The Analysis of Organometallic Compounds using SFC-ICPMS

by

Earl Blake,
BSc(Hons)

Submitted in fulfilment of the requirements for the degree of Master of Science in the Department of Chemistry and Applied Chemistry, University of Natal, 1994

Department of Chemistry and Applied Chemistry,
Division of Applied Chemistry,
University of Natal,
Durban,
4001,
South Africa

Abstract

Supercritical fluid chromatography has recently been coupled to an ICP-MS detector. The method has been shown to be suitable for the speciation and analysis of organometallic compounds at trace levels. This study has attempted to further the research initiated by other groups in this field by developing a new interface for coupling these two instruments. The new interface makes use of a modified join between the nebuliser and the torch in the ICP unit. The effect of the mobile phase on the plasma with time has been investigated and little spectral background interference has been observed.

The chromatographic conditions were optimised using a flame ionisation detector and a series of tin, arsenic, iron, and mercury compounds were analysed using SFC-ICPMS. After focusing the ICP-MS on the element of interest, each compound was evaluated in terms of the change in peak intensity with change in concentration and the theoretical detection limits were compared to the practical detection limit.

The restrictor temperature was determined using a rough calibration procedure with bench top experiments. The effect of the restrictor temperature on the peak intensity of each compound was then studied. All results were plotted and a theory for the observed trends and observations is proposed. The results obtained and the interface used have been

compared to the results and interfaces of other groups and differences have been explained. Attempts to extract relevant compounds from topsoil using supercritical fluid extraction were made.

Finally, sediment samples were collected from relevant points in Durban Bay and an attempt was made to extract these samples using supercritical fluid extraction. The extracted samples were analysed using SFC-ICPMS although little success was obtained. Reasons for the failure of this method on the real samples have been proposed. In the concluding section of this study SFC-ICPMS has been evaluated in terms of its future applicability and use as a viable analytical method.

Table of Contents

Acknowledgments

I would like to thank Mr. R. Stanton (CSIR), Mrs. G. Ross, my supervisor, Dr. M. W. Raynor, and co-supervisor, Prof. D. Cornell, for their help and many interesting and useful discussions during this study. Colleagues Bradley Bricknell, Magan Govender, Colin Manikum, Natalie Ingouville, William Modie, Rajan Moodley, Jane Broadbent and Charmaine Hogan also provided some useful input and discussion during this study.

I would especially like to thank Stefan Nicolescu for his help with the maintenance and operation of the ICP-MS even as it became clear in the latter stages of the study that our efforts at keeping the instrument running were useless.

This study would have been a lot less successful without the aid and efforts of the technicians Mr. I. Houston (Geology), Mr. S. Peterson and Mrs. S. Subramoney (Applied Chemistry), Mr. R. Singh (Chemistry) and Mr. P. Forder from the glassblowing workshop on the Pietermaritzburg campus. I appreciate the loan of the sampling cone from Tim McClurg and Ronnie Sibbald of the CSIR.

This study would not have been possible without the financial assistance of the Foundation for Research and Development, the University Research Fund and the Graduate Assistant Program. I would like to thank Mrs. J. Hardman for her help with many administrative tasks during the study.

Finally I would like to thank my parents for their help and patience during this study and the loan of the car for moving the SFC from one end of the campus to the other on numerous occasions.

Preface

This study was initiated after preliminary research in this area was carried out by a post-doctorate research group under the leadership of Dr. J. A. Caruso at the University of Cincinnati. This study was aimed at confirming, and perhaps bettering, the results obtained by that group. In addition this study developed a different interface for coupling SFC to ICP-MS and made studies of the effect of the mobile phase on the plasma with time rather than pressure as was the case with the other group. The work presented here is entirely that of the author (except where text makes reference to published material) and has not been presented in full or in part to any other institution for examination. The work has been presented in part as an oral presentation by the author at:

***The 32nd Convention of the South African Chemical Institute*, Escom Conference Centre, Halfway House, South Africa, 30 January - 3 February 1994 (O-WED-A4).**

***The Third International Symposium on Hyphenated Techniques in Chromatography*, University of Antwerp, Antwerp, Belgium, 22-25 February, 1994 (A08).**

The work has been presented in part as a poster presentation on behalf of the author by Dr. M. W. Raynor at:

***The 5th International Symposium on Supercritical Fluid Chromatography and Extraction*, Baltimore, Maryland, USA, 11-14 January 1994.**

***The Sixteenth International Symposium on Capillary Chromatography*, Palazzo dei Congressi, Riva del Garda, Italy, 26-30 September 1994.**

The work has also been presented as publications by the author, Dr. M. W. Raynor and Prof. D. Cörnell, in:

***American Laboratory*, 26 (1994) pp 46-50.**

***The Journal of Chromatography*, 683 (1994) p 223**

The Journal of High Resolution Chromatography and Chromatographic Communications, **18 (1995) p 33.**

It is hoped that this work will complement that of Caruso's group and will stimulate interest and further study in this field of analysis.

Glossary

Terms and abbreviations used in this document.

Anthropogenic - arising from industry or other human related pollution.

Atm - atmospheres

Bu - butyl.

cps - counts per second

Et - ethyl

Gosio's gas - trimethylarsine as produced from fly ash and arsenical pigments by the mould *Penicillium brevicaule.*

Me - methyl.

Metalloenzyme - an enzyme which contains one or more metal atoms in its structure (e.g. cytochrome).

Metalloid - an element which has properties intermediate between those of the metallic and non-metallic elements.

MPa - megapascals

MPa/min - megapascals per minute

ng - nanograms (10^{-9} g)

pg - picograms (10^{-12} g)

Ph - phenyl

ppb - parts per billion

ppm - parts per million

Radiofrequency -a wave frequency between 10 kHz and 30 000 MHz.

In addition to the above terms and abbreviations standard IUPAC and SI units and terms have been used.

The following terminology has been adopted when referring to elements and ICP-MS:

For example - ^{120}Sn refers to the isotope of tin having a mass of 120 amu. $^{120}Sn^{+}$ refers to the cation of this isotope detected by the ICP MS.

CHAPTER 1

1.1 Introduction and aims of the project

In a study of the analysis of organometallic compounds it is necessary to consider a number of factors. These include a survey of the nature of organometallic compounds and why it is necessary to analyse them, the nature of the analytical techniques presently used for these compounds, and the nature of the chosen analytical technique. The applicability of the chosen method to real samples must also be considered. In this study the nature, use and toxicity of organometallic compounds is discussed and reasons for analysing them at trace levels are given. A survey of the most common analytical techniques used for analysing organometallic compounds is presented and a detailed study of supercritical fluid chromatography (SFC) and inductively coupled plasma - mass spectrometry (ICPMS) is made. In this investigation a new method for coupling SFC to ICPMS has been developed and this will be presented in detail and evaluated for the analysis of organometallic compounds. Results for the supercritical fluid extraction (SFE) and analysis of sediment samples from Durban bay will also be presented. In addition the optimisation of SFC conditions and ICPMS conditions will be presented and the results obtained using SFC-ICPMS will be compared to results obtained by other groups working in this field. Finally the method will be evaluated in terms of ease of use, cost, maintenance and applicability to real samples.

1.2 Organometallic Compounds

The class of compounds known as organometallics contain one or more organic groups covalently bonded to a metal atom. There may also be inorganic elements, usually halides, and small organic ions, such as

acetate, attached to the metal atom. Although the term organometallic is generally applied to any compound in which there is a metal atom bonded to a carbon atom either ionically or covalently [1], in this study it will be considered as a metal atom covalently bonded to the carbon atom of a particular organic group. This assumption is made as only a small proportion of metals form ionic organometallic compounds (that is the alkali metals) and moreover SFC is not capable of analysing highly polar or ionic compounds. Furthermore this study has not considered carbonyl compounds which, in terms of the general definition, can also be considered as organometallics. The metal atom is not limited to only a few elements but can be almost any metal with the most common ones being the transition metals. In general vanadium and nickel occur as metalloporphyrins. Iron can occur naturally as a porphyrin or synthetically in compounds such as ferrocene. Chromium may also occur in some metalloproteins and metalloenzymes while tin, antimony, mercury, bismuth, and lead usually are present as synthetic organometallics. Arsenic, although classed as a metalloid rather than a metal, is also capable of forming a number of synthetic and natural organometallic compounds.

The most common and well known organometallic compounds are those of the *d*-block transition metals where σ-, π- and δ-bonding can occur. These different types of bonding and the different ligands bonded to the metal atom affect the chemistry of the compound as a whole. In general, compounds that have a number of inorganic groups and only one small organic ligand attached to the metal atom are more likely to have inorganic characteristics such as greater solubility in water and a more ionic nature. However, if there are a number of large organic ligands attached to the metal, then the compound would be more organic in nature with low water solubility. The most common type of bonding in the transition metals is σ- and π-bonding where σ-bonding consists of a covalent metal-carbon bond and π-bonding has a covalent bond between a

metal and an unsaturated organic ligand. Thus, σ-bonding can occur for all metals except the most electropositive metals which form ionic bonds while π-bonding is more prevalent for the *d*-block metals.

As organometallic compounds vary widely in their structure, bonding and physical and chemical properties, clearly they will have varying degrees of stability. However, when considering their stability, it is important to distinguish between kinetic and thermal stability. The importance of this is shown in the environmental chemistry of many of these compounds. For example, tetramethyltin has a heat of formation of approximately -19.2 kJ/mol. That is exothermic and favours formation of this compound. However, dimethylmercury has a heat of formation of 93.3 kJ/mol. That is distinctly endothermic yet this compound is formed in the environment by methylation of mercury metal by methylating bacteria. Moreover, this compound is more resistant to breakdown in the environment than tetramethyltin [1]. The reason for this is dimethylmercury's thermodynamic instability towards the breakdown reaction:

$$Me_2Hg \rightarrow CH_4 + C_2H_6 + C_2H_4 + Hg$$

However, tetramethyltin is prone to photodegradation and in a relatively short time (approximately one year) can decay to form trimethyltin chloride, dimethyltin dichloride, methyltin trichloride and tin tetrachloride. Thus, in dimethylmercury, thermodynamic stability prevents decomposition while in tetramethyltin photokinetics are able to overcome thermodynamic stability and cause breakdown to other products. Although photodegradation of dimethylmercury does occur it is less prevalent than with the tin species.

The metalloporphyrins of nickel and vanadium are of lesser interest to the chemist but are significant to the geologist and geochemist as they mostly occur in shale, coal and to a certain extent in some oils. Nevertheless,

Table 1.1: Some common organometallic compounds and their uses.

Compound	Uses
Ph_3SnOH	Fungicides, Anti-fouling paints
Ph_3SnCl	Anti-fouling paints
Bu_3SnCl	Rodent repellent
$(Bu_3Sn)_2O$ (TBTO)	Fungicides, wood and stone preservatives
Bu_3Sn benzoate	Germicide
$(Bu_3Sn)_3PO_4$	Wood preservatives (Bacteriostatics)
$(c\text{-}C_6H_{11})_3SnOH$	Orchard insecticide
$(CH_2{=}CH)_3SnCl$	Herbicide
$[n\text{-}Oct_2Sn(C_4H_2O_4)]_n$	PVC stabiliser (food contact)
$Bu_2Sn(SC_{12}H_{25})_2$	PVC stabiliser (no food contact)
$[Bu_2SnO]_n$	Catalysis
Me_2SnCl_2	Glass strengthening, precursor for SnO_2
Me_6Sn_2	Insecticides
$BuSn(OH)_2Cl$	Catalyst for transesterification
Et_4Pb	Anti-knock agent
Me_4Pb	Anti-knock agent
pyHgX X = halide	Catalyst for urethane production
Thiomersal (derivative of Hg)	Antiseptic
Mercurochrome (Hg fluorescein compound)	Antiseptic
Chlormerodrin (Hg alkoxyalkyl derivative)	Diuretic
$(p\text{-}NH_2PhAsO(OH)_2)$	Veterinary medicine
$Me_2AsOONa$	Herbicide
$p\text{-}NO_2PhAsO(OH)_2$	Animal feed additive
ferrocene	fuel additive, iron fertiliser

many chemical studies on the analysis of metalloporphyrins have been carried out [2-4]. The metalloproteins of chromium are of interest to the food and pharmaceutical industry while the synthetic organometallic compounds of iron, tin, antimony, bismuth and lead are mainly used as intermediates or additives. Generally, the arsenic and mercury organometallics, and some synthetic organometallic compounds of lead and tin, are of primary importance to the environmental chemist. A more detailed list of some common organometallic compounds and their uses is given in Table 1.1.

