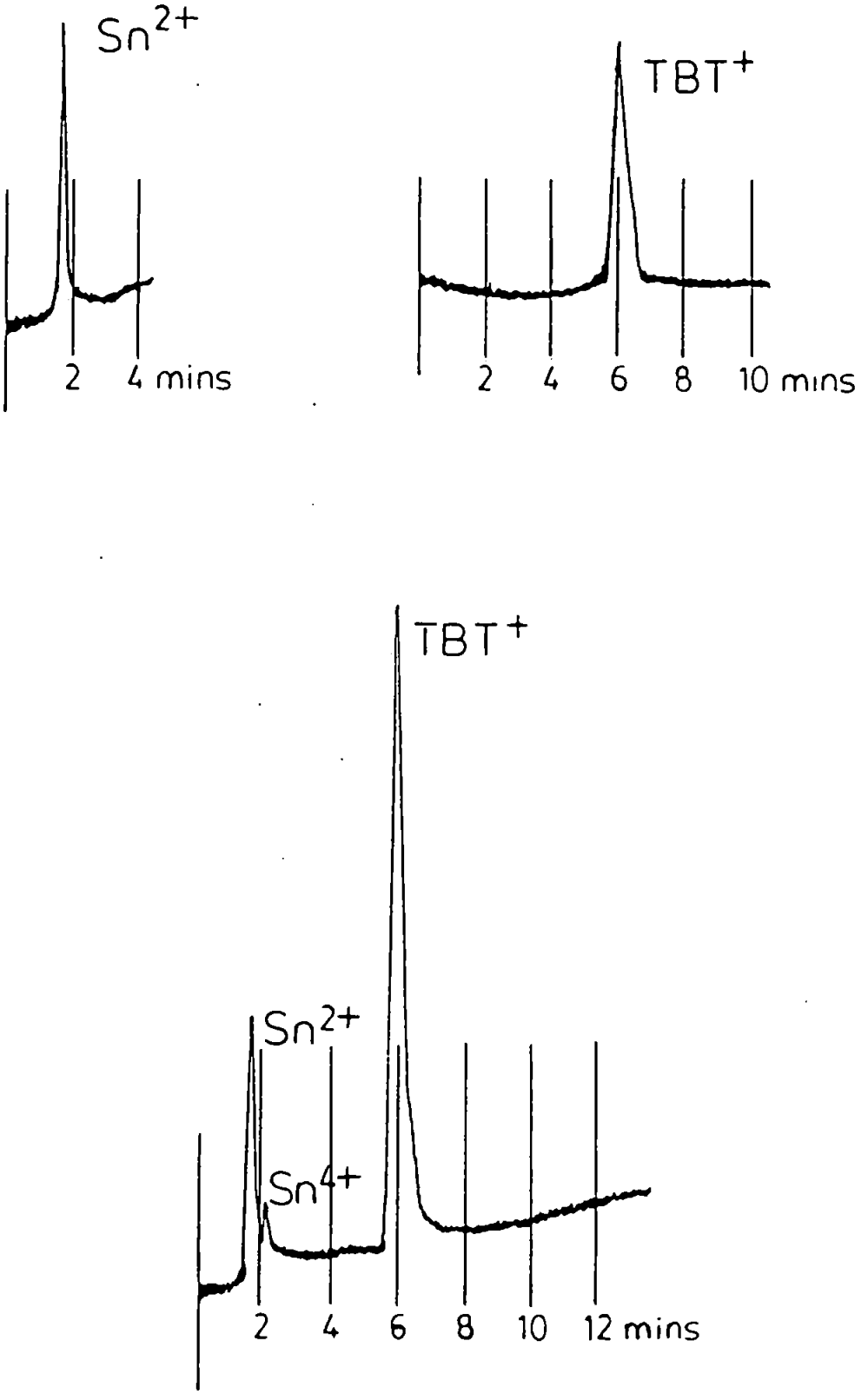


Figure 29

Identification of tin species in local coastal waters

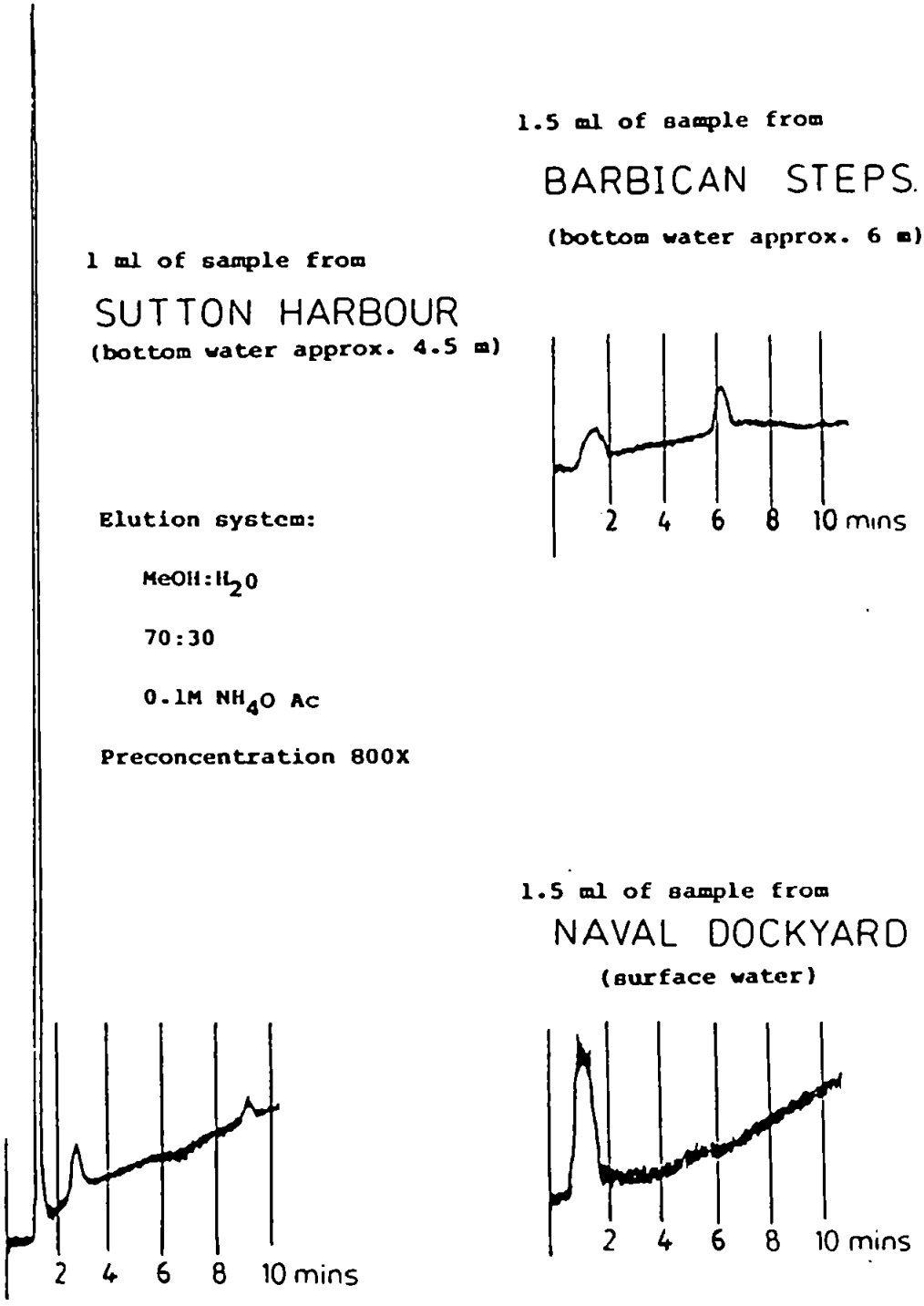
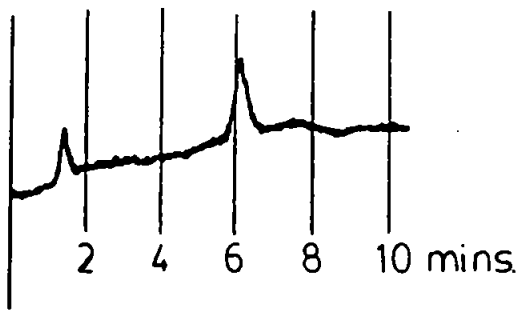


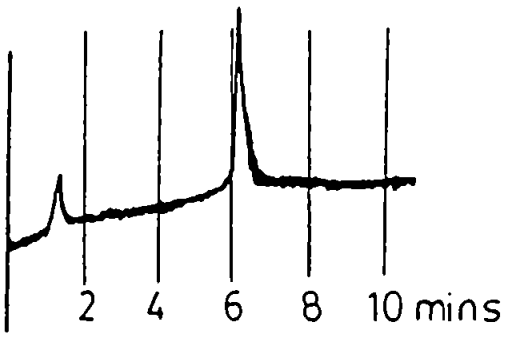
Figure 30

Co-injection of TBTC with samples collected at the Barbican Steps



1 ml of sample

(800X preconcentration)



1 ml of sample

500 ng TBTC

(20 µl of 25 ppm)

state atoms.

The use of pulse nebulisation has been well documented as a means of directly coupling a high performance liquid chromatograph to an atomic absorption spectrometer via the nebuliser. However, the method described above provides an elegant means for speciation studies at lower levels than previously possible using this technique. Further, the application of the system to the determination of organotin compounds, such as tributyltin, has provided a simple alternative to GC-MS for the routine determination of such compounds, since the instrumentation required is readily available in most laboratories.

CHAPTER 7

COUPLED HPLC-HYDRIDE-ATOMIC ABSORPTION SPECTROSCOPY

7.1 Introduction

The covalent hydrides are a series of compounds whose elements are of the C, N and O groups, where the number of valency electrons is equal to, or greater than, the number of orbitals. Of the thirteen elements which come into this category, eight have been induced to form covalent hydrides in sufficient amounts to be of practical analytical use; these are As, Bi, Ge, Pb, Se, Sb, Sn and Te (314).

Although hydride generation has been utilized for over 100 years for the determination of arsenic, in both qualitative and quantitative procedures (the Marsh reaction and the Gutzeit test, respectively) it was not until the 1970's that techniques were developed to overcome many of the interference problems encountered with the early systems. An account of the historical development of hydride generation techniques has been reported by Godden and Thomerson (314). Today, hydride generation has been interfaced with a variety of detection techniques, particularly atomic spectrometry, for the determination of the above metals and metalloids. The technique offers several advantages over conventional solution nebulisation, including the capability for preconcentration of the analyte, the elimination of chemical and spectral interferences and the presentation of the analyte as a desolvated moiety to the atomisation source (162). These advantages have led to as much as a thousand-fold improvement in detection limits (315).

In essence, hydride generation is based on the formation of the hydride by chemical reduction of the sample which is then entrained in a current of inert gas and led into the observation zone. Here it is decomposed by heat to form the atomic vapour. A number of different methods have been reported based on this principle, although they differ in the ways both the reduction and the atomisation are performed. The extensive review by Godden and Thomerson (314) covers the design of reaction vessels, methods of atomisation, interferences and applications in some detail. Conflicting opinions (316, 317) exist regarding the selectivity and efficiency of various reductants. Some authors (316, 318) have preferred sodium tetrahydroborate (IV) solution. Others have used a mixture of titanium (III) chloride/magnesium powder (319) and potassium iodide/zinc powder/tin(II) chloride (317, 320). Although $NaBH_4$ reduction was not used in atomic spectroscopic analysis until 1972 (321) it has now virtually replaced the metal/acid reaction. Advantages claimed for this technique include speed of hydride evolution, simplicity, higher conversion efficiency, lower blank levels, and the co-evolution of hydrogen which helps to purge the hydride. The $NaBH_4$/acid reaction is shown below, where E is the element of interest.

$$BH_4^- + 3H_2O + H^+ \longrightarrow H_3BO_3 + 8H^-$$

$$\xrightarrow{Em^+} EH_n + H_2(\text{excess})$$

(m may or may not equal n)

Various collection devices for storing the hydrides prior to transfer to the atom cell have been used although more recent systems (322, 323) have excluded such devices by sweeping the hydride directly into

the atom cell. These systems give speedier analysis, improved precision and greater freedom from interferences (324) compared to manual injection.

Flames and tubes, either flame (322, 323) or electrically heated (325, 326) have been used to generate free atoms from the gaseous hydrides. The high absorbance of the air/acetylene flame (327) at the arsenic and selenium resonance lines (193.7 and 196.0 nm, respectively) results in poor signal-to-noise ratios. In contrast, the argon or nitrogen/hydrogen/entrained air diffusion flames are markedly more transparent at these wavelengths and have found many applications (316, 317, 327, 328). The interferences arising from compound formation that are sometimes encountered in these relatively cool flames are largely overcome when gaseous samples of covalent hydrides are introduced directly into the flame. Many authors have reported using heated tubes as the atom cell, which improves sensitivity by eliminating flame absorption and increasing residence times. Graphite tube atomisers have also been used, though interference effects are apparently more pronounced (324).

7.2 Coupled HPLC-hydride generation

The use of hydride generation after separation by HPLC has also been reported by several groups of workers. In a comprehensive study of organotin compounds, Thornburn Burns et al. (165) found that their coupled hydride-ETA-AAS system readily enabled the determination of tin in organotin compounds after mineralisation, and had the advantage over gas-liquid chromagraphic systems for the methyltin series in that redistribution reactions did not take place (330). For tetramethyl- and tetraethyltin the response was similar to, but not identical with,

that of inorganic tin, but for other compounds the response, although linear, was a function of the thermal stability and volatility of the alkyltin hydrides produced, namely R_3 Sn H, R_2 Sn H_2 and R Sn H_3 - see section 7.4.2.

The use of hydride generation after separation by liquid chromatography has also been used to monitor reducible arsenic species. Ricci et al. (331) used ion chromatography in conjunction with hydride generation into an electrothermally heated quartz tube for atomic absorption detection. The separation of reducible arsenic species required either a gradient elution necessitating column restabilization for one hour between determinations, or two separate isocratic separations. With the isocratic approach the column required re-equilibration for one hour after every 10-15 samples. Thus, although satisfactory sensitivity had been achieved, the analysis time offered no improvement on existing LC-ETA AAS systems (see section 4.3.2), indeed the repeated column equilibration makes this technique less desirable for routine work.

A simple and sensitive continuous flow hydride system based on two peristaltic pumps has been developed by Ebdon et al. (332). This consisted of a modified form of the system developed by Thompson et al. (333) for use with an inductively-coupled plasma. A small nitrogen/hydrogen/entrained air flame was used as the atom cell and sodium tetrahydroborate (III) as the reductant for the reasons outlined above. The continuous generation of the hydride improves precision by avoiding the need for discrete injections whilst the accuracy is also improved since the zero level is unambiguously defined. Such a system is thus well suited for use with coupled HPLC

systems and was employed in the work described below.

7.3 Application to arsenic speciation

Arsenic resonance lines lie in the far ultraviolet spectral region where flame absorption can produce unfavourable signal-to-noise ratios. A conventional air/acetylene flame absorbs 62% of the incident radiation at 193.7 nm, whereas the argon-hydrogen diffusion flame absorbs only about 15%. The hydrogen diffusion flame is cooler than the air/acetylene flame, and interferences due to molecular absorption and incomplete salt dissociation are common. Thus by forming volatile hydrides of arsenic, matrix separation is possible thus minimising interferences in the flame. The major advantage of hydride generation is, however, in the increased efficiency in sample transport.

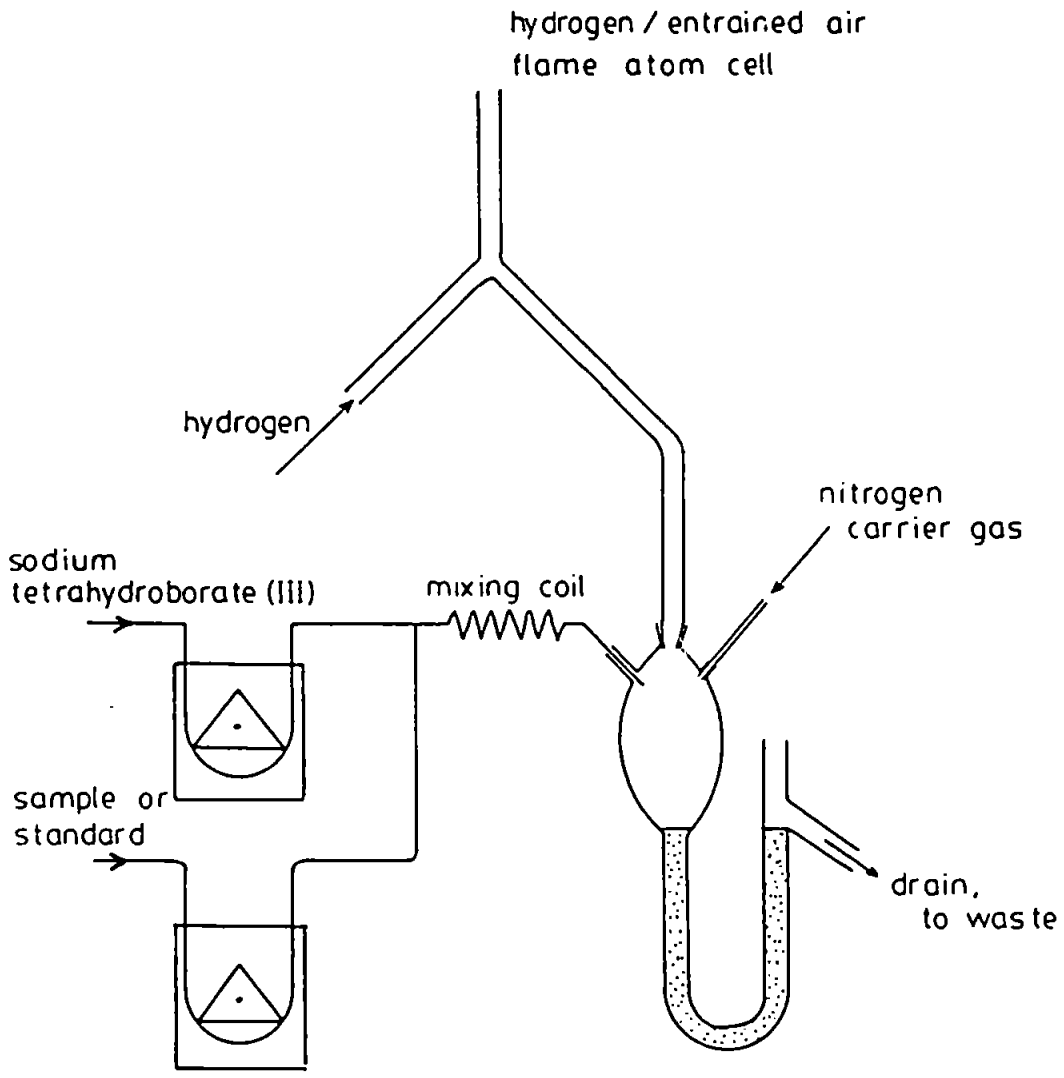
As stated above, by careful design the use of flames for atomisation gives detection limits comparable to those obtained with more complex systems. The system described and optimised by Ebdon *et al.* (332) and modified by Ward (34) was therefore used here in a directly coupled HPLC-AAS system for the determination of arsenic.

7.3.1 Experimental

The basic system consisted of two peristaltic pumps which delivered the acidified sample (5M HCl) at 7.0 ml min^{-1}, and sodium tetrahydroborate solution, at 2.5 ml min^{-1}, to a mixing coil, and then into a conventional gas-liquid separator, Figure 31. Argon purge gas (120 ml min^{-1}) then carried the volatile hydrides into a small hydrogen diffusion flame burnt on an inverted "Y" glass burner (8.5 mm i.d., 100 mm high). The side arms of the burner acting as the gas

Figure 31

Continuous flow system for the generation of gaseous hydrides



inlets, a fuel gas flow of 180 ml min^{-1} was used. The burner was then located vertically in the spectrometer, replacing the conventional burner/nebuliser assembly, and at the focal point of the entrance slit lens. The eluent from the high performance liquid chromatograph was introduced into the reaction manifold just prior to the introduction point of the tetrahydroborate (III) solution. The complete system is shown schematically in Fig. 31.

Various separation systems have been reported in the literature for arsenic species. The technique used here was developed at Plymouth Polytechnic and reported by Tye et al. (304). A resin based strong anion exchange (SAX) BAX 10 column was used with an ammonium carbonate (0.1 M) elution. This is a polystyrene-based material (mean particle diameter 3 μm) with quaternary ammonium groups. Although the system gives acceptable separations, the use of a precolumn packed with Zipax, a silica based anion exchange material (40 μm) and a step elution system of sulphuric acid (10^{-4}%)/ammonium carbonate (0.1 M), results in a preconcentration step on the Zipax, which in addition gives the protection of a general column.

The conditions used for both hydride generation and separation of the arsenic species are summarised in Table 15.

7.3.2 Results and Discussion

The results obtained from the above system for the separation of arsenite, arsenate, dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) are shown in Figure 33. The complete separation is achieved in less than four minutes although because of the arbitrary nature of switching over eluents, the retention times for the last

Table 15

Instrumental parameters for the HPLC-hydride generation atomic absorption spectrometric analysis of alkylarsenic compounds

SPECTROMETER CONDITIONS

Lamp:	Hollow cathode lamp 7.0 mA
Wavelength:	193.7 nm
Background correction:	OFF
Bandpass:	1.0 nm
Air flow:	4.5 l min^{-1}
Acetylene flow:	1.0 l min^{-1}

ELUTION SYSTEM

Solvent	time	Flowrate
Sulphuric Acid (10^{-4}%)	0-2 mins	3.5 ml min^{-1}
Ammonium Carbonate (0.1M)	2-12 mins	3.5 ml min^{-1}

PERISTALTIC PUMP RATES

Sodium tetrahydroborate (III) (4%), 1.6 ml min^{-1}

Hydrochloric acid (5M), 1.6 ml min^{-1}

Auxillary flows

N_2 purge flow: 0.2 l min^{-1}

COLUMNS

Zipax ion-exchange resin 40 μm (100 mm x 5 mm i.d.) in series with strong anion-exchange resin BAX10 5 μm (250 mm x 5 mm i.d.)

Figure 32

Schematic diagram of HPLC-Hydride-FAAS/FAFS coupling

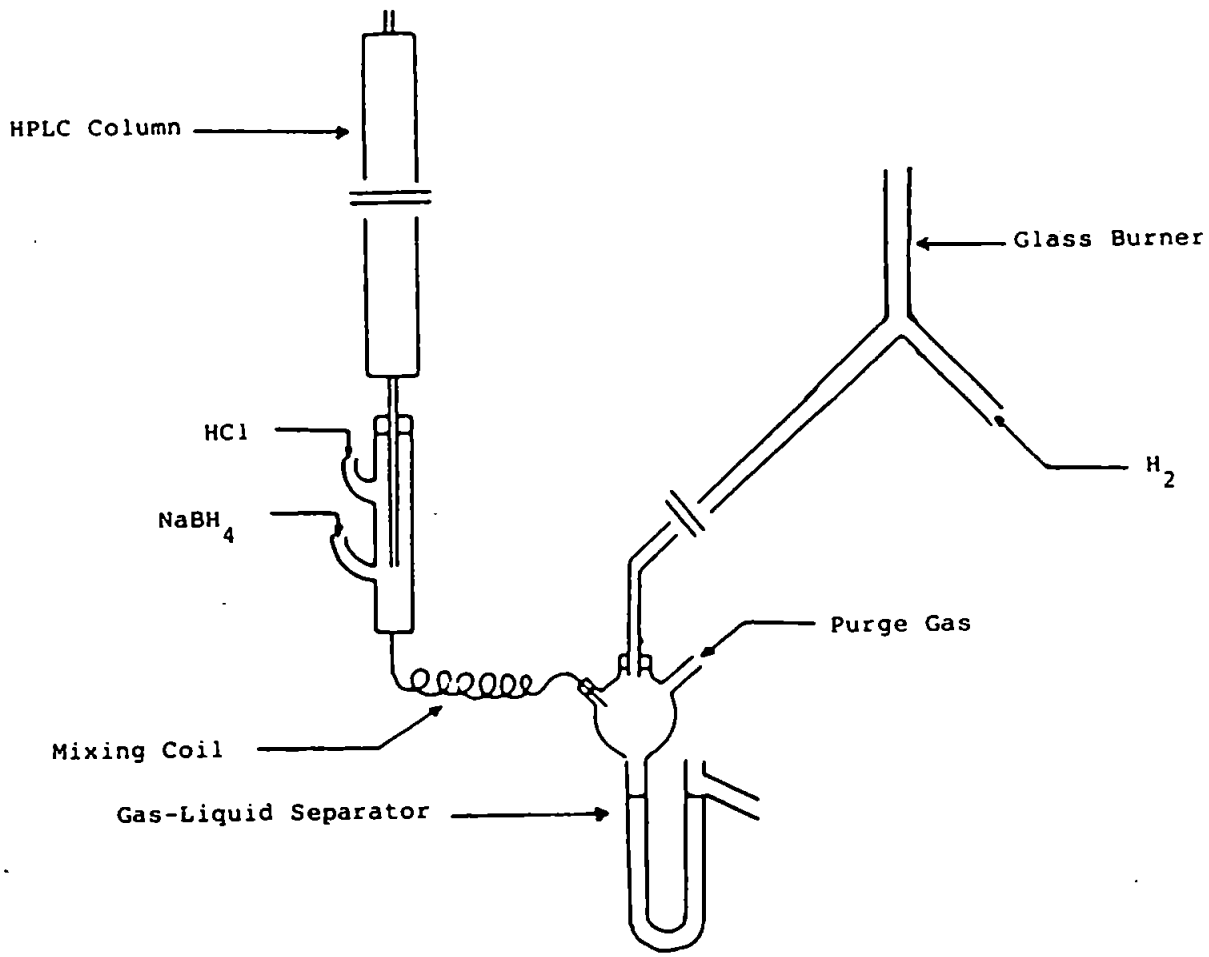
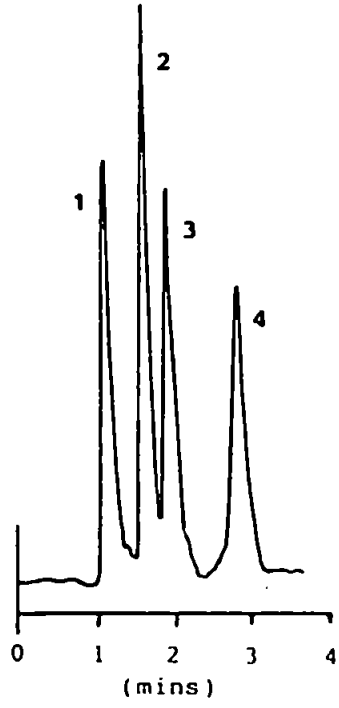


Figure 33

Separation of arsenite, arsenate, dimethylarsinic acid and monomethylarsonic acid by directly coupled HPLC-Hydride-PAAS



Peak

1 arsenite

2 dimethylarsinic acid

3 monomethylarsonic acid

4 arsenate

10 ng of each species injected

three peaks are best measured with respect to the last peak. Arsenite has been shown to pass through both columns without retention, even when a sulphuric acid elution system is used.

For the separation of arsenic species the above system utilising hydride generation has thus been shown to avoid the problems of low nebulisation efficiency normally encountered with FAAS, enabling sensitive detection along with "real-time" detection. However such a technique is only suited to the determination of species which form volatile hydrides. In many instances this may not be the case, even though the associated metal may be readily reduced. One example of such species is tributyltin compounds. Whilst Sn^{4+} will readily form a volatile hydride, the tributyltin species will not (b.p. of Bu_3SnH 80°C at 0.4 mm), and so is not detected by the above system. Thus it is necessary to modify the technique to incorporate some means of degrading the tributyltin prior to the hydride generation stage. One means of doing this is described below.

7.4 Determination of tributyltin compounds

The rationale employed here was the same as in section 6.2 i.e. to devise a system enabling direct injection of sample onto the HPLC column for separation of the organotin species without pretreatment, and then detection by FAAS keeping the interface as simple as possible. The arrangement used in the preliminary experiments therefore consisted of a simple continuous flow hydride system as described above directly coupled to the HPLC column.

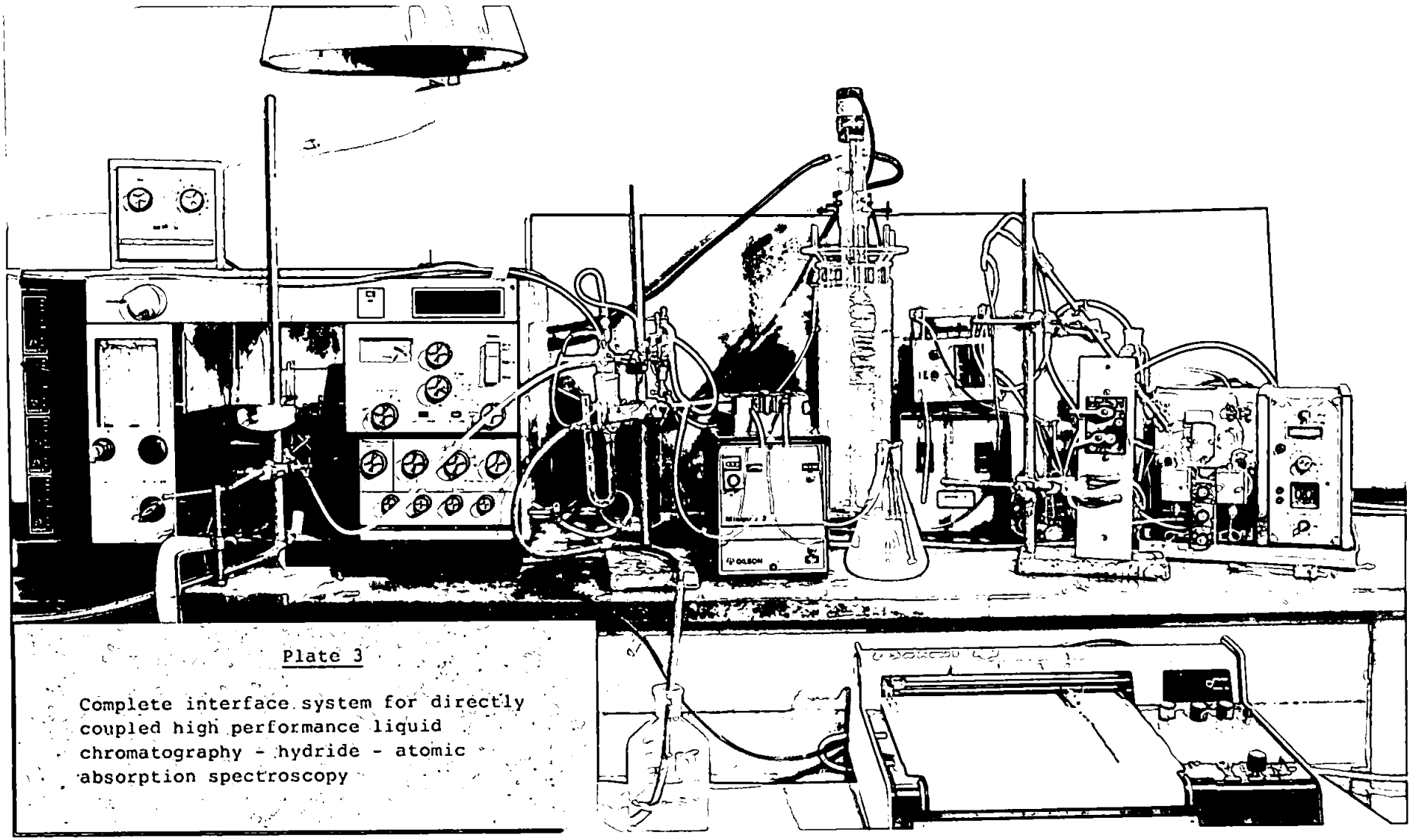
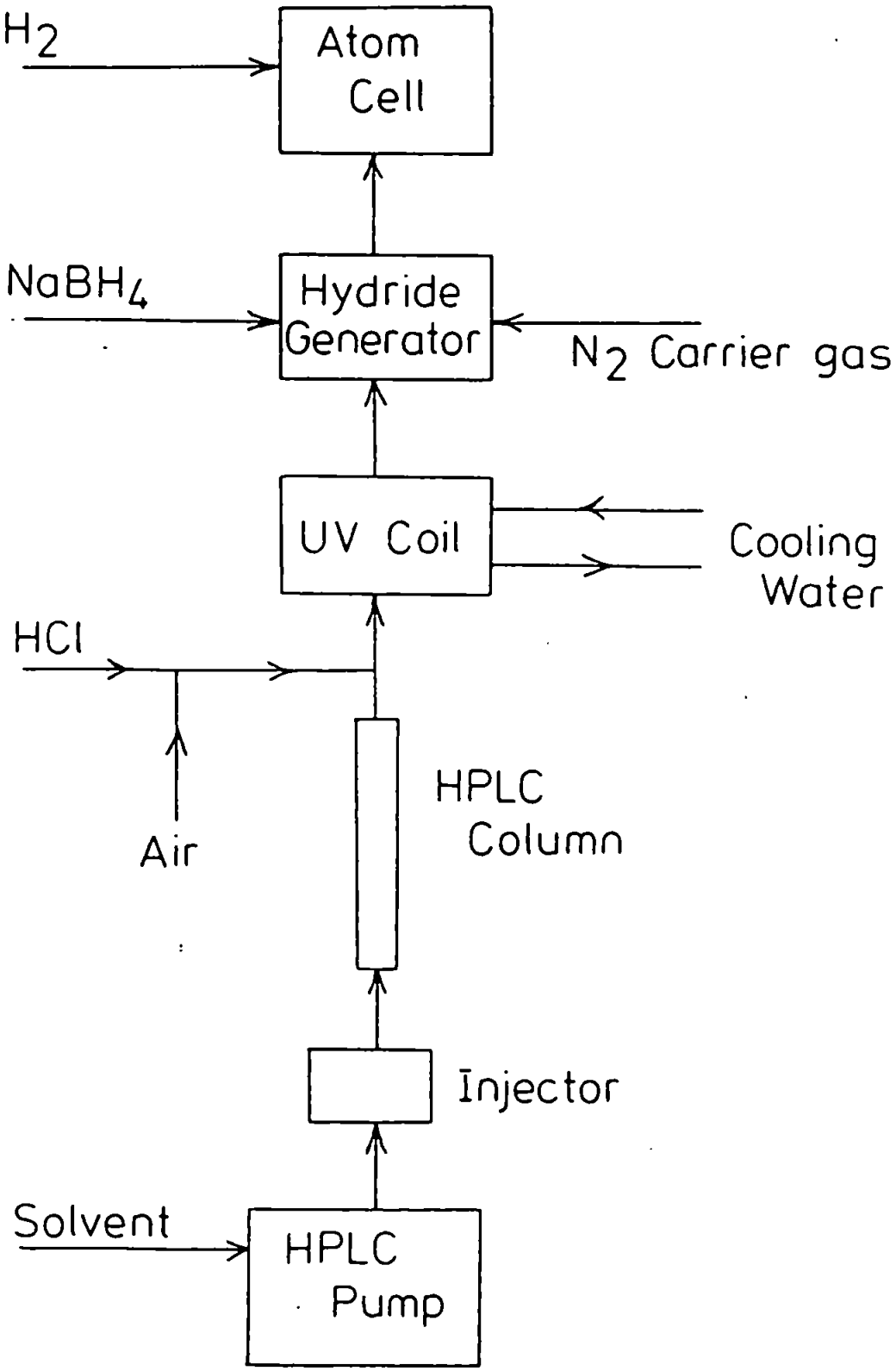


Plate 3

Complete interface system for directly coupled high performance liquid chromatography - hydride - atomic absorption spectroscopy

Figure 34

Schematic diagram of complete interface incorporating the UV photolysis coil



7.4.1 Apparatus

The basic continuous flow system and atom cell described in Section 7.3.1 (Figure 31) was used, although the parameters were changed to facilitate the generation of the tin hydrides. A tin hollow-cathode lamp was used as the spectral source for atomic absorption measurements, the light beam irradiating an area about 10 mm above the burner. The 286.3 nm tin line was used.

In later work (Section 7.5) the interface was modified to incorporate an on-line UV photolysis system. This consisted of a coil of quartz tubing (1.5 mm i.d.) mounted on a glass former. A large UV lamp (Englehard Hanovia Lamps, Slough) was placed at the centre of the coil, thus irradiating the eluent before it reached the hydride generation unit. To retain sample integrity in the coil, air segmentation was utilised by pumping air into the eluent channel prior to reaching the photolysis coil A peristaltic pump (LBK, Produkter AB, Bromma, Sweden) was used for this purpose. The complete interface is shown in Figure 34, and in more detail in Plate 3.

7.4.2 Reagents

Unless otherwise stated, all reagents used were analytical reagent grade.

Sodium tetrahydroborate (III) solution

Dissolve sodium tetrahydroborate (III) (1g; G.P.R.; Fisons Scientific) in sodium hydroxide solution (0.1 M, 100 ml). This solution remains usable for 2-3 days.

Hydrochloric acid

Diluted to 0.2 M solution. (BDH Chemical Ltd., Poole, Dorset).

Tributyl tin chloride (Aldrich Chemical Co. Ltd., Gillingham)

Tin (IV) Stock solution prepared as in Section 6.2

Preservation of standards

It has been shown (309) that tin (IV) ions adsorb on to glass surfaces within a very short time but the same solution stored in polythene appeared to be stable. The reverse is true for organotin compounds, especially the R_3 Sn X compounds, where it has been shown (308) that solutions of these compounds were stable when stored in borosilicate glass for 1 year.

7.4.3 Generation of tributyltin hydride

The hydride system described above was initially optimised for inorganic tin (Sn^{4+}) using the parameters stated in the literature (322) - Table 16.

Table 16

Optimal conditons for the generation of Sn H_4

Concentration of $NaBH_4$	Concentration of HCl (M)	Nitrogen flow-rate $l\ min^{-1}$
1% m/v in 0.1 M NaOH	0.2	1.2

Using the conditions given above a detection limit of 0.005 $\mu g\ ml^{-1}$ was obtained.

The input of tributyltin chloride (TBTC) into the hydride generator was facilitated by using both discrete injections of sample and continuous flow via a peristaltic pump. However in both cases no response was detected to indicate that a volatile hydride had been formed, even when samples containing 100 ppm TBTC were used. Indeed, after a series of injections the tributyltin hydride was observed in the gas/liquid separator as an oily layer.

7.4.4 Chemical degradation of organotin compounds

To overcome the fact that tributyl tin hydride is a non volatile liquid a method was sought to degrade the TBTC by removal of the butyl groups to yield Sn^{4+}, and hence allow the hydride generation technique to be used successfully. Several chemical methods were considered but finally rejected due to the reaction time involved and the desire to keep a continuous-flow on-line system. A brief summary of the chemical degradation of organotin is given below.

Carbon-tin bonds are thermally stable below 200°C, but are capable of polarization by attacking species in either direction. Organotin compounds are thus susceptible to attack at the carbon-tin bond by both nucleophilic and electrophilic reagents, leading to hydrolysis, solvolysis, acidic and basic reactions, halogenation, etc. (335 - 337). The results of kinetic studies scattered in the literature on the cleavage of aklyl-, unsaturated and aromatic groups from tin by hydrogen chloride and metal halides, CrO_3 in glacial acetic acid, alkali metal hydroxides in water and aqueous perchloric acid are reviewed by Zuckerman et al. (192). Homolytic reactions involving organotin compounds with free radicals have also been reviewed (339). All these studies were carried out in homogenous media where it is

found that the cleavage of the organic groups from tin is always first order in each reactant. In polar solvents there is probably initial solvation of the tin compound, followed by electrophilic attack (S_E2) on a carbon atom adjacent to tin. With alkali there can be nucleophilic attack (S_N2) on the tin atom with expulsion of a carbon ion. Although some organotin compounds will undergo unimolecular photolysis or thermolysis under mild conditions, free organotin radicals are usually formed by biomolecular reaction with some other radical. The attack can be at the tin-carbon bond, or elsewhere in the molecule (339). From these and other studies (340 - 341) it can be generalized that the progressive cleavage of organic groups from tin is dependent upon the type of organotin compound, the number of organic substituents, and the solvolytic conditions. The relative ease of removal of aliphatic groups decreases with increasing size of the group, but unsaturated and aromatic groups are cleaved more rapidly. For the series:

$$R_4Sn \xrightarrow{k_4} R_3\,Sn\,X \xrightarrow{k_3} R_2\,Sn\,X_2 \xrightarrow{k_2} R\,Sn\,X_3 \xrightarrow{k_1} Sn\,X_4$$

the reaction rates are $k_4 >> k_3 >> k_2 \approx k_1$. Laboratory solvolytic reactions generally represent extreme pH conditions (pH < 1 or > 14). Half-lives range from one minute to 115 days, depending upon the conditions and specific organotin compounds studied. The solvolysis of tetraalkyltins carried out under less severe conditions (pH = 4 to 10), may be several orders of magnitude slower (10^{-4} to 10^{-6}), and these tetraalkyltins will react 10 to 100 times faster than trialkyltins. The solvolysis rates of dialkyltins again approach those of the tetraalkyltins.

The inorganic anionic groups in the organotin compounds react with

Figure 35

Schematic diagram of continuous flow TBTC detection system

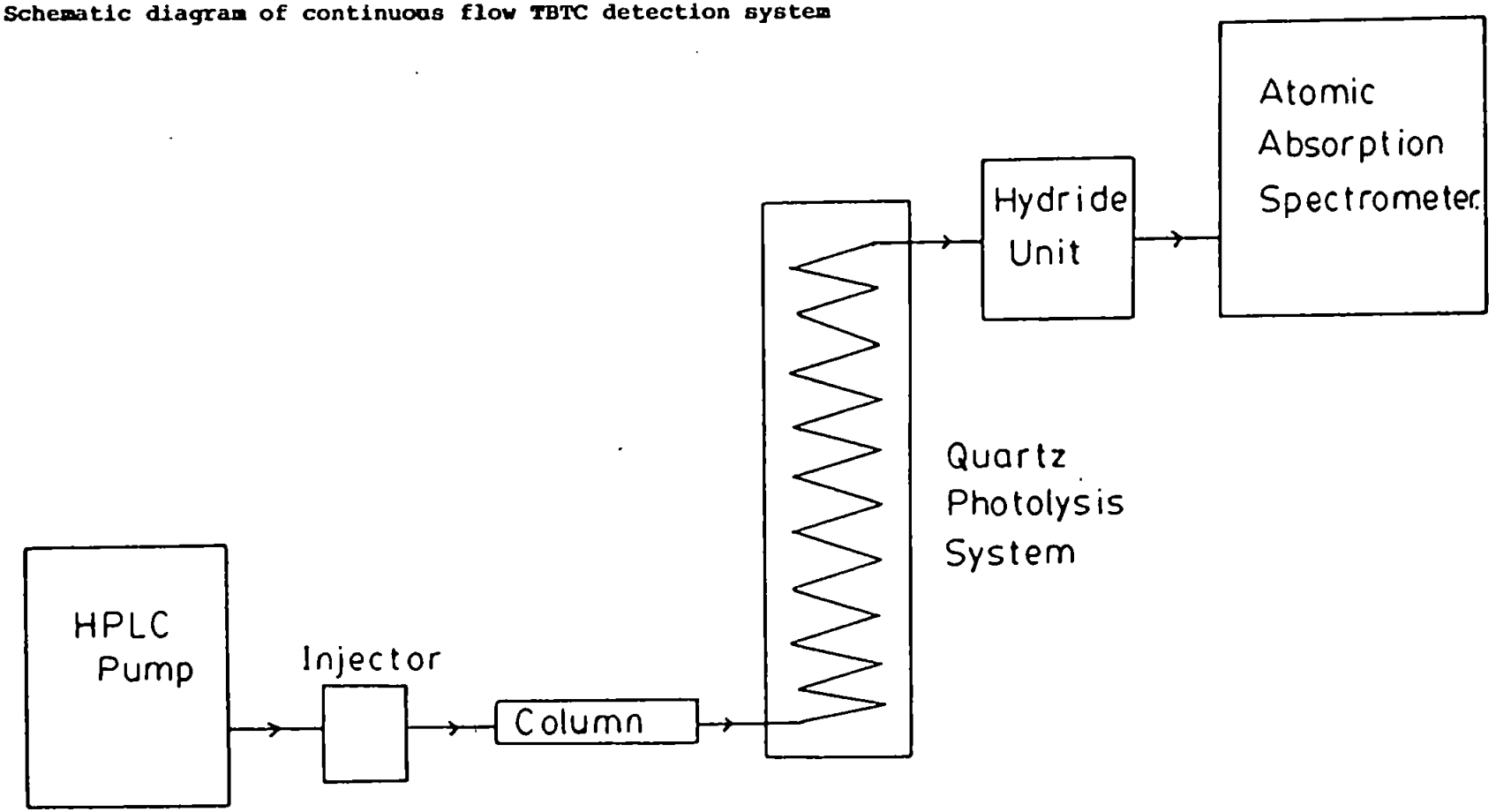
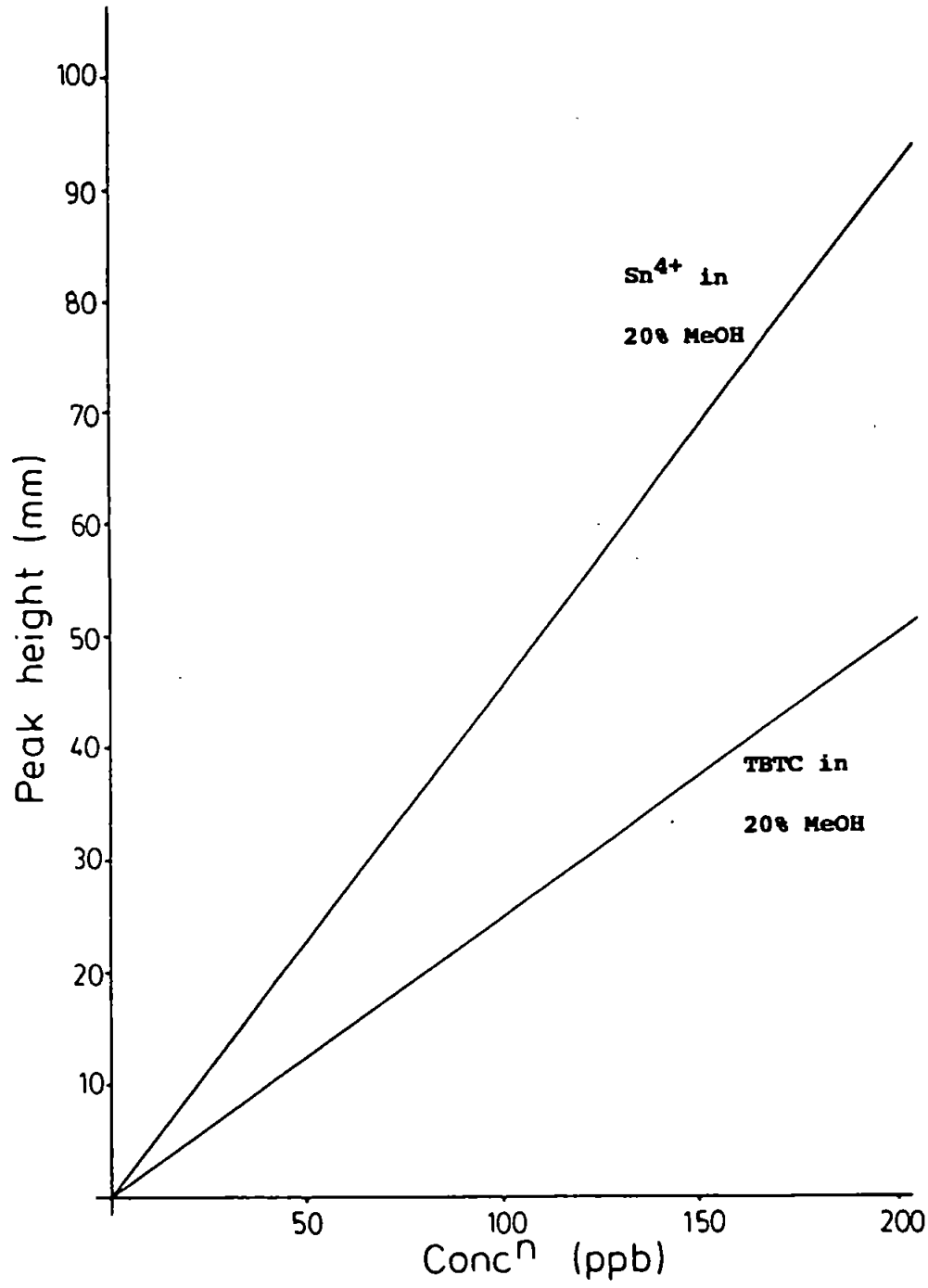


Figure 36

Initial results for the determination of TBTC following UV photolysis and hydride generation



moisture and air to cleave from tin in an hydrolysis oxidation to give stannols and oxides. In this way successive reaction of both parts of the molecule leads eventually to complete inorganic hydrated tin oxides.

7.5 Development of ultra-violet photolysis system

Since degradation of the TBTC by chemical means proved to be difficult, the use of ultra-violet irradiation was investigated. Initial studies used a quartz coil wound onto a glass former, the diameter of the coil being large enough to enable a u.v. lamp to be inserted down the centre. It was envisaged at this stage that if the photolysis of the TBTC proved successful, then such a system would enable the input of the coil to be connected directly to the end of the HPLC column, and the output, once the TBTC had been irradiated, directly to the input of the hydride generator thus allowing continuous flow operation - Figure 35.

The results of these first studies indicated that whilst the above technique was a viable method of degrading TBTC to give tin in a form suitable for producing a volatile hydride, Figure 36, the system needed various modifications. One of the main problems resulted from the wide bore (~ 1.5 mm) of the quartz coil which allowed back diffusion of the sample. This was overcome by segregating the flow through the coil using air bubbles in a similar manner to the air segmentation system of Skeggs. It was found, however, that the size of the bubbles is also important, since if too large they tend to break-up, and if too small will leave pockets of solution behind. The ideal size appears to be 2-3 times the bore of the tubing.

No attempt was made to precisely control temperature in the UV photochemical reactor system, although a fan unit was used to provide a flow of cooling air around the silica coil. This was done by installing the fan into the bottom of the reactor housing, the top of the housing being vented to allow the warm air and ozone from the UV reactor to escape and be drawn away via the laboratory extraction system. A scheme for precise thermostatic control of the reaction temperature using a thyristor to control the fan speed has however been described by Mantoura and Woodward (329) who used a similar UV photochemical reactor in an automatic dissolved organic carbon analyser.

To optimise the system the HPLC instrumentation was disconnected and the sample supplied by means of a peristaltic pump. The results obtained from the optimisation of acid and sodium tetrahydroborate IV solution flow rates to the hydride generator, flow rates of N_2 carrier gas, and pH of sample for maximum degradation of TBTC in the quartz coil are shown in Figures 37, 38, 39 and 40 respectively. The optimal conditions are summarised in Table 17.

7.5.1 Results and discussion

It can be seen from the optimisation studies above that the optimum pH for the sample, to achieve maximum degradation by the UV irradiation, is far lower than the optimum acid pH required for maximum hydride generation efficiency. However, it was found from the kinetic curves plotted to observe the efficiency of degradation in the coil e.g. Figure 41, that using 1 M acid solution to improve the degradation efficiency still gave 86% conversion into the hydride. These curves were obtained by filling the coil with a 10 ppb solution of TBTC and

Figure 37

Univariate search for acid concentration

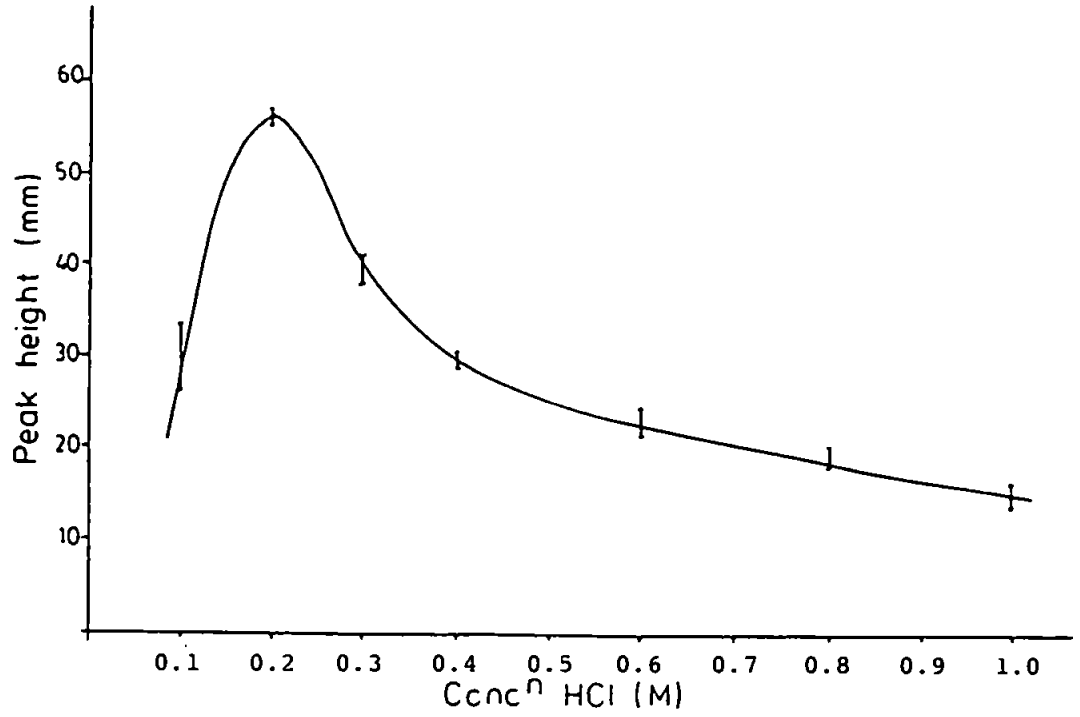


Figure 38

Univariate search for $NaBH_4$ flowrate

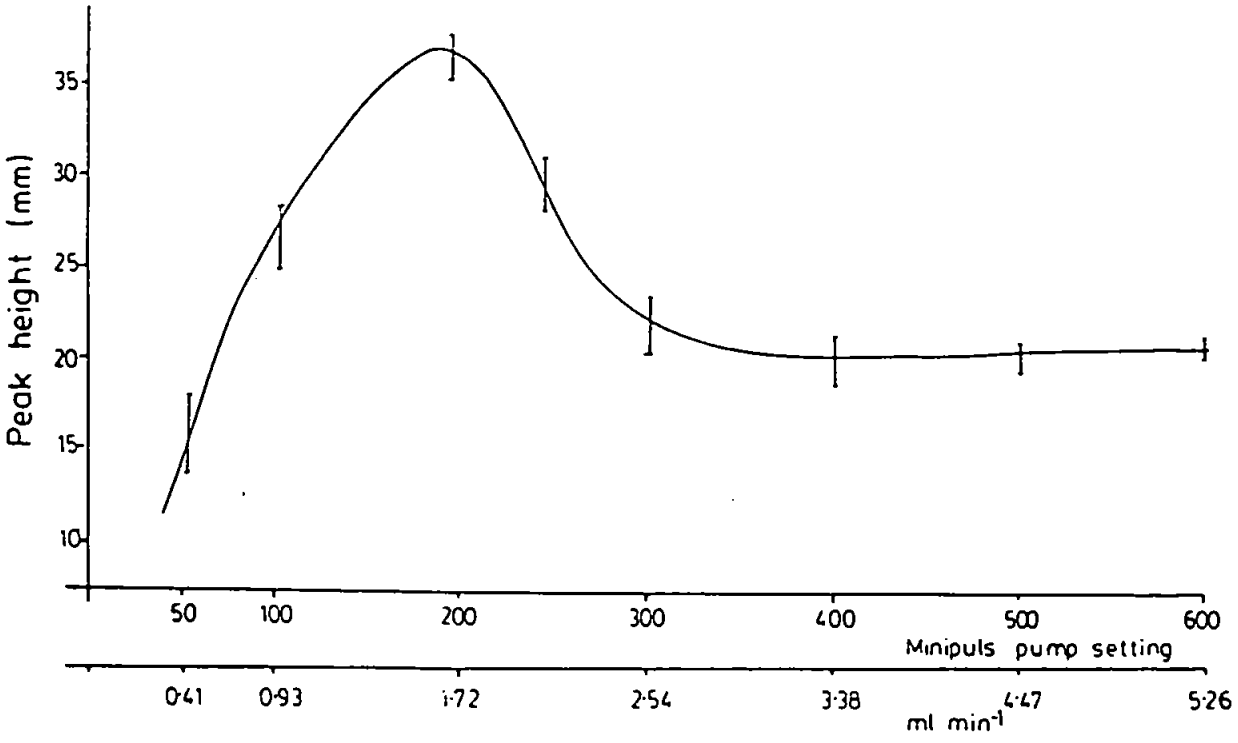


Figure 39

Univariate search for carrier gas flowrate

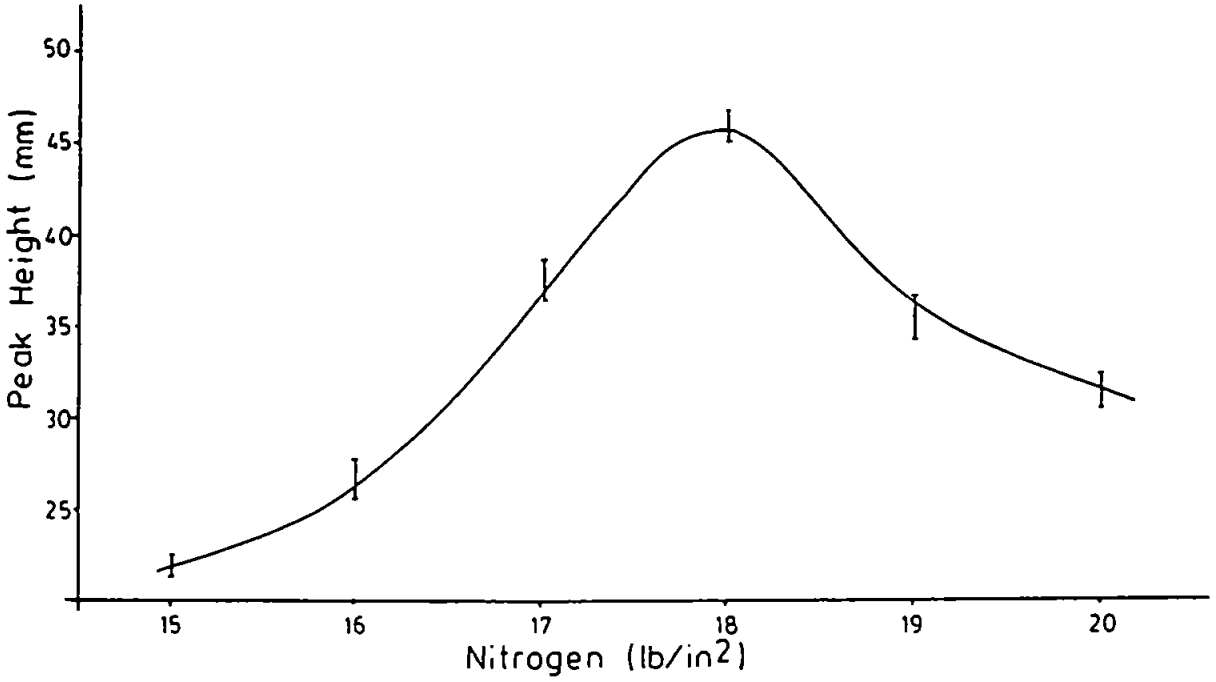


Figure 40

Univariate search for pH of sample in the quartz photolysis coil

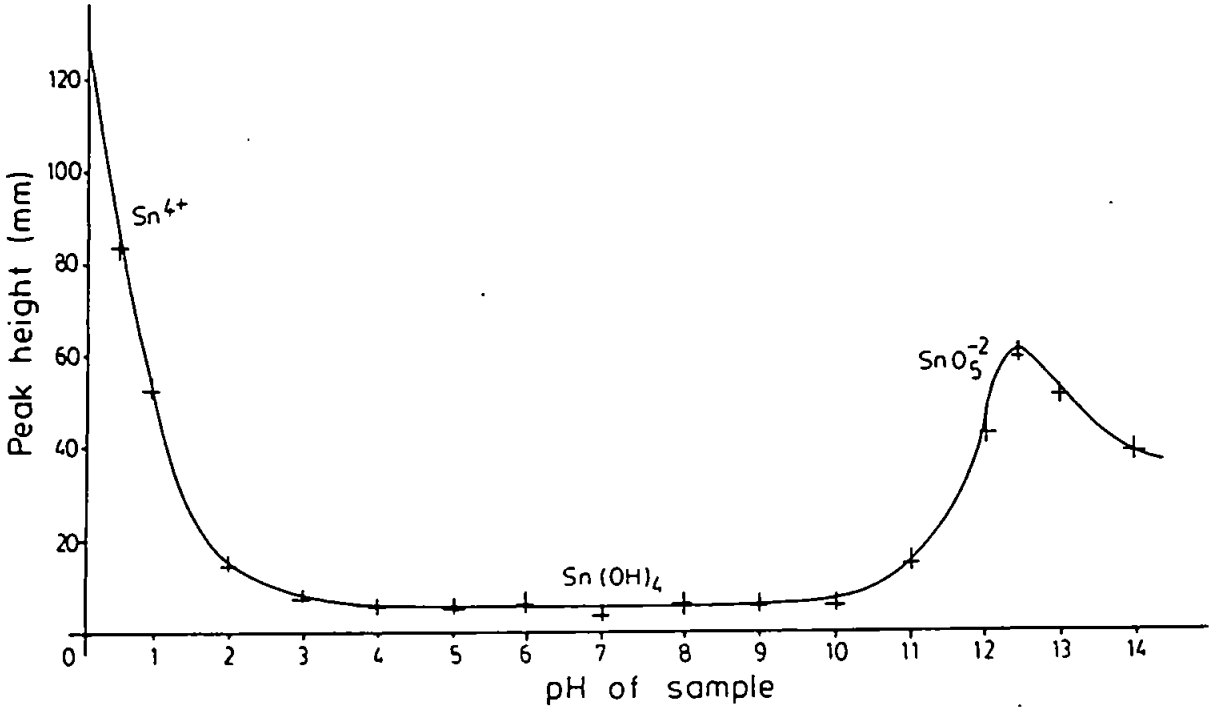


Table 17

Optimum conditions for the hydride generation determination of TBTC after degradation by u.v. photolysis

Hydride generator

Acid	0.2 M HCl
Flow-rate	1.5 ml min^{-1}
Reductant	1% solution of $NaBH_4$ in 0.1 M NaOH
Flow-rate	1.8 ml min^{-1}
Carrier gas flow rate	120 ml min^{-1}

Sample degradation

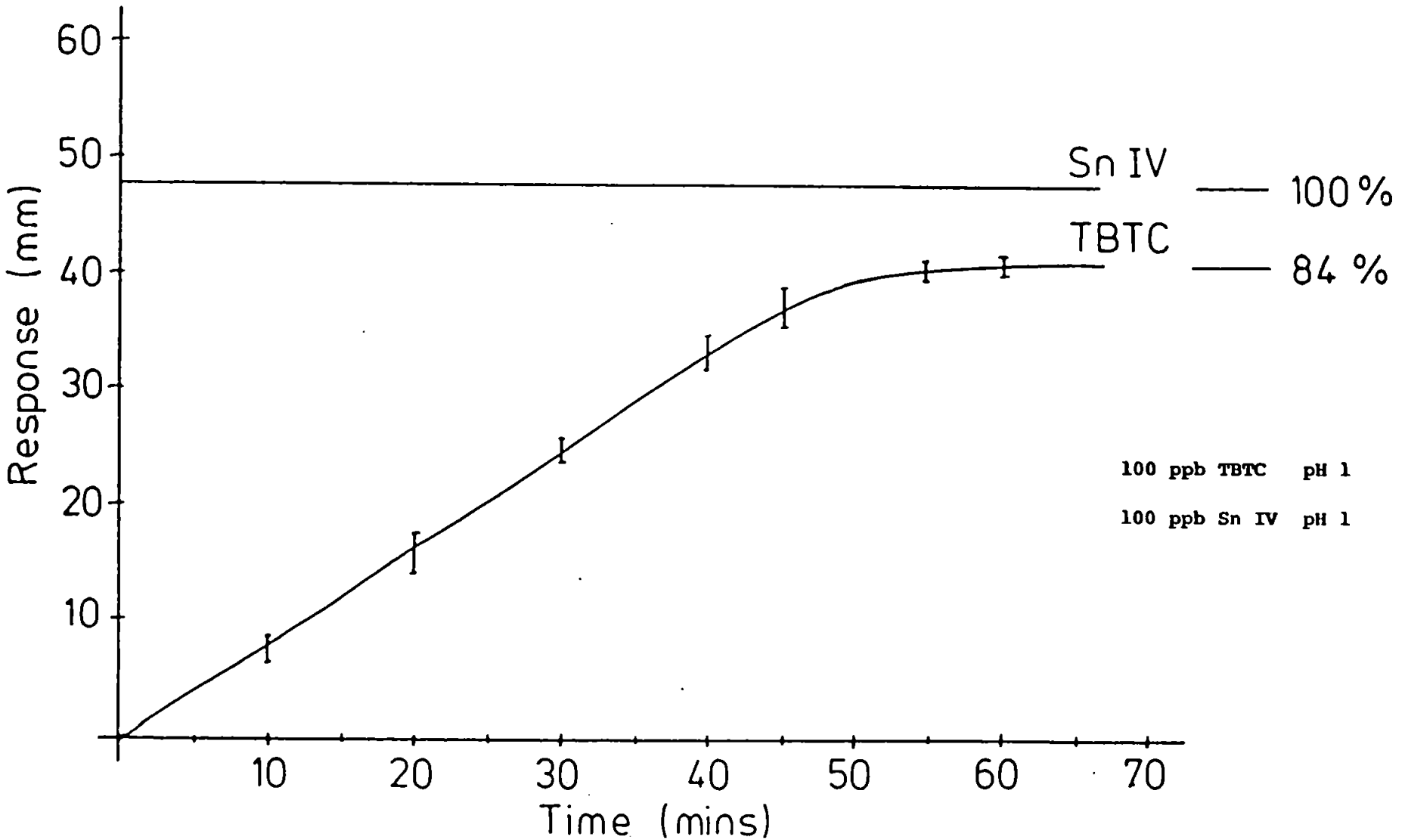
pH < 1 for maximum u.v. degradation

Detector

All atomic absorption spectrometer parameters as in section 7.4.1

Figure 41

Degradation efficiency of UV irradiation on TBTC



then turning the UV lamp and chart recorder on simultaneously so that the degradation with time could be plotted. The possible use of pH adjustment to further improve the hydride formation was considered at this point, although not incorporated into the system, since it was thought that the disadvantage of sample dilution would counteract any possible improvement on the 86% conversion rate already obtained. However, although it can also be seen from Figure 41, that maximum degradation of the TBTC, is achieved within 16 minutes, later studies revealed that the degradation efficiency was severely degraded with extended use. This was the result of inefficient cleaning of the quartz coil thus reducing the u.v. irradiation of the sample. The quartz coil when thoroughly cleaned with chromic acid once again gave maximum degradation of the sample in just under 20 minutes. In all later work the coil was routinely cleaned with chromic acid to avoid the build up of deposits which could again reduce the effective irradiation.

The results obtained from studies on the optimum pH for the sample in the UV coil to achieve maximum degradation - Figure 40, are also interesting. As the pH changes, so will the form of the tin in solution. Thus the curve obtained in Figure 40 may well reflect the solubility of the tin species present. The soluble stannic forms would be expected at low pH, whilst at high pH values the soluble stannate form will exist. As the solution tends towards pH 7, less soluble tin species such as $Sn(OH)_4$ will exist. Thus the efficiency of the photolysis system may well depend on the solubility of the tin species present. In all later work acid conditions were used.

Once the above parameters had been optimised, the chromatograph was

reconnected. Ideally a completely aqueous elution system would be employed to minimise u.v. absorbance by the organic solvent. However, the existing HPLC system optimised in section 5.2 was used and found to give acceptable results, a detection limit of 2.0 ng TBTC being obtained. The chromatograms obtained also had well defined peaks indicating that sample integrity had been maintained within the system. Figure 42 shows the calibration curve obtained for TBTC using the complete system as described earlier in Figure 34. It should be noted that the detection limit for TBTC obtained by this method is 100x better than that obtained using the HPLC-STAT system described in section 6.2.

Finally a number of real samples were investigated. Natural water samples were collected from a range of coastal sites in the Plymouth area and extracted using the procedure described in Section 5.2. In most cases only inorganic tin species were detected, although, in one harbour water sample a number of species were detected - see Figure 43. From the use of standards, two of these peaks have been identified as the inorganic and tributyltin species, although the identity of the third species is unknown.