As would be expected in such a large group of compounds, the physical and chemical properties within this group varies widely. Even within a given series of compounds there can be large differences in the chemical and physical properties with no trend within the series being apparent. For example, in the series of tetrabutyltin, tributyltin chloride, dibutyltin dichloride and butyltin trichloride all are liquids except for dibutyltin dichloride which is a crystalline solid. Thus, there does not appear to be a trend for increasing or decreasing volatility within the series. Therefore, when analysing these compounds it is necessary to take into account the wide variations that can occur and try and use a method that is universal in its application. Before discussing the methods that are currently available and the method used in this study it is first necessary to examine why it is necessary to analyse organometallic compounds in the environment.

1.3 The importance of analysing organometallic compounds

Organometallic compounds are used in a variety of applications as shown in Table 1.1. Organometallic compounds are used to a lesser extent in the food industry where compounds such as *chlorophyll a*[1] are used as

[1] The structure of chlorophyll a is shown in Appendix A.

colorants. The food industry is often involved in research of proteins and enzymes and these may include metalloproteins and enzymes such as cytochrome. Moreover, organometallic compounds such as organolead compounds may occur in samples such as wine [5]. Thus, it would be useful for this industry to have a chromatographic method that is able to analyse these compounds irrespective of their volatility. To a greater extent organometallic compounds are used in the pharmaceutical industry where many organomercury compounds are used as antiseptics (i.e. mercurochrome) and organoarsenic compounds have a variety of uses (as shown in Table 1.1). More importantly, haemoglobin[2], which aids in oxygen transport in blood, is an organoiron compound. Thus, the analysis of organometallic compounds is also of some importance to the pharmaceutical industry.

However, by far the greatest interest in analysing organometallic compounds is in environmental chemistry. Table 1.1 illustrates the wide variety of uses for organometallic compounds and closer inspection reveals that many of these may impact in some way on the environment. Additives to anti-fouling paints and PVC are often leached out into the surrounding environment while bacteriostatics, fungicides, herbicides and insecticides are often introduced into the environment directly. Catalysts and other industrial compounds may also be introduced into the environment through industrial waste disposal. Moreover, organometallic compounds in the environment may arise from methylation of metals by certain methylating bacteria. There are many examples of this and these include methylation of mercury [6], lead [7,8] and arsenic [9,10]. Mercury metal is methylated to form methylmercuric chloride and dimethylmercury while lead metal and various organic and inorganic lead compounds are methylated to tetramethyllead. Methylation of arsenic usually occurs in moist environments on or near fly ash where bacteria methylate the arsenic to form Gosio's Gas (Me_3As).

[2] The structure of haemoglobin is shown in Appendix A.

The majority of these organometallic compounds, whether from natural or anthropogenic sources, accumulate in the aquatic environment. However, due to their lipophilic nature they are easily absorbed by fish, shellfish and other marine organisms. As many of these compounds are toxic, even at trace levels, they can deplete large fish populations or they can accumulate in the food chain to cause harm to mammals reliant on fish or shellfish as their staple diet. A good example of this is Japan where fish catches total 11 800 000 metric tons per annum which is the largest consumption in the world [11]. The danger of organometallic compounds in the environment is illustrated by the outbreak of Minamata disease in Japan in 1952[3]. It is estimated that in this outbreak alone over 2000 cases were treated with nearly 50 deaths related directly to this disease [12]. Another metal believed to be responsible for serious health risks is cadmium which can cause itai-itai disease[4]. Over 100 deaths were attributed to this disease in Japan at approximately the same time as the Minamata disaster. Other metals such as copper, iron and tin are also responsible for poisoning aquatic life but are less dangerous to mammals. Nevertheless, if there is large scale depletion of the fish population, this can impact negatively on the economy if the fishing industry is significantly large enough as in Japan. Alkyllead compounds are also known to be toxic to mammals but these compounds are of more concern in the air than in the marine environment [12].

Many of these organometallic species are toxic or harmful even at trace levels [13] and so an efficient method with low detection limits needs to be used. If these compounds are not detected in time there may be depletion of aquatic life, accumulation of harmful substances in the food chain or, as illustrated in Minamata, serious health risks to human life. It has

[3] Minamata disease is the often fatal paralysis of the entire nervous system caused by methyl mercury formed in the gut of fish by methylation of inorganic forms of mercury.
[4] Itai-itai disease is a debilitating disease of the bones and joints.

recently been shown that SFC-ICPMS is a useful method for analysing organometallic compounds at trace levels [14-19]. However, before continuing further it is necessary to give relevant background information on the instrumental techniques involved in this study.

1.4 Supercritical fluid chromatography

In general, chromatography can be described as a physical method of separation of an analyte between a stationary and a mobile phase where the mobile phase flows through the column, percolating through the stationary phase. In all forms of chromatography there are a number of basic principles which are used in the analysis. The time at which a compound elutes is the retention time, t_r. The first peak usually has a relatively small retention time and is due to a compound such as nitrogen in GC or the organic solvent in SFC. As the retention time is so low this is referred to as the unretained peak, t_o. Thus, by subtracting the retention time of the unretained peak from the retention time of the analyte it is possible to find the retention time of the analyte relative to the unretained peak. This new retention time is labelled t_r'. The capacity factor is defined as

$$k' = \frac{t_r'}{t_o} = \frac{m_s}{m_m}$$

and is a measure of how much longer a component will stay in the stationary phase compared to the mobile phase where m_s is the number of moles in the stationary phase and m_m is the number of moles in the mobile phase. The partition coefficient under equilibrium conditions is defined as

$$K = \frac{\text{conc. solute in stat. phase}}{\text{conc. solute in mobile phase}}$$

However, the concentration is moles per cubic decimetre and the new partition coefficient will be

$$K = \frac{m_s/V_s}{m_m/V_m} = \frac{V_m m_s}{V_s m_m}$$

The mole ratio of the component in the stationary and mobile phase has already been defined as the capacity factor and so

$$K = \frac{V_m}{V_s} k'$$

The ratio of the relative volumes of mobile phase and stationary phase is defined as the phase ratio, β, and so the partition coefficient can be given as

$$K = \beta k'$$

The partition coefficient is constant for a particular separation at a particular temperature. Thus, as k' increases β decreases and there is better resolution. Resolution is a measure of the peak separation or the relationship between peak width and interpeak distance. It is measured using the formula

$$R = \frac{t_{r2} - t_{r1}}{\frac{1}{2}(w_{b1} + w_{b2})} = \frac{2\Delta t}{w_{b1} + w_{b2}}$$

where w_{b1} and w_{b2} are the peak widths at the base of each peak.

The primary feature of supercritical fluid chromatography is its use of a supercritical fluid as the mobile phase. A supercritical fluid exists in the region above the critical temperature and pressure of a chemical substance. This is illustrated in the phase diagram of CO_2 shown in Figure 1.1. CO_2 is chosen as it is the most common mobile phase used in SFC. However, there are a number of other compounds which can be used and these include N_2O, NH_3, MeOH, $CClF_3$, ethane and ethylene [20]. Within the supercritical region these compounds are neither a liquid nor a

gas but have properties intermediate between the two. Thus, by altering conditions of temperature and pressure within the supercritical region it is possible to alter properties such as density and viscosity from a near gas-like state to a near liquid-like state. However, if considering density, the change is not linear as temperature is altered. This is more clearly seen in Figure 1.2 which shows that the density profile for CO_2 will vary depending on the change in pressure at different temperatures. Another important property of supercritical fluids is that they have molecular closeness and thus solvating power and by altering the temperature and density of the supercritical fluid it is possible to alter their solvating power. Normally, separation is achieved by pressure or density programming the mobile phase at a constant temperature. Although a number of compounds can be used as supercritical mobile phases, CO_2 is the most common as it is has a low critical temperature and pressure (31 °C and 7.3 MPa). Thus, because of the low critical temperature of mobile phase, and the high temperatures which can be tolerated, it is possible to analyse compounds with a wide range of volatilities. The relatively low critical temperature allows analysis of thermally labile compounds. Moreover, by altering the density and solvating power of the mobile phase it is possible to optimise the conditions of separation for a particular compound or series of compounds. This makes this method ideal for analysis of organometallic compounds as they have such a wide range of melting points, boiling points and solubilities in organic solvents. With regard to the chromatographic factors SFC is slightly different to gas chromatography (GC). The partition coefficient is given as follows:

$$K = k'\beta$$

where $$k' = \frac{t_r'}{t_0} \qquad \textbf{(a)}$$

and $$\beta = \frac{\text{volume of mobile phase}}{\text{volume of stationary phase}}. \qquad \textbf{(b)}$$

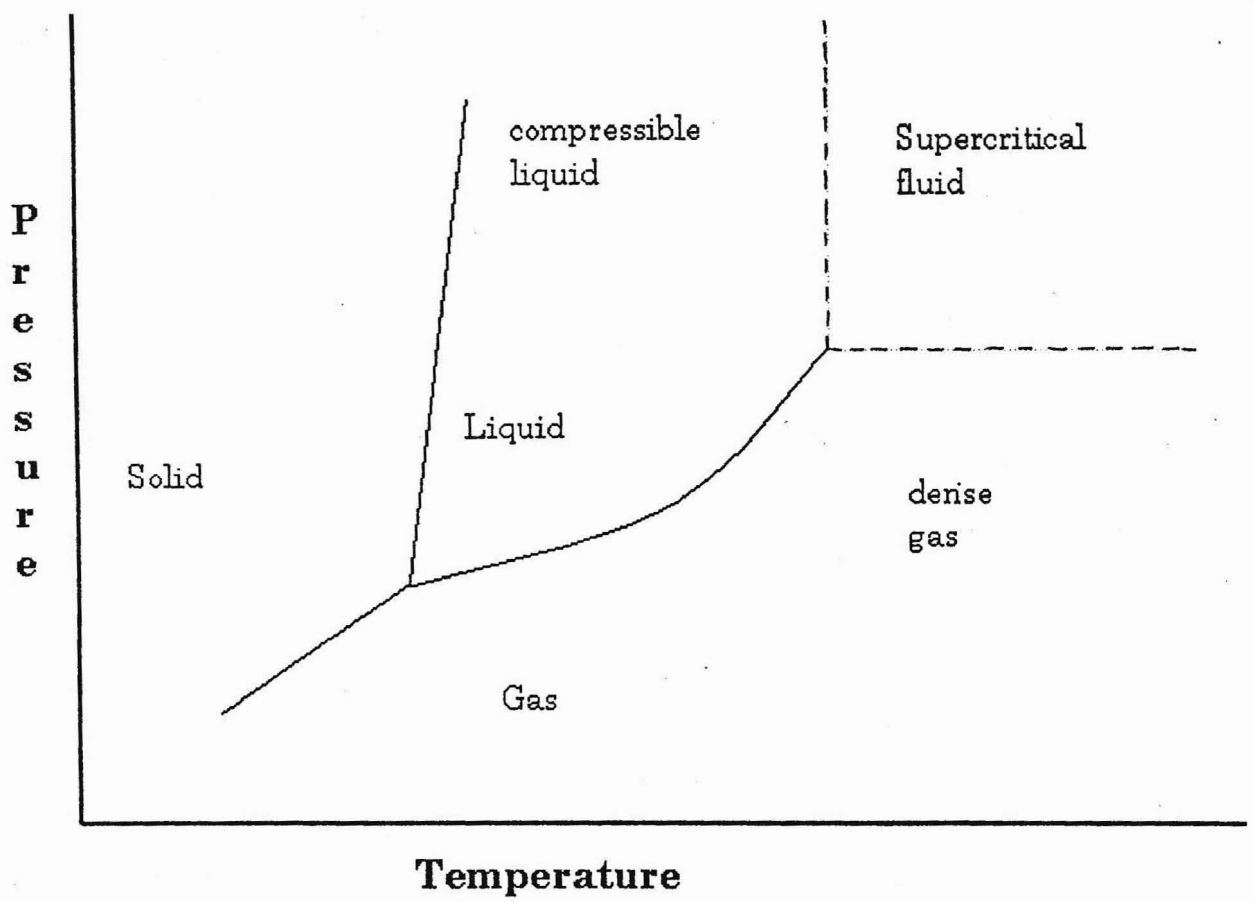


Figure 1.1: The phase diagram of CO_2.