The use of UV photolysis has thus been shown to extend the range of species which may be determined using coupled HPLC-hydride generation atomic absorption spectrometric analysis. However, although such a technique is very sensitive, it is limited to the eight elements listed in Section 7.1. The next section describes a novel sample transport system which again avoids the inefficiencies associated with sample introduction via the nebuliser, but which may be extended to a much wider range of species, and applied to several interesting trace

Figure 42

Calibration curve for TBTC using coupled HPLC-hydride-AAS

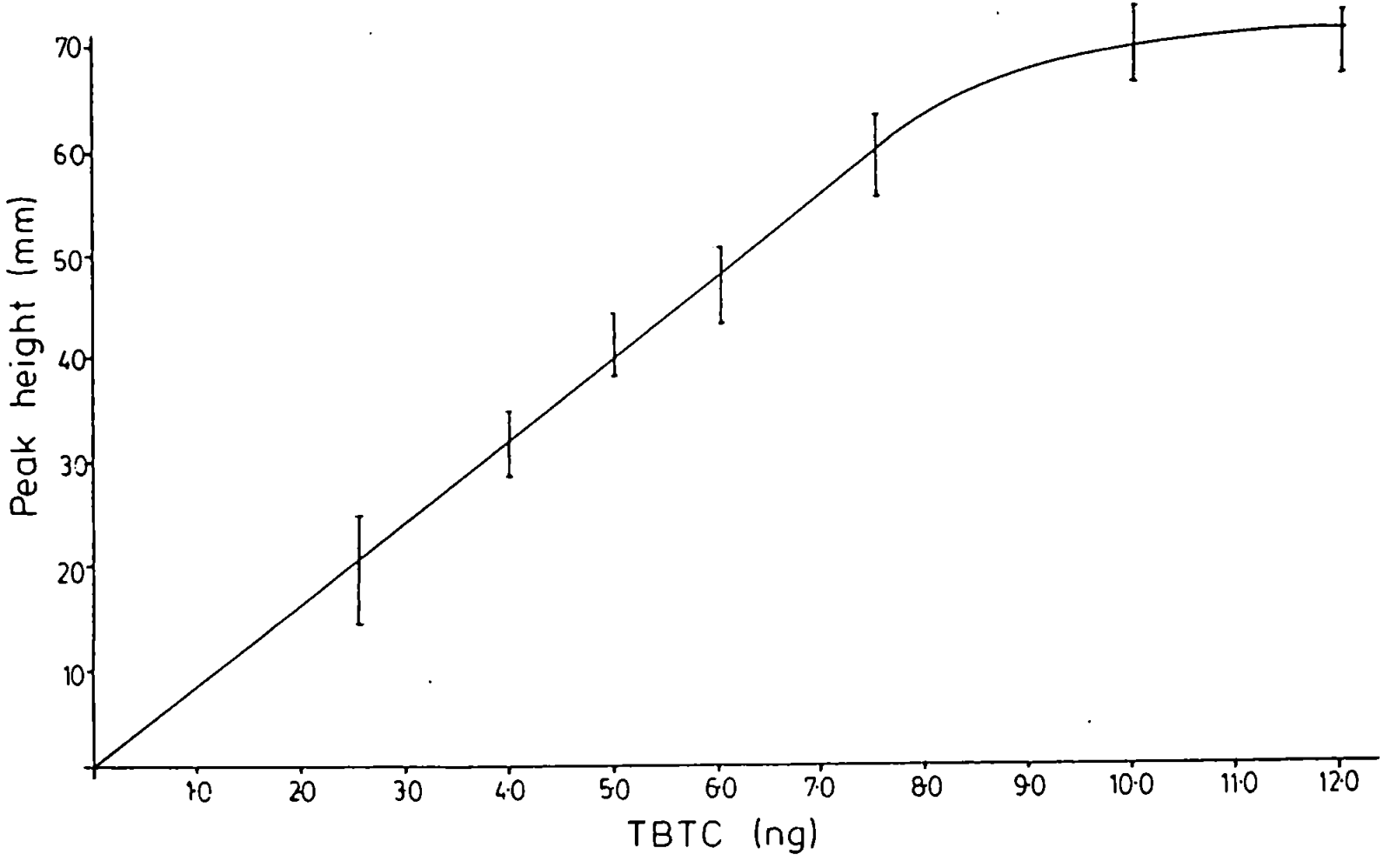
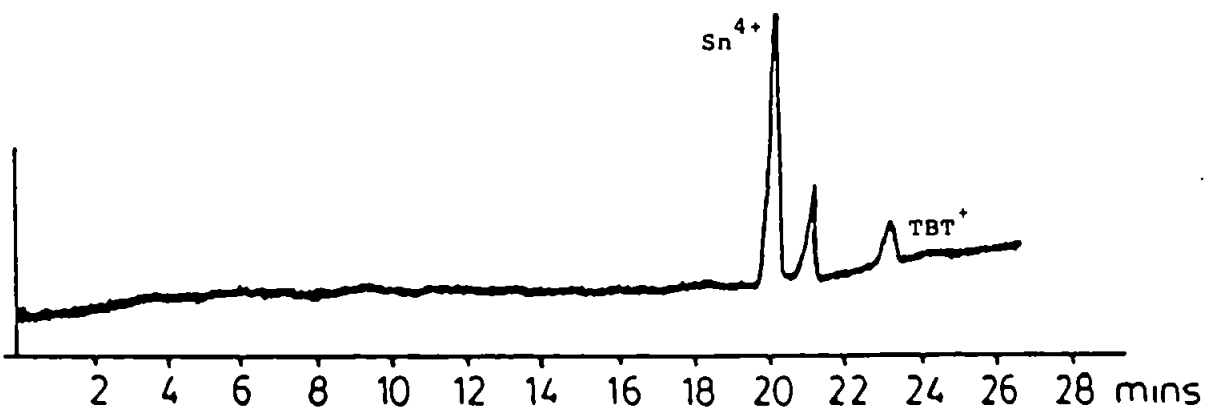


Figure 43

Chromatogram obtained from harbour water sample using hydride generation after UV photolysis



metal speciation studies.

CHAPTER 8

NOVEL SAMPLE TRANSPORT SYSTEMS

8.1 Introduction

Transport detectors represent an independent category of detectors in liquid chromatography. The most widely reported use of such detectors is where the sample is conveyed, e.g. by a moving wire, to a flame ionisation detector FID (342-348). The substantial difference between this and more conventional detectors, is that the effluent does not enter the sensing element of the detector directly from the column but is transported through a thermal zone which removes the mobile phase and through a zone where either vaporization or pyrolysis of the sample takes place. Vapours of the substance under analysis or its pyrolysis products are then detected by sensing elements commonly used in gas chromatography. A wire transporter (343-345) has been used in most designs, (and also used in the only commercially available detector of this nature - now discontinued), although chains (346), belts (347), discs (348) and wire helix (349) have also been used. The non-selective flame ionisation detector (343-348) has been the most widely used sensing element.

More recently moving-belt interfaces have been used for coupled liquid chromatography - mass spectrometry. In early systems plastic belts were used although thermal desorption was found to be a problem. Since the early 1980's however improvements have been made using spray deposition and micro-HPLC equipment. In addition, new ionisation methods are now applied directly to non-volatile samples on the belt. These are laser desorption (355), fast atom bombardment (FAB) of a

glycerol matrix (356), or secondary ion mass spectrometry (SIMS) from a metallic ribbon (357).

Many of the moving wire detectors outlined above however proved unsuccessful principally for two main reasons - the ineffective pyrolysis of the solutes, and difficulties in coating the moving carrier with effluent. The problems with the pyrolysis step were to some extent overcome (e.g. for highly oxygenated compounds) by the method of Scott and Lawrence (350), in which the solute was burnt in a stream of oxygen to carbon dioxide and water, followed by reduction of the carbon dioxide to methane, which can be detected by the flame. However, the coating step remained a problem, in most cases only a small part of the effluent being coated on the moving carrier. This seriously diminished the sensitivity of the FID since it is mass and not concentration sensitive. Although other forms of transport device e.g. a moving chain (351) can handle larger quantities of eluate, they are also noisier, and little, if any, sensitivity is gained. A further shortcoming of the moving wire is the fact that aqueous solutions are poorly coated on the stainless steel wires commonly employed (352).

Two other problems with the moving wire technique are also encountered. Firstly, the amount of coated effluent on the wire remains the same, irrespective of effluent flow rate, in other words, the split ratio (part of the solute deposited on the wire) will vary with the flow rate. Secondly, there is a tendency for the solute to creep during the period of drying, thus causing, at irregular intervals, places of extremely high concentration along the moving carrier. This effect is seen in the chromatogram as sharp "spikes"

superimposed on the solute peaks.

A number of methods have been devised to overcome the above problems, such as spraying the column effluent on the wire instead of coating from a block (353), coating the wire with a layer of porous absorbent (354), and feeding the wire directly into a combustion chamber, the total combustion products being swept by a stream of air into the FID (343). However the technique remained unreliable and is now little used, the last reports of the technique appearing in the literature during the mid 1970's.

The basic principle behind moving wire techniques is to collect and transport a continuous stream of effluent. However, if the effluent is regarded as a series of discrete aliquots, the nature of the interface may be modified to take advantage of the successful procedures used in atomic absorption for analysis of microsamples. Such techniques have the advantage that sample introduction avoids the use of a nebuliser with its inherently low efficiency, so that the detection limits are one to two orders of magnitude better than those of conventional flame AAS (358). In recent years various direct sample insertion devices have been reported, employing platinum loops (359), nickel cups (360) and tantalum boats (361). The best known of these is probably the use of a nickel micro-crucible, the so called 'Delves cup' (360), for the determination of lead in biological and environmental solutions. Other devices have also been reported for flameless operation in graphite furnaces such as carbon rod, carbon filament and tantalum strip atomisers (362).

This chapter describes the development of an HPLC-FAAS interface which

utilises the benefits of microsample insertion devices by collecting the HPLC effluent as a series of discrete aliquots which are then transported via a series of rotating spirals into the flame. Although based on a similar principal, most of the disadvantages described above for the moving wire technique are overcome, although certain features have been retained, such as a desolvation stage prior to atomisation for the reasons discussed below. The result is a very sensitive and yet versatile HPLC-FAAS interface, described here for use with conventional, and minibore HPLC systems. Applications to more specialist systems such as Fast Protein Liquid Chromatography (FPLC) are also described.

8.2 Development of rotating spiral interface

8.2.1 Apparatus

Atomic absorption spectrometer (SP192, Pye Unicam, Cambridge) with a rapid response interface (SP198). The instrument was fitted with background correction and one of four hollow cathode lamps - Zn, Cd, Cu and Pb.

The solvent delivery system (PU4010, Pye Unicam, Cambridge) was equipped with an injector (U6K Waters Associated Inc., Massachusetts) with a 200 µl sample loop. Analytical columns were self-packed with Spherisorb ODS 2 (Phase Separations Ltd., Queens Ferry, Clwyd). Samples were injected directly into the column using a 100 µl syringe (Scientific Glass Engineering, Melbourne, Australia).

A Gilson Minipuls 2 (Villiers, Le Bel, France) peristaltic pump was used for some of the development work.

Output from the atomic-absorption spectrometer was displayed on a chart recorder (AR25, Pye Unicam).

The interface was constructed in house, all electronic components being obtained from R.S. Components (Corby, England). The computer used to control the second interface was a BBC microcomputer system (Acorn Computers Ltd., Cambridge).

The platinum wire and all reagents used were supplied by BDH Chemicals Ltd. (Poole, Dorset).

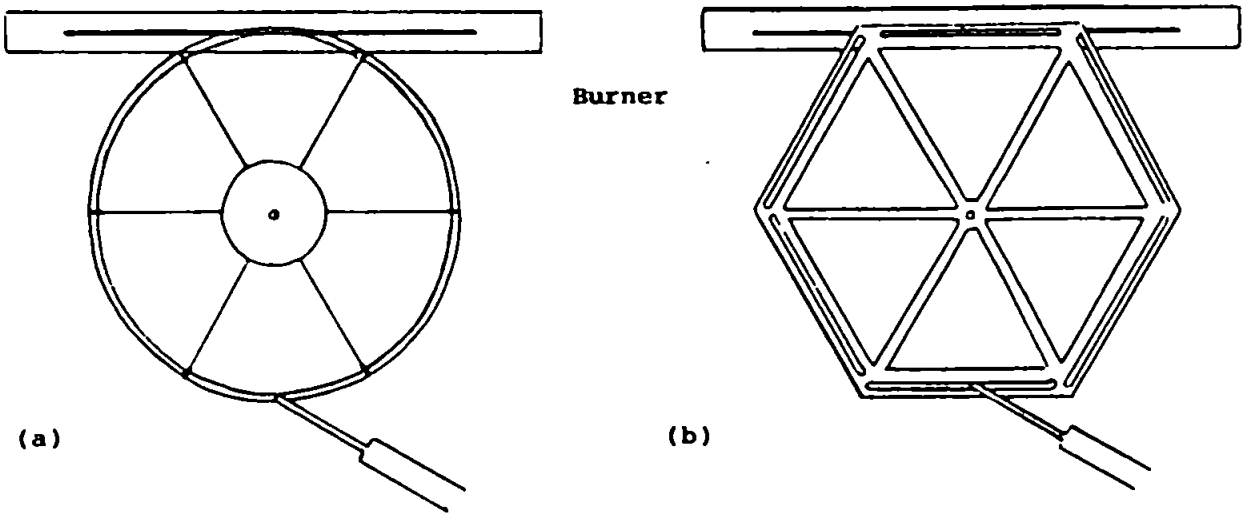
8.2.2 Design of the basic system

The rationale behind this development was to construct a simple interface system capable of collecting the HPLC effluent as it leaves the column and transporting it into the flame of an atomic absorption spectrometer on some form of rotating collection device.

The initial system constructed was crudely based on the moving wire detector describedin Section 8.1, although two parallel wires were used in place of the single strand. The idea here was to collect and hold the effluent by surface tension effects between the two wires which would then rotate the sample into the flame. The wire supports holding the rotating spindle served to segment the sample and hence stop the sample spreading as well as give the complete device a degree of rigidity - see Figure 44a. This system proved unsatisfactory however since the wires tended to distort and buckle in the flame resulting in loss of sample. The second construction was similar in concept to the first in that the sample was again held between two parallel edges. In this design however a hexagonal stainless steel

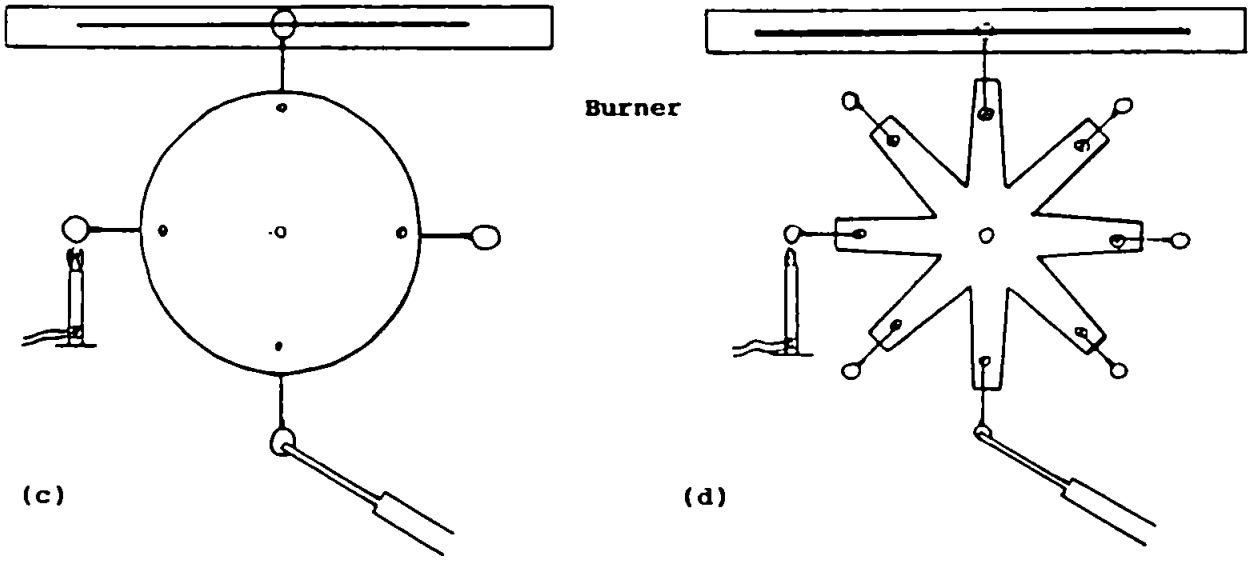
Figure 44

Plan view of parallel wire and loop schemes evaluated for the continuous flow interface



Parallel wires

slots cut in
1/8" stainless steel plate



loops located in holes drilled into the side of the aluminium disc and held by screw

loops held under washer on top of main arm

plate was used with parallel slots cut carefully along each edge. The length of each side of the hexagon (6 cm) was selected so that once rotated each slot lay directly above the slot in the burner. This design had the advantage that each section was completely inserted into the flame, as opposed to the earlier design where only a small section of the sector was located over the burner slot at any one time - see Figure 44b. Once again however when heated, the sample was lost as the plate buckled. Reducing the mass of the plate did not alleviate this problem.

At this stage it was decided to completely change the design of the collection device and a series of miniature crucibles and sampling boats were evaluated. Although such devices are well documented in the literature for single discrete samples, it quickly became apparent that such designs were ill suited to the requirement of the HPLC-FAAS interface sought here without extensive modification. In spite of the use of deuterium background correction, samples were found to generate three absorbance peaks. The first and last peaks were spurious and coincided with the introduction and withdrawal of the crucible from the flame. These effects have been reported by Bahreyni-Toosi et al. (363) and attributed to changes in flame temperature arising from the large mass of the crucible and its holder. Attempts were made to reduce the mass of the device although the spurious peaks were not completely eliminated.

The basic principle behind using the sample boats above was to atomise the sample as completely and quickly as possible in order to record a high narrow absorption peak. Since this is based on reducing the thermal capacity of the holder, a series of wire loops were finally

evaluated for holding the sample. A set of four single loops were mounted at 90° intervals around an aluminium disc (11 cm dia., 3 mm thick). At this stage the loops were constructed from nichrome wire since the objective of this set of experiments was to determine the feasibility of such an interface. The loops were found to hold up to 60 µl of sample and reproducible peaks (within 8% rsd) were obtained by desolvating the sample over a gentle Bunsen burner flame prior to manually rotating the loop into the atomising flame - Figure 44c.

The use of a small microburner to desolvate the sample required the use of a heat shield above the flame to protect the spectrometer. Other methods to desolvate the sample were considered, such as the use of an infrared lamp, although these were rejected due to the practical problems of arranging such a system in the confined space between the spray chamber and top of the instrument. The heat shield which was finally constructed consisted of a stainless steel plate with a water cooled copper winding. At first further problems were encountered since the system tended to disturb the laminar flow of the atomising flame. Gas barriers were considered to help prevent this, although it was found that the problem could be rectified by angling the heat shield towards the chimney above the burner.

Until this stage, the sample had been loaded onto the loops using a precision pipette. Consequently a variable speed peristaltic pump was incorporated into future work to represent the HPLC and supply a continuous flow of sample. This revealed that a number of modifications to the basic system were necessary. Firstly the number of loops mounted on the rotating disc was increased from four to eight. This enabled the loops to be rotated faster and hence ensure

that all the sample was being collected. Radiation heating from the atomisation flame helped further desolvate the sample in the position prior to reaching the burner and thus reducing the time required to atomise the sample.

The final modification at this stage was to the main body of the disc holding the loops. With the solid disc described above, each of the loops were located in a small hole drilled in the edge of the disc, and held in position by a small grub screw from above - see Figure 44c. To facilitate ease in positioning the loop, and renewal when necessary, the mountings were changed so that the arm from each loop was secured under a washer held by a small bolt. In addition a thinner sheet of aluminium (1 mm) was used to fabricate the disc and unwanted sections cut away to reduce the weight of the support. This proved particularly important in later work when the position of the disc was controlled by a stepper motor - see Section 8.2.5. The final construction is shown in Figure 44d.

8.2.3 Construction of loops and spirals

Having determined that the basic system worked in principle, a variety of different shaped loops and spirals were evaluated. This was done in order to find the most effective design for holding the sample securely during rotation, and yet give a single, narrow absorption peak. The various designs and the resulting absorption peaks obtained are shown in Figure 45.

Each of the devices illustrated was loaded with 25 µl of 1 ppm zinc solution and then manually rotated into the flame. The 213.9 nm zinc line was used with background correction. Various responses were

Figure 45

Design of the various loops and spirals evaluated, and resulting atomic absorption signals

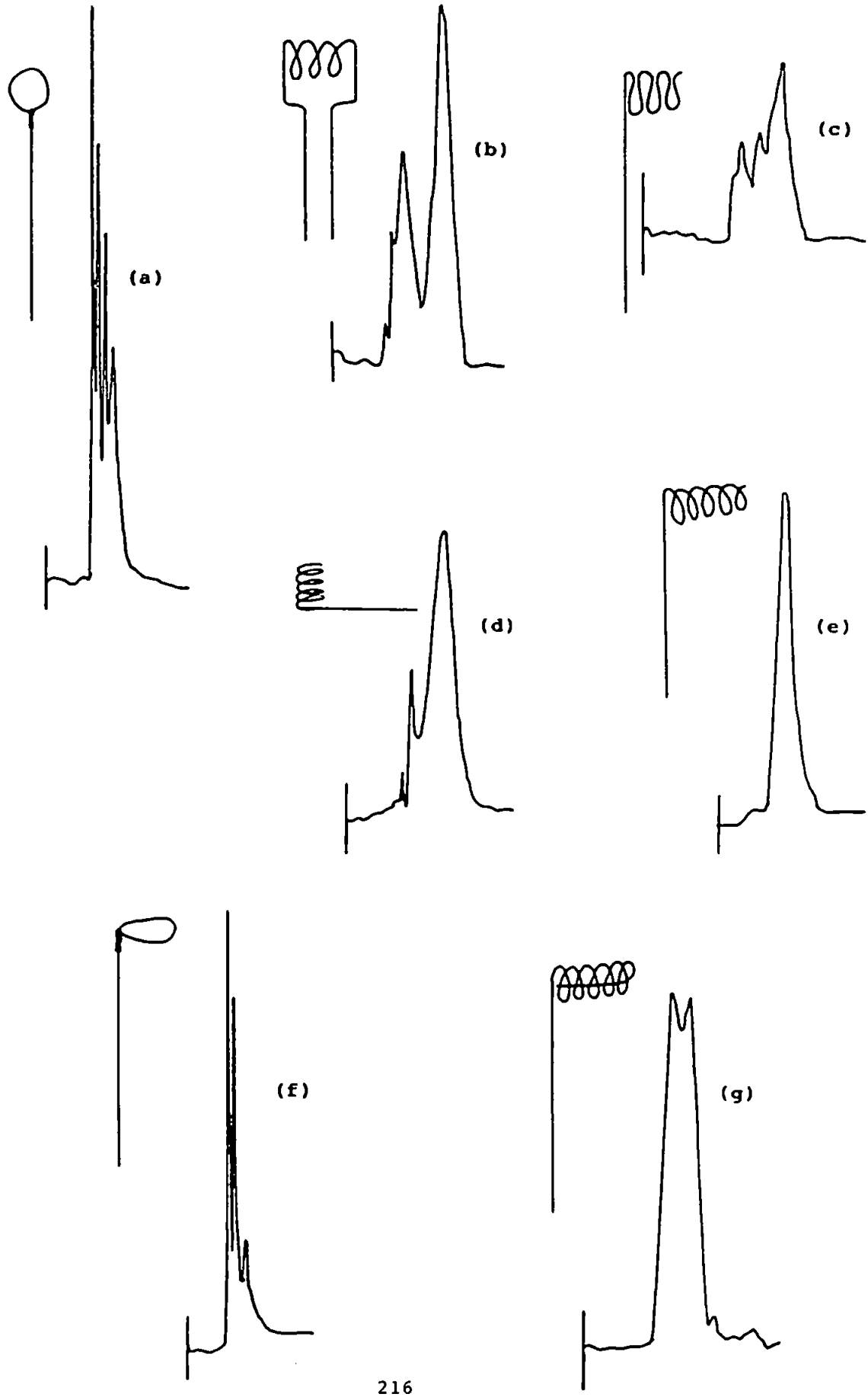
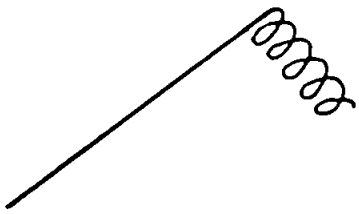
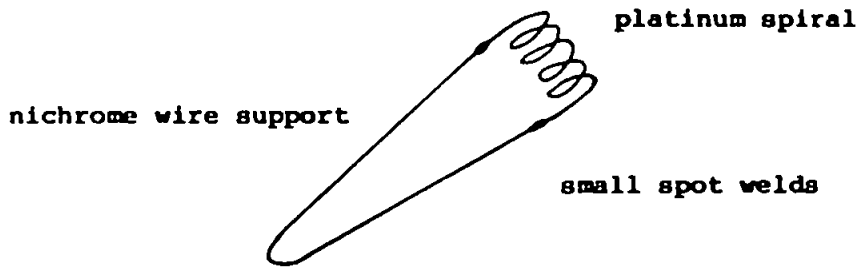


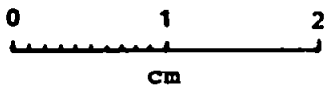
Figure 46

Construction of platinum spirals



One piece construction





Also see Plate 4

obtained reflecting the degree of atomisation of the sample from various areas of the loop or platform. The spiral devices in Figures 45c and 45g, not only give a large, single peak, but also allow a greater sample loading than the other designs and so were used in further work.

Once the spiral had been adopted, consideration was given to various materials for its construction. A range of materials have been reported in the literature, although no single material would appear ideal. Thin tungsten wire has been tried (363) although found to oxidise rapidly and thus has a short working life. Tantalum has also been examined (363), but although more resistant to flame oxidation, it is very brittle and inconvenient to work with. The use of loops constructed with iridium have been reported (364), although once again it is brittle and hard to shape into a loop or spiral.

The material eventually selected for this work was platinum (SWG26 - 0.46 mm) due to its chemical resistance and excellent thermal conductivity. Spirals made of pure platinum however, are easily deformed and so care is required in their construction and maintenance. During early investigations the spirals were made with a single supporting arm. In use however, the platinum softened in the flame resulting in a twisting of the spiral. This was overcome by fusing the platinum spiral to a nichrome wire support at both ends using miniature spot welds - see Figure 46. In this way the spiral was fully supported so that once the optimum height in the atomising flame had been determined, the position of the spirals on rotation was reproducible.

Various lengths of spiral were also examined. It was found that to facilitate collection of an HPLC effluent at 1 ml min^{-1}, three drops (a total of 31.5 µl) had to be collected on each spiral if sufficient time was to be allowed for the sample to be completely atomised. A number of spirals were constructed at different lengths and consisting of a varying number of turns. It was found that by winding the spiral on a threaded support (dia. 2.5 mm), reproducible spirals could be made and easily removed. From this study a spiral 0.7 cm long consisting of 6 turns was found to be able to support the required loading. The lifetime of the spirals is in excess of 500 hours, although spirals may occasionally need remounting due to oxidation of the nichrome wire support.

8.2.4 Optimisation for maximum sensitivity

The use of the slotted tube atom trap and various modified forms of 'Delves Cup' have been shown in previous chapters to give a significant increase in sensitivity. It was decided therefore to adopt a similar system here, using a quartz tube with a single hole in the centre. The tube was mounted on two knife edges so that the hole was directly above the spiral as it came to rest in the flame.

Three different size tubes were evaluated with diameters 8, 10 and 13 mm. The 13 mm diameter tube was ceramic, the two small tubes being made of quartz. Each tube was aligned carefully on the burner so that the light beam passed directly along the centre without touching the walls. The detection limits obtained with each arrangement was then determined for five elements, Zn, Cu, Pb, Sn and Cd, using 50 µl samples loaded onto the spiral using a precision pipette. The results obtained are shown in Table 18.

Table 18

The effects of various tube diameters on sensitivity

	Detection Limit using pulse nebulisation 60 µl/ng	Spiral without Tube/ng	Fold Improvement	13 mm Tube/ng	Fold Improvement	10 mm Tube/ng	Fold Improvement	8 mm Tube	Fold Improvement
Zn	7.0	2.8	2.5	0.175	36	0.0785	89		
Cu	5.3	2.4	2.2	0.56	9.5				
Pb	28	10	2.8	0.430	65	0.404	69		
Sn Air/C_2H_2	2200	395	5.7	55.5	40.5	50	45		
Cd	5.8	454	12.7	0.098	59	0.060	96	78 pg	74

Figure 47

Calibration graphs for zinc using the rotating spiral (a) without the flame adaptor (b) with tube flame adaptor

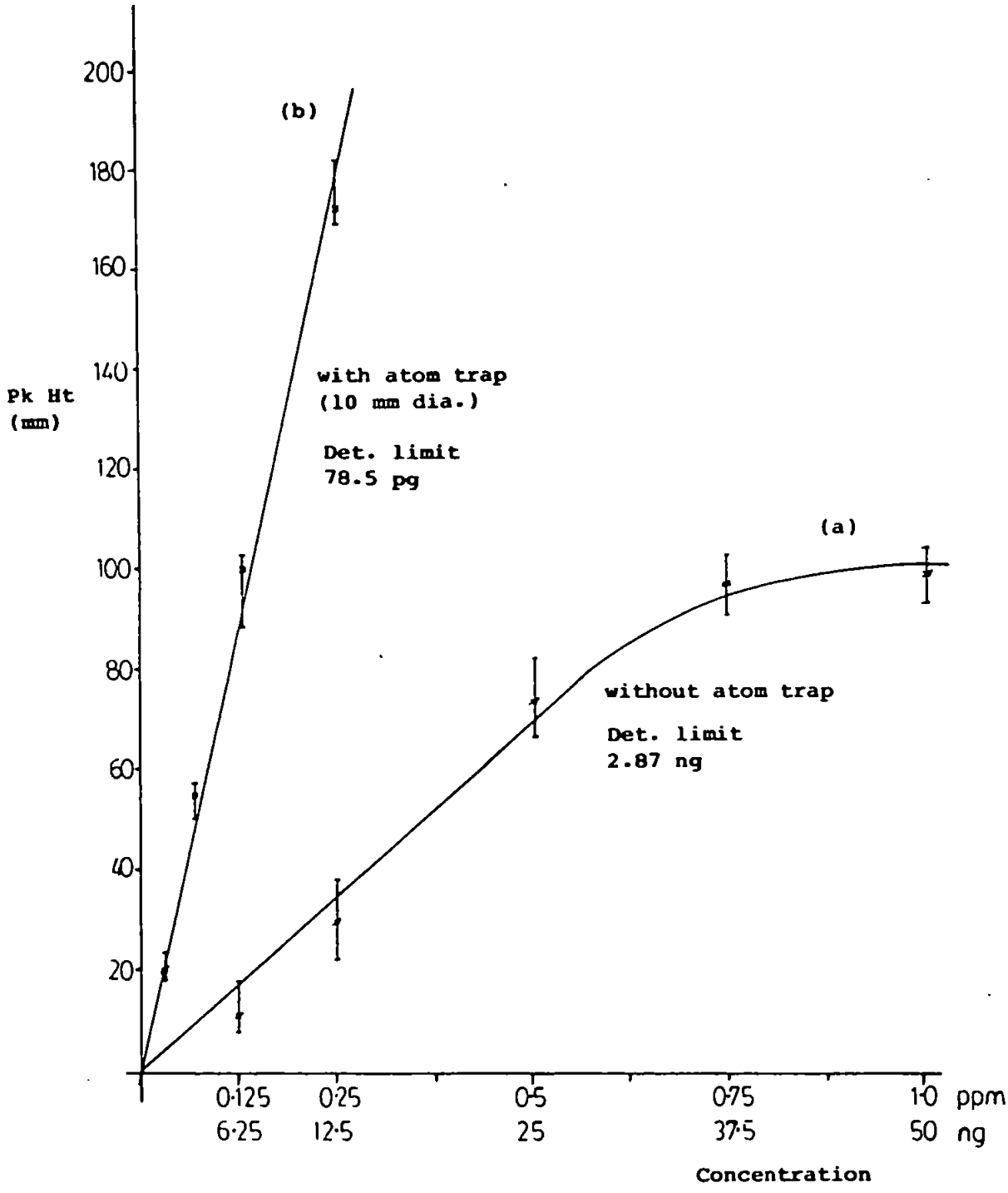
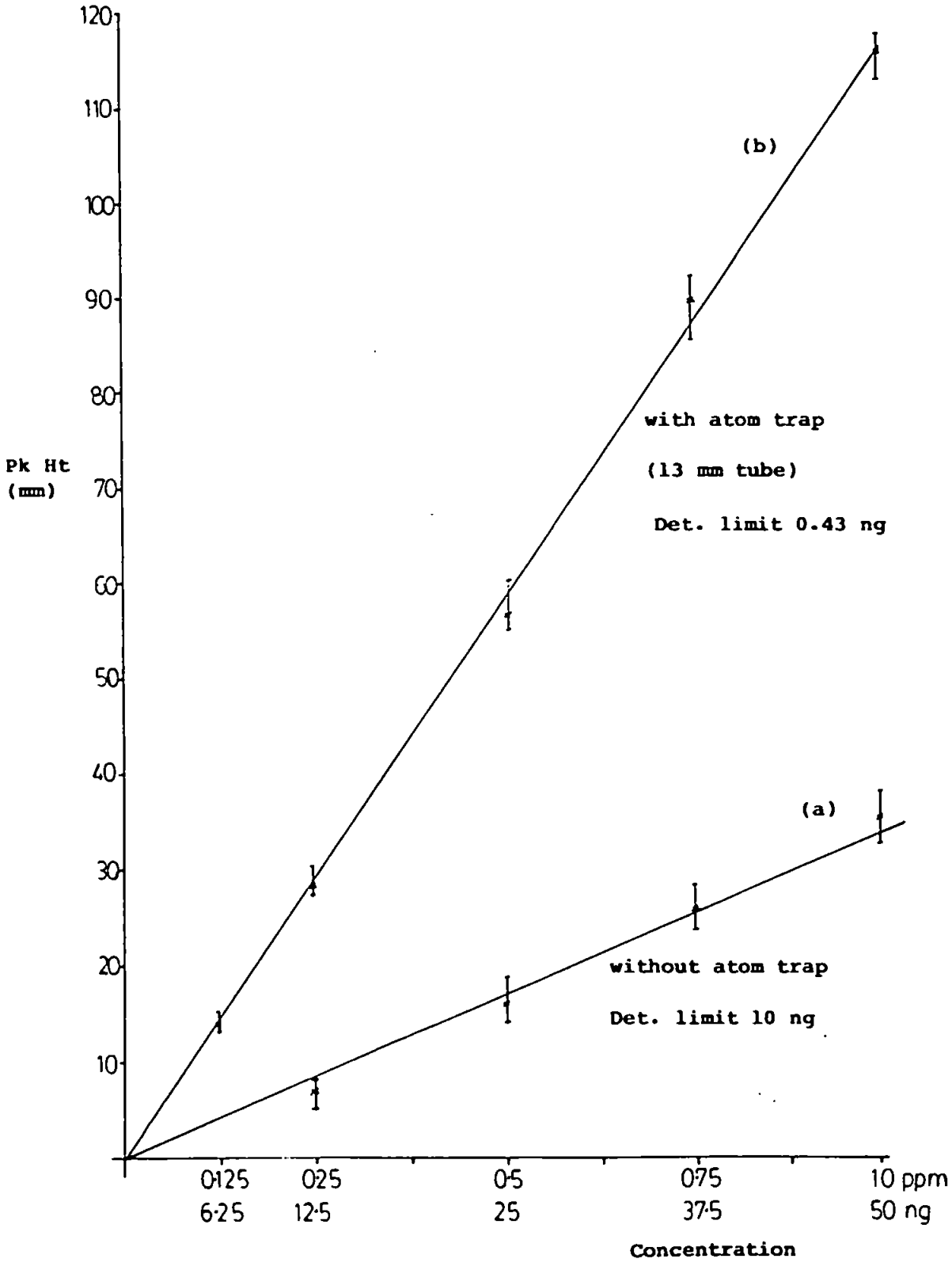


Figure 48

Calibration graphs for lead using the rotating spiral - (a) without tube flame adaptor (b) with tube flame adaptor



It can be seen from Table 18 that a significant improvement in sensitivity was obtained using the tubes. The calibration curves obtained for Zn and Pb (with and without the tubes) are shown in Figures 47 and 48 respectively. Of the three sizes evaluated, the 10 mm tube offered the greatest enhancement and so was selected for use in future work. The disappointing improvement in sensitivity obtained for copper reflects the limitations of this technique for elements which require higher vaporisation temperatures, e.g. Ag, Ca, Co, Cu, Mg, Mn, and Ni. (However it should be noted that it is possible to determine copper using this technique and not from the alternative approaches based on 'Delves Cup'.) By substituting iridium for the platinum as the spiral material and using ceramic tubes, in a manner similar to Bernt and Messerschmidt (364), this problem may be alleviated.