For the usual partitioning process:

$$\log k' = -\log\beta - \frac{0.43\Delta G^\circ}{RT} \qquad \textbf{(c)}$$

In GC ΔG° can be replaced by the heat of solution of solute in the stationary phase ΔH_s. However, in SFC the mobile phase must be considered and $\Delta G^\circ = \Delta H_s - \Delta H_m$. Thus, equation (c) becomes

$$\log k' = -\log\beta - \frac{0.43\Delta H_s}{RT} + \frac{0.43\Delta H_m}{RT} \qquad \textbf{(d)}$$

In equation (d) the ΔH_s part of the equation represents the volatility contribution while the ΔH_m part of the equation represents the solvation contribution. Thus, a plot of $^1/_T$ versus log k' will give a straight line increasing with log k' at high to intermediate temperatures as the volatility factor is more important. However, at intermediate to low temperatures the mobile phase solvation becomes more important and log k' will decrease. Thus, as opposed to the straight line obtained for GC, in SFC there will be deviation from a straight line caused by the mobile phase solvation.

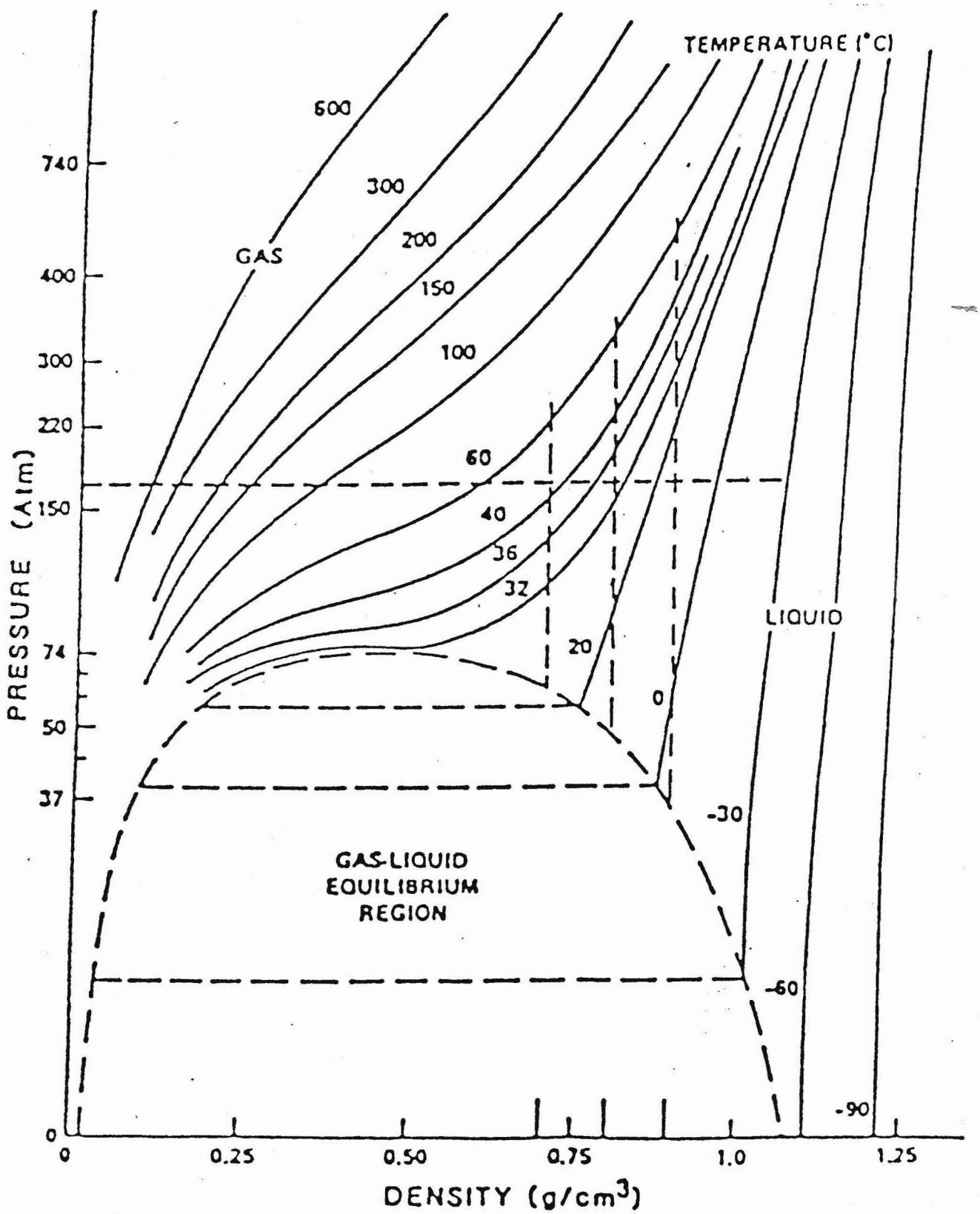


Figure 1.2: Phase diagram of CO_2 showing the density-temperature relationship.

1.4.1 SFC instrumentation

The SFC instrumentation consists of an oven to maintain the temperature of the column above the critical temperature; a syringe pump to pressurise the mobile phase above its critical pressure and a computer loaded with the correct software to control the analytical conditions. The supercritical mobile phase is sensitive to even small changes in the pressure and so it is important that a syringe pump is used as any other pump, such as a peristaltic pump, may give small but unacceptable changes in pressure as the mobile phase is passed through the column. Moreover, as there is a lot of heat generated by the pump it is important that it is linked to a cooling unit to prevent overheating. At the column inlet the sample is injected through a direct micro-valve injector similar to those used in high performance liquid chromatography (HPLC). The sample is injected into the sample loop, which has a fixed volume of 60-300 nl, and any excess is passed to waste. When the injector is activated by the activator gas (usually helium) it is rotated so that the mobile phase passes through the sample loop and carries the sample onto the column. A disadvantage of this injection system is the small volume of sample which is injected. In trace analysis it is important to have as much sample as possible injected onto the column and small volumes may cause difficulty in detecting the analytes. The amount of sample which moves along the column can be controlled by using either a splitter assembly or time split injection. If a splitter assembly is used, a split ratio is used to pass the desired amount of sample onto the column while the rest of the sample is bled off to waste. If time split injection is used, the desired injection time (usually < 200 ms) is entered into the controlling software and thus only a fraction of the sample is passed onto the column from the sample loop. The detector can be virtually any detector normally used with HPLC or GC provided that the mobile phase does not interfere with detection of the analytes. The detectors which have been used so far include the flame ionisation detector (FID) [21], the flame photometric detector (FPD) [22], the

ultraviolet detector (UV) [23], electrochemical detection [24] and hydrogen atmosphere flame ionisation detectors (HAFID) [25]. A schematic diagram of the instrumentation used in SFC is shown in Figure 1.3.

In SFC the columns may be either capillary columns or packed columns depending on the analysis. Although 50 μm i.d. capillary columns have been used in this work [76, 77, 78] there is still a use for packed columns and some novel stationary phases can be used. For example, recently Buckminsterfullerene (C_{60}) has been investigated in liquid chromatography and may find application in packed column SFC [26]. Although the stationary phases and packings used in SFC are similar to those used in GC and HPLC, they need to be very well deactivated and bonded to prevent them from being extracted by the mobile phase and from interacting with the analytes. Moreover, the capillary columns must be as narrow as possible to prevent the slow diffusion processes from degrading chromatographic efficiency. Thus, typical column diameters are in the 50 - 100 μm region. The column must also have a restrictor at the outlet. The restrictor creates the necessary pressure within the column to maintain supercritical conditions throughout the column, to minimise the pressure drop along the column and it also serves to control the linear velocity of the mobile phase which preserves chromatographic efficiency. The most common types of restrictor are shown in Figure 1.4 below. It was earlier stated that the mobile phase is a supercritical fluid such as CO_2 and this may create the impression that only a single, pure fluid can be used. However, it is possible to modify the mobile phase by adding a small amount (usually < 20 %) of another substance to the mobile phase. This modifier is usually an organic compound such as methanol or formic acid and is able to alter mobile phase properties such as density and solvating power. A modifier may be important as a polar modifier such as methanol in a non-polar mobile phase such as CO_2 is able to widen the scope of the method to more polar analytes. However, adding a mobile phase modifier

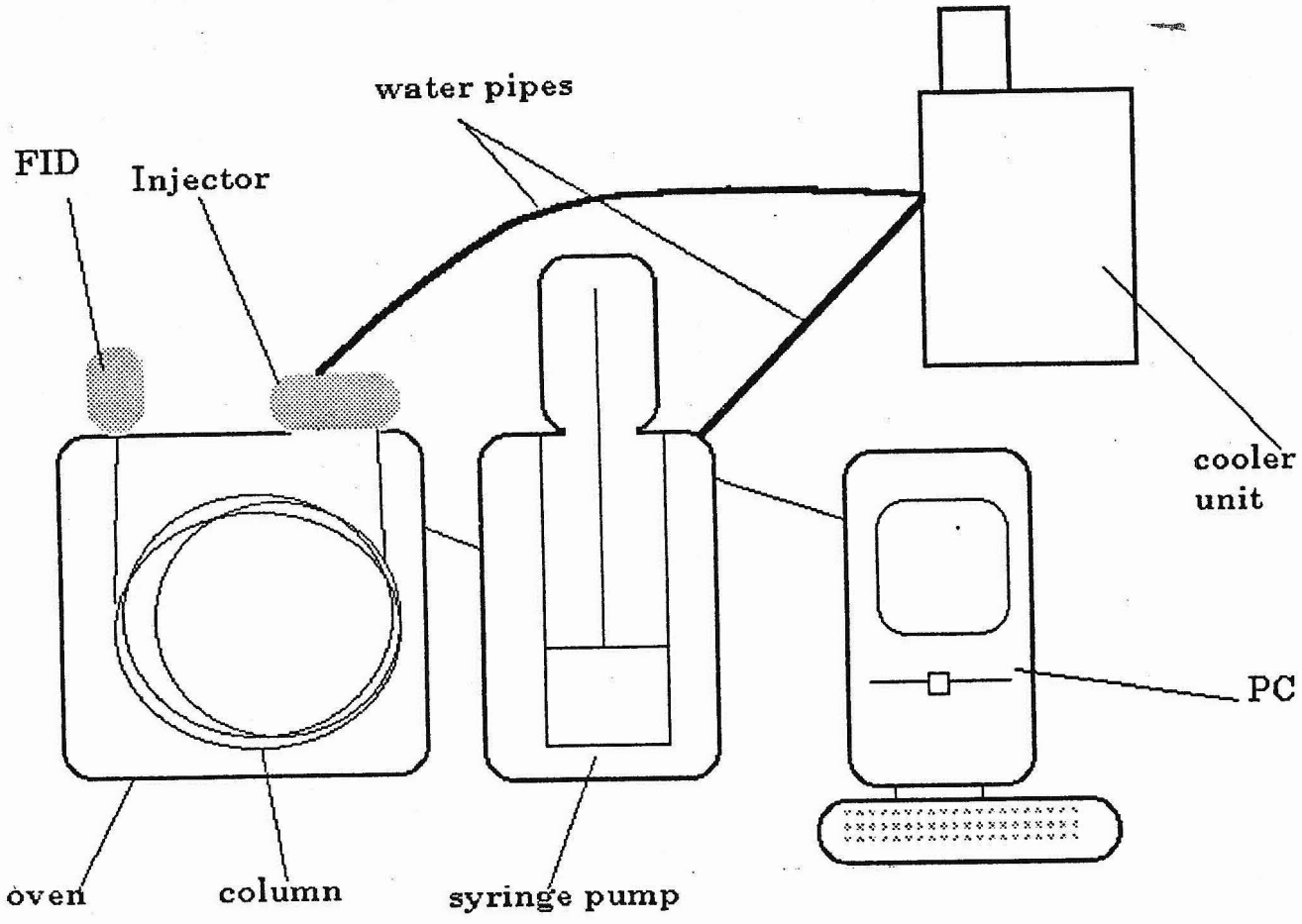


Figure 1.3: Schematic diagram of the SFC instrumentation.

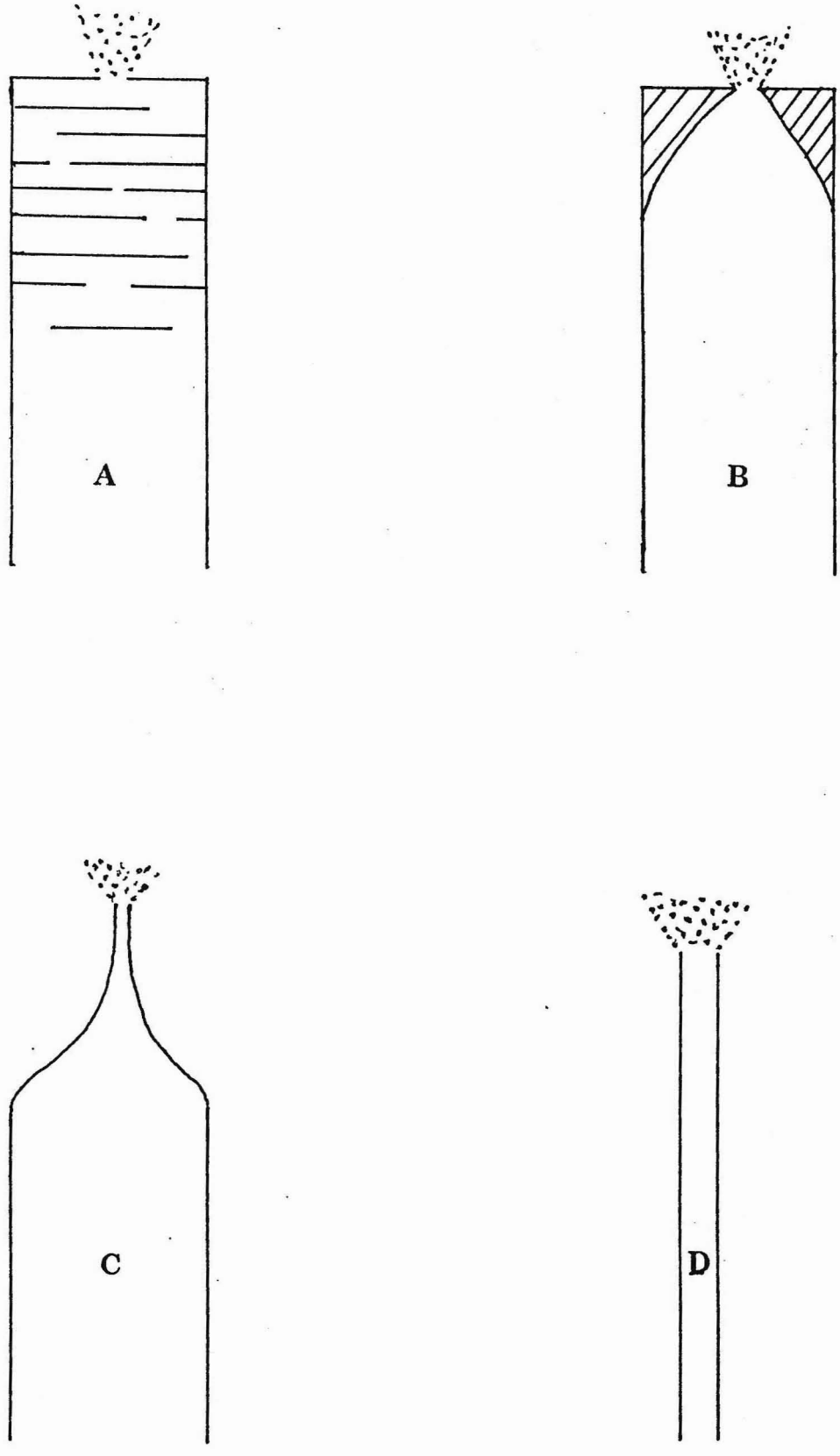


Figure 1.4: Diagram showing A) a frit restrictor, B) & C) tapered restrictors and D) a linear restrictor.

may limit the detection methods which can be employed. There have been many methods developed to add modifiers to mobile phases [27,28], methods have been developed to determine the densities of these fluids [29] as well as solubility parameters in these fluids [30]. Experiments have also been designed to study the phase behaviour of these fluids [31]. Modified mobile phase can also be purchased in aluminium cylinders but the composition variation within these cylinders can affect the results of the analysis [32].

1.5 Analysis of organometallic compounds by SFC

As previously stated organometallic compounds have a wide range of physical and chemical properties. Thus, a method is needed for analysing thermally labile compounds, such as triphenylarsine, and involatile compounds, such as tetraphenyltin. SFC using pure CO_2 as the mobile phase can perform relatively low temperature analyses (< 100 °C) for thermally labile compounds and high temperature analyses (> 300 °C) for involatile compounds. Moreover, through effective control of temperature and pressure in the supercritical region the solvating power of the mobile phase can be optimised for these compounds. The addition of a modifier to the mobile phase could increase the scope of this method to all but the most polar of organometallic compounds.

The majority of GC methods used to analyse organometallic compounds require the sample to be derivatised to more volatile species before analysis [33-43]. The most common derivatisation procedures are alkylation via the Grignard reaction using pentylmagnesium bromide or hydridisation using $NaBH_4$. For example:

$$Bu_2SnCl_2 + 2PeMgBr \rightarrow Bu_2SnPe_2 + 2ClMgBr$$

This may be a source of experimental error as problems may arise at any point during the derivatisation or during transfer of the derivatised sample from the reaction vessel to the analytical instrument of choice. Even if an on-line derivatisation is used experimental error can still occur. SFC is able to analyse compounds directly without derivatisation. This eliminates lengthy derivatisation procedures thus shortening analysis time and minimising the opportunity for experimental error.

Environmental samples are usually extracted into an organic solvent thus generating large amounts of waste solvent which may be difficult to dispose of. Many organic solvents, such as benzene and chloroform, are hazardous to work with as they are carcinogenic and thus special safety procedures may need to be introduced. Thus, SFE has gained popularity and is now coupled to a number of techniques [38, 44]. However, as SFE and SFC are both based on the same principles they are easily coupled and hence extraction and analysis of samples can be carried out on-line using ideally suited systems.

1.6 Supercritical fluid extraction

Supercritical fluid extraction involves the extraction of one or more analytes by a supercritical fluid. The principles of variable density and solvating power of the supercritical fluid are the same as those already discussed. Extraction is usually carried out in one of two ways - static extraction or dynamic extraction. In static extraction the sample is placed in the extraction vessel and the vessel is then filled with the supercritical fluid. In principle the analytes dissolve into the supercritical fluid and after a set period the extraction vessel is depressurised and the supercritical fluid and analyte are collected in one of the ways to be discussed later. In dynamic extraction the supercritical fluid is constantly passed through the extraction vessel collecting the analytes as it passes through. A schematic diagram of the SFE system is shown in Figure 1.5

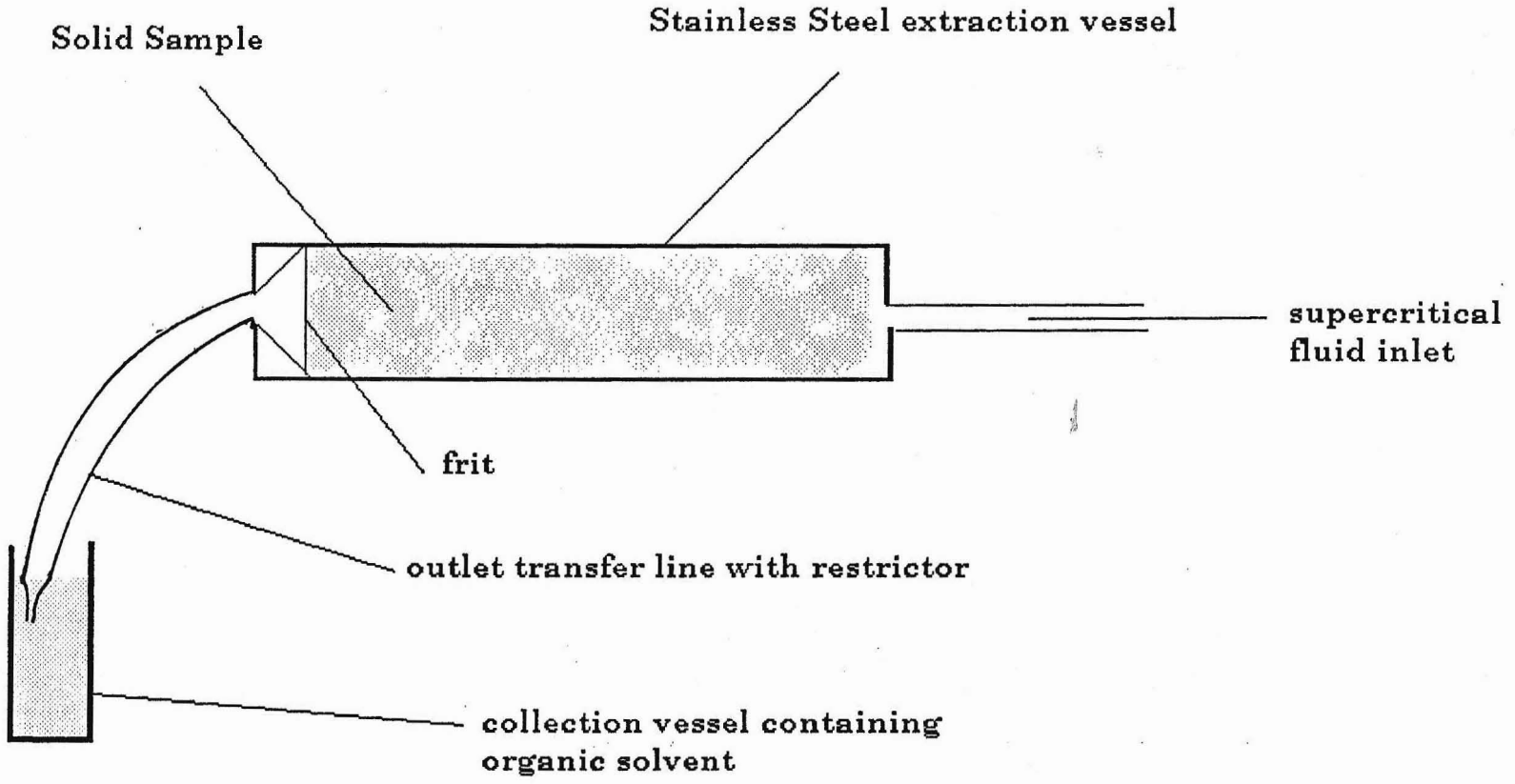


Figure 1.5: A schematic diagram of the typical SFE system for solid samples.