Studies were also made on the optimum position of the spiral in the flame below the tube. It was found that if the spiral was placed directly over the burner slit and as close to the tube as possible, the largest signal was obtained, Figure 49. An attempt was also made to modify the tubes in order to increase the sensitivity. Dawson's group (363) have reported that by reducing the speed of the gas flow through the tube, by encouraging a counter flow of flame gases, greater sensitivity may be obtained. This was achieved by creating additonal apertures in the underside of the tube. However attempts to repeat this success using the spiral system were unsuccessful, no significant difference between tubes with or without the additional hole being observed.

In later work on optimising the spectrometer conditions for various

Figure 49

Optimisation of height of spiral above burner

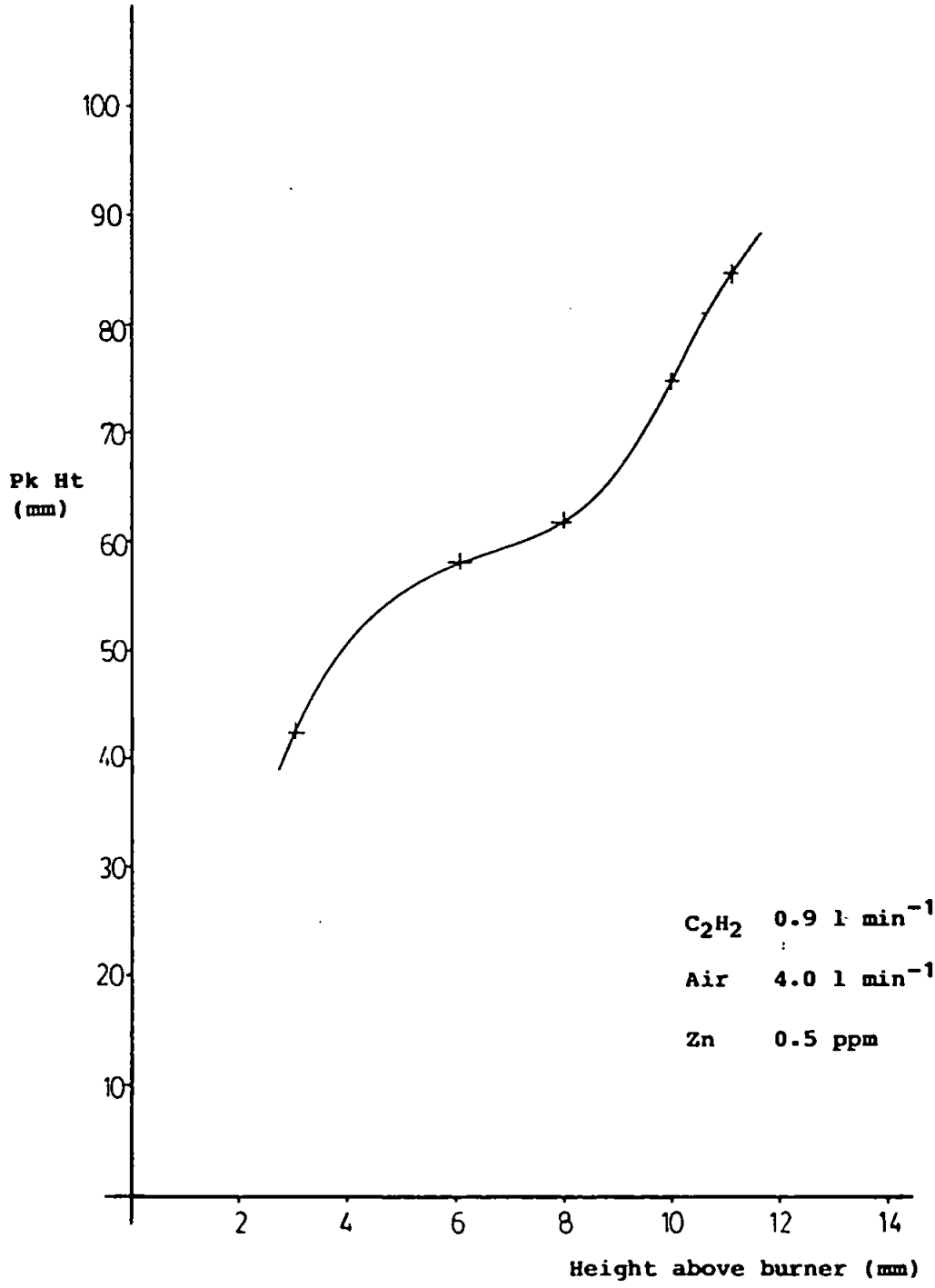


Figure 50

Effect of varying the flow rate of air on the response for zinc.

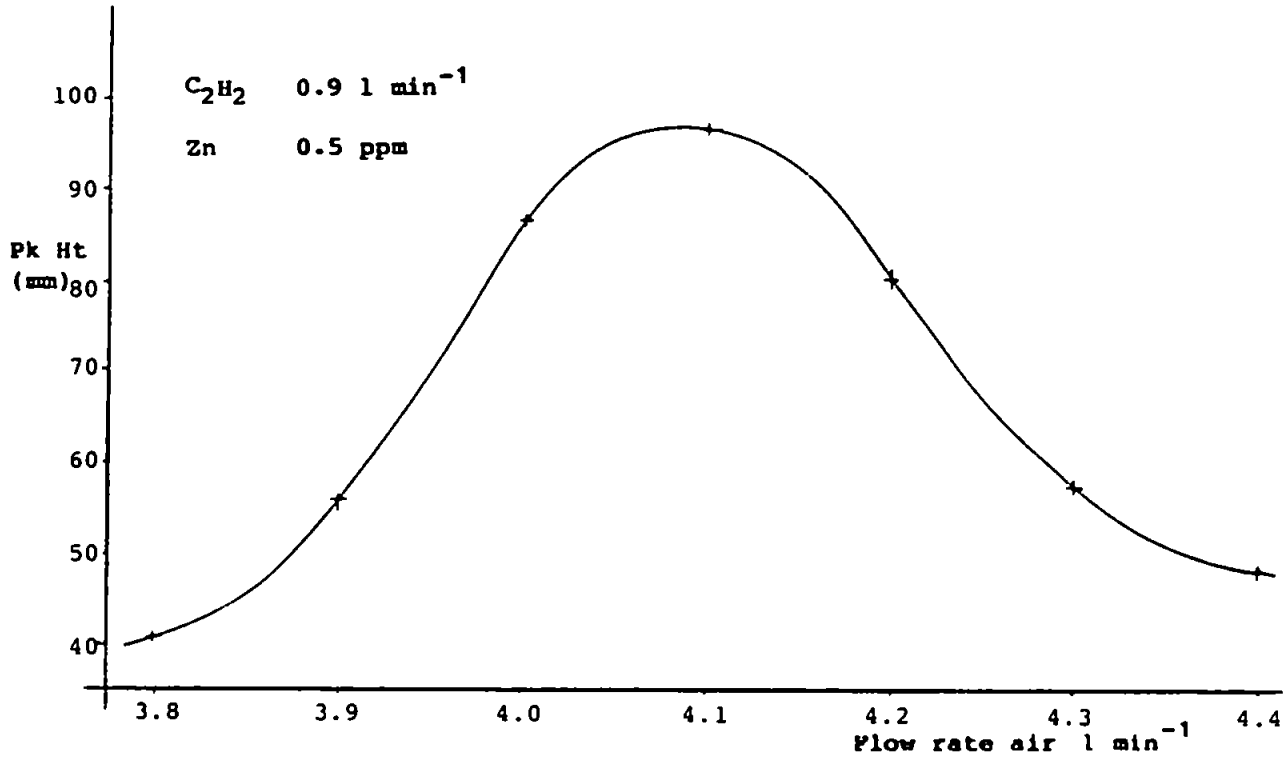


Figure 51

Effect of varying flow rate of C_2H_2 on the response obtained for zinc

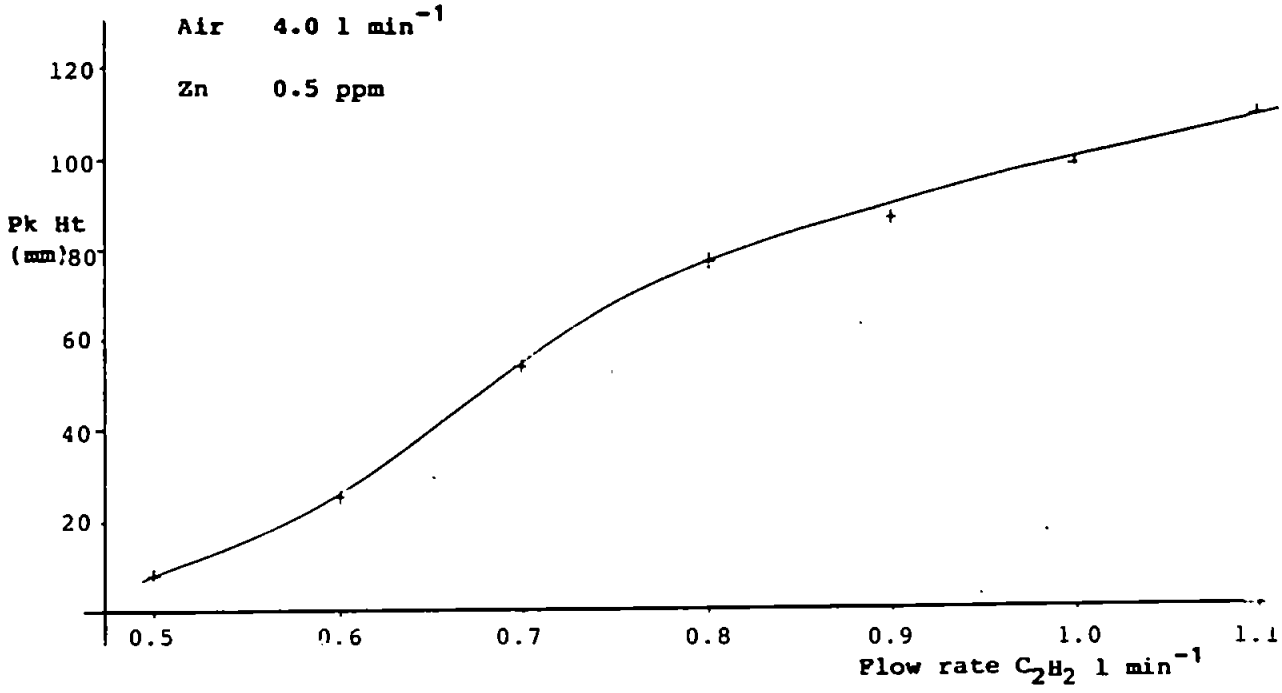


Figure 52

Calibration curves for Zn obtained at various C_2H_2 flow rates

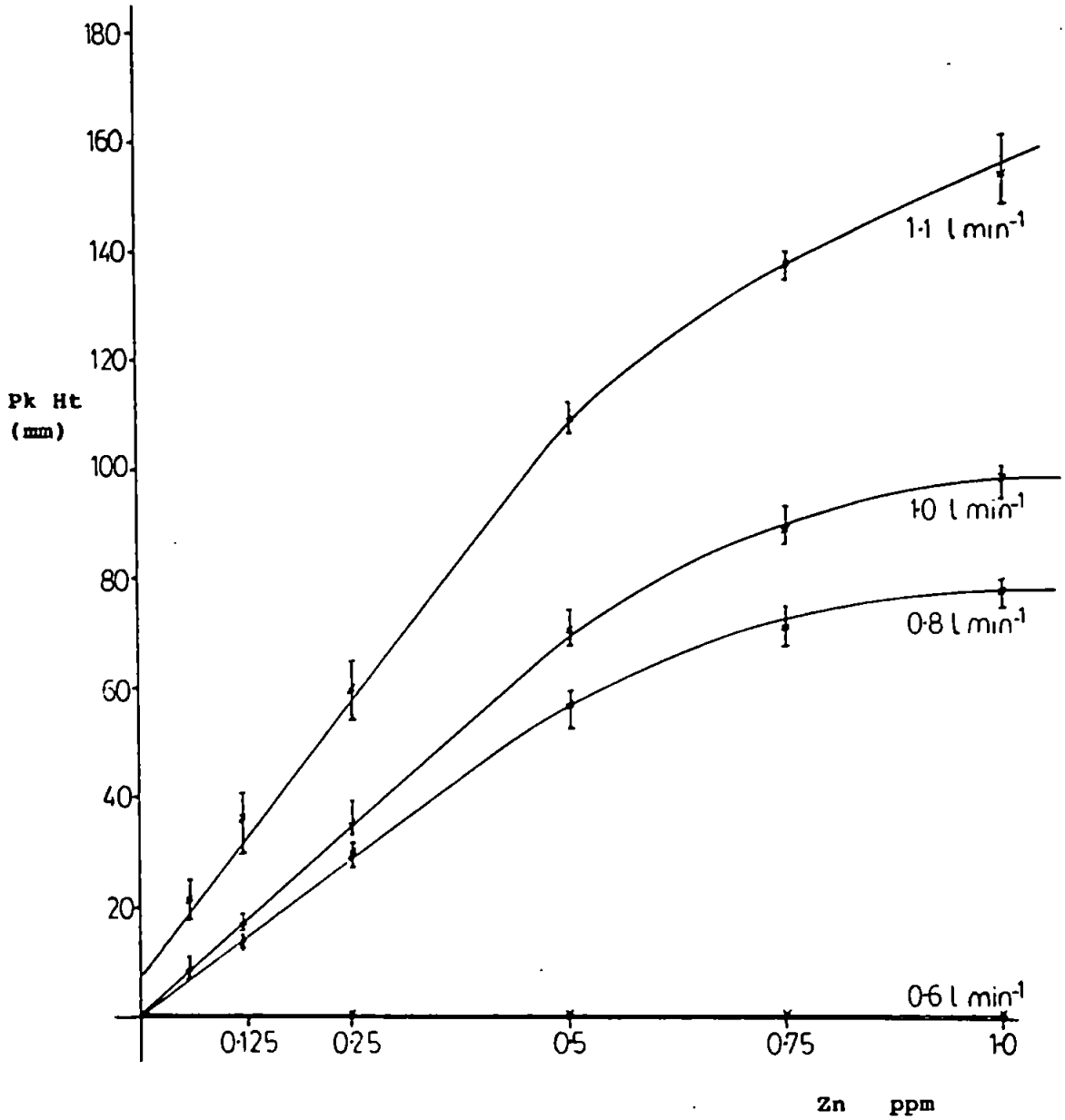


Figure 53

Apparent memory effects observed with high C_2H_2 flow rates

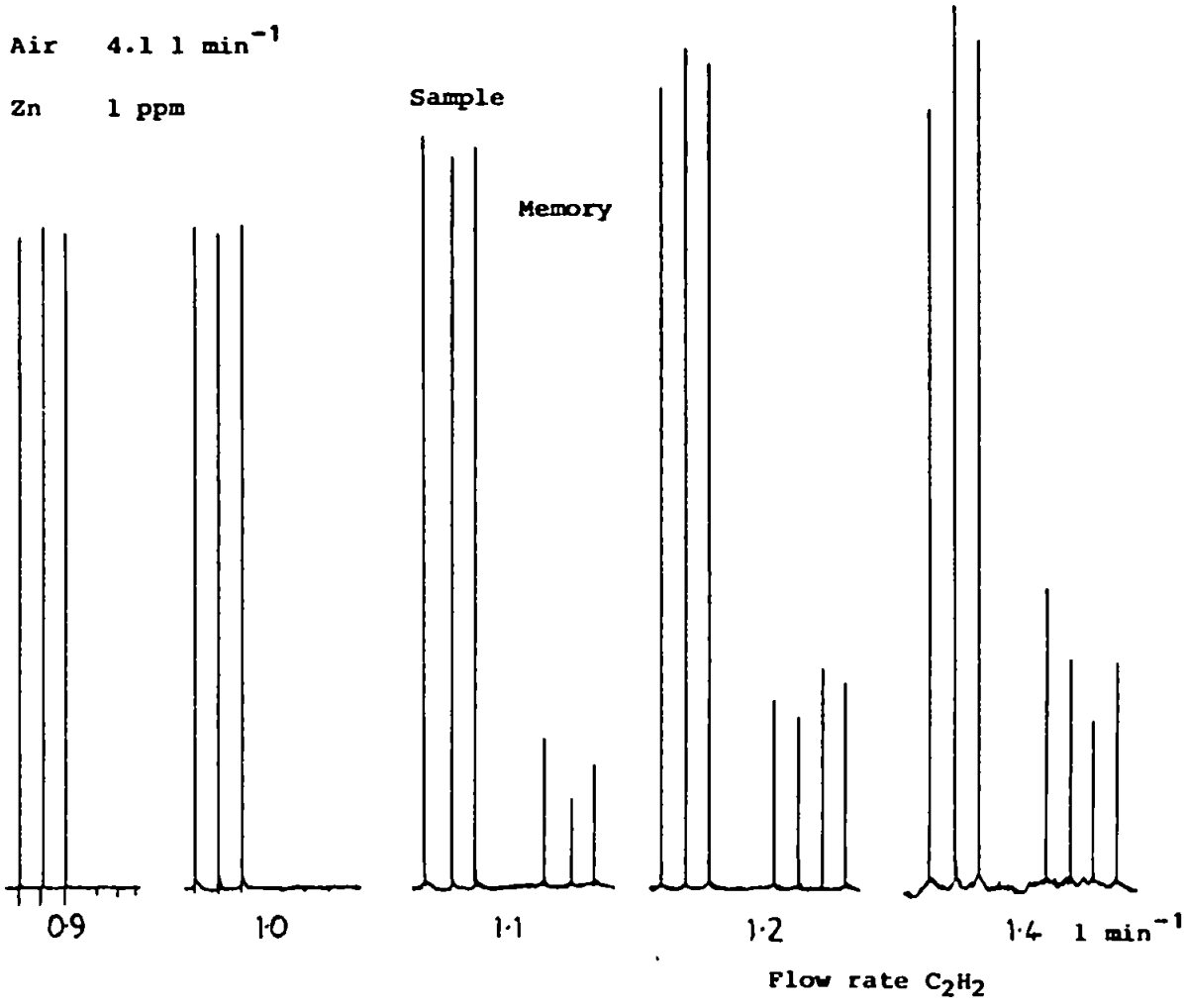
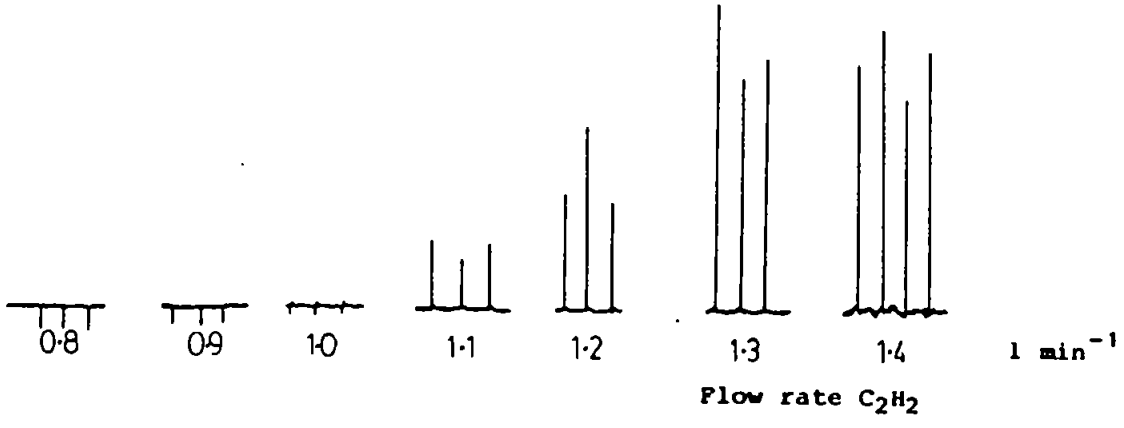


Figure 54

Response obtained from a new platinum spiral with no sample



elements it was noted that the use of the tube removed some of the baseline noise, presumably by reducing the effect of disturbing the flame gases as the spiral was rotated into position. However it was also noted that when high acetylene flow rates were used there was an apparent memory effect from the spirals. This was most clearly observed with zinc, the response for lead and cadmium being less affected by flame conditions.

The effects of varying the air and acetylene flow rates to the burner are shown in Figures 50 and 51 respectively. Although an optimum air flow was obtained, the results obtained in Figure 51 would suggest that a very fuel rich flame is required. However, when calibration graphs are made at increasing acetylene flow rates, high flow rates are found to give high blank values, Figure 52. To investigate this further the spiral was loaded three times with sample and then the response obtained for following blank values observed. A range of fuel flow rates between 0.9 and 1.4 l min^{-1} were used. As can be seen in Figure 53 apparent memory effects are seen at flow rates above 1.1 l min^{-1}. To investigate if this represented a true memory effect, the same experiment was repeated with a new platinum spiral. The spiral was rotated into the flame, again at a range of flow rates, although no sample was loaded onto the loops. Once again a similar effect was observed at high flow rates as shown in Figure 54. It was concluded from this that incomplete atomisation of the sample from the loop was not responsible, and that the artefact peaks were produced by disturbance of the flame by the spiral. Since it was found that the response for lead and cadmium in various flame conditions was similar to that for zinc, a set of standard flame conditions were used. These and the other spectrometer conditions are summarised in Table 19.

Table 19

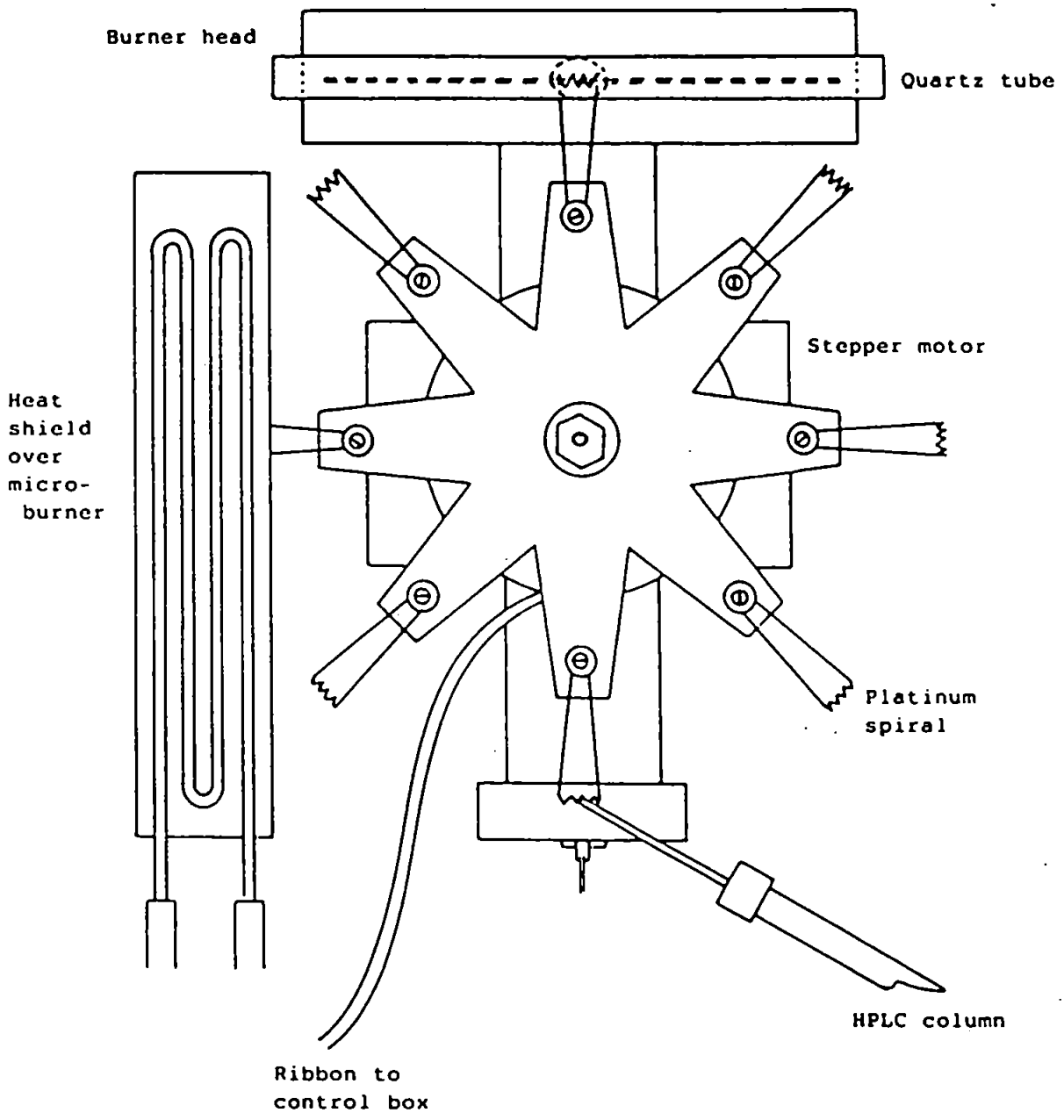
Spectrometer conditions for use with the rotating spiral interface

Spectrometer: Pye Unicam SP192 with SP198 rapid response interface

	Zn	Pb	Cd	Cu
Air	4.1 l min^{-1}	4.1 l min^{-1}	4.1 l min^{-1}	4.1 l min^{-1}
Acetylene	0.9 l min^{-1}	0.9 l min^{-1}	0.9 l min^{-1}	0.9 l min^{-1}
Wavelength	213.9 nm	283.3 nm	228.8 nm	324.7 nm
Lamp Current	3 mA	5 mA	3 mA	5 mA
Bandpass	0.8 nm	0.8 nm	0.8 nm	0.8 nm
Scale Exp.	1	1 - 2.5	1 - 2.5	1

Figure 55

Plan view of rotating spiral interface



A plan view of the completed interface is shown in Figure 55.

8.2.5 Automation and control of rotation

The final stage in the development of the interface was to automate the rotation of the system. Four important functions for the mechanism were identified:

(a) to rotate the spiral;

(b) to stop at eight reproducible locations;

(c) to be able to vary the speed of rotation and time spent at any single location;

(d) to give a smooth movement to avoid loss of sample.

Various mechanical devices were considered using either a screw or belt drive, although designs incorporating the above requirements proved complicated. The system finally adopted was a microprocessor controlled stepper motor unit.

The block diagram of the first system developed is shown in Figure 56. A 7.5^{o}, 12 V bi-directional stepper motor with permanent magnet rotors and 4-phase unipolar construction was used. This was controlled by a stepper control I.C. (SAA 1027) which was programmed to give steps in one direction. The speed of rotation was controlled by varying the number of clock pulses from the 556 I.C., using a potentiometer. A custom built logic control board determined the stopping locations, these being fixed at the end of each six pulse. The time in each location could also be varied between 3.0 and 23.0 seconds (Table 20) by controlling the input ot the 556 I.C. using a second potentiometer. The complete circuit diagram of the control unit is shown in Figure 58.

Figure 56

Block diagram of microprocessor controlled automation system

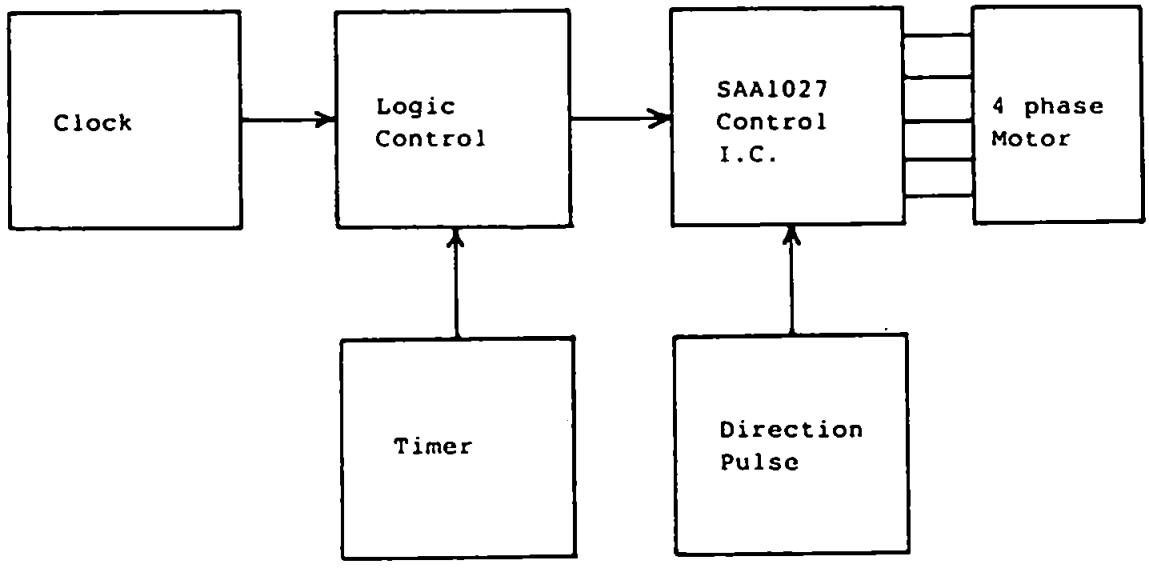
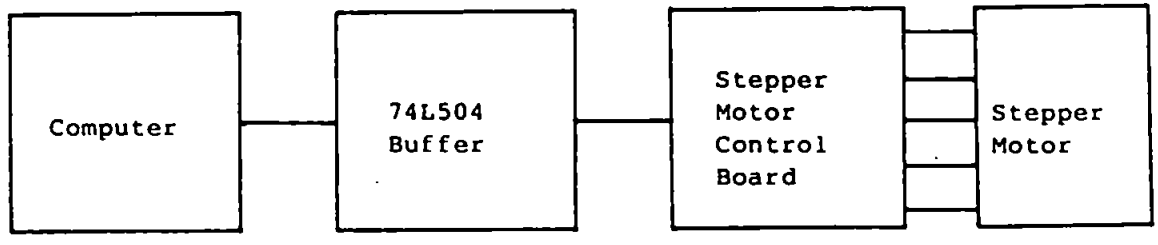
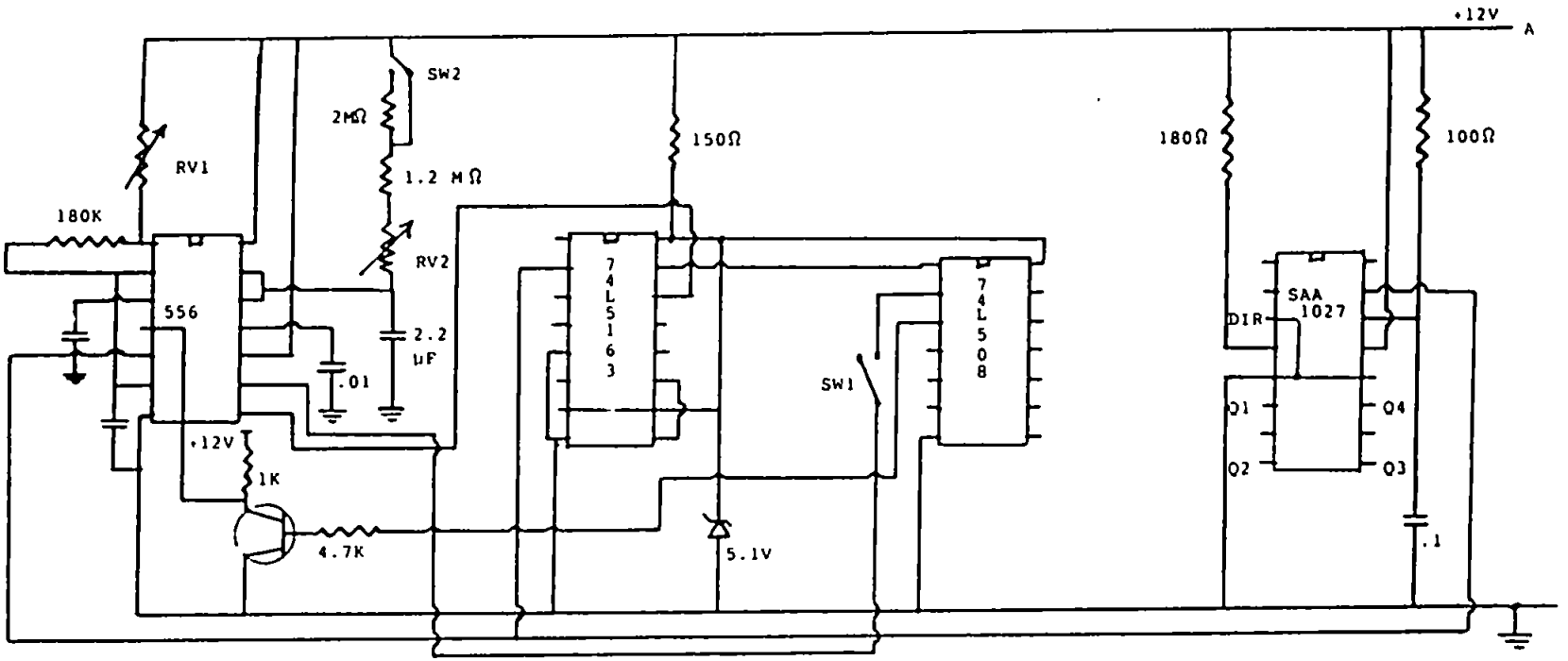


Figure 57

Block diagram of computer controlled automation system





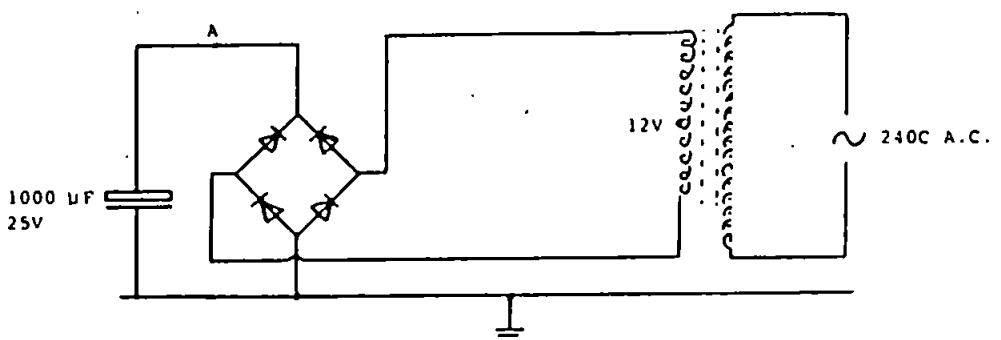


Figure 58

Circuit diagram for control box

Table 20

Calibration of control box

Position	Time for one revolution/s	Time in each location/s	
		Setting	
		1	2
0	84	10.5	23.0
1	84	10.5	23.0
2	82	10.25	22.5
3	81	10.00	22.5
4	78	9.5	22.0
5	65	8.0	20.5
6	45	5.5	18.0
7	30	4.0	16.0
8	25	3.0	15.5

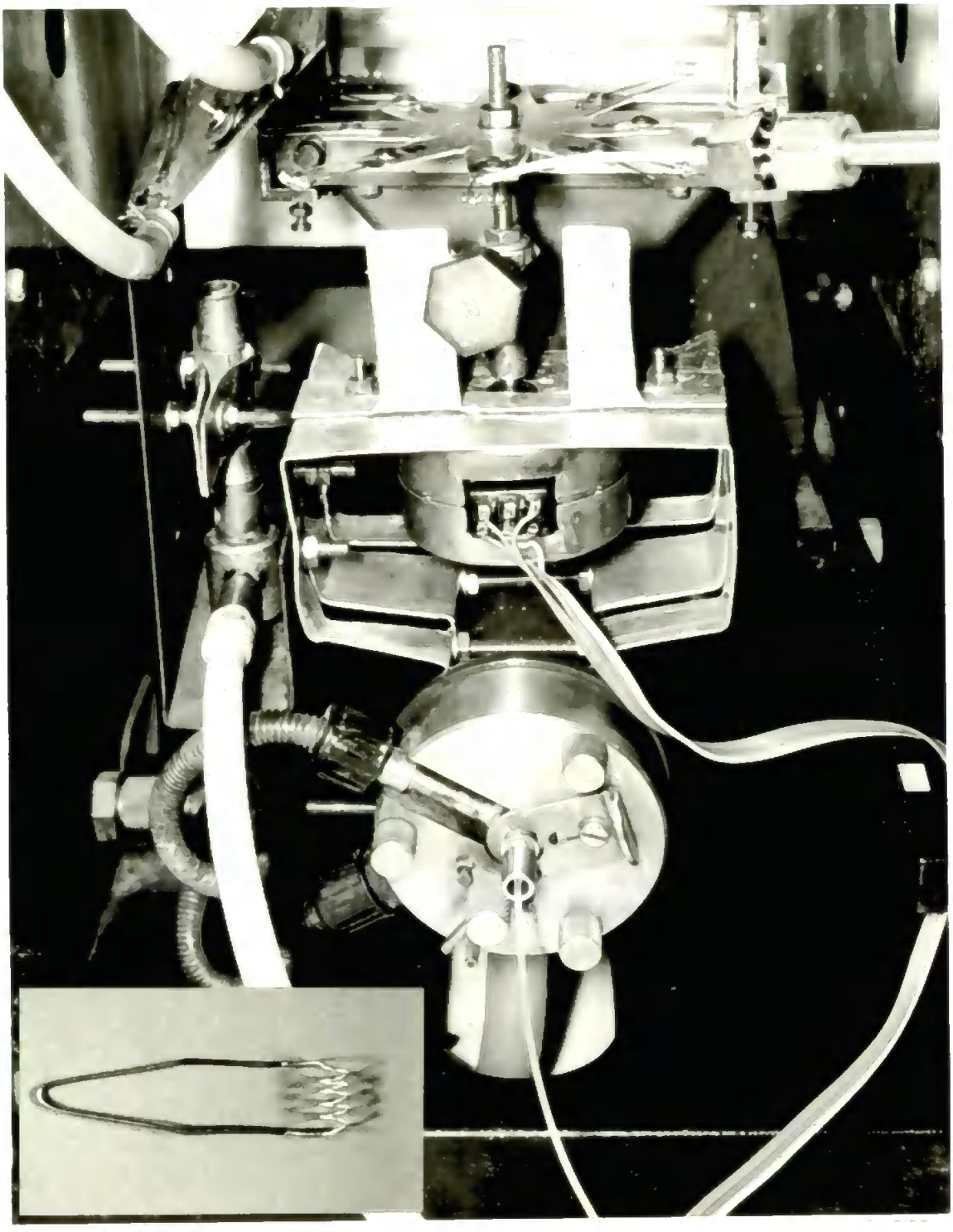


Plate 4

Rotating spiral interface for directly coupled high performance liquid chromatography - flame atomic absorption spectroscopy

The above system was found to work well except that the large stepping angle of the stepper motor resulted in a jerky movement. This was reduced to some extent by lowering the mass of the rotating wire, however further damping was still required. This was achieved by using a simple Terry clip around the drive spindle (see Plate 4), the clip being adjusted so that it was as tight as possible without preventing the spindle from rotating. Although this crude damping mechanism allowed the interface to be used successfully, a second system was developed giving a much smoother rotation by employing a better stepper motor.

In the second system constructed, Figure 57, a 1.8°, 5 V bi-directional hybrid stepper motor with 4-phase construction was used resulting in a higher stepper rate than available from permanent magnet types, while at the same time maintaining very high resolution due to the small step angle. The motor was driven by a 332-098 motor drive board, and the necessary clock pulses, direction, and choice of half or full step selected via a BBC microcomputer. This system provided much greater control over the movement of the spirals, without the need for extensive hardware development since all parameters were controlled via the computer program, Figure 59.

8.3 Application to the determination of organolead compounds

Once the complete interface system had been constructed it was used for the determination of a range of organolead compounds, including diethyl- and dimethyl lead species which cannot be determined directly by other techniques such as coupled GC-AAS. In the first instance, chromatographic conditions based on those reported by Vickrey *et al.* (244) for the separation of alkylleads were used, Table 21. This

Figure 59

Computer program for control unit

```
LIST
    1REM PROGRAM BY A.F.HOPKINS.
   10REM STEPPER
   30VDU 23,1,0;0;0;0;
   50*FX4,1
   70CLS
   90?&FC61=0
  110?&FC60=255
  130?&FC61=4
  150?&FC63=0
  170?&FC62=255
  190?&FC63=4
  210?&FE62=255
  230*KEY 0 "!!!0"
  250*KEY 1 "!!!1"
  270*KEY 2 "!!!2"
  290*KEY 3 "!!!3"
  310*KEY 4 "!!!4"
  330*KEY 5 "!!!5"
  350*KEY 6 "!!!6"
  370*KEY 7 "!!!7"
  390*KEY 8 "!!!8"
  410*KEY 9 "!!!9"
  430REM FE60=255 PUTS 1 ON PB0-7
  450W=0:X=0:Y=0:Z=0 :CLS
  550A=0
  570H=0
  590B=0
  610J=0
  620 R=25
  625D=0
  628PRINT TAB(32,10);X:PRINT TAB(38,12);D:PRINT TAB(35,14);
R
  630PRINT TAB(10,2);J
  650 PRINT TAB(13,6);"**MENU**"
  670PRINT TAB(0,8);"PRESS f0 TO RETURN TO MENU"
  690PRINT TAB(0,10);"PRESS f1 TO SET STEP SPEED"
  710PRINT TAB(0,12);"PRESS f2 TO SET DELAY BETWEEN STEPS"
  730PRINT TAB(0,14);"PRESS f3 TO SET NUMBER OF STEPS"
  735PRINT TAB(0,16);"PRESS f4 TO SET FULL/HALF STEP"
  740PRINT TAB(0,18);"PRESS f5 TO MOVE STEPPER MOTOR"
  745PRINT TAB(0,20);"PRESS f6 TO RESET SYSTEM"
  746PRINT TAB(0,22);"PRESS f7 TO RUN "
  748 PRINT TAB(0,24);"PRESS f8 TO STOP. f9 TO START"
  750I=INKEY(1)
  760IF I=32 THEN J=0 :PRINT TAB(10,1);"     ":PRINT TAB(10,1
);J
  770IF I=176 THEN 450
  790IF I=177 THEN 1525
  810IF I=178 THEN 3000
  820IF I=183 THEN 870
  830IF I=179 THEN 4000
  835 IF I=180 THEN 6000
  838IF W=1 THEN PRINT TAB(35,15);"HALF"
  839IFW=0 THEN PRINT TAB(35,15);"FULL "
  840IF I=181 THEN 1290
  850 GOTO 750
  870I=INKEY(10)
  890IF I=185 THEN PRINT TAB(1,1);"START":GOTO 950
```

```
 910IF I=176 GOTO750
 930GOTO 870
 950 H=0
 970REPEAT
 990H=H+1
1010?&FE60=A+W
1030 FOR C=0 TO X:NEXTC
1050?&FE60=A+2+W
1070FOR C=0 TO X:NEXTC
1090IF W=0 THEN Q=1
1110IF W=1 THEN Q=2
1130UNTIL H=R*Q
1150L=INKEY(20)
1170IF L=184 THEN PRINT TAB(1,1);"STOP  ":GOTO 5000
1190FTIME=TIME
1195IF TIME=FTIME+(D*100) THEN 1210
1196 L=INKEY(1)
1197IF L=182 THEN 10
1199GOTO1195
1210J=J+1
1230PRINT TAB(10,1);J
1250 GOTO 950
1270PRINT TAB(1,1);"STOP    "
1290K=INKEY(1)
1350IF K=136 THEN ?&FE60=1:?&FE60=3
1370IF K=137 THEN ?&FE60=5:?&FE60=7
1390IF K=176 THEN 750
1430GOTO1270
1450D=INKEY(10)
1470PRINTD
1490FOR T=0 TO 1000:NEXTT
1510GOTO1450
1525 PRINT TAB(5,8);"                          "
1530INPUT TAB(5,4)"SET STEP SPEED "X   :CLS
1550GOTO 628
3000 INPUT TAB(5,4)"SET DELAY TIME IN SEC "D :CLS
3020 GOTO 628
4000PRINT TAB(5,4)"                                    "
4005INPUT TAB(5,4)"NUMBER OF STEPS "R  :CLS
4010GOTO 628
5000K=INKEY(1)
5010IF K=185 THEN PRINT TAB(1,1);" CONT      ":GOTO 1190
5015 IF K=176 THEN 750
5020 GOTO5000
6000 INPUT TAB(5,4)"HALF OR FULL  "W$ :CLS
6010IF W$="HALF" THEN W=1
6020 IF W$="FULL" THEN W=0
6050 GOTO 628
```

Table 21

Chromatographic conditions for the determination of alkyllead compounds by directly coupled HPLC-PAAS

Column:	Whatman 10 μm C-18 ODS	(i) (25 cm x 4.6 mm i.d.)
		(ii) (20 cm x 2.0 mm i.d.)
Mobile phase:	80:20 Methanol:Water	
Flow rate:	(i) 0.5 ml min^{-1}	
	(ii) 0.3 ml min^{-1}	
Injection size:	Up to 1 ml facilitated	

study was made primarily to evaluate the interface in terms of response, peak shape and reproducibility.

The first chromatogram produced from the system is shown in Figure 60. As can be seen from the chromatogram the separation took over 45 minutes and gave tailing peaks. The flow rate was increased up to 1 ml min^{-1}, to reduce the retention times and improve peak shape, although it was found that at this flow rate, sample was lost due to overloading the spirals. To overcome this problem and remove flow-rate restrictions in any further development of the chromatography a minibore HPLC column was employed instead of the conventional standard column.

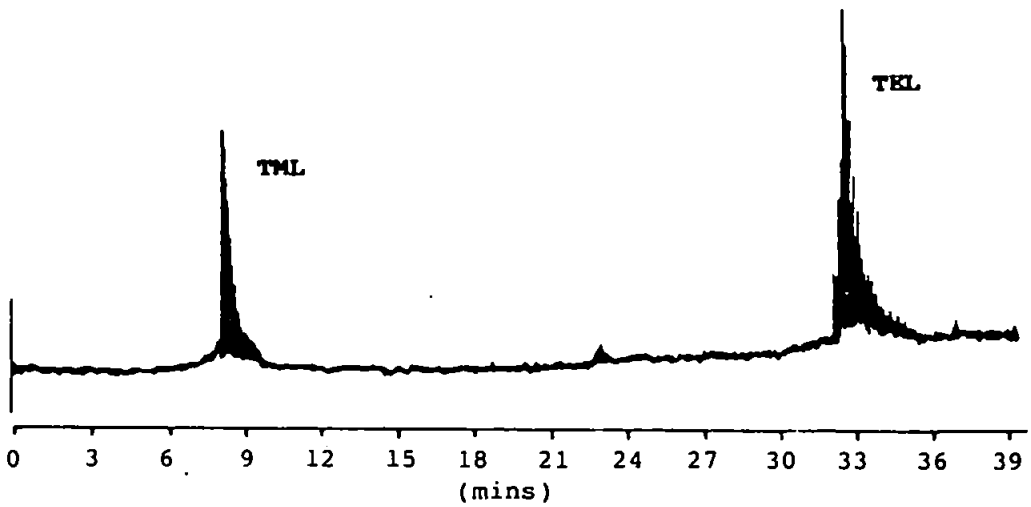
8.3.1 Use of minibore HPLC

The term minibore here refers to columns with an internal diameter of 2 mm and thus falls between conventional columns (4.6 mm i.d.) and true microbore (1 mm i.d.). In recent years considerable interest has been shown in the use of small bore and HPLC columns and a wide variety of microbore columns are now commercially available. The subject has also received considerable coverage in the literature (365-366).

There are a number of benefits in using minibore columns for HPLC. These include: (a) higher mass sensitivity arising from the smaller sample size; (b) lower solvent consumption due to the lower eluent flow rates; (c) better separation of complex mixtures because of the ease of increasing column length simply by connecting shorter columns in series; (d) higher speed of separation for simple mixtures using short columns at higher eluent flow rates; and (e) improved ability to

Figure 60

Chromatogram showing the separation of alkyllead compounds in petrol.



interface with coupled techniques, again due to lower eluent flow rates. Several of these factors make the use of minibore columns ideal for the HPLC-FAAS interface described above.

The minibore column (2 mm i.d.) used for this work was packed in the conventional way with Whatman 10 µm C-18 ODS. However, before commencing the experimental work some concern was felt over the reproducibility obtainable from conventional HPLC pumps when used to deliver such low flow rates. To check this the pump was calibrated from 0.1 - 0.6 ml min^{-1}. The results are shown in Table 22. Although the actual flow rates obtained showed a bias of up to 32% when compared with the pump setting, the error associated with the results obtained was < 1%. The conventional HPLC pump was therefore retained.

The complete system incorporating the minibore column was finally optimised to determine the best chromatographic conditions required for the determination of alkyllead compounds in petrol. The chromatograms produced and the reproducibility obtained using the interface were then critically assessed.

8.3.2 Results and Discussion

To optimise the separation of TML and TEL in petrol, the effect of eluent composition and flow rate were evaluated. A range of methanol:water eluents were used, and the retention times and peak shapes determined for 100 µl injections of a 10 ppm TEL standard. The results obtained are shown in Table 23. Clearly, the eluent composition has a marked effect on both parameters. The effect of eluent flow rates on the retention time is shown in Table 24. In order to maintain baseline resolution of the two species a flow rate

Table 22

Calibration of HPLC pump

Back pressure psi	Pump Setting /ml min^{-1}	Time to collect 5 mls /s	Actual flow rate /ml min^{-1}	Bias %
500	0.1	4420	0.07	- 32
1400	0.2	1792	0.17	- 15
2100	0.3	1119	0.27	- 10
2600	0.4	815	0.37	- 8
3000	0.5	640	0.47	- 6
5000	0.6	527	0.57	- 5

Table 23

Effect of eluent composition (MeOH:H_2O) on the retention time of TEL

	Retention time (mins)	Peak Height (mm)	Time to elute (mins)
80%	37.5	9	8.4
85%	24.0	20	5.4
90%	10.8	35	3.3
95%	7.5	52	1.5
100%	4.4	75	1.1

Injection size 100 µl

Flow rate 0.3 ml min^{-1}

Table 24

Effect of eluent flow rate on the retention times of TML and TEL

ml min^{-1}	TML	TEL
0.1	14.8	10.9
0.2	7.1	5.0
0.3	3.2	4.4
0.4	2.6	3.6
0.5	2.2	3.1
0.6	1.6	2.2

of 0.3 ml min^{-1} was selected for further work. The chromatographic conditions selected for the separation are summarised in Table 21.

The results obtained from this study were also used to evaluate the interface. Figure 61 shows how the actual chromatogram is produced. By increasing the chart recorder speed the response obtained from each spiral can be seen as a discrete peak. At normal chart speeds however, the individual peaks are not observed and a more conventional looking chromatogram is obtained. The reproducibility between injections was also investigated. Figure 62 shows the results obtained from a series of six 100 µl injections of a petrol sample diluted 50 times with methanol. The peak heights obtained were reproducible within 5% RSD. Finally the response obtained from a standard calibration series of TEL was determined, Figure 63. Once again a conventional looking curve was obtained, the calibration for TEL being linear up to 400 ng.

The results above demonstrate the effectiveness of the interface, although the determination of alkylleads in petrol is already well served by coupled GC-AAS, see Section 3.2. To demonstrate one of the advantages of the directly coupled HPLC system, the above study was extended to diethyl and triethyl lead compounds. These compounds cannot be determined directly by coupled GC-AAS, since they are not sufficiently volatile to be eluted from the column.

Initial studies gave a response for Et_3PbCl after 5.3 minutes. However Et_2PbCl_2 was not observed probably due to absorption onto the stainless steel HPLC column and connecting tubing. The inside of the HPLC system was therefore flushed through with a 5% solution of

Figure 61

Formation of chromatograms using the rotating spiral interface

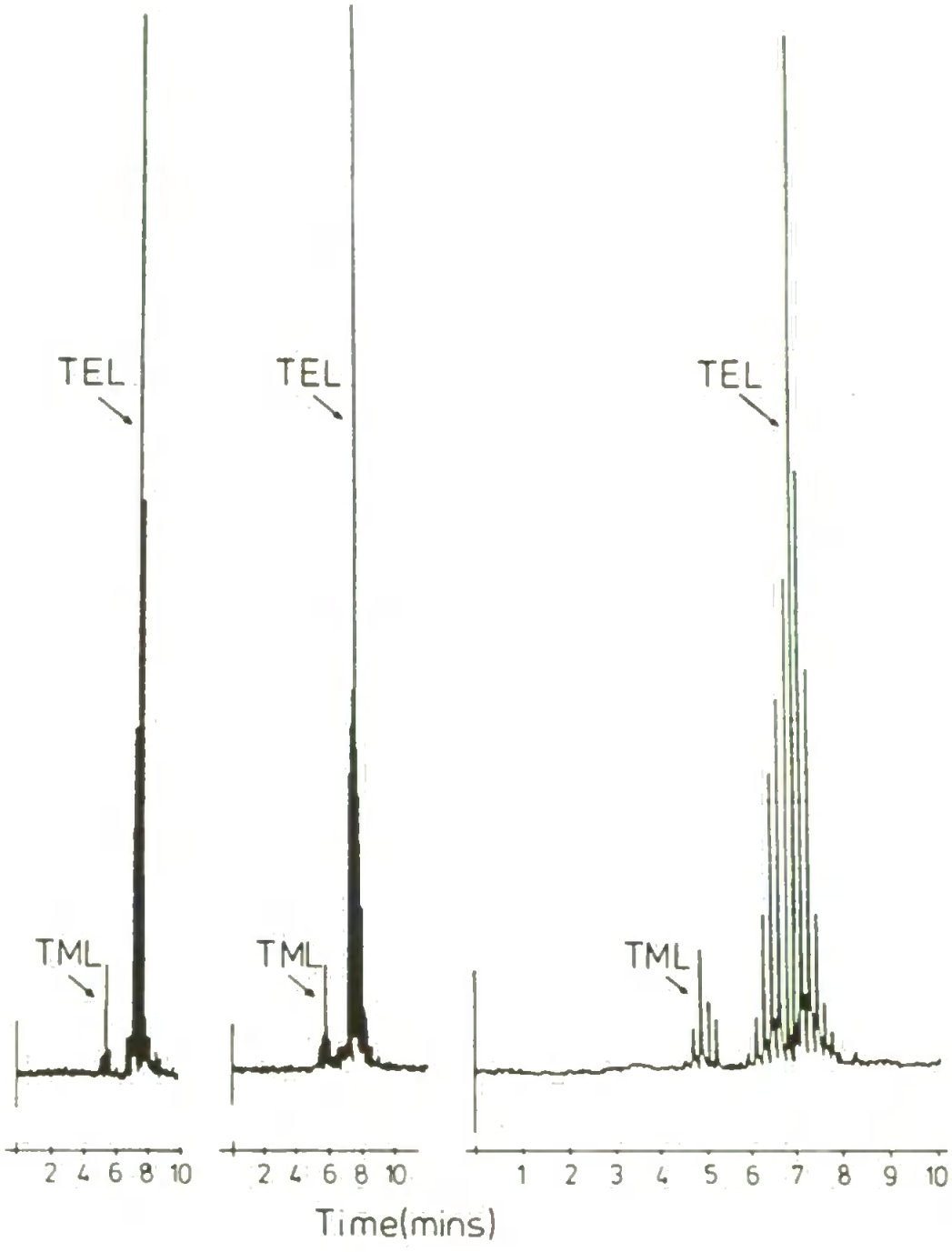
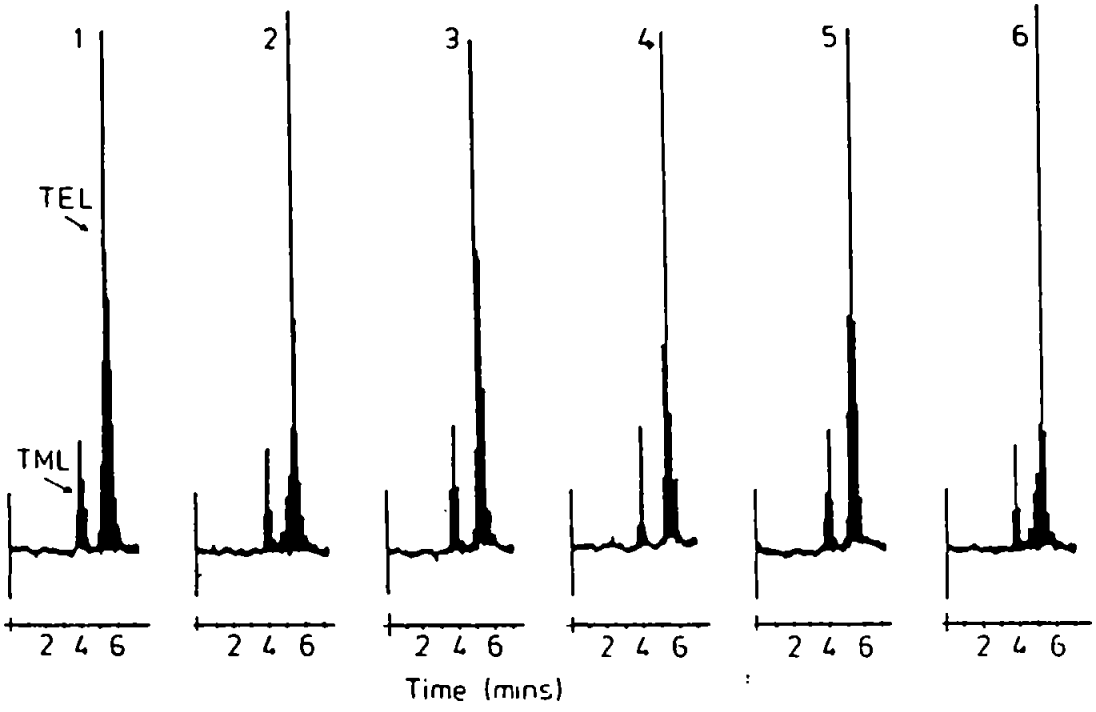


Figure 62

Reproducibility of injections



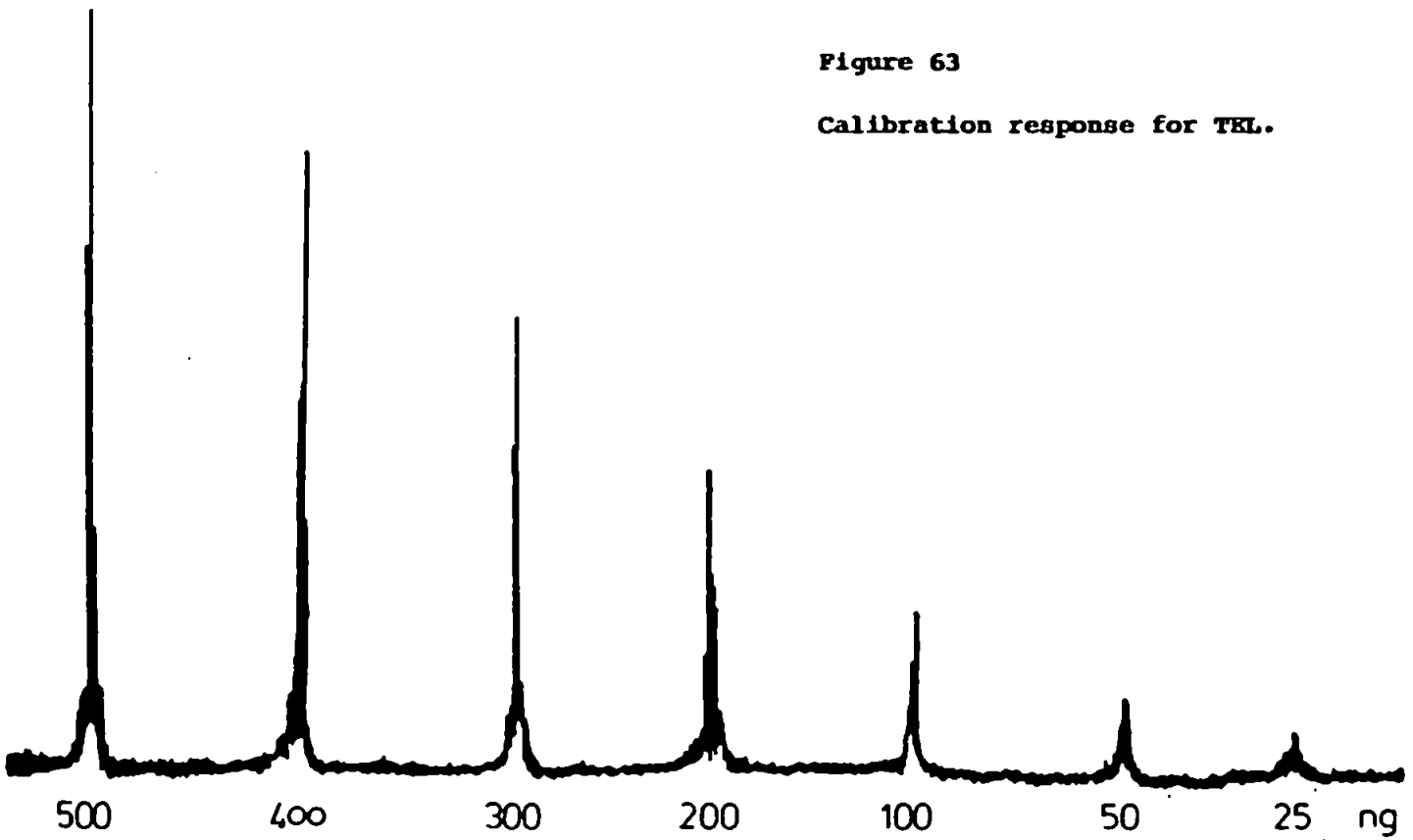


Figure 63

Calibration response for TKL.

dimethyldichlorosilane in an attempt to silanise any absorption sites. To do this, this system was washed through with acetone and then hexane, before the 5% solution of dimethyldichlorosilane in hexane was used. After flushing through for 10 minutes, the reverse procedure was followed i.e. washing with hexane and acetone before reconnecting the elution system above.

After silanising the system a sharp peak was obtained for Et_2PbCl_2 and a substantial improvement in the original response for Et_2PbCl obtained, the detection limit being reduced from 400 ng (before silanisation) to 10 ng. The detection limits for each species obtained with the silanised column are shown in Table 25.

The separation of Pb^{2+}, TEL, Et_2PbCl_2, Et_2PbCl and $(CH_3COO)_3Pb$ on a single chromatogram is shown in Figure 64. After about twenty injections however, the sharp peak obtained for Et_2PbCl_2 was found to deteriorate, presumably because the dimethyldichlorosilane was stripped from the column. This effect can be seen in Figure 65. Re-silanisation of the column using the above procedure helped regain a sharp peak although once again the peak shape quickly deteriorated.

The speciation of diethyl and triethyllead compounds is thus shown to be possible using the directly coupled HPLC-FAAS system developed. However further work is obviously required if this separation is to be achieved routinely. One possible improvement would be the replacement of stainless steel with PTFE lined columns and connecting tubes. This should overcome the need for silanisation, although the system may also benefit from further investigations into the use of other mobile phases.

Figure 64

Separation of lead species by directly coupled HPLC-FAAS.

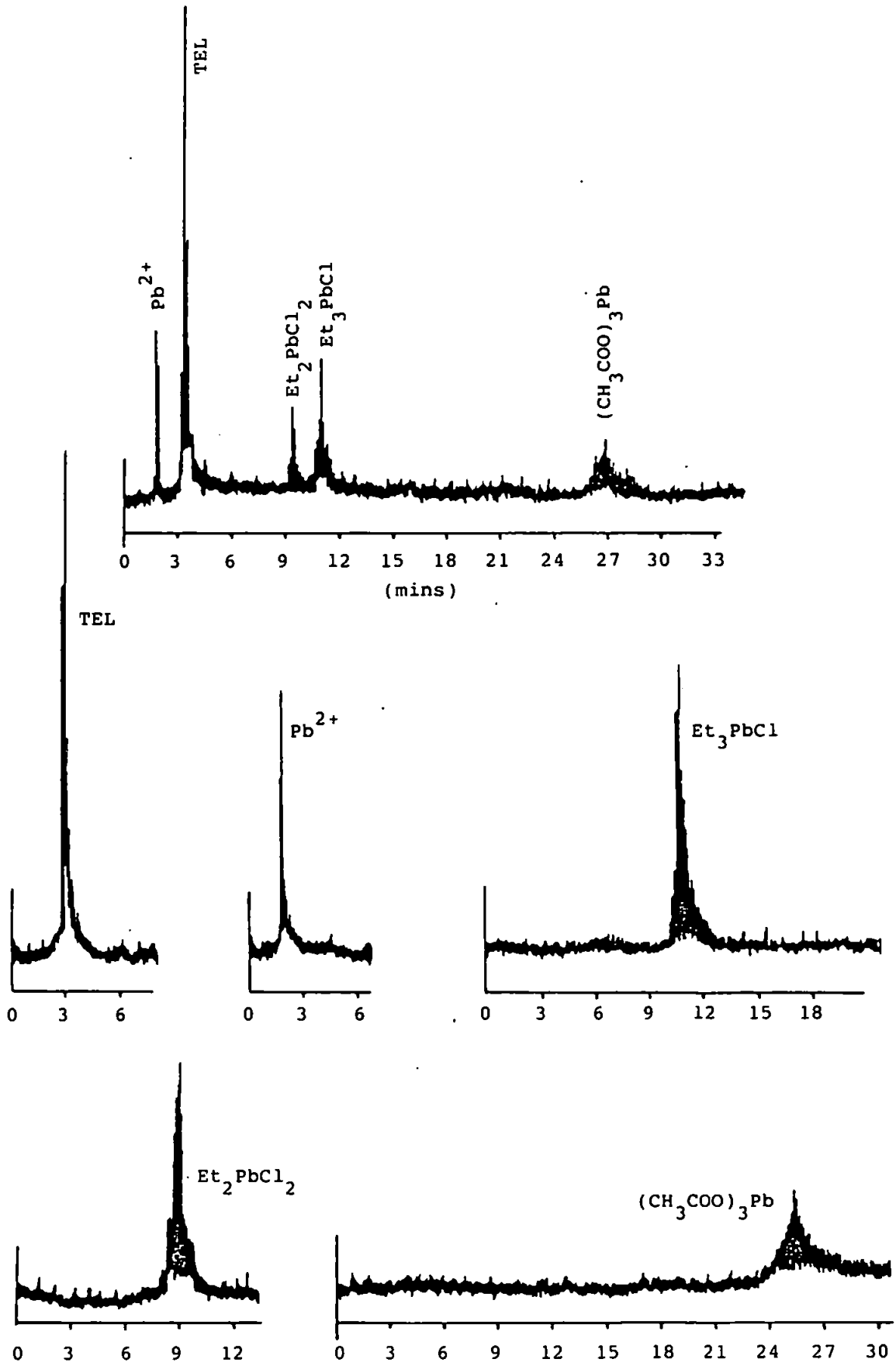
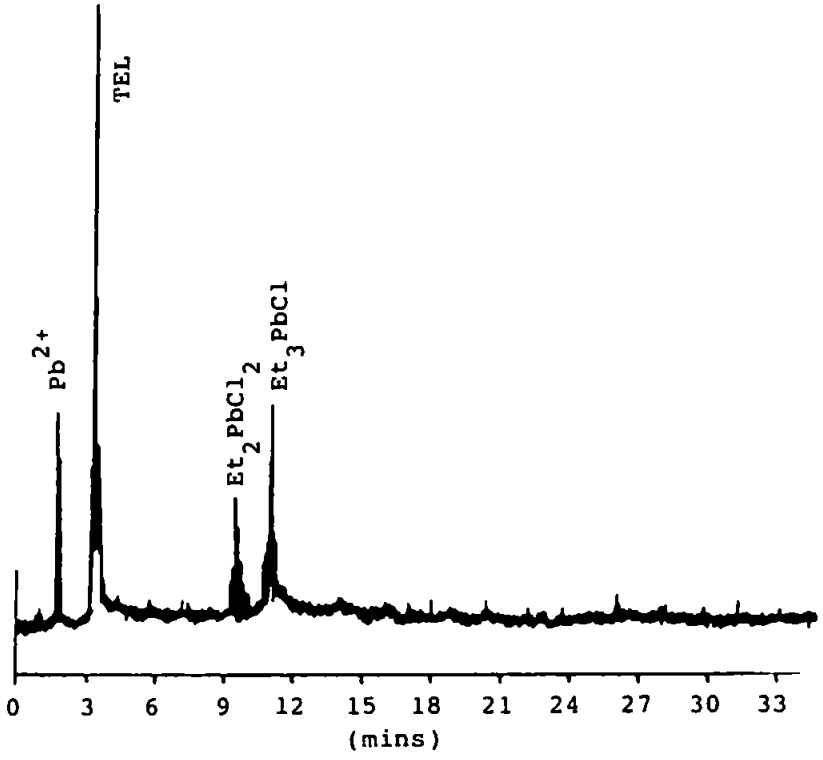


Figure 65

Deterioration of silanised columns for the determination of diethyl lead compounds.