The analytes can be collected in two different ways. The most common and easiest way is to place the restrictor in a sample vial containing a small volume (say 5 ml) of organic solvent. As the supercritical fluid leaves the restrictor it depressurises to a gas and bubbles out of the organic solvent while the analytes pass into the organic solution. Provided the restrictor is placed deep enough in the organic solvent, it should remain clear. However, if the restrictor is placed near the surface of the solvent (that is too near the liquid/air interface) the bubbling may become too vigorous thus allowing air to encompass the restrictor. The adiabatic expansion of the mobile phase is not effectively counteracted and the restrictor becomes blocked with solid CO_2. Another problem associated with this method of collection is evaporation of the organic liquid. The vigorous bubbling may encourage evaporation of the organic solvent and so it is often desirable to place the collection vial in an ice bath. The less common method is to collect the analytes on a solid sorbent material as the CO_2 is vented to the atmosphere. The analytes are then washed from the sorbent material using a liquid solvent. However, this method increases the problem of the restrictor becoming blocked.

During SFE, separation of the analytes from the matrix depends on three important factors: solubility of the analyte in the supercritical fluid, diffusion of the analyte out of the matrix and into the fluid and the sample matrix. A number of studies have been carried out to quantify the contribution made by each of these factors and to investigate matrix/extraction relationships [60-70]. Although solubility and diffusion are important, the most important factor is the sample matrix. If the matrix is complex and strongly absorbs the analyte then the solubility in the mobile phase may not be sufficient as the matrix prevents diffusion of the analyte out of the sample.

Once the sample has been extracted it is analysed by a suitable method. However, when considering organometallic compounds, it has already been stated that SFC is ideal. However, a sensitive, selective detector is needed. A good detector satisfying these requirements is inductively coupled plasma mass spectrometry.

1.7 Inductively Coupled Plasma - Mass Spectrometry

1.7.1 Inductively Coupled Plasmas

Mass spectrometry of inductively coupled plasmas was pioneered by Gray and Date in the early 1970's [45]. A diagram of the ICPMS system is shown in Figure 1.6. The ionisation source is the plasma which is generated by applying a radiofrequency to argon or helium flowing through the torch towards the mass spectrometer. The radiofrequency is generated by an RF generator which passes the current through a load coil. This ignites the argon or helium flowing through the torch and a plasma is formed. Although either argon or helium can be used, most instruments are designed for an argon plasma although low pressure plasmas often use helium. Moreover, the design of the torch is important as it must be able to withstand the high temperatures of the plasma (> 6000 K). Thus, the torch is designed to carry three gas flows within one unit. Assuming that the plasma is an argon plasma, there is an inner argon flow from the nebuliser and this is primarily to introduce the sample into the plasma. Surrounding the nebuliser flow is the auxiliary argon flow which forms the plasma when the radiofrequency is applied. Flowing around the outside is the cooling argon which has a high flow rate and which acts as a shield to prevent the torch from melting. It is important to note that the three flows each have different flow rates best suited to their own function. The torch is constructed of quartz and can have a variety of designs with the most common being the Fassel torch and the Greenfield torch. The torch used will depend on the flow rates

which will be used as the Greenfield torch can handle higher flow rates than the Fassel torch. The sample is introduced into the plasma from the nebuliser where it has been nebulised to form minute droplets of liquid. The general design of the torch is shown in Figure 1.7.

Once the sample has entered the plasma it is atomised and ionised by the high temperatures within the plasma. Even non-metallic elements and metalloids can be efficiently ionised by the plasma [46]. Thus, the ICP provides an effective ion source for mass spectrometry. However, it is necessary to have an interface which allows efficient extraction of the ions from the plasma into the mass spectrometer.

1.7.2 The Inductively coupled plasma - mass spectrometry interface

The mass spectrometer operates under vacuum while the ICP is at atmospheric pressure. The interface is designed so that the tip of the plasma flows around a cone known as the sampling cone. This is usually made of nickel and has a small circular hole drilled into the tip. The diameter of this hole is important and is usually between 0.5 and 1.0 mm. Behind this sampling cone is the first vacuum chamber which is typically at 133 Pa. In this chamber most of the gas is evacuated while the remaining gas passes through a central orifice in the skimmer cone situated at an appropriate point behind the sampling cone. The position of the skimmer cone is crucial as it must allow as much gas remaining in the first chamber as possible to pass into the second chamber where the pressure is typically 0.03 Pa. In this chamber the low pressure ensures that the mean free path is long enough for the ions to be collected and focused by a series of ion lenses. The ions are then transferred into a third chamber containing the mass analyser or quadrupole. The pressure in the third chamber is typically at 2.6×10^{-4} Pa. A schematic diagram of the ICP-MS interface is shown in Figure 1.8. The main feature of this system

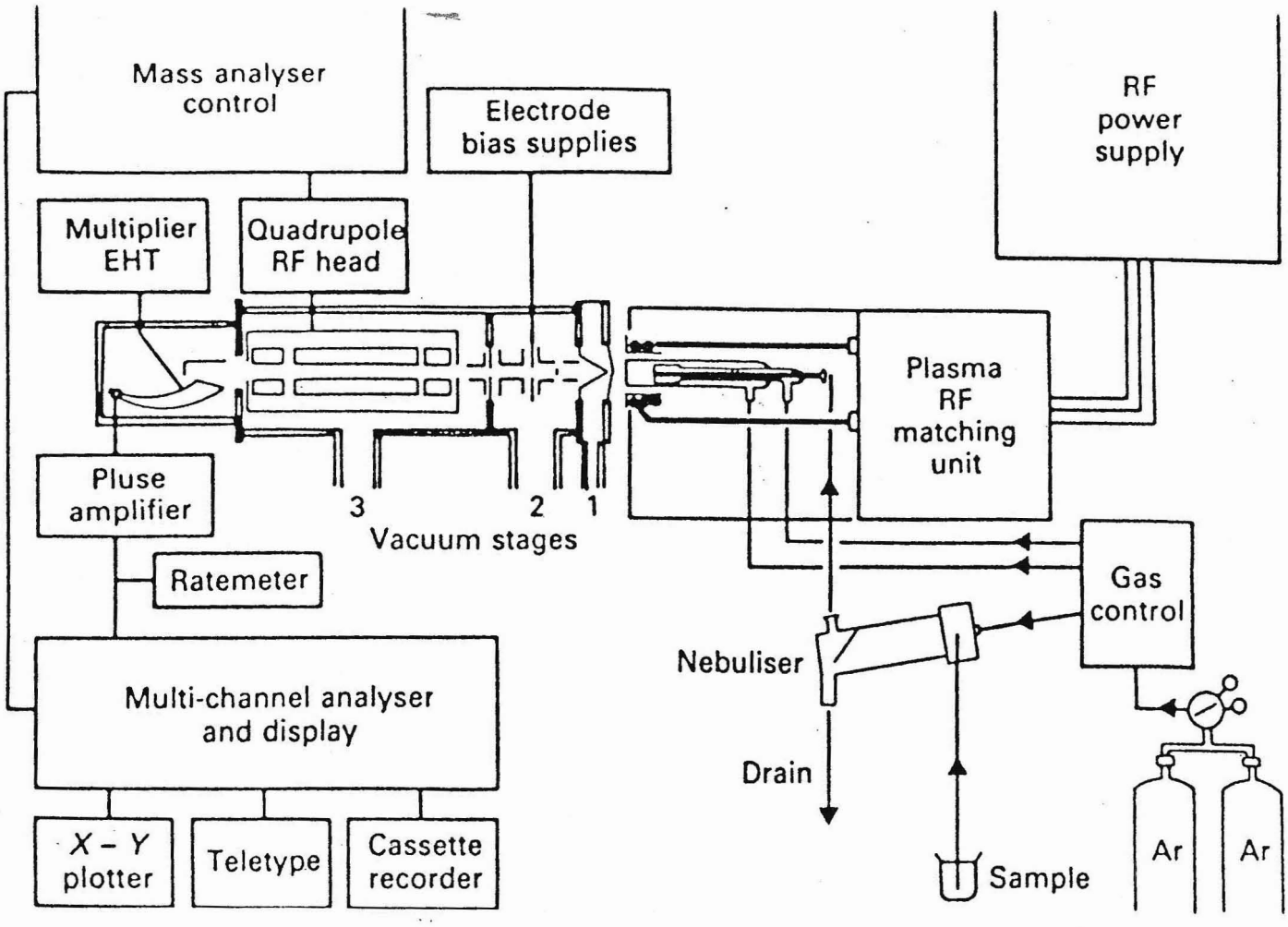


Figure 1.6: Schematic diagram of conventional ICPMS instrumentation.

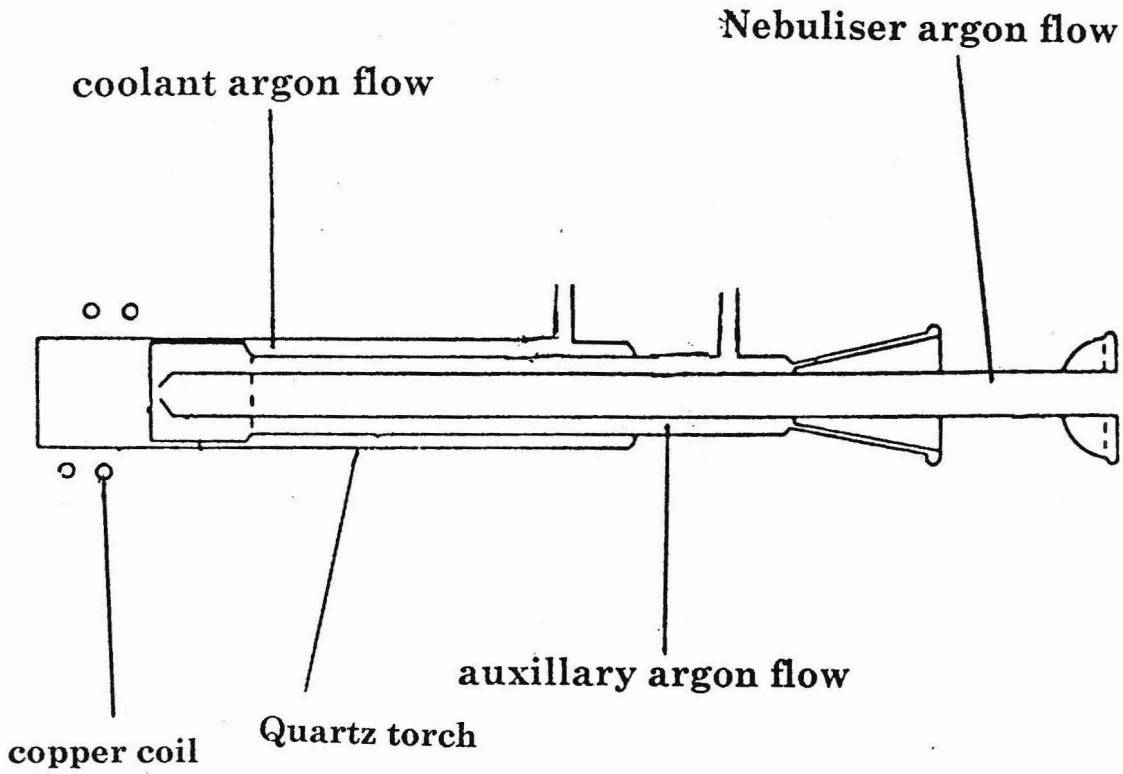


Figure 1.7: Schematic diagram of the ICPMS torch.

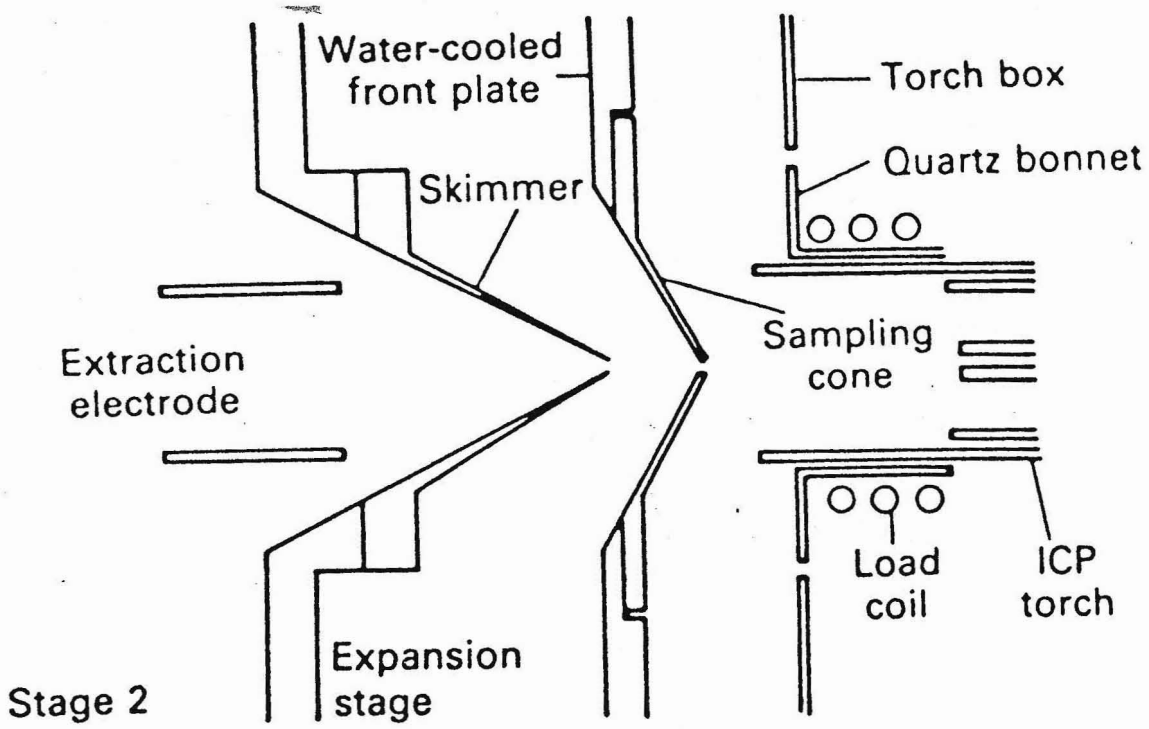


Figure 1.8: Schematic diagram showing the ICP-MS interface region.

is that there is no ionisation source within the MS unit and the ions analysed must be generated in the plasma and survive the extraction and transfer through the various vacuum chambers. The system is controlled by the necessary software loaded on a personal computer.

1.7.3 Mass spectrometry

The mass spectrometer contains a quadrupole which consists of four metal bars which are fixed at the angles of a square. These bars are ceramically isolated and alternately connected to form two couples. Direct current and radiofrequency potentials are applied to the couples and as a mass fragment ion enters the quadrupole it will oscillate in a unique and complex manner according to its mass to charge ratio (m/z) and the RF/DC ratio. Thus, only one mass is able to impinge on the collector at a time while all other mass fragments will impinge on one of the four bars in the quadrupole. By altering the direct current and the radiofrequency it is possible select the ions of interest and hence carry out a selective analysis.

1.7.4 Interference in ICP - MS

The main problems associated with ICPMS are the interferences which can occur. These may be spectroscopic interferences arising from reaction within the plasma or from isotopes of one element having the same mass ions as isotopes of another element. Non-spectroscopic interferences usually arise from matrix effects within the sample. A detailed explanation of these interferences and how to overcome them has been given by Evans and Giglio [47]. The most important of the spectroscopic interferences are reactions of some ions with the argon to form species such as $ArCl^+$, ArO^+, ArC^+ and ArN^+. Moreover, argon can form a cationic dimer, Ar_2^+. These interferences occur in the low mass region and can prevent efficient analysis of low mass metals such as magnesium, aluminium and the first row of the transition metals. Despite these interferences ICPMS is a suitable detector for SFC in the analysis of organometallic compounds.

1.8 ICPMS as a detector for SFC of organometallics

ICPMS has the advantage of being an element specific detector and more specifically a metal selective detector. Thus, it is possible to detect specific organometallic compounds in a sample without showing all of the other organic matter which is usually present. Thus, in analysing an extracted sample of river sediment ICPMS will only show the metals which are present and will not show all the organic compounds as would be shown using FID detection. Thus, in a complex sample, identification is easier as organometallic peaks can still be detected even if they coelute with peaks from organic compounds.

ICPMS also has low detection limits (at the sub-picogram level) suitable for analysis of compounds at trace levels. Thus, organometallic compounds which are toxic at low levels, such as dimethylmercury, can still be analysed with a high degree of accuracy. This ensures adequate monitoring of polluted environments and allows introduction of the necessary preventative measures before other environments become polluted. Moreover, ICPMS is able to monitor a number of ions simultaneously or one single ion. Thus, one metal or different metals can be studied depending on the requirements of the analyst.

Coupling SFC to ICPMS provides a synergistic effect between the two instruments. SFC is capable of separating a variety of compounds at temperatures unacceptably low for GC. The solvating power of the mobile phase ensures specificity for the compounds of interest and careful control of the pressure ramp can provide a good compromise between separation and time. Thus, SFC provides high separating power for the organometallic compounds. ICPMS is a selective detector with low detection limits. Thus, by coupling the two instruments it is possible to

obtain a hyphenated technique for the analysis of organometallic compounds which not only has a high separating ability but is also selective and has low detection limits. Each method alone would be unacceptable for analysing organometallic compounds but together they produce a powerful analytical tool.

Chapter 2

2.1 Introduction

The importance of organometallic speciation and detection at trace levels has already been discussed. However, a survey of the literature reveals the methods which have developed for organometallic analysis and evaluates the advantages and disadvantages of each method. It is important to look at the development of SFC-ICPMS as an acceptable method and discuss its advantages over the other methods currently used. It should be noted at this stage that all the methods currently employed are coupled techniques as no one method is able to offer adequate speciation and good detection limits. Thus, this chapter will examine the current speciation methods (and detection methods) which have been used so far for organometallic speciation and detection. The two main fields of interest have been detection of the separated compounds using a plasma based detector and speciation of organometallic compounds using gas chromatography (GC). Although HPLC has been used with detectors other than plasma based detectors [58], this is not common.

2.2 Gas chromatography with a metal selective detector

The speciation of organometallic compounds using GC has been used for a variety of compounds, with mercury, lead and tin being the most prominent compounds analysed. Baeyens [33] gives a detailed account of the GC methods available for organomercury speciation and stresses the importance of derivatisation to fully substituted organomercury compounds before analysis by GC (preferably *in situ*). The speciation of lead using GC has been studied quite extensively by Lobinski and Adams [48, 49, 34], Chau *et al.* [36, 50], Thompson and Crerar [8] and Harrison and Hewitt [37]. As in the speciation of mercury, all of these studies stress

the need for derivatisation of the samples to the fully alkylated form prior to analysis by GC. Environmental samples have been analysed for organotin compounds by a number of different teams including Jackson *et al.* [39], Chau *et al.* [40], Matthias *et al.* [41], Forster and Howard [42] and Liu *et al.* [38]. As before, all groups have emphasized derivatisation before GC analysis.

As most of the compounds of interest have been partially alkylated compounds, such as triethyllead chloride or dibutyltin dichloride, they must be derivatised to the fully alkylated metal (that is the tetralkyllead or tetralkyltin) before analysis. The two derivatisation methods used are ethylation or pentylation using the Grignard reaction or hydridisation using $NaBH_4$. The purpose of the derivatisation procedure is to increase the volatility of the compounds of interest and hence render them suitable for analysis by GC as the underivatised compounds are usually involatile and would require temperatures beyond the range of normal GC instruments for suitable separation. Moreover, many of these compounds have similar boiling points and for a complex mixture good separation may be difficult.

If the hydridisation technique is used it can be performed in-line with the extraction and analysis as discussed by Matthias. This can eliminate possible experimental error which may arise during off-line derivatisation and transfer of the derivatised sample to the GC. If the Grignard reaction is used it must be performed off-line and hence there is an increased possibility of experimental error. Apart from experimental errors which may arise from the person performing the derivatisation, there may be other unknown factors influencing the result. These include the extent to which all of the compounds have been reacted and side reactions which may also occur. The latter problem is particularly relevant in environmental samples where the composition is unknown and the possibility of reaction of the derivitising agent with purely organic

compounds is greater. This may lead to incomplete derivatisation of the organometallic compounds and thus low results. Even if studies are performed on spiked samples, this usually emulates an ideal sample and results can be inaccurate or imprecise.

Despite the difficulties associated with derivatisation, GC offers good resolution of peaks and is more than adequate for the speciation of organometallic compounds. However, the FID method of detection normally used for GC is not suitable as it is a universal detector for organic compounds and for the analysis of a real sample it will show the many organic compounds present and these may obscure the peaks generated by the organometallic compounds of interest. Thus, GC must be coupled to a sensitive, metal selective detector. The most common detectors used with GC are the atomic emission detector (AED) [48, 38], the atomic absorption spectrometer (AAS) [42, 40, 48 36, 50, 37, 33], the FPD [39, 41], the mass spectrometer (MS) [8], the atomic emission spectrometer (AES) [33, 34], the atomic fluorescence spectrometer (AFS) [33] and the graphite furnace atomic absorption spectrometer (GFAAS) [37]. With all of these detectors there is the difficulty of coupling the detector to the GC.

However, although all of these detectors give adequate results with detection limits in the parts per billion (ppb) range this may not be sufficient for some of the compounds of interest. A series of detectors which is able to detect these compounds at the sub ppb level is the plasma based detectors.

2.3 Various speciation methods with plasma detectors

The plasma based detectors most commonly used are plasma - AED, plasma - AES and plasma - MS where the plasma is either an inductively coupled plasma or a microwave induced plasma (MIP). AED systems have

been used by Liu and Lopez - Avila [51] who coupled capillary zone electrophoresis to MIP-AED. An AES system has been used by Lobinski and Adams (CGC - MIPAES) [1, 35] and Hill (HPLC - ICPAES) [52]. MS systems have been used by Shum *et al.* (LC - ICPMS) [53], Hill (GC - ICPMS) [52], Peters and Beauchemin (GC - ICPMS) [54, 55], Pretorius *et al.* (HPLC - ICPMS and GC - ICPMS) [3, 56] and Ebdon [57].