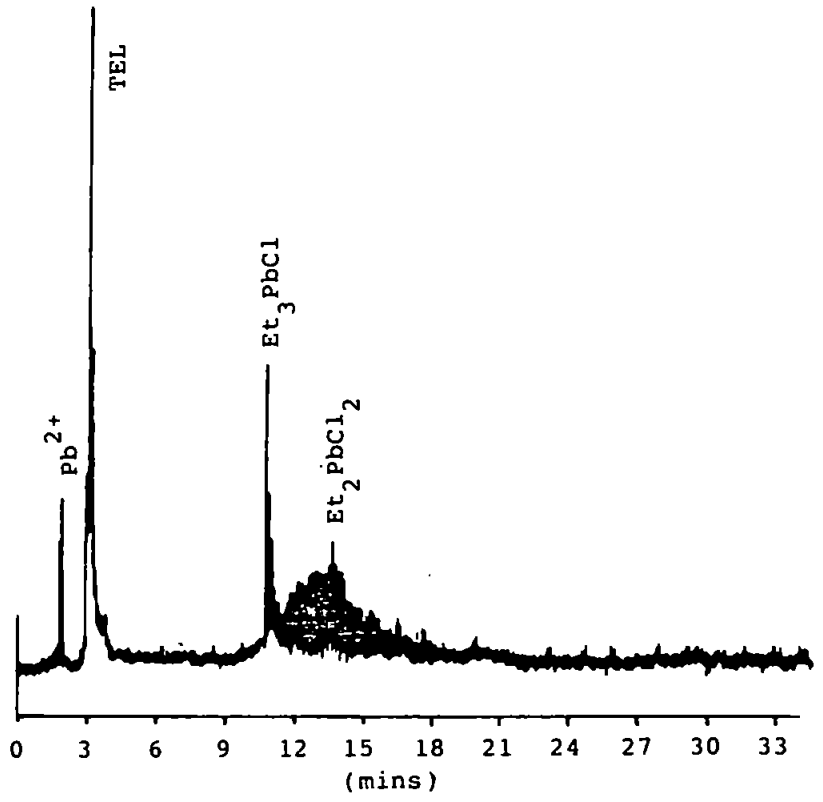


Table 25

Detection limits for lead species by directly coupled HPLC-FAAS

Pb^{2+}	0.4	ng
TEL	20	ng
Et_2PbCl_2	20	ng
Et_3PbCl	10	ng

8.4 Application to the determination of protein bound metals using Fast Protein Liquid Chromatography

Measurements of the total concentration of zinc in plasma or serum are of limited value in the assessment of zinc deficiency in clinical medicine. The zinc content of plasma is almost entirely bound to proteins with approximately 50-60% bound to albumin and 30-40% to α_2-macroglobulins. The remainder may be present as low relative molecular mass species (367). As the concentrations of these carriers are themselves subject to change, the relevance of an abnormal total plasma zinc concentration requires consideration of the zinc content of the individual zinc-binding species. Various techniques have been proposed for the fractionation of zinc-binding proteins, including anion exchange chromatography (368), affinity chromatography (367), gel filtration (368, 369), sucrose density-gradient centrifugation (370), salt fractionation (371), and electrophoresis (372). Some of these procedures may disrupt the binding of zinc to protein whereas others are time consuming thus limiting this application to clinical laboratories.

In one of the few applications of directly coupled systems in this field, Morita et al. (290) employed gel permeation HPLC interfaced with ICP. The system used a 0.9% NaCl aqueous solution as the mobile phase, at a flow rate of 1 ml min^{-1} and ambient temperature. Each chromatogram took just under an hour to run, the results indicating a range of metals associated with common proteins. However, with the recent developments in high performance ion-exchange chromatography (373-375), it is now possible to achieve higher resolution separation in a matter of minutes. The system described below utilises one of the new commercial "fast protein liquid chromatography" (FPLC) ion

exchange columns directly coupled to a flame atomic absorption spectrometer via the interface described above.

8.4.1 Apparatus

The ion-exchange chromatography was performed with a Pharmacia FPLC system (Pharmacia Fine Chemicals AB, Uppsala, Sweden), consisting of a GP 250 gradient programmer, two P-500 solvent pumps, a V-7 injection valve, a solvent mixer, a prefilter, a sample loop of 50 µl, a UV-1 UV monitor with an HR low-dead-volume flowcell, and a Rec-1 recorder. The Mono Q HR 5/5 used is a strong anion exchanger based on a beaded hydrophilic resin (10 µm). The charged group on the gel is $-CH_2-N+(CH_3)_3$.

The atomic absorption spectrometer and interface were as described in Section 8.2.1.

All solvents were HPLC grade (BDH Chemicals Ltd., Poole, Dorset). Protein standards were supplied by Sigma Chemical Company Ltd (Poole, Dorset).

The pooled human blood serum was supplied by Dr. T. Hardwell, Torbay Hospital.

8.4.2 Separation of proteins by FPLC

Conventional chromatographic techniques have been used in biomedical research for many years and yet have not been recognised as a substitute for electrophoresis due mainly to their comparatively low resolution and slow separations. FPLC however is not only faster than electrophoresis, but also preserves the biological activity of the

protein and allows easy collection of the separated material for subsequent analysis. The application of FPLC to the study of proteins in body fluids is still in its infancy, although a number of methods have already been reported for the separation of proteins in plasma (376), serum (377) and urine (378).

Two approaches have been used to resolve proteins present in human plasma/serum by FPLC: (a) fast chromatofocusing (separation according to their isoelectric points) on columns of Mono PHR 5/20 using broad pH gradients 6.0 - 3.8 and 9.0 - 60 (376) and (b) anion exchange on columns of Mono Q HR 5/5 at pH 8.6 with a linear gradient of NaCl (OH 0.5 M) (377). The optimum chromatographic conditions for protein separation using the latter technique have been investigated in detail by Tomono et al. (377). The effects of solvent composition, pH, flow-rates, sodium chloride concentration gradient and sample loading on the resolution and elution profile of protein standards and pooled human plasma were studied. The optimum conditions determined from this work were utilised in the following study.

8.4.3 Experimental

Before using the interface system for the determination the zinc in pooled human blood plasma, the technique was evaluated using standard solutions of albumin and ferritin. The first stage of this investigation involved developing an operating programme to control the injection sequence, flow-rate, solvent, gradient and data handling facilities of the chromatograph.

The conditions required for the separation of albumin and ferritin are shown in Table 26. These were incorporated into the operating

Table 26

Chromatographic conditions for the separation of albumin and ferritin

Column:	Mono Q HR 5/5
Flow rate:	0.8 ml min^{-1}
Buffer A:	Bis-Tris (0.02 M, pH 6.0)
Buffer B:	Buffer A + NaCl (0.35 M)
Gradient:	0 - 2 mins buffer A; 2 - 15 mins linear gradient to 100% B; 15 - 20 mins buffer B.
Detection:	280 nm, 1.0 AUFS
Injection:	size 100 µl

programme (Figure 66) which also includes facilities to take the elution system back to the gradient start conditions at the end of each run. Although this separation can be achieved in under eight minutes using a flow-rate of 2 ml min^{-1}, a slower elution time was tolerated here in order to comply with the flow-rate restrictions of the interface.

Once the separation of albumin and ferritin had been achieved using the standard FPLC UV detector (280 nm), the system was conncected to the atomic absorption interface. This was simply achieved by diverting the delivery tube to the fraction collector so that it deposited the sample directly onto the spirals. Thus element specific detection was facilitated in series with UV detection. The use of the two detectors in series was particularly useful in this work since use of the Mono Q column proved problematic (see Section 8.4.4), the UV chromatograms obtained allowing a check of the chromatography for each injection. Three chromatograms were obtained from the system to identify zinc, copper and cadmium associated with the two proteins. The spectrometer conditions for each element were given earlier in Section 8.2.4.

Finally a study of zinc associated with a sample of pooled human plasma was made. The optimum conditions for the separation of proteins in plasma as determined by Tamono et al. (see Section 8.4.2) were used. These conditions and those for the spectrometer are summarised in Table 27. The sample was deposited onto the spirals as above, although it was not possible to reduce the eluent flow-rate and retain resolution. To overcome this problem a second burner was incorporated to desolvate the sample. A gentle flame was used, and

Figure 66

FPLC control programme for the separation of proteins

0.0	CONE % B	0.0
0.0	ML/MIN	0.8
0.0	CM/MIN	0.33
0.0	LOOP TMS	1
0.0	VALVE.POS	1.1
0.0	ALARM	0.1
0.0	HOLD	
0.0	CONC % B	0.0
0.0	VALVE.POS	1.2
0.0	CLEAR DATA	
0.0	MONITOR	1
0.0	LEVEL %	4.0
0.0	MIN/MARK	1.0
0.0	INTEGRATE	1
2.0	CONC % B	0.0
15.0	CONC % B	100
15.0	INTEGRATE	0
18.0	CONC % B	100
20.0	CONC % B	0.0
20.0	END OF LOOP	

Table 27

Summary of conditions for the determination of zinc in pooled human plasma by directly coupled FPLC-FAAS

Spectrometer conditions:

Wavelength	213.9 nm
Air	4.0 l min^{-1}
C_2H_2	0.5 l min^{-1}
Scale Exp.	2
Background correction	ON

Chromatographic conditions:

Column:	Mono Q HR 5/5
Flow rate:	2 ml min^{-1}
Buffer A:	0.05 M Tris HCl, pH 8.6
Buffer B:	Buffer A with 0.5 M NaCl
Gradient:	linear gradient of NaCl to 0.5 M
Detection:	280 nm
Injection size:	100 µl

Interface:

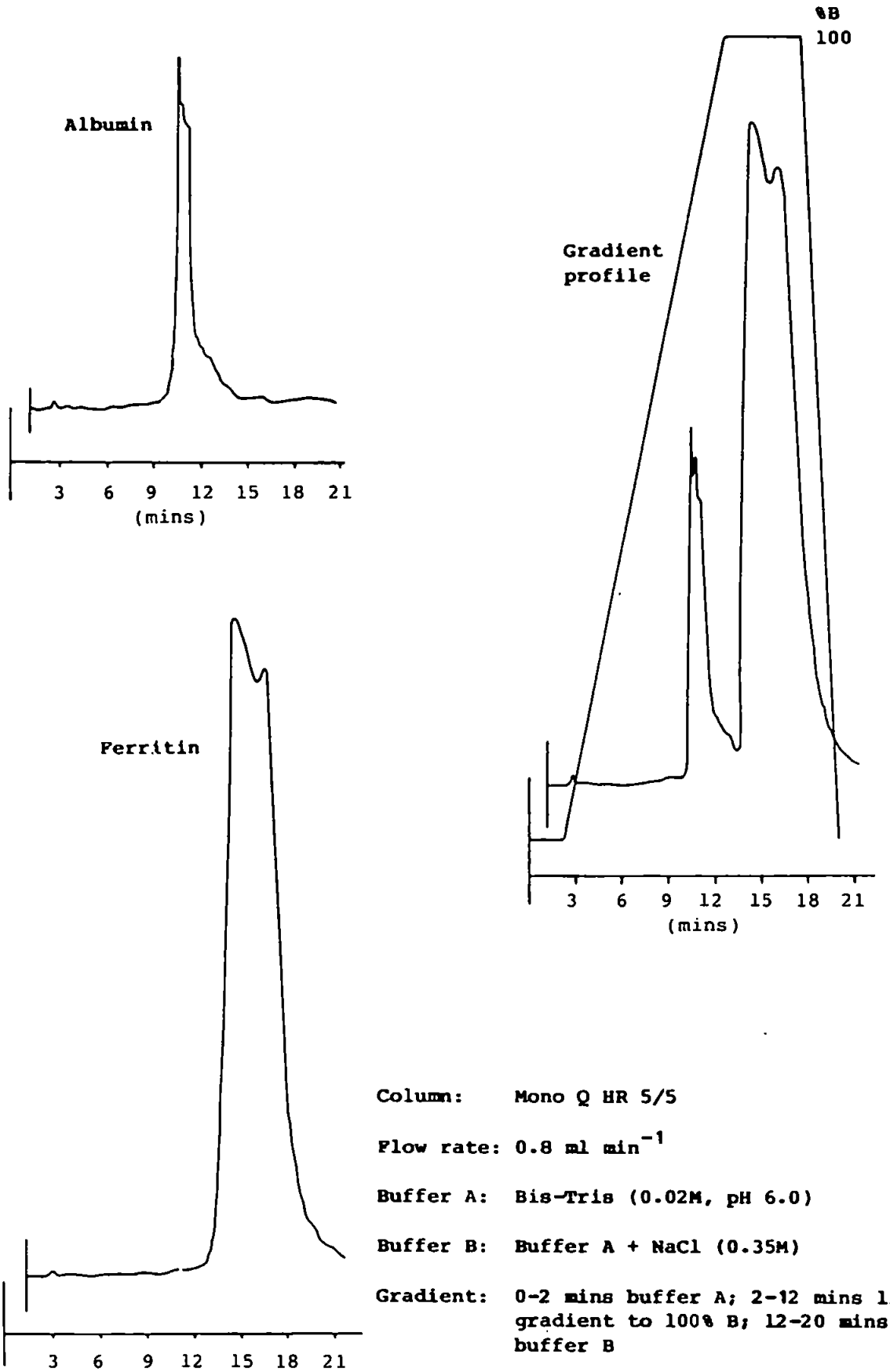
Location time	8.5 secs

Extra burner used to desoluate sample

Tube above burner removed

Figure 67

Separation of Albumin and Ferritin on Mono Q



Column: Mono Q HR 5/5

Flow rate: 0.8 ml min^{-1}

Buffer A: Bis-Tris (0.02M, pH 6.0)

Buffer B: Buffer A + NaCl (0.35M)

Gradient: 0-2 mins buffer A; 2-12 mins linear gradient to 100% B; 12-20 mins buffer B

Detection UV-1, HR Flow Cell, 280 nm, 1.0 AUFS

found to be sufficient to reduce the solvent loading on the spiral to a level at which the spiral could be rotated without losing the sample. Once again both the UV and element specific detectors were connected in series.

The use of background correction was found to be required to overcome the effects of high levels of NaCl (0.5 M) in the buffers. The use of these buffers also greatly reduced the lifetime of the quartz tubes, although since relatively high levels of zinc (1 µg/ml) were expected in the plasma, the use of the tubes was unnecessary.

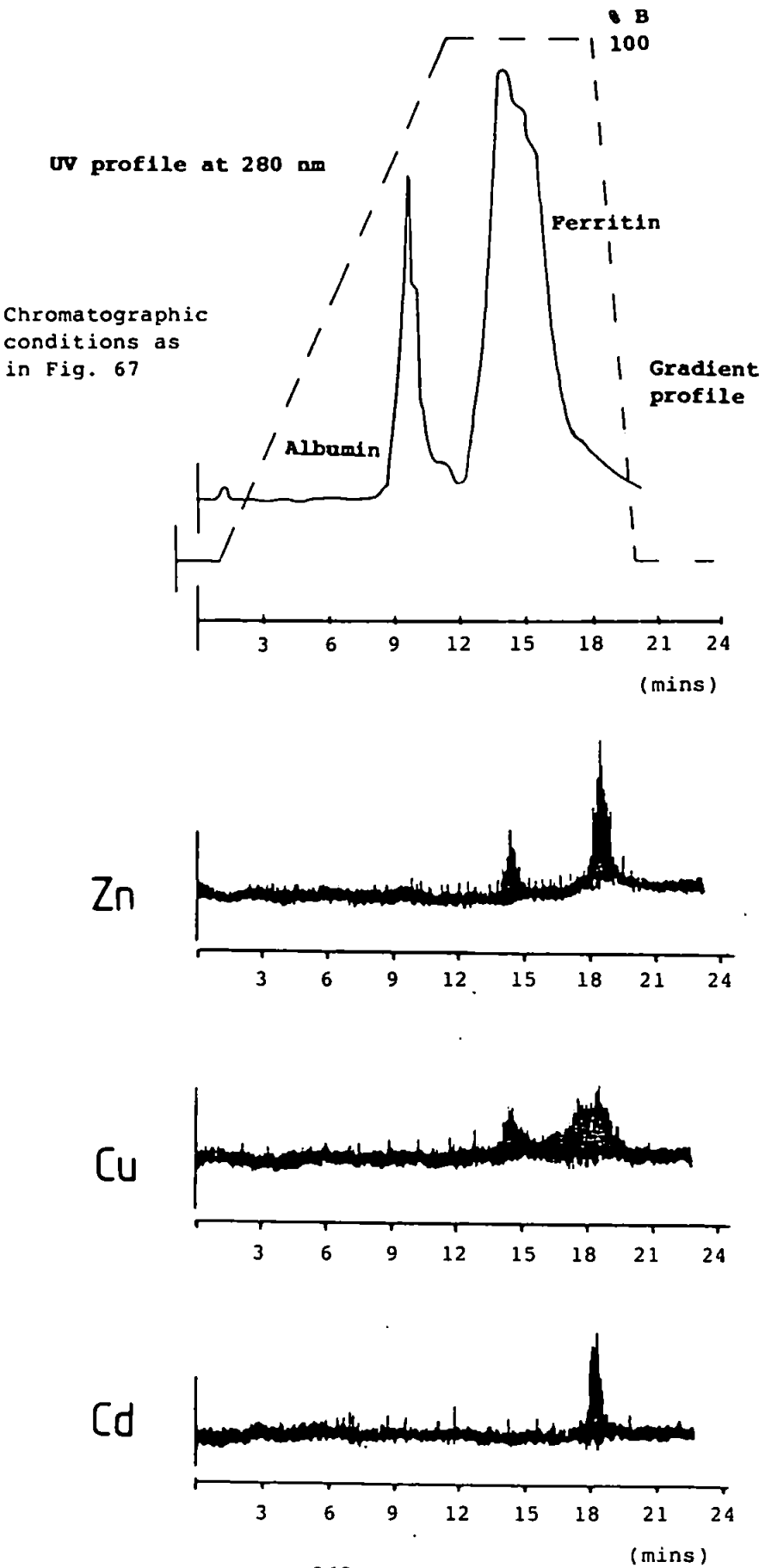
The sample of pooled human plasma, was diluted ten times with the start buffer, 100 µl being injected into the chromatograph. All samples were filtered through a 0.22 µm sterile filter before use. The usual precautions for dealing with biological samples were taken.

8.4.4 Results and Discussion

The chromatographic conditions in Table 26, separated the albumin and ferritin in under 18 minutes, Figure 67. The gradient profile throughout the run is also plotted and can be seen to return to the baseline, i.e. 0% Buffer B, after 21 minutes. The results obtained when monitoring zinc, copper and cadmium are shown in Figure 68. A time lag is observed for each peak representing the time for the sample to travel between two detectors. It can be seen from the results that zinc is associated with both the albumin and ferritin, cadmium with only the ferritin, and copper once again with both proteins. The chromatogram obtained for copper lacks the resolution obtained with zinc, the lack of sensitivity being expected from earlier work, see Section 8.2.4. Clearly however, the interface has enabled the metals

Figure 68

Chromatograms obtained from directly coupled FPLC - AAS system for Zn, Cu and Cd associated with albumin and ferritin



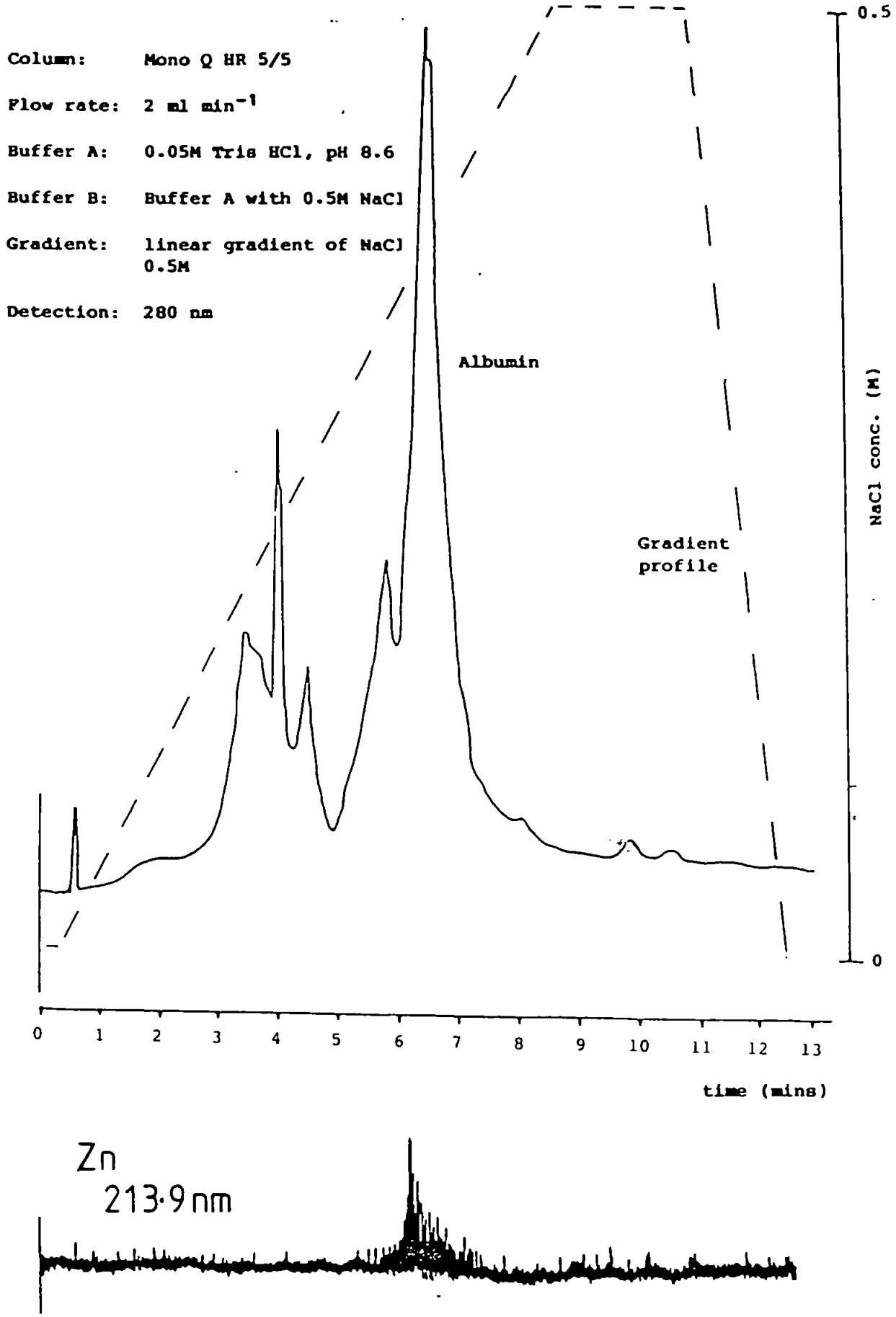
associated with each protein to be clearly identified.

The only problems encountered at this stage were connected with the Mono Q column. Even though care was taken to filter all samples before use, and re-equilibriate the column between injections, difficulty was experienced in obtaining reproducible chromatograms. An increase in backpressure on the column was noted, presumably resulting from precipitated or denatured proteins. Washing with two 1 ml injections of 75% acetic acid removed most of the problem, although the column was also washed with 80% methanol immediately after the acid wash. This is recommended by the manufacturers to remove low molecular weight contaminants e.g. fats, steroids or hydrophobic peptides which may be present. Although the above precedure successfully cleaned the column, the complete process was found to be time consuming and tedious.

The final experiment using pooled human plasma gave the chromatogram in Figure 69. Again the salt gradient is shown. The complete chromatogram was obtained in under 12 minutes. The work of Tomono et al. (376) referred to above, included the use of immunochemical methods and size exclusion HPLC for detailed component analysis. A total of 14 components were identified, the peak corresponding to albumin being shown in Figure 69. The response obtained using the interface for zinc, indicates that it is clearly associated with this albumin fraction. No further work was carried out on the sample although the study could have been extended to quantify the amount of zinc present (using peak area) and further extended to the determination of other metals. The interface, however, was proved to operate reliably, and shows much promise for future work in the area

Figure 69

Chromatogram of pooled human plasma showing associated zinc



of clinical analysis.

CHAPTER 9

FUTURE WORK AND CONCLUSIONS

9.1 Suggestions for future work

The range of applications used to demonstrate the various interfaces described in this work give some indication of the versatility of directly coupled systems for trace metal speciation. However, the examples given in the text have not been exhausted. One example of this is the speciation of organotin compounds by directly coupled HPLC-FAAS. In recent years, the shell-fish industry in certain parts of this country has become increasingly concerned about the poor quality and reduced meat yield of stocks. This has been attributed to the pollution of estuaries resulting from the leaching of organotin anti-fouling paints particularly from yachts and other small craft. There are currently moves to follow the French example and ban the use of certain antifoulants and introduce a 0.02 ppb quality limit for tributyltin through Government legislation. However, much work still needs to be done on mapping the concentration levels found in the estuaries thought to be particularly affected, and in investigating the environmental degradation of such compounds. The speciation of organotin compounds by directly coupled HPLC-FAAS as developed in this thesis, provides a sensitive yet simple, rapid and unequivable means of analysis, well suited to the large number of samples encountered in survey work and degradation studies.

The differentiation between more or less toxic species of arsenic has also been shown to be possible using directly coupled HPLC-FAAS. Two approaches are described in the text. The first of these utilising

on-line hydride generation is now well established for determining readily reducible species. However, in many studies non-reducible species have been determined by the difference between the total arsenic level (determined by graphite furnace AAS), and total reducible species (determined by hydride generation). Directly coupled HPLC-AAS however has the potential to provide a direct means for speciating arsenic, including non-reducible forms, such as arsenobetaine, which is of particular interest at the present time in food analysis. Little work has been carried out to determine the arsenic species in plants compared to marine organisms, presumably because of the analytical difficulties in dealing with such low concentrations using traditional techniques. The methods described in this work, either used directly or following further development, for example using cryogenic trapping prior to hydride generation and subsequent GC-AAS analysis, may be of great value in determining the actual forms of arsenic present in food, and thus help in assessing dietary risk.

The use of coupled HPLC-AAS for the speciation of arsenic and organoarsenic compounds in oil shale retort and process waters has already been reported (258). Such studies may however be extended to compounds of vanadyl and nickel to provide a fingerprint based on metal speciation (253). Despite numerous studies directed towards the identification of vanadyl and nickel non-porphyrin compounds (379), the effectiveness of this work has been limited by the lack of a suitable metal detector. Since metallo-non-porphyrin compounds, unlike metalloporphyrin compounds, do not have readily discernible UV-visible spectra, the identification of these organometallic compounds requires novel separation and detection techniques. Some work on this

has alreadp been done by Fish et al. (252, 253) using HPLC-ETA-AAS. However by utilising minibore HPLC separation techniques as described in this work, many of the problems associated with graphite furnace couplings could be overcome.

One further field in which the advantages of coupled HPLC-AAS can be readily identified is that of clinical analysis. It has been shown in the last chapter that by using a suitable interface, the determination of metals associated with individual proteins is possible, directly following separation by FPLC. The examples used in the text looked a copper, zinc and cadmium affiliated to various common proteins. However two other applications may be identified in clinical analysis which lend themselves to directly coupled speciation techniques.

The first of these is the determination and speciation of selenium in human urine. Since the discovery that selenium is an essential element (380), there has been an increasing interest in the element. In recent years selenium has been reported to inhibit carcinogenesis by chemical agents such as aminazo compounds, polycyclic aromatic hydrocarbons and nitrosamines (338., 322), and this has been extensively reviewed (250). Variations in dietary selenium intake have been suggested as an explanation for differences in the incidence of cancers in various human populations (200). In addition selenium may also play an important role in the prevention of cardiovascular diseases (201). The above literature indicates that there is a range of selenium intake that is consistent with health, and outside this range deficiency or toxicity effects can occur. Since selenium is the element with the narrowest difference between deficiency and toxicity, reliable methods are needed to check the selenium states of man and to

monitor occupational exposure to this element by measuring its concentrations in body fluids. Serum, plasma, or whole blood may be taken for measurement, although in cases of intoxication or to provide information on the balance between intake and output, the selenium level in the urine is an important indicator, since the kidney is an important feature in body homeostasis (202).

A second clinical application which would benefit from the use of directly coupled speciation techniques is in monitoring the use of anti-neoplastic drugs such as Cis-dichlorodiamine-platinum(II), (Cis DDP). Information concerning levels, distribution and half-life of the drugs may lead to their more effective use in the treatment of such diseases. In investigations of this nature, the technique employed should not only be sensitive and reliable, but also offer rapid analysis since this is often important in retaining the integrity of the sample (202). The directly coupled techniques described can readily meet such requirements in addition to providing the speciation data required.

The discussion above outlines a number of applications for the various interface techniques described in this work. However, one of the most versatile of these, the rotating spiral interface, may also benefit from further refinements if it is to be used in routine work. The computerised operating system described has been developed with an emphasis on controlling the movement of the spirals. One such refinement would therefore be to use the computer for data handling and display in addition to its primary function of control. This could provide information on peak identification and give simultaneous quantification in terms of both peak area and peak height. Such data

is obviously beneficial in many applications, although the potential also exists to include signal processing routines such as Kalman filtering. The desolvation system employed in the above system could also be improved by using electrically heated spirals. This could be achieved using small brushes attached to the end of each supporting arm. The advantage of this would be greater control over the desolvation process, both in terms of temperature, and duration of the heating period. An electrothermal atom cell could also be used instead of the flame. This would overcome the disadvantages associated with combustion flames such as the presence of highly reactive species within the atomic vapour, and help in studies on how mixtures of elements respond in absorption, or how non-absorbing species may affect or interfere with the elements to be measured.

One final area for future work is the development of chromatographic separations for the speciation of organometallic compounds, specifically for use with the coupled techniques described. Many of the separations achieved in this thesis are based on previously published work, although often such separations use non-ideal solvents or complicated solvent programs. The results obtained from the various interface systems developed indicate that an ideal system would use aqueous solvents at low flow rates. This has been achieved in this work using minibore columns, as described for the separation of organolead compounds in Chapter 8. However there is obviously much scope for development in this field.

9.2 Conclusions

The work presented in this thesis demonstrates the advantages of metal specific detection for trace metal speciation, including unequivocal chromatographic interpretation and the ability to withstand less than optimal chromatographic resolution. The use of flame atomic absorption is often quoted to suffer from poor detection limits compared to electrothermal atomising devices. However by careful consideration of the atom cell design, with particular attention to increasing atomic residence times, it has proved possible to devise systems which actually give better detection limits using flame atomisers. In addition the interface systems described are of relatively simple construction, robust, of low cost and readily demounted.

Each of the directly coupled techniques described in the text lends itself to particular applications. The coupling of gas chromatography with atomic spectroscopy is well suited to the separation of organometallic species with thermal stability and favourable gas-solution partition coefficients such as tetraalkyllead compounds. In this example a detection limit of 17 pg for lead as TML or TEL is obtained. In most situations however HPLC is a better separation technique, although direct couplings with flame instruments have in the past been limited to interfacing via the nebuliser. The simple interface reported here based on pulse nebulisation has proved superior to others reported in the literature since attention has also been given to the atom cell. The complete system offers a simple way of determining tributyl tin species which in the past were determined by techniques such as GC-MS.

The improvement in sensitivity with the suspended tube may be attributed to the increased residence time of the neutral atoms in the optical path of the spectrometer. This results from the longer optical path along which the atoms pass, and reduced velocity of the flame gases (not possible by lowering the flame gas-flow rates directly since these are dictated by the flame propagation velocities). The partial exclusion of entrained air may also effect the chemical environment in the tube since the portion of the flame burning in the tube probably consists largely of interconal gases and few oxidising species. This will result in a more reducing environment and consequently stabilise the neutral atoms in the optical path.

A second advantage may also be identified in using such tubes for the applications described in this thesis. Since the flame is a dynamic entity and the samples are passed through it in a quantized fashion, a series of transient signals are produced. Thus the recorder response must be faster and consistent with the amplifier time constant as in systems using electrothermal atomisation (183). The response of the amplifier/recorder system used in this work has been improved using a rapid response interface supplied with the spectrometer. However by increasing the residence time of the atoms in the optical path the demands made on the electronics are greatly reduced. Chart recorders rather than readings from a digital display have also been used since the pen measurements - including noise and drift from all sources - are recorded, and a more accurate estimate of an average position possible.

The limitations of low nebulisation efficiency encountered when

introducing liquids to a flame can be overcome by generating a volatile derivative prior to introduction to the atom cell. One common way of achieving this is hydride generation. The elements which form volatile hydrides include many of interest in speciation studies, the examples of arsenic and tin are given in the text. However, not all species of the hydride forming element form volatile hydrides. The system described in Chapter 7 utilising UV photolysis prior to hydride generation thus has considerable potential for expanding the use of directly coupled HPLC - hydride - AAS systems. The example of tributyl tin not only demonstrates this point, but also shows the increase in sensitivity obtained using hydride generation - the detection limit of 2 ng being two orders of magnitude better than that obtained introducing the sample via the nebuliser.

The most versatile interface developed in this work is probably that using the novel sample transport system. A number of applications are given in the text and further work using this system also suggested. This interface utilises the attractive features of using a flame atomiser, whilst avoiding sample introduction via the nebuliser. The technique can also be used for a wider range of elements than hydride generation. The use of minibore HPLC to achieve the separation at low eluent flow rates may also be of interest in re-evaluating coupled HPLC-ETA-AAS systems, shown in this work to be limited by the inability to handle a continuous flow of sample.

The peak dispersion characteristics of coupled LC-AAS systems have been discussed by Katz and Scott (263), who concluded that the important dispersion characteristics of the system are not those that occur in the column, but those associated with the interface and the

atomic absorption spectrometer. Clearly any such dispersion is important since it not only reduces element sensitivity but can also destroy the separation originally attained in the column. However, the work by Katz and Scott is based on using high-speed columns operated at the high flow rates necessary for efficient solvent aspiration when using direct connections to the AAS nebuliser. The work reported in this thesis shows that utilising minibore columns of much lower flow rates, and direct sample transport to the flame, provides a much better interface arrangement for many applications. In this case the dispersion in the interface and spectrometer is minimal since the collection of sample in discrete aliquots removes any possibility of peak spreading prior to atomisation in the flame.

The various systems described, although different in approach, demonstrate the potential and versatility of directly coupled chromatography - atomic spectroscopy. The use of a flame atom cell as an atomic absorption detector for liquid chromatography has the advantage of simplicity and well understood operation. The interfaces described are simple, reliable, enable real-time analysis, and produce continuous chromatograms. Although only a limited number of examples are given in the text, there are numerous applications in many fields including clinical, industrial, forensic and environmental analysis.