With all of these systems the detector has the necessary metal selectivity and good detection limits for trace organometallic analysis. Moreover, the methods of speciation are usually adequate for separating the organometallic compounds of interest. However, systems using GC as the speciation method suffer the same problems as previously and the samples must be derivatised before the analysis. Thus, although the detector may be sensitive, the method of speciation may be inaccurate or imprecise because of the reasons discussed in the previous section. With HPLC the need for derivatisation is eliminated and analytes can be analysed without pretreatment of the sample (apart from filtering). However, HPLC consists of normal phase HPLC, reversed phase HPLC, gel permeation HPLC and ion chromatography and it is necessary pay careful attention to the type of HPLC chosen. Moreover, the pH and elution gradient of the mobile phase must be carefully controlled. Although these problems are not insignificant, the greatest problem lies in coupling HPLC to the plasma based system. The introduction of large volumes of organic liquids and sometimes water into the plasma would extinguish the plasma and cause carbon buildup on the sampling cone thus blocking the orifice and preventing the sample from reaching the vacuum chambers (assuming the detector used is ICPMS). Thus, for those groups using HPLC speciation it has been necessary to design elaborate solvent removal systems prior to introduction of the sample into the plasma. Clearly this is undesirable as it can lead to loss of analyte with the solvent during the desolvation process.

Thus, although the current methods are usually adequate and have good detection limits, it is desirable to have a method which is capable of speciating organometallic compounds without excessive pretreatment of the sample. Moreover, it would be an advantage if this method could be coupled to an efficient method of extracting real samples and which has accurate and precise detection limits at the trace level. One possible method which has recently been developed and which has these properties is SFC - ICPMS.

2.4 Supercritical fluid chromatography with inductively coupled plasma - mass spectrometric detection

Although the idea of using a supercritical fluid as a solvent is an old one, its use as a form of chromatography is fairly recent and commercial instrumentation has only been available since 1987. ICPMS is also a relatively new technique and in 1991 Caruso *et al.* coupled ICPMS to SFC for the analysis of organotin compounds [17]. This group then made a detailed study of the method and produced a number of papers [14, 16-18] evaluating this new method.

Close inspection of the method reveals that it has a number of advantages over the other methods and is able to overcome some of the problems associated with them. Using SFC for the speciation eliminates the need for excessive pretreatment of the sample as there is no need for derivatisation and compounds can be analysed as is. If a modifier is added to the mobile phase then this method is universal except for the most polar organometallic compounds. It is also suitable for compounds with a wide range of boiling points and because it relies on density for separating compounds rather than temperature it can separate thermally labile and involatile compounds. SFC is usually fast compared to GC and HPLC and so a number of analyses can be done in a relatively short period of time.

The greatest advantage of SFC is its easy coupling to the SFE extraction system and many commercial systems have an SFE system built into the SFC. Thus, samples can be extracted and analysed on-line with minimal handling.

ICPMS offers good detection limits at trace levels and is a metal selective detector. Thus, by coupling the two instruments together it is possible to have on-line extraction and analysis of diverse compounds at trace levels. The metal selectivity of ICPMS allows for the possibility of isotope dilution analysis and monitoring of a range of metals or monitoring of various species of one metal. However, the greatest advantage of coupling the two instruments is the method of sample introduction into the plasma. In stand alone ICPMS the sample is first nebulised and < 2% reaches the plasma. However, in SFC-ICPMS the sample is introduced directly into the plasma and so a larger proportion of the sample enters the detector. Thus, the detection limits are improved even further and detection limits at the sub picogram level have been reported. [14, 16-18].

Thus, Caruso's work sparked an interest in SFC as a method for organometallic speciation although other detectors were usually used [21, 22]. However, the interface developed by Caruso's group for coupling SFC to ICPMS, although efficient and more than adequate, appears bulky and requires the nebuliser to be removed from the torch box and an external source of make-up argon to be introduced [15]. A diagram of Caruso's interface is shown in Figure 2.1. Thus, in this study a new interface was developed which it was hoped would improve on the interface presently used. The design and evaluation of a new interface and the use of SFC-ICPMS for the analysis of a number of organotin, organoarsenic and other organometallic compounds is presented in the following chapters.

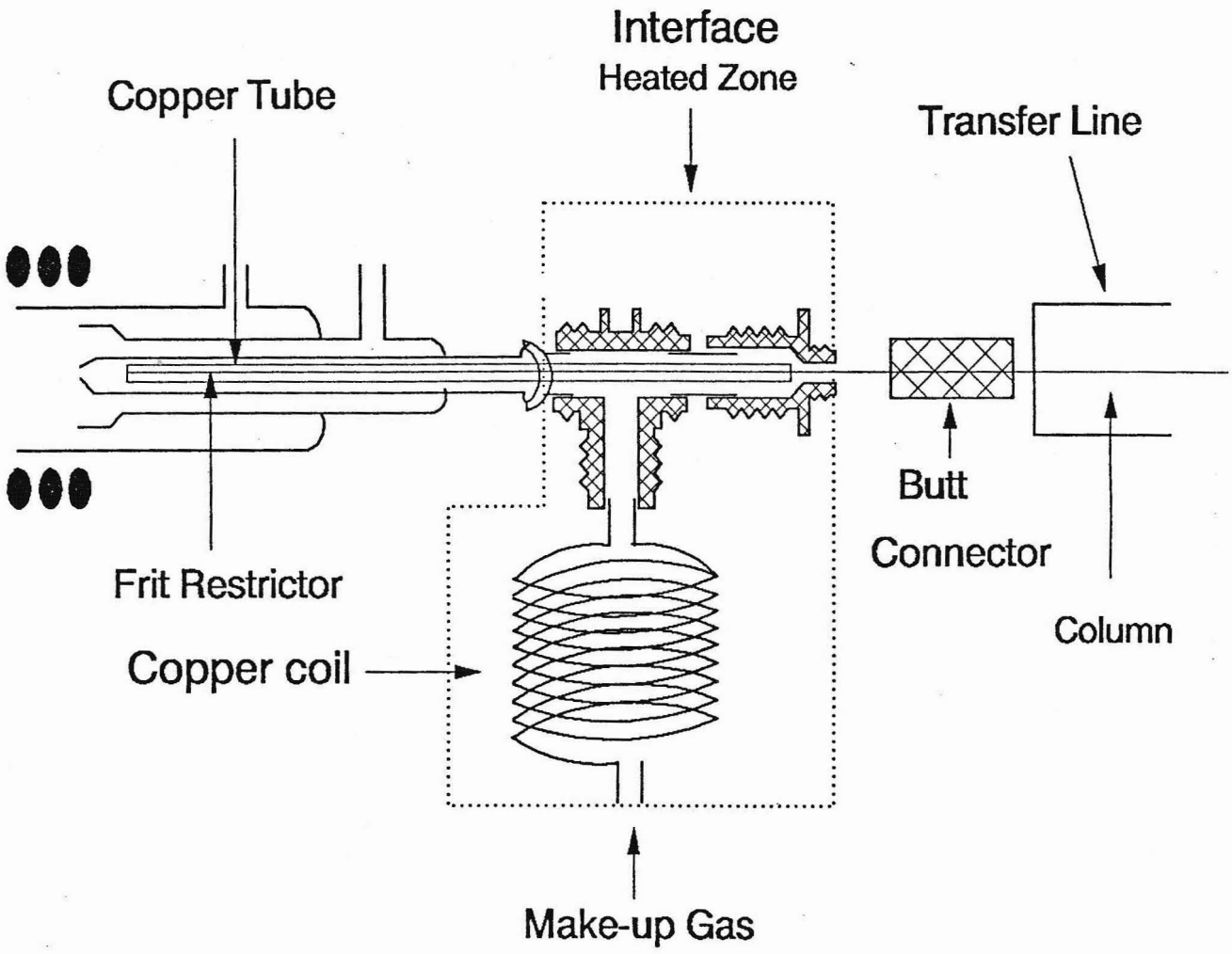


Figure 2.1: A schematic diagram of the interface used by Caruso to couple SFC to ICPMS.

Chapter 3

3.1 Supercritical fluid chromatography

3.1.1 Hardware Requirements

A schematic diagram of the supercritical fluid chromatograph is shown in Figure 1.3. The oven was a Lee Series 600 GC/SFC oven with SFE accessory from Dionex (Sunnyvale, CA, USA). The mobile phase was pressurised using a syringe pump and controlled by a computer. The whole system was a Lee Scientific Series 600 chromatograph from Dionex (Sunnyvale, CA, USA). The computer was loaded with DOS 3.3 (Microsoft Corporation, Redmond, USA) and the instrument was controlled by 600 Series Chromatograph Control Firmware v2.60 and Series 600 User/Instrument Interface Software v2.11 (Dionex, Sunnyvale, CA, USA).

The injector used to inject the sample onto the column was a Valco AC14UWP injector (Valco Instruments Co. Inc., Houston, USA) with a 200 nl internal volume rotor. All injections were time split injections with a 0.100 s injection time. The injector was activated by helium. The injector and pump were cooled to 7 °C by a Neslab Endocal RTE 110 cooler (Neslab Instruments, Newington, USA). Experiments for optimising SFC conditions were carried out using a flame ionisation detector heated to 350°C. The gas flows to the FID were approximately 300 ml/min air and 30 ml/min hydrogen with nitrogen make-up gas. Each day after igniting the flame, the detector was allowed to stabilise and it was then autozeroed. All tubing used to connect the oven, the pump and the mobile phase cylinder was 0.0016 m ($^1/_{16}$") or 0.0032 m ($^1/_8$") internal diameter stainless steel tubing. The tubing from the oven to the helium cylinder was made of poly(tetrafluoroethylene) (PTFE).

3.1.2 System Reagent Requirements

The helium used to activate the injector was pure helium (> 99% purity) from Fedgas (Durban, South Africa). The cooling fluid used by the cooler to cool the injector and the pump was 1% methanol in water. The mobile phase was SFC grade carbon dioxide from Air Products (Allentown, USA). The synthetic air and the hydrogen for the FID were reagent grade gases from Fedgas (Durban, South Africa).

3.1.3 The Capillary Column

Two columns were used in this study. In optimising the SFC conditions, a 10 m SB-Biphenyl-30 capillary column from Dionex (Sunnyvale, CA, USA) was used. This had an internal diameter of 50 μm and a film thickness of 0.25 μm. As will be shown in the results this column showed significant activation during the study and all subsequent analyses using the ICPMS as a detector were performed using a 2 m SB-Biphenyl-30 column (Dionex, Sunnyvale, CA, USA) with a 50 μm internal diameter and a 0.25 μm film thickness. The same problems encountered with the 10 m column were experienced with the 2 m column (that is activation with time). The end of the column which entered the injector was housed in a $^1/_{16}$" o.d. 200 μm i.d. polyetheretherketone (PEEK) tube approximately 120 mm long and it was secured in the injection port by a nut fitted with a PEEK ferrule (Rheodyne, Cotati, CA, USA).

3.2.4 The Restrictor

The restrictor was made of 50 μm internal diameter deactivated fused silica capillary tubing (SGE, Australia) by drawing out the end of a piece of the tubing in a bunsen flame [59]. The end drawn out in this way formed a tapered restrictor similar to type C shown in Figure 1.4. The

tapered end of the restrictor was covered with 320 μm internal diameter deactivated fused silica capillary tubing (SGE, Australia) and this covering was sealed to the restrictor contained within using polyimide resin (Chemical Research Supplies, Addison, USA). The restrictor was connected to the column using a zero-dead volume butt connector (SGE, Australia). For SFC optimisation experiments the restrictor was placed into the FID and secured with a nut and ferrule. The distance from the point where the restrictor was fixed with the nut to the end of the restrictor within the FID was approximately 55 mm.