References

1. Thayer, J.S., J. Organometal. Chem., 1974, 76, 265.
2. Iverson, W.P., Brinckman, F.E. in 'Water pollution Microbiology Vol. 2', R. Mitchell (ed.), 1978, Wiley & Sons.
3. Saxena, J., Howard, P.J., Adv. Appli. Microbiol., 1977, 21, 165.
4. Ridley, W.P., Dizikes, L.J., Wood, J.M., Science, 1977, 197, 329.
5. Zuckerman, J.J. (ed.) 'Organotin Compounds: New Chemistry and Applications'. Adv. in Chem. Ser. No. 157 Amer. Chem. Soc. 1976.
6. Woolson, E.A., "Arsenical Pesticides", ACS Symposium, Ser. No. 7. Amer. Chem. Soc. 1975.
7. Parris, G.E., Brinckman, F.E., Environ. Sci. Technol., 1976, 10, 1128.
8. Craig, P.J. in 'Pollution: Causes, Effects and Control'. R.M. Harrison (ed.), 1983, Royal Society of Chemistry.
9. Schwarz, K., in 'Clinical Chemistry and Chemical Toxicology of Metals', S.S. Brown (ed.), 1978, Elsevier, New York.
10. Sterritt, R.M. and Lester, J.N. Sci. Total Environ., 1980, 14, 5.
11. Jenne, E.A. and Luoma, S.N. in 'Biological Implications of Metals in the Environment', R.E. Wildung and H. Drucker (eds) CONF-750929. NTIS Springfield, Virginia, 1977.
12. Florence, T.M., Talanta, 1982, 29, 345.
13. Cooley, T.N. and Martin, D.F., J. Inorg. Nucl. Chem., 1980, 42, 151.
14. Florence, T.M. and Belew, W.L., J. Electroanal. Chem., 1969, 21, 157.

15. Whitfield, M. and Turner, D.R., in 'Chemical Modelling in Aqueous Systems', E.A. Jenne (ed.), ACS Symposium Series 93, p.657. Am. Chem. Soc., Washington D.C., 1979.

16. Huang, C.P., Elliott, H.A. and Ashmead, R.M., J. Water Pollut. Control Fed., 1977, 49, 745.

17. Hall, K.H. and Lee, G.F., Water Res., 1974, 8, 239.

18. Florence, T.M., Water Res., 1977, 11, 681.

19. Buffle, J., Deladoey, P., Greter, F.L. and Haerdi, W., Anal. Chim. Acta, 1980, 116, 255.

20. Lee, F.G., in 'Heavy Metals in the Aquatic Environment', P. A. Krenkel (ed.), p.137. Pergamon Press, Oxford, 1974.

21. Salmons, W., Environ. Sci. Tech. Lett., 1980, 1, 356.

22. Batley, G.E. and Gardner, D., Estuar. Coast. Mar. Sci., 1978, 7, 59.

23. Brewer, P.G. and Hao, W.M., in 'Chemical Modelling in Aqueous Systems'. E.A. Jenne (ed.), ACS Symposium Series 93, p.261. Am. Chem. Soc., Washington D.C., 1979.

24. Florence, T.M. and Batley, G.E., Talanta, 1976, 23, 179.

25. Förstner and Wittman in 'Metal Pollution in the Aquatic Environment'. Springer-Verlag, 1981.

26. Brezonik, P.L. in 'Aqueous environmental chemistry of metals', p.167. A.J. Rubin (ed.) Ann. Arbor. Science Pub. Inc., 1974.

27. Mancy, K.H., Proc. Int. Water Pollution Research Conf., Vol. 6. New York: Pergamon Press, 1973.

28. Van den Berg, C.M.G., Anal. Proc., 1984, 21, 359.

29. Meyer, A. and Neeb, R., Fresenius' Z. Anal. Chem., 1983, 315, 118.

30. Davidson, W., J. Electroanal. Chem., 1978, 87, 395.

31. Guy, R.D. and Chakrabarti, C.L. in 'Proc. Int. Conf. Heavy Metal Environ. Vol. 1', T. Hutchinson (ed.) p.275 University of Toronto, Ontario, 1975.

32. Florence, T.M., Batley, G.E., Anal. Chem., 1980, 52, 1962.

33. Ebdon, L., Ward, R.W., Leathard, D.A., Analyst, 1982, 107, 129.

34. Ward, R.W., Ph.D. Thesis (Plymouth Polytechnic), CNAA, 1982.

35. McNair, H.M., Bonelli, E.J., 'Basic Gas Chromatography'. Varian Aerograph 1968.

36. Gittins, R. in 'Chromatography', D.R. Browning (ed.) McGraw-Hill 1969.

37. Dal Nogare, S. and Juvet, R.S., 'Gas-liquid Chromatography', Wiley-Interscience, New York, 1962.

38. Van Deemter, J.J., Zuiderweg, F.J. and Klinkenbrg, A., Chem. Eng. Sci., 1956, 5, 271.

39. Gluechauf, E., 'Ion Exchange and Its Applications', p.34. Soc. of Chem. Ind., London, 1955.

40. Klinkenberg, A., Sjenitzer, F., Chem. Eng. Sci., 1956, 5, 258.

41. McCormack, A.J., Tong, S.C., Cooke, W.D., Anal. Chem., 1965, 37 147.

42. Bache, C.A., and Lisk, D.J., Anal. Chem., 1965, 37, 1447.

43. Bache, C.A., and Lisk, D.J., Anal. Chem., 1966, 38, 783.

44. Bache, C.A., and Lisk, D.J., Anal. Chem., 1966, 38, 1756.

45. Bache, C.A., and Lisk, D.J., Anal. Chem., 1967, 39, 786.

46. Moye, H.A., Anal. Chem., 1967, 39, 1441.

47. Bache, C.A., and Lisk, D.J., J. Gas Chromatogr., 1968, 6, 301.

48. Dagnall, R.M., Pratt, S.J., West, T.S. and Deans, D.R., Talanta, 1969, 16, 797.

49. Dagnall, R.M., Pratt, S.J., West, T.S. and Deans, D.R., Talanta, 1970, 17, 1009.

50. Bache, C.A., and Lisk, D.J., J. Assoc. Off. Anal. Chem., 1967, 50, 1246.

51. Westöö, G., Acta Chem. Scand., 1967, 21, 1790.

52. Westöö, G., Acta Chem. Scand., 1968, 22, 2277.

53. Bache, C.A. and Lisk, D.J., Anal. Chem., 1971, 43, 950.

54. Dagnal, R.M., West, T.S. and Whitehead, P., Anal. Chim. Acta, 1972, 60, 25.

55. Dagnal, R.M., West, T.S. and Whitehead, P., Anal. Chem., 1972, 44, 2075.

56. Grossman, W.E.L., Eng. J. and Tong, Y.C., Anal. Chim. Acta, 1972, 60, 447.

57. Lowings, B.J., Analysis, 1972, 1, 510.

58. Dagnall, R.M., West, T.S. and Whitehead, P., Analyst, 1973, 98, 647.

59. Dagnall, R.M., Johnson, D.J. and West, T.S., Spectrosc. Lett., 1973, 6, 87.

60. Kawaguchi, H., Sakamoto, T. and Mizuike, A., Talanta, 1973, 20, 321.

61. McLean, W.R., Stanton, D.L. and Penketh, G.E., Analyst, 1973, 98, 432.

62. Serravello, F.A. and Risby, T.H., J. Chromatogr. Sci., 1974, 12, 585.

63. Talami, Y. and Andren, A.W., Anal. Chem., 1974, 46, 2122.

64. Jones, D.R., Tung, H.C. and Manahan, S.E., Anal. Chem., 1976, 48, 7.

65. Luckow, V. and Russel, H.A., J. Chromatogr., 1977, 138, 381.

66. Talami, Y., Anal. Chim. Acta, 1975, 74, 107.

67. Talami, Y. and Norvell, V.E., Anal. Chem., 1975, 47, 1510.

68. Talami, Y. and Bostick, D.D., Anal. Chem., 1975, 47, 2145.

69. Black, M.S. and Sievers, R.E., Anal. Chem., 1976, 48, 1872.

70. Houpt, P.M., Anal. Chim. Acta, 1976, 86, 129.

71. Sakomoto, T., Kawaguchi, H. and Mizuike, A., J. Chromatogr. 1976, 121, 383.

72. Talami, Y. and Norvell, V.E., Anal. Chim. Acta, 1976, 85, 203.

73. Beenakker, C.I.M., Spectrochim. Acta Part B, 1977, 32, 173.

74. Bostick, D.T. and Talamic, Y., J. Chromatogr. Sci., 1977, 15, 164.

75. Van Dalen, J.P.J., de Lezenne, P.A. and de Galan, L., Anal. Chim. Acta, 1977, 94, 1.

76. Bonnekessel, J. and Klier, M., Anal. Chim. Acta, 1978, 103, 29.

77. Brenner, K.S., J. Chromatogr., 1978, 167, 365.

78. Fricke, F.L., Robbins, W.D. and Caruso, J.A., J. Assoc. Off. Anal. Chem., 1978, 61, 1118.

79. Quimby, B.D., Uden, P.C. and Barnes, R.M., Anal. Chem., 1978, 50, 2112.

80. Reamer, D.C., Zoller, W.H. and O'Haver, T.C., Anal. Chem., 1978, 50, 1449.

81. Schwarz, F.P., Anal. Chem., 1978, 50, 1006.

82. Schwarz, F.P., Braun, W. and Wasik, S.P., Anal. Chem., 1978, 50, 1903.

83. Hobbs, J.S., Mannan, C.A. and Beeston, B.E.P., Amer. Lab., 1979, 11, 43.

84. Hobbs, J., Euro. Spectrosc. News, 1979, 26, 40.

85. Mulligan, K.J., Hahn, M.H., Caruso, J.A. and Fricke, F.L., Anal. Chem., 1979, 51, 1935.

86. Quimby, B.D., Delaney, M.F., Uden, P.C. and Barnes, R.M., Anal. Chem., 1979, 51, 875.

87. Robbins, W.B., Caruso, J.A. and Fricke, F.L., Analyst, 1979, 104, 35.

88. Robbins, W.B. and Caruso, J.A., J. Chromatogr. Sci., 1979, 17, 360.

89. Schwarz, F.P., Anal. Chem., 1979, 51, 875.

90. Estes, S.A., Uden, P.C., Rauch, M.D. and Barnes, R.M., J. High Res. Chromatogr. Comun., 1980, 3, 471.

91. Estes, S.A., Poirier, C.A., Uden, P.C. and Barnes, R.M., J. Chromatogr., 1980, 196, 265.

92. Mulligan, K.J., Caruso, J.A. and Fricke, F.L., Analyst, 1980, 105, 1060.

93. Moisen, M., Leprince, P., Beaudry, C. and Bloyet, E., U.S. Patent No. 4,049,940.

94. Hanie, T., Coulombe, S., Moisan, M. and Hubert, J., In 'Developments in Atomic Plasma Spectrochemical Analysis', Barnes, R.M. (Ed.), Heyden, 1981, pp. 337-344.

95. Quimby, B.D., Delaney, M.F., Uden, P.C. and Barnes, R.M., Anal. Chem., 1980, 52, 259.

96. Reamer, D., Ph.D. Thesis, University of Maryland, 1975.

97. Reamer, D.C. and Zollar, W.H., Science, 1980, 208, 500.

98. Wasik, S.P. and Schwartz, F.P., J. Chromatogr. Sci., 1980, 18, 660.

99. Beenakker, C.I.M., Boumans, P.W.J.M. and Rommers, P.J., Git. Fach. Lab., 1981, 25, 1979.

100. Dingjan, H.A. and de Jong, H.J., Spectrochim. Acta Part B, 1981, 36, 325.

101. Estes, S.A., Uden, P.C. and Barnes, R.M., Anal. Chem., 1981, 53, 1336.

102. Sarto, L.G. Jr., Estes, S.A., Uden, P.C., Siggia, S. and Barnes, R.M., Anal. Lett., 1981, 14, 205.

103. Tanabe, K., Haraguchi, H., Fuwa, K., Spectrochim. Acta Part B, 1981, 36, 633.

104. Uden, P.C., Anal. Proc., 1981, 18, 181.

105. Chiba, K., Yoshida, K., Tanube, K., Ozuki, M., Haraguchi, H., Winefordner, J.D. and Fuwa, K., Anal. Chem., 1982, 54, 761.

106. Estes, S.A., Uden, P.C. and Barnes, R.M., J. Chromatogr., 1982, 239, 181.

107. Eckhoff, M.A., Ridgway, T.H., Caruso, J.A.. Anal. Chem., 1983, 55, 1004.

108. Cerbus, C.S., Gluck, S.J., Spectrochim. Acta., 1983, 38B,387.

109. Delaney, M.F., Vincent Warren, F., Spectrochim. Acta, 1983, 38B, 399.

110. Mulligan, K.J., Zerezhgi, M., Caruso, J.A., Spectrochim. Acta, 1983, 38B,369.

111. Hagen, D.F., Belisle, D.F., Marhevka, J.S., Spectrochim. Acta, 1983, 38B, 377.

112. Gardiner, P.E., Brätter, P., Spetrochim. Acta, 1983, 38B, 427.

113. Riska, G.D., Estes, S.A., Beyer, J.O., Uden, P.C., Spectrochim Acta, 1983, 38B, 407.

114. Haas, D.L., Caruso, J.A., Anal. Chem., 1985, 57, 846.

115. Windsor, D.L. and Denton, M.B., Appl. Spectrosc., 1978, 32, 366.

116. Windsor, D.L. and Denton, M.B., J. Chromatogr. Sci., 1979, 17, 492.

117. Windsor, D.L. and Denton, M.B., Anal. Chem., 1979, 51, 1116.

118. Sommer, D. and Ohls, K., Fresen. Z. Anal. Chem., 1979, 295, 337.

119. Ohls, K. and Somer, D., in 'Developments in Atomic Plasma Spectrochemical Analysis', Barnes, R.M. (Ed.), Heyden, 1981, pp. 321-326.

120. Brown, R.M. and Fry, R.C., Anal. Chem., 1981, 53, 532.

121. Fry, R.C., Northway, S.J., Brown, R.M. and Hughes, S.K., Anal. Chem., 1980, 52, 1716.

122. Eckhoff, M.A., McCarthy, J.P. and Caruso, J.A., Anal. Chem., 1982, 54, 165.

123. Uden, P.C., Barnes, R.M. and DiSanzo, F.P., Anal. Chem., 1978, 50, 852.

124. Lloyd, R.J., Barnes, R.M., Uden, P.C. and Elliott, W.G., Anal. Chem., 1978, 50, 2025.

125. Coker, D.T., Anal. Chem., 1975, 47, 386.

126. Uden, P.C., Henderson, D.E., Disanzo, F.P., Lloyd, R.J. and Tetu, T., J. Chromatogr., 1980, 196, 403.

127. Treybig, D.S. and Ellebracht, S.R., Anal. Chem., 1980, 52, 1633.

128. Kolb, B., Kemmner, G., Schleser, F.H. and Wiedeking, E., Fresen, Z. Anal. Chem., 1966, 221, 116.

129. Morrow, R.W., Dean, J.A., Shults, W.D. and Guerin, M.R., J. Chromatogr. Sci., 1969, 7, 572.

130. Hey, H., Fresen. Z. Anal. Chem., 1971, 256, 361.

131. Gonzalez, J.G. and Ross, R.T., Anal. Lett., 1972, 5, 683.

132. Dressman, R.C., J. Chromatogr. Sci., 1972, 10, 472.

133. Longbottom, J.E., Anal. Chem., 1972, 44, 1111.

134. Longbottom, J.E. and Dressman, R.C., Chromatogr. Newsl., 1973, 2, 17.

135. Blair, W., Inverson, W.P. and Brinkman, F.E., Chemosphere, 1974, 4, 167.

136. Katou, T. and Nakagawa, R., Bull. Inst. of Environ. Sci. Technol., 1974, 1, 19.

137. Segar, D.A., Anal. Lett., 1974, 7, 89.

138. Chau, Y.K. ,Wong, P.T.S. and Goulden, P.D., Anal. Chem., 1975, 47, 2279.

139. Wong, P.T.S., Chau, Y.K. and Luxon, P.L., Nature, 1975, 253, 263.

140. Chau, Y.K., Wong, P.T.S. and Saitoh, H., J. Chromatogr. Sci., 1976, 14, 162.

141. Chau, Y.K., Wong, P.T.S. and Goulden, P.D., Anal. Chim. Acta, 1976, 85, 421.

142. Chau, Y.K. and Wong, P.T.S., in 'Environmental Analysis', Ewing, G.W. (Ed.), Academic Press (New York), 1977.

143. Van Loon, J.C. and Radziuk, B., Can. J. Spectrosc., 1976, 21, 46.

144. Radziuk, B. and Van Loon, J.C., Sci. Tot. Environ., 1976, 6, 251.

145. Van Loon, J.C., Radziuk, B., Kahn, N., Lichwa, J., Fernandez, F.J. and Kerber, J.D., At. Absorpt. Newsl., 1977, 16, 79.

146. Wolf, W.R., Anal. Chem., 1976, 48, 1717.

147. Chau, Y.K., Wong, P.T.S. and Goulden, P.D., in 'Lead in the Marine Environment', Branica, M. and Konrad, Z. (Eds.) Pergamon Press, 1980, pp. 72-82.

148. Andreae, M.O., Anal. Chem., 1977, 49, 820.

149. Grimm, P., Libs, S., Cressely, J. and Deluzarche, A., Ann. Fals. Exp. Chim., 1977, 70, 523.

150. Parris, G.E., Blair, W.R. and Brinckman, F.E., Anal. Chem., 1977, 49, 378.

151. Robinson, J.W., Kiesel, E.L., Goodbread, J.P., Bliss, R. and Marshall, R., Anal. Chim. Acta, 1977, 92, 321.

152. Wolf, W.R., J. Chromatogr., 1977, 134, 159.

153. Bye, R., Paus, P.E., Solberg, R. and Thomassen, Y., At. Abs. Newsl., 1979, 17, 131.

154. Bye, R. and Paus, P.E., Anal. Chem. Acta, 1979, 107, 169.

155. Chau, Y.K., Wong, P.T.S., Bengert, G.A. and Kramar, O., Anal. Chem., 1979, 51, 186.

156. Radziuk, B., Thomassen, Y., Van Loon, J.C. and Chau, Y.K., Anal. Chim. Acta, 1979, 105, 255.

157. Radziuk, B., Thomassen, Y., Butler, L.R.P., Van Loon, J.C. and Chau, Y.K., Anal. Chim. Acta, 1979, 108, 31.

158. Robinson, J.W., Kiesel, E.L. and Rhodes, I.A.L., J. Enviorn. Sci. Health, 1979, 14A, 65.

159. Chau, Y.K., Wong, P.T.S., Kramar, O., Bengert, G.A., Cruz, R.B., Kinrade, J.O., Lye, J. and Van Loon, J.C., Bull. Environ. Contam. Toxicol., 1980, 24, 265.

160. Coe, M., Cruz, R. and Van Loon, J.C., Anal. Chim. Acta, 1980, 120, 171.

161. Van Loon, J.C., Anal. Chem., 1979, 51, 1139A.

162. Hahn, M.H., Mulligan, K.J., Jackson, M.E. and Caruso, J.A., Anal. Chim. Acta, 1980, 118, 115.

163. De Jonghe, W.R.A., Chakrabarti, D. and Adams, F.C., Anal. Chim. Acta, 1980, 115, 89.

164. De Jonghe, W.R.A., Chakrabarti, D. and Adams, F.C., Anal. Chem., 1980, 52, 1974.

165. Burns, D.T., Glockling, F. and Harriott, M., Analyst, 1981, 106, 921.

166. Chakraborti, D., Jiange, S.G., Surkign, P., De Jonghe, W. and Adams, F., Anal. Proc., 1981, 18, 347.

167. Chan, L., Foren. Sci. Int., 1981, 18, 57.

168. Chau, Y.K., Wong, P.T.S., Dramar, O. and Bengert, G.A., Proc. 3rd Int. Conf. on Heavy metals in the Environ., September 1981, Amsterdam, Pub. CEP Consultants Ltd. (Edinburgh), 1981, p. 641-643.

169. Baker, M.D., Wong, P.T.S., Chau, Y.K., Mayfield, C.I., and Innis, W.E., Proc. 3rd Int. Conf. on Heavy metals in the Environ., September 1981, Amsterdam, Pub. CEP Consultants Ltd. (Edinburgh), 1981, p. 645-647.

170. De Jonghe, W.R.A., Chakrabarti, D. and Adams, F.C., Proc. Int. Conf. Heavy Metals in the Environ., Amsterdam, Sept. 1980.

171. Thompson, J.A.J., Proc. 3rd Int. Conf. on Heavy metals in the Environ., September 1981, Amsterdam, Pub. CEP Consultants Ltd. (Edinburgh), 1981, p. 653-656.

172. Brueggemeyer, T.M. and Caruso, J.A., Anal. Chem., 1982, 54, 872.

173. Chau, Y.K., Wong, P.T.S. and Bengert, G.A., Anal. Chem., 1982, 54, 246.

174. Ohta, K., Smith, B.W., Winefordner, J.D., Anal. Chem., 1982, 54, 321.

175. Chakraborti, D., De Jonghe, W.R.A. Van Mol, W.E., Van Cleavenbergen, C., Adams, F.C., Anal. Chem., 1984, 56, 2692.

176. Forsyth, D.S., Marshall, W.D., Anal. Chem., 1985, 57, 1279.

177. Hewitt, C.N., Harrison, R.M., Anal. Chim. Acta, 1985, 167, 277.

178. Gaydon, A.G. and Wolfhard, H.G., "Flames their structure, Radiation, and Temperature", Chapman and Hall (London) 3rd Ed., 1970.

179. Gaydon, A.G., 'Spectroscopy of Flames', Chapman and Hall (London 2nd Ed., 1974.

180. Alkemade, C. and Herrmann, R., 'Fundamentals of Analytical Flame Spectroscopy', Adam Hilger (Bristol), 1979.

181. Kirkbright, G.F. and Sargent, M., 'Atomic Absorption and Fluorescence Spectroscopy', Academic Press (London), 1974.

182. De Galan, L. and Samaey, G.F., Spectrochim. Acta Part B, 1970, 25, 245.

183. Price, W.J., 'Spectrochemical Analysis by Atomic Absorption', Heyden (London), 1979.

184. Ebdon, L., 'An Introduction to Atomic Absorption Spectroscopy', Heyden (London), 1982.

185. L'Vov, B.V., 'Atomic Absorption Spectrochemical Analysis', Adam Hilger, London, 1970. 186. Walsh, A., Spectrochim. Acta, 1955, 7, 108.

187. IP Standards for Petroleum and its Products Pt. 1. 'Methods for Analysis and Testing', Heyden, 1978.

188. Hodge, V.F., Seidel, S.L. and Goldberg, E.D., Anal. Chem., 1974,51, 1256.

189. Maguire, R.J., Chau, Y.K., Bengert, G.A., Hale, E.J., Wong, P.T.S. and Kramer, O., Environ. Sci. Technol., 1982, 16, 698.

190. Braman, R.S. and Tompkins, M.A., Anal. Chem., 1979, 51, 12.

191. Jackson, J.A., Blair, W.R., Brinckman, F.E. and Iverson, W.P., Environ. Sci. Technol., 1982, 16, 110.

192. Zuckerman, J.J., Reisdolf, R.P., Ellis III, F.V. and Wilkinson, R.R., in 'Organometals and Organometalloids, Occurrence and Fate in the Environment', F. E. Brinckman and J. M. Bellama (Eds), Symposium Series 82, Am. Chem. Soc. 1978.

193. Chau, Y.K., Wong, P.T.S. and Bengert, G.A., Anal. Chem., 1982, 54 246.

194. Burns, D.T., Glockling, F. and Harriott, M., Analyst, 1981, 106, 921.

195. Maguire, R.J. and Huneault, H., J. Chromatogr., 1981, 209, 458.

196. Maguire, R.J., Tkacz, R.J., J. Chromatogr., 1983, 268, 99.

197. Wolfe, W.R., J. Chromatogr., 1977, 134, 159.

198. Talmi, Y., Bostick, D.T., Anal. Chem., 1975, 47, 2145.

199. Braman, R.S., Foreback, C.C., Science, 1973, 182, 1247.

200. Schrauzer, G.N., Adv. Exp. Biol. Med., 1977, 91, 323.

201. Cawley, G.D., Bradley, R., Vet. Rec., 1978, 103, 239.

202. Robberecht, H.J., Deelstra, H.A., Talanta, 1984, 31, 497.

203. Ahmad, I., Chau, Y.K., Wong, P.T.S., Carty, A.G. and Taylor, L., Nature, 1980, 287, 716.

204. Cruz, R.B., Lorouso, C., George, S., Thomassen, Y., Kinrade, J.D., Lye, J. and Van Loon, J.C., Spectrochim. Acta Part B, 1980, 35, 775.

205. Van Loon, J.C., At. Absorpt. Newsl., 1976, 15, 72.

206. Ebdon, L., Ward, R.W. and Leathard, D.A., Anal. Proc., 1982, 19, 110.

207. Willeford, B.R., Veening, H., J. Chromatogr., 1982, 251, 61.

208. Enos, C.T., Geoffrey, G.L. and Risby, T.H., J. Chromatogr. Sci., 1976, 12, 213.

209. Lawrence, J.H. and Frei, R.W., "Chemical Derivatisation in Liquid Chromatography", Elsevier, Oxford, 1976.

210. Buchta, R.C. and Papa, L.J., J. Chromatogr. Sci., 1976, 12, 213.

211. Delumye, R. and Hartkopf, A.V., Anal. Chem., 1976, 48, 1402.

212. McLafferty, F.W., Knutti, R., Vankataraghaven, R., Arpino, P.J. and Dawkins, B.G., Anal. Chem., 1975, 47, 1503.

213. Szakasits, J.J. and Robinson, R.E., Anal. Chem., 1974, 46, 1648.

214. Chamberlain, A.T. and Marlow, J.S., J. Chromatogr. Sci., 1977, 15, 29.

215. Rokushika, S., Taniguchi, H. and Hatano, H., Anal. Letts., 1973, 6, 745.

216. Engelhardt, H., 'High Performance Liquid Chromatography', Springer-Verlag, New York, 1979.

217. Fernandez, F.J., At. Absorpt. Newsl., 1977, 16, 33.

218. Van Loon, J.C., Anal. Chem., 1979, 51, 1139A.

219. Hill, S. J. and Ebdon, L. Euro. Spectrosc. News, 1985, 58, 20.

220. Manahan, S.E. and Jones, D.R., Anal. Lett., 1973, 6, 745.

221. Jones, D.R. and Manahan, S.E., Anal. Chem., 1976, 48, 1897.

222. Jones, D.R. and Manahan, S.E., Anal. Chem., 1976, 48, 503.

223. Jones, D.R. and Manahan, S.E., Anal. Lett., 1975, 8, 569.

224. Pankow, J.R. and Janauer, G.E., Anal. Chim. Acta, 1974, 69, 97.

225. Yoza, N. and Ohashi, S., Anal. Lett., 1973, 6, 595.

226. Yoza, N., Kouchiyama, K., Miyajima, T. and Ohashi, S., Anal. Lett., 1975, 8, 641.

227. Bottre, C., Cace, F., and Cozzani, R., Anal. Lett., 1976, 9, 825.

228. Messman, J.D., and Rains, T.C., Anal. Chem., 1981, 53, 1632.

229. Cassidy, R.M., Hurteau, M.T., Mislan, J.P. and Asheley, R.W., J. Chromatogr. Sci. 1976, 14, 444.

230. Van Loon, J.C., Radziuk, B., Kahn, N., Lichwa, J., Fernandez, F.J. and Kerber, J.D., At. Absorpt. Newsl., 1977, 16, 79.

231. Kahn, N. and Van Loon, J.C., Anal. Lett., 1978, 11, 991.

232. Slavin, W. and Schmidt, G.J., J. Chromatogr. Sci., 1979, 17, 610.

233. Berndt, H. and Slavin, W., At. Absorpt. Newsl., 1978, 17, 109.

234. Renoe, B.W., Shideler, C.E., Savory, J., Clin. Chem., 1981, 27, 1546.

235. Jones, D.R., Tung, H.C. and Manahan, S.E., Anal. Chem., 1976, 48, 7.

236. Koropchak, J.A. and Coleman, G.N., Anal. Chem., 1980, 52, 1252.

237. Haswell, S.J., O'Neill, P., Bancroft, K.C.C., Talanta, 1985, 32 (1) 69.

238. Brinckman, F.E., Blair, W.R., Jewett, K.L. and Inverson, W.P., J. Chromatogr. Sci., 1977, 15, 493.

239. Parks, E.J., Brinckman, F.E. and Blair, W.R., J. Chromatogr., 1979, 185, 563.

240. Koizumi, H., McLaughlin, R.D. and Hadeishi, T., Anal. Chem., 1979, 51, 387.

241. Vickrey, T.M., Buren, M.S. and Howell, H.E., Anal. Lett., 1978, 11, 1075.

242. Vickrey, T.M. and Eue, W., J. Autom. Chem., 1979, 1, 198.

243. Vickrey, T.M., Howell, H.E. and Paradise, M.T., Anal. Chem., 1979, 51, 1880.

244. Vickrey, T.M., Howell, H.E., Harrison, G.V. and Ramelow, G.R., Anal. Chem., 1980, 52, 1743.

245. Stockton, R.A. and Irgolic, K.J., Intern. J. Environ. Anal. Chem., 1979, 6, 313.

246. Brinckman, F.E., Jewett, K.L., Inverson, W.P., Irgolic, K.J., Ehrhardt, K.C. and Stockton, R.A., J. Chromatogr., 1980, 191, 31.

247. Woolson, E.A. and Ahronson ,N., J. Assoc. Offic. Anal. Chem., 1980, 63, 523.

248. Iadevaia, R., Aharonson, N. and Woolson, E.A., J. Assoc. Offic. Anal. Chem., 1980, 6, 742.

249. Waters Technical Bulletin, 82291/J91/Jan. 1982.

250. Griffin, A.C. in 'Molecular Interrelations of nutrition and cancer'. Arnott, M.S., Van Eys, J., Wong, Y.M. (eds) p.401. Raven Press, New York, 1982.

251. Ricci, G.R., Shepard, L.S., Colovos, G. and Hester, N.E., Anal. Chem., 1981, 53, 611.

252. Fish, R.H., Komlenic, J.T., Anal. Chem., 1984, 56, 510.

253. Fish, R.H., Komlenic, J.J., Wines, B.K., Anal., Chem., 1984, 56, 2452.

254. Kouchiyama, K., Yoza, N. and Ohashi, S., J. Chromatogr., 1978, 147, 271.

255. Kahn, N. and Van Loon, J.C., J. Liq. Chromatogr., 1979, 2, 23.

256. Grabinski, A.A., Anal. Chem., 1981, 53, 966.

257. Vijan, P.N. and Wood, G.R., At. Absorpt. Newsl., 1974, 13, 33.

258. Fish, R.H., Brinckman, F.E. and Jewett, K.L., Environ. Sci. Technol., 1982, 16, 174.

259. Takamatsu, T., Aoki, H. and Yoshida, T., Soil Sci., 1982, 133, 239.

260. Yoshimura, K. and Taratani, T., J. Chromatogr., 1982.

261. Brown, L., Haswell, S.J., Rhead, M.M., O'Neill, P. and Bancroft, K.C.C., Analyst, 1983, 108, 1511.

262. Katz, E.D., Scott, R.P.W., Analyst, 1985, 110, 253.

263. Chakraborti, D., Hillman, D.C.J., Irgolic, K.J., Zingaro, R.H., J. Chromatogr., 1982, 249, 81.

264. Ebdon, L., Hill, S.J., Jones, P., Analyst, 1985, 110, 515.

265. Radzuik, B., Thomassen, Y., Van Loon, J.C. and Chau, Y.K., Anal. Chim. Acta, 1979, 105, 255.

266. Van Loon, J.C., and Radziuk, B., in 'Environmental Analysis' Ewing, G.W. (Ed.) Academic Press (New York) 1977.

267. Van Loon, J.C., Lichwa, J., and Radziuk, B., J. Chromatogr., 1977, 136, 301.

268. Siemer, D.D., Kotell, P., Haworth, D.T., Taraszewski, W.J. and Lawson, S.R., Anal. Chem., 1979, 51, 575.

269. Mackey, D.J., J. Chromatogr., 1982, 236, 81.

270. Larkins, P.L., Spectrochim. Acta, Part B, 1971, 26, 477.

271. McGuffin, V.L. and Novotny, M., Anal. Chem., 1981, 53, 946.

272. Cope, M.J. and Townshend, A., Anal. Chim. Acta, 1982, 134, 93.

273. Veillon, C. and Margoshes, M., Spectrochim. Acta Part B, 1968, 23, 553.

274. Lichte, F.E. and Skogerboe, R.K., Anal. Chem., 1973, 45, 399.

275. Skogerboe, R.K. and Coleman, G.N., Appl. Spectrosc., 1976, 30, 504.

276. Uden, P.C., In 'Developments in Atomic Plasma Spectrochemical Analysis', Barnes, R.M. (Ed.), Heydon, 1981, pp. 302-32.

277. Uden, P.C. and Bigley, I.E., Anal. Chim. Acta, 1977, 94, 29.

278. Uden, P.C., Quimby, B.D., Barnes, R.M. and Elliot, W.G., Anal. Chim. Acta, 1978, 101, 99.

279. Uden, P.C., Bigley, I.E. and Walters, F.H., Anal. Chim. Acta, 1978, 100, 555.

280. Koropchak, J.A. and Coleman, G.N., In 'Developments in Atomic Plasma Spectrochemical Analysis', Barnes, R.M. (Ed.), Heyden, 1981, pp. 345-350.

281. Mazzo, D.J., Elliott, W.G., Uden, P.C., Barnes, R.M., App. Spec. 1984, 38, (4), 585.

282. Krull, I.S., Panaro, K.W., Gershman, L.L., J. Chromatogr. Sci. 21, 1983, 460.

283. Biggs, W.R., Guno, J.T., Brown, R.J., Anal. Chem. 56, 1984, 2653.

284. Whaley, B.S., Snable, K.R. and Browner, R.F., Anal. Chem., 1982, 54, 162.

285. Meinhard, J.E., ICP Inf. Newsl., 1976, 2, 163.

286. Novak, J.W., Lillie, D.E., Boorn, A.W. and Browner, R.F., Anal. Chem., 1980, 52, 579.

287. Fraley, D.M., Yates, D. and Manahan, S.E., Anal. Chem., 1979, 51, 2225.

288. Fraley, D.M., Yates, D., Manahan, S.E., Stalling, D. and Petty, J., Appl. Spectrosc., 1981, 35, 525.

289. Gast, C.H., Kraak, J.C., Poppe, H. and Maessen, F.J.M.J., J. Chromatogr., 1979, 185, 549.

290. Morita, M., Uehiro, T. and Fuwa, K., Anal. Chem., 1980, 52, 349.

291. Kurosawa, S., Yasuda, K., Tguchi, M., Yamazaki, S., Toda, S., Morita, M., Uehiro, T. and Fuwa, K., Agric. Biol. Chem., 1980, 44, 1993.

292. Hausler, D.W. and Taylor, L.T., Anal. Chem., 1981, 53, 1223.

293. Hausler, D.W. and Taylor, L.T., Anal. Chem., 1981, 53, 1227.

294. Gardner, W.S., Landrum, P.F. and Yates, D.A., Ibidem, 1982.

295. Jinno, K., Tsuchida, H., Nakanishi, S., Hirata, Y. and Fujimoto, C. App. Spec. 37 (3), 1983, 258.

296. Jinno, K. and Tsuchida, H., Anal. Lett. 15 (45), 427, 1982.

297. Jinno, K., Nakanishi, S., Nagoshi, T., Anal. Chem., 1984, 56 (11) 1977.

298. Lawrence, K.E., Rice, G.W. and Fassel, V.A., Anal. Chem., 1984, 56, 292.

299. Yoshida, K., Hasegawa, T., Haraguchi, H., Anal. Chem., 1983, 55, 2106. 300. Irgolic, K.J., Stockton, R.A., Chakraborti, D. and Beyer, W., Spectrochim. Acta, 38B, 1983, 437.

300. Irgolic, K.J., Stockton, R.A., Chakraborti, D. and Beyer, W., Spectrochimica Acta, 1983, 38B, 437.

301. Yoshida, K. and Haraguchi, H., Anal. Chem., 1984, 56, 2580.

302. Fernandez, F.J., Chromatogr. Newsl., 1977, 5, 17.

303. Haswell, S.J., Ph.D. Thesis (Plymouth Polytechnic), CNAA, 1983.

304. Tye, C.T., Haswell, S.J., O'Neill, P., Bancroft, K.C.C., Anal. Chim. Acta 1985, 169, 195.

305. Sebastinai, E., Ohls, K. and Riemer, G., Z. Anal. Chem., 1973, 105.

306. Watling, R.J., Anal. Chim. Acta, 1978, 91, 395.

307. Horvåth, C., Melander, W. and Nahum, A., J. Chromatogr., 1979, 186, 371.

308. Jewett, K.L. and Brinckman, F.E., J. Chromatogr. Sci., 1981, 19, 583.

309. Meinema, H.A., Burger-Wiersma, T., Versluis-de Haan, G. and Gevers, E.C., Environ. Sci. Technol., 1978, 12, 288.

310. Suderquist, C.J. and Crosby, D.G., J. Agric. Food Chem., 1980, 28, 111.

311. Algieu, C., Thiband, Y., Héral., M. and Boutier, F. La Revue des Travaux de ISTM, 1980, 4 Fas. 4, 301.

312. Evans, J.C. and Smith, P.J., J. Oil. Col. Chem. Assoc., 1975, 58, 160.

313. Thain, J.E. and Waldock, M.J., 'Estuarine and Coastal Pollution International Conference', Plymouth, 1985.

314. Godden, R.G. and Thomerson, D.R., Analyst., 1980, 105,1137.

315. Robbins, W.B. and Caruso, J.A., Anal. Chem., 1979, 51, 889a.

316. Schmidt, F.J. and Royer, J.L., Anal. Lett., 1973, 6, 17.

317. Maruta, T. and Sudoh, G., Anal. Chim. Acta, 1973, 77, 37.

318. Evans, W.H., Jackson, F.J. and Dellar, D., Analyst, 1979, 104, 16.

319. Pollock, E.N. and West, S.J., At. Absorpt. Newsl., 1973, 12, 6.

320. Korenaga, T., Analyst, 1981, 106, 40.

321. Braman, R.S., Justen, L.L. and Foreback, C.C., Anal. Chem., 1972, 44, 2195.

322. Thompson, K.C. and Thomerson, D.R., Analyst, 1974, 99, 595.

323. Fleming, D.E. and Taylor, G.A., Analyst, 1978, 103, 101.

324. Pierce, F.D. and Brown, H.R., Anal. Chem., 1977, 49, 1417.

325. Thompson, K.C., Analyst, 1975, 100, 307.

326. Wauchope, R.D., At. Absorpt. Newsl., 1976, 15, 64.

327. Kahn, H.L. and Schallis, J.E., At. Absorpt. Newsl., 1968, 7, 5.

328. Guimont, J., Pichette, M. and Rhéaume, N., At. Absorpt. Newsl., 1977, 16, 53.

329. Mantoura, R.F.C. and Woodward, E.M.S., Geochim. et Cosmochim. Acta, 1983, 47, 1293.

330. Thornburn Burns, D., Glockling, F. and Harriott, M., J. Chromatogr., 1980, 200, 305.

331. Ricci, G.R., Shepart, L.S., Colovor, G. and Hester, N.E., Anal. Chem., 1981, 53, 610.

332. Ebdon, L., Wilkinson, J.R. and Jackson, K.W., Anal. Chim. Acta, 1982, 136, 191.

333. Thompson, M., Pahlavanapour, B., Walton S.J. and Kirkbright, G.F., Analyst, 1978, 103, 705.

334. Harbach, P.R. and Swenberg, J.A., Carcinogenesis, 1982, 2, 575.

335. Zuckerman J.J. (ed) 'Organotin Compounds: New Chemistry and Applications'. Adv. in Chem. Ser No. 157 Amer. Chem. Soc. 1976.

336. Neumann, W.P., 'The Chemistry of Tin', Wiley - Interscience, New York, 1970.

337. Poller, R.C., 'The Chemistry of Organotin Compounds', Academic Press, New York, 1970.

338. Griffin, A.C., Adv. Cancer Res., 1979, 29, 419.

339. Davis, A.G., in 'Organic Compounds': New Chemistry and Applications, ed. by J. J. Zuckerman, Advances in Chem. Series, No. 157 p 26. Am. Chem.Soc. 1976.

340. Matteson, D.S., 'Organometallic Reaction Mechanisms of the Nontransition Elements', Academic Press, New York, 1974.

341. Gielen, M., Dehouck, C., Mokhtan-Jamai, H. and Topart, J., Rev. Silicon, Germanium, Tin, Lead Compd., 1974, 1, 9.

342. Stolywo, A., Privett, O.S., Erdahl, J. Chromatogr. Sci., 1973, 11, 263.

343. Slais, K., Krejei, M., J. Chromatogr., 1974, 91, 181.

344. James, A.T., Ravenhill, J.R., Scott, R.P.W., Chem. Ind. (Lond.), 1964, 18, 746.

345. Young, T.E. and Maggs, R.T., Anal. Chim. Acta, 1967, 38, 105.

346. Hahti, E., Nikkari, T., Karkainen, 'Gas Chromatography, 1964', A. Goldup, Ed., Institute of Petroleum, London, 1965, p.190,

347. Coll, H., Johnson, H.W., Polgar, A.G., Seibert, E.E. and Stross, K.H., J. Chromatogr. Sci., 1979, 7, 30.

348. Dubsky, H., J. Chromatogr., 1972, 71, 395.

349. Lapidus, B.M., Karmen, A., J. Chromatogr., 1972, 10, 103.

350. Scott, R.P.W., Lawrence, J.G., J. Chromatogr. Sci., 1970, 8, 65.

351. Karmen, A., Anal. Chem., 1966, 38, 286.

352. James, A.T., Ravenhill, J.R. and Scott, R.P.W., 'Gas Chroamtography, 1964', A. Goldup, Ed., Institute of Petroleum, London, 1965, p.205.

353. Van Dijk, J.H., J. Chromatogr., 1972, 10, 31.

354. Pretorius, V., Van Rensburg, J.F.J., J. Chromatogr. Sci., 1973, 11, 355.

355. Hardin, E.D., Fan, T.P., Blakley, C.R. and Vestal, M.L., Anal. Chem. 1984, 56, 2.

356. Dobberstein, P., Korte, E., Meyerhoff, G. and Pesch, R., Int. J. Mass. Spectrom. Ion. Phys., 1983, 46, 201.

357. Smith, R.D., Burger, J.E. and Johnson, A.L., Anal. Chem., 1981, 53, 1603.

358. Berndt, H., Messerschmidt, J., Spectrochim. Acta, 1979, 34B, 241.

359. Gücer, S. and Berndt, H., Talanta, 1981, 28, 334.

360. Delves, H.T., Analyst, 1970, 95, 431.

361. Kahn, H.L., Peterson, G.E., Schallis, J.E., At. Abs. Nsl., 1968, 7, 35.

362. Kahl, M., Mitchell, D.G., Kaufman, G.I., Aldous, K.M., Anal. Chim. Acta., 1976, 87, 215.

363. Bahreyni-Toosi, M.H., Dawson, J.B., Duffield, R.J., Analyst, 1984, 109, 943.

364. Bernt, H., Messerschmidt, J., Anal. Chim. Acta., 1982, 136, 407.

365. Scott, R.P.W., J. Chromatogr. Sci., 1980, 18, 49.

366. Oliver, R.W.A., Anal. Proc. 1984, 21, 424.

367. Foote, J.W., Delves, T., Analyst, 1982, 107, 121.

368. Dawson, J.B., Bahreyni-Toosi, M.H., Ellis, D.J. and Hodgkinson, A., Analyst, 1981, 106, 153.

369. Gardner, P.E., Ottaway, J.M., Fell, G.S. and Burns, R.R., Anal. Chim. Acta, 1981, 124, 28.

370. Song, M.K. and Adham, N.F., Clin. Chim. Acta, 1979, 99, 13.

371. Prasad, A.S. and Oberleus, D., J. Lab. Clin. Med., 1970, 76, 416.

372. Dennes, E., Tupper, R. and Wormall, A., Biochim. J., 1962, 82, 466.

373. Alpert, A.J. and Regnier, F.E., J. Chromatogr., 1978, 185, 375.

374. Chang, S.H., Noel, R. and Regnier, F.E., Anal. Chem., 1976, 48, 1839.

375. Kato, Y., Komiya, K. and Hashimoto, T., J. Chromatogr., 1982, 246, 13.

376. Fägerstam, L., Lizuna, J., Fredriksson, U., J. Chromatogr., 1983, 266, 523.

377. Tamono, T., Ikeda, H., Tokunage, E., J. Chromatogr., 1983, 266, 39.

378. Lindblom, H., Söderberg, L., Cooper, E.H., J. Chromatogr., 1983, 266, 107.

379. Spencer, W.A., Galobardes, J.F., Rogers, L.B., Sep. Sci. Technol., 1983, 17, 797.

380. Schwarz, K. and Foltz, C.M., J. Am. Chem. Soc., 1957, 79, 3292.

Lectures and associated studies

i. SERC Short Course at Imperial College, London, June 1983, 'Microcomputers in Chemical Instrumentation'.

ii. Update course on 'ICP Emission Spectrometry' at SAC 83 International Conference on Analytical Chemistry, Edinburgh University, July 1983.

iii. Series of lectures on 'Advanced Analytical Atomic Spectrosocpy', Plymouth Polytechnic, June - August 1984.

iv. Weekly meetings of the Chemistry of Natural and Polluted Environments Research Group, Plymouth Polytechnic, September 1983 - September 1986.

v. RSC Lecture, 8th October 1982, Plymouth Polytechnic, Prof. F. McCapra, 'The Chemistry of Bioluminescence'.

vi. RSC AGM and Lecture, 22nd October 1982, Chepstow, Prof. D. Betteridge, 'Flow Injection Analysis'.

vii. RSC Lecture, 11th February 1983, Plymouth Polytechnic, Dr. T. Delves, 'Clinical Aspects of Trace Element Analysis'.

viii. RSC Lecture, 10th March 1983, Bath University, Prof. E. Bishop, 'Fire in Flight'.

ix. Departmental Colloquium, 15th March 1983, Plymouth Polytechnic, Dr. P. craig, 'Methylation of Heavy Metals in the Environment'.

x. IMER Seminar, 7th September 1983, IMER Plymouth, Dr. M. Andreae, 'Organometalloid Compounds in Natural Waters'.

xi. Harwell Seminar, 20th October 1983, Dr. L. Ebdon, 'Trace Metal Speciation by Coupled Chromatography - Atomic Spectroscopy for Toxicological and Environmental Monitoring'.

xii. RSC Meeting, 28th October, 1983, Plymouth Polytechnic, Prof. J.P. Riley, 'Analytical Chemistry Applied to Marine Pollution Problems'.

xiii. IMER Seminar, 24th November 1983, IMER Plymouth, Dr. R. Loughlin, 'Physiochemical Factors Governing the Toxicity of Organotins'.

xiv. RSC AGM and Lecture, 13th January 1984, Bristol University, Mr. J.G. Jones, 'Death of the Analyst'.

xv. RSC Lecture, 17th February, 1984, Plymouth Polytechnic, Prof. J.N. Miller, 'Hunting the Snark - Recent developments in ultra sensitive molecular spectroscopy'.

xvi. RSC Lecture, 16th November 1984, Plymouth Polytechnic, Dr. D. Woodcock, 'Fungicides and the Environment'.

xvii. RSC AGM and Lecture, 14th December, 1984, Chepstow, Prof. S. Greenfield, 'The future, if any, for ASIA'.

xviii. Departmental Colloquium, 8th February, 1985, Plymouth Polytechnic, Dr. C. Corfield, 'Gas Chromatography - Mass Spectrometry, Useful Applications of a Technique Come of Age'.

xix. RSC Lecture, 15th March 1985, Plymouth Polytechnic, Dr. P.S. Liss, 'The Role of the Oceans in the Chemistry of the Atmosphere'.

xx. MBA/IMER Joint Seminar, 22nd July 1985, MBA Plymouth, Dr. F. Millero, 'The kinetics of inorganic redox reactions in natural waters'.

xxi. Departmental Colloquium, 10th September 1985, Plymouth Polytechnic, Dr. J. Harnly, 'Recent developments in Analytical Atomic Spectroscopy'.

xxii. NERC conference 16th - 19th July 1985, Plymouth Polytechnic, 'Estuarine and coastal pollution - detection, research and control'.

Meetings of the Royal Society of Chemistry

i. Analytical Division Current Awareness Symposium on 'Electrothermal Atomisation in Analytical Atomic Spectroscopy', 4th November 1982, Bristol University.

ii. Joint meeting with The Institute of Physics - Spectroscopy Group, 'Other Plasmas', 17th February 1983, Polytechnic of North London.

iii. Analytical Division meeting, 28th and 29th March 1983, 'Research and Development Topics in Analytical Chemistry', University of Technology Loughborough.

iv. Analytical Division Symposium, 14th April 1983, The Tenth Annual Reports on Analytical Atomic Spectroscopy Symposium 'Refractory Elements and Refractory Matrices', University of Sheffield.

v. Analytical Division meeting, 24th May 1983, 'Atomic Spectroscopy', Scientific Societies Lecture Theatre London.

vi. Analytical Division, 17th - 23rd July 1983, 'SAC 83 International Conference and Exhibition on Analytical Chemistry', Edinburgh University.

vii. Analytical Division Symposium, 12th April 1984, Annual Reports on Analytical Atomic Spectroscopy 'Automation - is it cost effective?', Sheffield City Polytechnic.

viii. Analytical Division, 2nd and 3rd May 1984, 'Novel Instrument Design in Atomic Spectroscopy', University of Strathclyde.

ix. Analytical Division, 26th and 27th June 1984, 'Research and Development Topics in Analytical Chemistry', UMIST, Manchester.

x. Analytical Division, 10th - 13th July 1984, 'Second Biennial National Atomic Spectroscopy Symposium', University of Leeds.

xi. Analytical Division Symposium, 19th and 20th March 1985, 'Computer aided methods in chromatography', University of Edinburgh.

xii. Analytical Division Symposium, 2nd April 1985, 'Annual Reports on Analytical Atomic Spectroscopy', Sheffield City Polytechnic.

xiii. Analytical Division Symposium, 18th and 19th April 1985, 'The Bishop Symposium', University of Exeter.

xiv. Analytical Division, 26th - 28th June 1985, 'Research and Development Topics in Analytical Chemistry', Belfast. 1985.

Presentations and Publications

Resulting from the work reported in this thesis the following papers have been presented and published.

(a) **Presentations**

1. 'A coupled gas chromatography - atomic absorption spectrometry interface'

   Poster presentation presented at the Novel Instrument Design in Atomic Spectroscopy Meeting, University of Strathclyde, May 1984.

2. 'Trace metal speciation using coupled high performance liquid chromatography - flame atomic absorption spectrometry'

   Poster presentation at the Second Biennial National Atomic Spectroscopy Symposium, University of Leeds, July 1984.

3. 'Novel approaches to directly coupled high performance liquid chromatography - flame atomic absorption spectroscopy for trace metal speciation'

   Paper presented at the RSC Research and Development Topics in Analytical Chemistry Meeting, University of Belfast, July 1985.

(b) **Publications**

1. Ebdon, L., Hill, S., Jones, P.

   'The speciation of tin in natural waters using coupled high performance liquid chromatography - flame atomic absorption spectrometry'

   Analyst, 1985, 110, 515.

2. Ebdon, L., Hill, S., Jones, P.

'Novel approaches to directly coupled high performance liquid chromatography - flame atomic absorption spectroscopy for trace metal speciation'

Anal. Proc. - in press.

Speciation of Tin in Natural Waters Using Coupled High-performance Liquid Chromatography - Flame Atomic-absorption Spectrometry

Les Ebdon, Steve J. Hill and Philip Jones
Department of Environmental Sciences, Plymouth Polytechnic, Drake Circus, Plymouth, Devon PL4 8AA, UK

A simple yet effective high-performance liquid chromatography - flame atomic-absorption spectrometry coupling utilising pulse nebulisation and a slotted tube atom trap is described for the speciation of tin in natural waters. The effects of the various parameters on analytical performance are discussed. A detection limit of 200 ng for tin was obtained and the separation of Sn(II), Sn(IV) and tributyltin was possible within 8 min. Tributyltin compounds have been quantified in local harbour waters, and in one harbour situation were observed at levels up to 0.47 μg l^{-1}.

Keywords: *High-performance liquid chromatography; flame atomic-absorption spectrometry; trace metal speciation; organotin determination*

There has been a growing realisation that the form in which a trace metal occurs, so called "speciation," is of vital importance in a number of fields, *e.g.*, toxicology and environmental monitoring. This has led to an increased interest in methods for trace metal speciation. A promising analytical approach to such speciation is to couple the capability of chromatography, for species separation, to the selectivity and sensitivity of atomic spectrometry for detection. The use of such specific detection allows less than optimum chromatographic separation to be tolerated with a consequent saving in time for sample clean-up and analysis. If two species co-elute and only one contains the metal of interest, the use of metal-specific detection means that only the metal-containing species is detected. Hence complete chromatographic separation is not required, only separation of the species containing the metal of interest being necessary, and complex samples can be analysed.

Although the success of coupled gas chromatography - flame atomic-absorption spectrometry (FAAS) has already been reported,[1] gas-chromatographic separation is only effective where thermal stability and favourable gas - solution partition coefficients exist, and often this is not the case. Therefore, for most interesting trace metal speciation studies, high-performance liquid chromatography (HPLC) is the separative technique of choice. Further impetus is also given to metal-specific HPLC detection systems where conventional UV or fluorescence detectors are not suitable, owing to the absence of an active chromophore or fluorophore, respectively—a common property of all non-aromatic derivatives of organotin.[2]

The demand for analytical techniques capable of speciating organotin compounds has increased markedly in recent years. The presence of butyltin[3] and methyltin[4] species with concentrations from nanograms to micrograms per litre has been reported in a variety of natural waters, and is probably due to the increased use of organotin compounds as stabilisers for poly(vinyl chloride), as catalysts and as pesticides.[5] The use of organotin compounds in the formulation of antifouling paints [*e.g.*, bis(tributyltin) oxide and tributyltin fluoride[6]], although economically attractive, has received particular attention with relation to effects on shellfish and a series of bans on organotin use have been instigated by the French Government. In addition, more is now known about the marked variation in toxicity of different species, thus stressing the importance of such studies.

Slavin and Schmidt[7] described a simple, directly coupled HPLC - FAAS system in which the column eluent is introduced directly into an injection cup. Detection limits have been improved in this work by the additional use of a double slotted quartz tube as an atom trap. The use of such tubes was first described by Watling,[8] although the tubes used here have been modified in design so that the slots are at 180° to each other.[9] This modification has been found to give improved detection limits for some elements, although because the slots are superimposed and directly in-line with the burner slot the atomic residence time in the tube is probably reduced, resulting in a decrease in sensitivity. The improvement in detection limits can probably be attributed to the decreased turbulence of the hot gases in the 180° slot configuration.

In this work tin ions, including organotin ions, have been separated by HPLC using a silica base cation-exchange column and then introduced directly into an injection cup for aspiration into an air - hydrogen flame, beneath and surrounding the silica tube atom trap. Conventional instrumentation operating on-line in real-time has been used throughout. The method developed has been applied to a variety of local harbour waters.

Experimental

Apparatus

An atomic-absorption spectrometer (Model SP9; Pye Unicam, Cambridge, UK), provided with a deuterium hollow-cathode lamp background corrector, was fitted with a 5-cm slot-burner with a slotted-tube atom trap (STAT) accessory. The separation of the tin species was achieved using a solvent-delivery system (PU 4010; Pye Unicam) equipped with a Waters U6K injector with either a 200-μl or 2-ml sample loop, and connected to a Partisil 10 SCX analytical column (10-μm particle size, 25 cm × 4.6 mm i.d.) (Whatman, Englewood Cliffs, NJ, USA). Samples were injected directly on to the column using a 100-μl syringe (Scientific Glass Engineering, Melbourne, Australia). The output from the atomic-absorption spectrometer was displayed on a chart recorder (AR25; Pye Unicam). The hollow-cathode lamp (Pye Unicam) current used was 6.0 mA and the 224.6-nm tin line was monitored with a slit width of 0.5 nm. The optimum operating parameters for the spectrometer and high-performance liquid chromatograph are shown in Tables 1 and 2, respectively.

Reagents

Standards were prepared from stock solutions of both tributyltin chloride and tributyltin fluoride (Aldrich, Gilling-

Table 1. Spectrometer conditions

Spectrometer	Pye SP9 with 5-cm burner and STAT accessory
Air flow-rate	4.0 l min^{-1}
Hydrogen flow-rate	2.6 l min^{-1}
Burner height	16 mm (fixed)
Lamp current	6.0 mA
Wavelength	224.6 nm
Slit width	0.5 nm

Table 2. High-performance liquid chromatograph conditions

Column	Whatman Partisil 10 SCX (10-μm particle size, 25 cm × 4.6 mm i.d.)
Mobile phase	Methanol - water (70 + 30)
Buffer	0.1 M ammonium acetate solution
Flow-rate	3 ml min^{-1}
Injection volume	Up to 2 ml facilitated

ham, Kent, UK) by solution with HPLC-grade methanol. All other chemicals used were of analytical-reagent grade (BDH Chemicals, Poole, Dorset, UK).

Construction of Interface

Sample introduction was facilitated using discrete volume nebulisation, *i.e.*, allowing the nebuliser to draw air between aliquots of sample to balance the flow from the analytical column with that of the natural uptake to the nebuliser. To achieve this, the sample uptake capillary from the nebuliser was connected to a wider bore tube into the side of which was fitted a second tube left open to allow air to enter. The connection between the two pieces of tubing was made with care to avoid forming a liquid trap that would cause cross-contamination. The connecting tube was then attached to the analytical column and the arrangement mounted in front of the spectrometer (Fig. 1). The instrument was set up in the normal way for flame analysis except that the spray chamber and burner were allowed to run dry when no sample was being passed. The damping of the spectrometer was adjusted to 0.5 s, thus removing the pulsed response otherwise observed.

Results and Discussion

The use of discrete volume nebulisation overcomes the problems of incompatible uptake rates, *e.g.*, natural uptake for the spectrometer of 5 ml min^{-1} and HPLC delivery of 1–3 ml min^{-1}, and of small sample uptake, *e.g.*, less than 0.5–1.0 ml, which may not give conventional systems time to attain equilibrium and produce a steady reading. In most such situations,[7] analysis would be performed by transferring 50- or 100-μl aliquots of the sample into some form of small conical funnel mounted vertically in front of the spectrometer. The sensitivity obtained using this method was found to be only marginally lower than that for continuous aspiration (Fig. 2). In this system, however, the funnel was replaced with a vented tube and the sample supplied directly from the HPLC column, as this offered equivalent sensitivity without the practical problems caused by "gulping" of the sample.

Although discrete volume nebulisation allows a simple yet effective way of getting the sample into the nebuliser, the detection limit for tin using conventional FAAS with an air - hydrogen flame was only 0.06 mg l^{-1}. The use of the slotted tube atom trap lowered the detection limit by a factor of four, *i.e.*, to 0.015 mg l^{-1}. The tube was supported above the flame from a conventional burner with one of the slots aligned directly above the flame and adjusted so that the burner

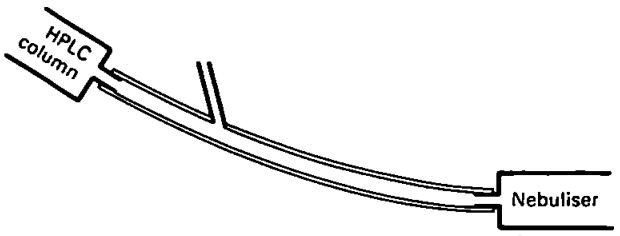


Fig. 1. Schematic diagram of HPLC - nebuliser interface

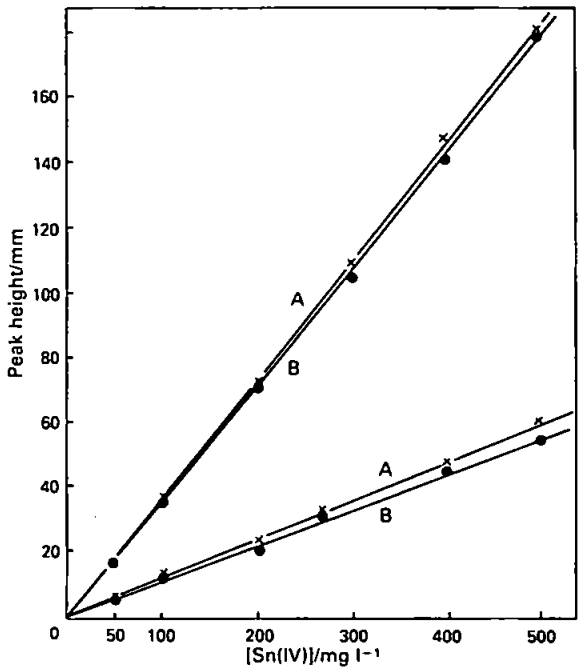


Fig. 2. Comparison of discrete volume nebulisation with direct uptake. A, Direct uptake to nebuliser; B, discrete volume nebulisation. Upper lines: acetylene at 2.2 l min^{-1}, air at 5.0 l min^{-1}; lower lines, acetylene 2.0 l min^{-1}, air 5.0 l min^{-1}

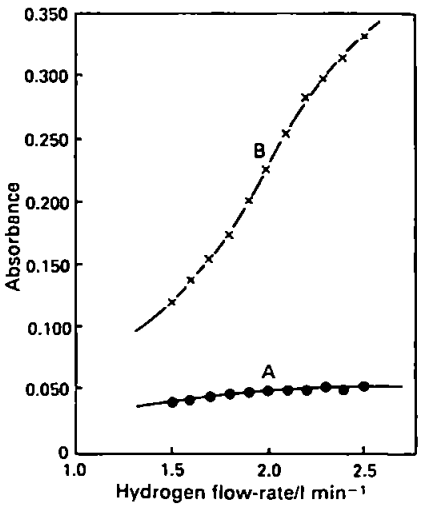


Fig. 3. Effect of changing the hydrogen flow-rate when using the slotted-tube atom trap and an air - hydrogen flame for (A) tin(IV) solutions and (B) tributyltin chloride in 20% methanol

height, rotation and lateral alignment ensured that maximum radiation passed through the tube. The improvement in sensitivity obtained when using the STAT is generally confined to elements that are readily atomised in the flame, and is thus well suited to the determination of tin.

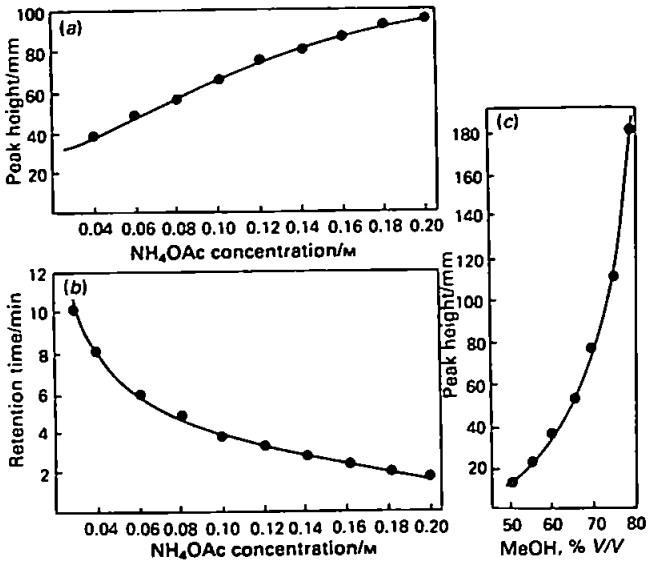


Fig. 4. Optimisation of chromatographic conditions. (*a*) Effect of ammonium acetate concentration on response for tributyltin ions; (*b*) effect of ammonium acetate concentration on retention time for tributyltin ions; and (*c*) effect of eluent composition on response for tributyltin ions

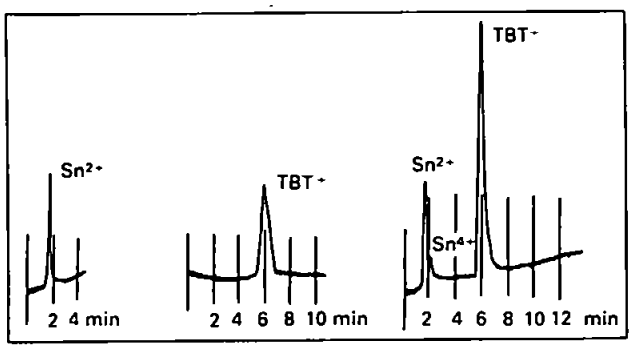


Fig. 5. Separation of tin species by coupled HPLC - FAAS

The gas flows to the burner STAT assembly were optimised for both Sn(IV) and tributyltin chloride (TBTC). The effect of changing the hydrogen flow-rate whilst keeping the air flow constant and aspirating solutions of both standards is shown in Fig. 3. Changing the air flow did not greatly affect the response, although at lower flow-rates excessive noise was observed. To overcome this problem, the air was pre-set at 4.0 l min^{-1} and background correction was used, which reduced the noise to negligible levels.

The analytical column selected was a Partisil silica based cation-exchange column. The column properties and proposed mechanism for separation involving a "free" tributyltin cation separation have been reported in detail by Jewett and Brinckman.[10] By varying the mobile phase composition and ionic strength, suitable conditions for speciation of a mixture of organotins were obtained, as demonstrated in Fig. 4. In addition, increasing the proportion of methanol in the eluent results in a decrease in retention time and a marked decrease in peak width. Using a mobile phase of 80 + 20 methanol - water in 0.1 M ammonium acetate solution a detection limit of 200 ng (2σ) of TBT was obtained. However, the conditions shown in Table 2 were selected in order to permit a better separation of real samples, and speciation of Sn(II), Sn(IV) and TBT^+ in 6 min (see Fig. 5).

Real samples were collected at a number of local coastal sites. The organotin compounds were quantitatively extracted from sea water into chloroform and pre-concentrated prior to

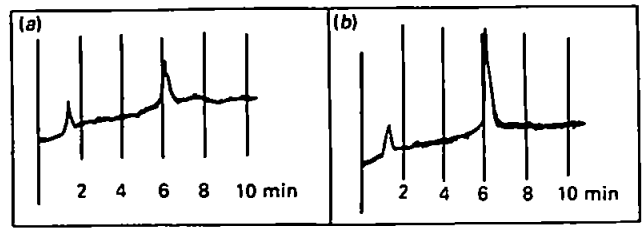


Fig. 6. Analysis of water samples collected at a harbour site in Plymouth, with and without co-injection of tributyltin chloride. (*a*) 1 ml of sample (800-fold pre-concentrated); (*b*) 1 ml of sample (800-fold pre-concentrated) + 500 ng of tributyltin chloride

analysis using the coupled HPLC - FAAS system. The efficiency of this process was determined from the recovery of standards spiked into blank solution and found to be 92 ± 6%. To facilitate injection on to the HPLC column, the chloroform was evaporated and the sample redissolved in methanol. The recovery from this second stage was 91 ± 5%. The results obtained from the analysis at one such site are shown in Fig. 6. The identification of tributyltin was supported by using co-injection, giving evidence of levels of up to 0.47 μg l^{-1} in the original samples.

Conclusion

The use of a flame atom cell as an atomic-absorption detector for liquid chromatography has the advantages of simplicity and a well understood operation. The continuous mode of operation is ideal for dealing with continuous sample streams. The use of the slotted-tube atom trap above the flame increases atom residence times and thus increases sensitivity. The components of this simple system are commercially available and do not require modification of existing instrumentation. The system is also readily decoupled. The interface has been used here for the determination of tributyltin, a compound that has proved difficult to determine by other techniques, although obviously the system lends itself to many other applications.

We thank Pye Unicam Ltd. for support of this work and particularly Dr. A. A. Brown for many helpful suggestions and the SERC for the provision of a Studentship (to S.J.H.).

References

1. Ebdon, L., Ward, R. W., and Leathard, D. A., *Analyst*, 1982, **107**, 129.
2. Brinckman, F. E., Blair, W. R., Jewett, K. L., and Iverson, W. P., *J. Chromatogr. Sci.*, 1977, **15**, 493.
3. Hodge, V. F., Seidel, S. L., and Goldberg, E. D., *Anal. Chem.*, 1974, **51**, 1256.
4. Maguire, R. J., Chau, Y. K., Bengert, G. A., Hale, E. J., Wong, P. T. S., and Kramer, O., *Environ. Sci. Technol.*, 1982, **16**, 698.
5. Zuckerman, J. J., Reisdolf, R. P., Ellis, H. V., and Wilkinson, R. R., *in* Brinckman, F. E., and Bellama, J. M., *Editors*, "Organometals and Organometalloids, Occurrence and Fate in the Environment," Symposium Series 82, American Chemical Society, Washington, DC, 1978.
6. Evans, J. C., and Smith, P. J., *J. Oil. Colour Chem. Assoc.*, 1975, **58**, 160.
7. Slavin, W., and Schmidt, G. J., *J. Chromatogr. Sci.*, 1979, **17**, 610.
8. Watling, R. J., *Anal. Chim. Acta*, 1978, **91**, 395.
9. Taylor, A., and Brown, A. A., *Analyst*, 1983, **108**, 1159.
10. Jewett, K. L., and Brinckman, F. E., *J. Chromatogr. Sci.*, 1981, **19**, 583.

Paper A4/288
Received August 20th, 1984
Accepted October 10th, 1984