3.1.5 Optimisation of the Chromatographic Conditions

In order to determine the optimum chromatographic conditions for evaluating the SFC-ICPMS interface, a series of experiments were first carried out using FID detection. Individual 10 000 μg/g standards of tetrabutyltin, tributyltin chloride, dibutyltin dichloride, tetraphenyltin, triphenyltin chloride, bis(tributyltin)oxide (TBTO), methylmercuric chloride, ferrocene, triphenylarsine and bis(1,2-diphenylarsino)ethane (BDPAE) in dichloromethane were injected to determine whether these compounds could be analysed and if so what their retention times would be. Samples of tributyltin hydride, dibutyltin dihydride, butyltin trihydride and diethylmercury were prepared according to the procedures discussed later and these compounds were also injected. After each compound had been checked individually a series of mixtures which were 10 000 μg/g in each compound were also prepared and analysed. The mixtures which were considered are shown in Table 3.1. Standards and mixtures were prepared approximately one hour before analysis and were discarded at the end of each day. Each mixture was analysed in duplicate at pressure ramps of 0.5, 1, 2, 2.5, 3, 3.5, 4 and 5 MPa/min. After studying the effects of the pressure program on the mixtures, pressure programs of 3 and 3.5 MPa/min were selected and analyses were carried out for each pressure program at temperatures of 40, 50, 60, 70, 75, 80 and 100 °C.

Table 3.1: The different mixtures used in optimising the SFC conditions.

Mixture	Components
1	Bu_4Sn, Bu_3SnCl
2	Bu_4Sn, Bu_3SnCl, Ph_4Sn, Ph_3SnCl
3	Bu_3SnH, Bu_2SnH_2, $BuSnH_3$
4	Bu_4Sn, Bu_3SnCl, Bu_3SnH, Bu_2SnH_2, $BuSnH_3$, Ph_4Sn, Ph_3SnCl, BDPAE

3.1.6 Supercritical Fluid Extraction

The line carrying the mobile phase from the pump to the extraction vessel was $^1/_{16}$" i.d. stainless steel tubing. The extraction vessel was made of stainless steel and had a volume of approximately 400 mm^3. The mass being analysed will depend on the sample. The extraction vessel was held in the extraction unit contained on the oven. All SFE experiments were performed off-line. The SFC was set up so that the CO_2 could be passed through the extraction vessel without eluting on to the column. The restrictor was a piece of 10 μm i.d. fused silica capillary tubing which also served to transfer the analytes to the collection solvent. The collection solvent (dichloromethane) was contained in a 10 ml volumetric flask which was placed in an ice bath and the restrictor was placed approximately 20 mm into the solvent. A schematic diagram of the SFE is shown in Figure 3.1

The extraction vessel was filled with topsoil samples spiked with tetrabutyltin. Dynamic and static extractions were carried out at a temperature of 70 ^0C and pressures of 35, 40 and 41 MPa. Each extract was then made up to 10 ml and analysed using a pressure program of 3.5

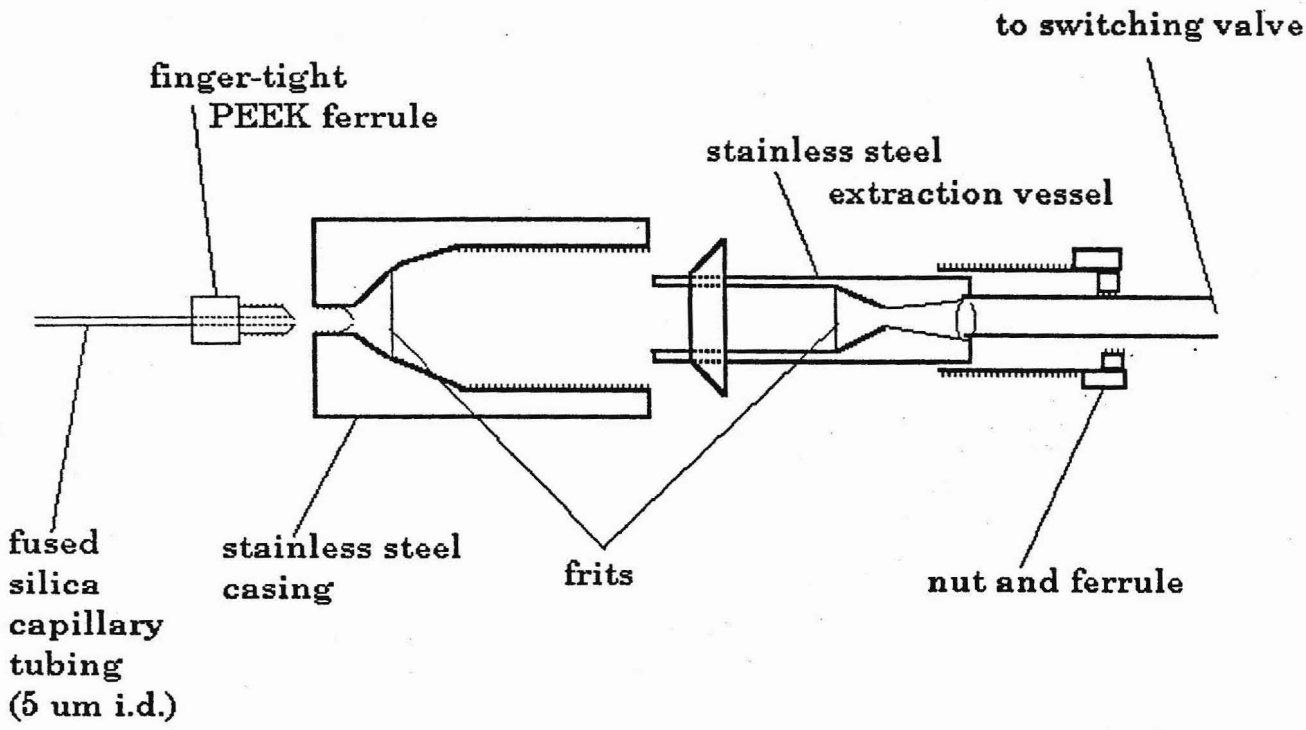


Figure 3.1: Schematic diagram of the supercritical fluid extraction system.

MPa/min at 70 °C. Similar extractions were attempted for bis(1,2-diphenylarsino)ethane and ferrocene. All analyses were performed using the FID as detector.

3.1.7 Analysis of Metalloporphyrins

A series of nickel, vanadium and tungsten metalloporphyrins were obtained from Warren Pretorius of the University of Plymouth. These compounds (1000 μg/ml standards) were analysed at 70 °C with a pressure program of 3.5 MPa/min. The initial pressure was 12 MPa (hold for 1 min) and the final pressure was 41 MPa (hold for 5 min). The column used was a 10 m SB-methyl column (Lee Scientific, Salt Lake City, USA) with a 50 μm internal diameter and a 0.25 μm film thickness. All analyses used flame ionisation detection at 350 °C.

3.2 Inductively Coupled Plasma - Mass Spectrometry

3.2.1 Hardware Requirements

The ICPMS was a VG PlasmaQuad from VG Elemental (Cheshire, UK). The argon temperature was controlled by a Neslab Endocal RTE 100 water bath (Neslab Instruments, Newington, USA). The system was controlled by an IBM personal computer loaded with the VG PlasmaQuad software. The argon was > 99% purity and was supplied by Fedgas (Durban, South Africa). A quartz Fassel design torch was used. A schematic diagram of the system is shown in Figure 1.6.

3.2.2 Analysis Conditions

The forward power was maintained throughout the study at 1.35 kW which is within the normal operating range of 1.2 to 1.5 kW. The reflected power was constantly monitored and was usually < 5 W but never more than 20 W. The nebuliser argon (used as make-up gas) had a flow of 0.8 L/min, the auxillary argon had a flow of 0.5 L/min and the coolant argon flowed at 14 L/min. The carrier pressure was 0.16 MPa (1.6 bar). The expansion chamber was maintained at 200 Pa (2 x 10^0 mbar), the intermediate stage was maintained at 0.02 Pa (2 x 10^{-4} mbar) and the analyser was maintained at 4 x 10^{-4} Pa (4 x 10^{-6} mbar). All analyses were performed in single-ion monitoring mode at 2047 channels with varying dwell times. Altering the dwell time while keeping the number of channels constant allows the scan time to be altered according to the analysis time of the SFC. The lens settings were optimised as described in the following section.

3.2.3 Focusing on the Element of Interest

In order to maximise the response of the detector, it was necessary to focus the ICPMS on the element of interest. At the beginning of the study the lens settings were returned to their preset values as suggested by the supplier and shown in Table 3.2. A 100 μg/ml standard solution of indium in HNO_3 was then passed through the instrument (which was disconnected from the SFC) and the lenses were focused on the largest of the two indium peaks. The settings obtained for indium are shown in Table 3.3. The multiplier, cone bias, extraction 2, pre-filter and differential curvature were always kept constant and their settings are shown in Table 3.4.

Table 3.2: The preset values for the ICPMS lenses as suggested by the suppliers.

Lens	Setting
L3	100
L1	770
Extraction 1	100
L2	540
L4	380
Collector	770
Front plate	800
Pole bias	600

Table 3.3: The ICPMS lens settings obtained for 100 μg/ml indium.

Lens	Setting
L3	474
L1	494
Extraction 1	200
L2	411
L4	349
Collector	480
Front plate	483
Pole bias	530

Table 3.4: Lens settings which remain unaltered throughout the study.

Lens	Setting
Multiplier	650
Cone bias	0
Extraction 2	46
Pre-filter	227
Differential curvature	0

If the ICPMS was to be used to detect tin containing compounds, then a few sacrificial SFC-ICPMS runs of one of the standard mixtures of tin compounds were made with the lenses on their indium settings. Immediately after the last peak in the tin mixture had eluted the lenses were adjusted in the order L3, L1 (these two are adjusted using an iterative procedure), extraction 1, L2, L4, collector, front plate and pole bias. As the lens settings were adjusted, there was a corresponding increase or decrease in the baseline and on the ratemeter. The lenses were therefore adjusted until no further increase in the baseline or on the ratemeter was observed. As the focusing is carried out on the back of a peak (that is during peak tailing) there is not much time to optimise these conditions in one analysis. Thus, three to four sacrificial analyses were carried out before moving on to the samples of interest. Focusing on indium was carried out at about six week intervals whereas focusing on the element of interest was carried out each day, even if the element being analysed was the same as the previous day.

3.3 The SFC-ICPMS Interface

3.3.1 Materials Required

The interface was a modification of the elbow joint between the nebuliser and the torch in the ICP torch box. This was assembled from standard concave and convex ground glass joints. The assembly is shown in Figure 3.2. A stainless steel metal tube for holding the transfer line was made from an SFC splitter assembly with the side arm removed. The restrictor was housed in a glass melting point tube onto which was wound a nichrome wire filament (GM Heating, Durban, South Africa) attached to a low voltage DC power supply. The restrictor was heated by applying a low DC voltage across the filament. The filament was insulated with polyimide resin (Chemical Research Supplies, Addison, USA) and the wires leading from the filament were coated with shrinksleeve. The transfer line was an extension of the restrictor and was attached to the column using a butt connector.

3.3.2 Design and Assembly

The two ground glass joints were assembled as a T-join as shown in Figure 3.2. The stainless steel tube was inserted into this T-join so that it protruded approximately half way into the inner tube of the torch. The melting point tube was wound with the nichrome wire filament and fixed to the plasma end of the stainless steel tube using polyimide resin. The wires from the filament were placed flush with the stainless steel tube and exited the T-join where the stainless steel tube was inserted. This end of the T-join was then sealed with 372 epoxy to create a gas-tight seal. This prevented loss of make-up argon and prevented air from entering the torch and extinguishing the plasma. The restrictor was inserted into the

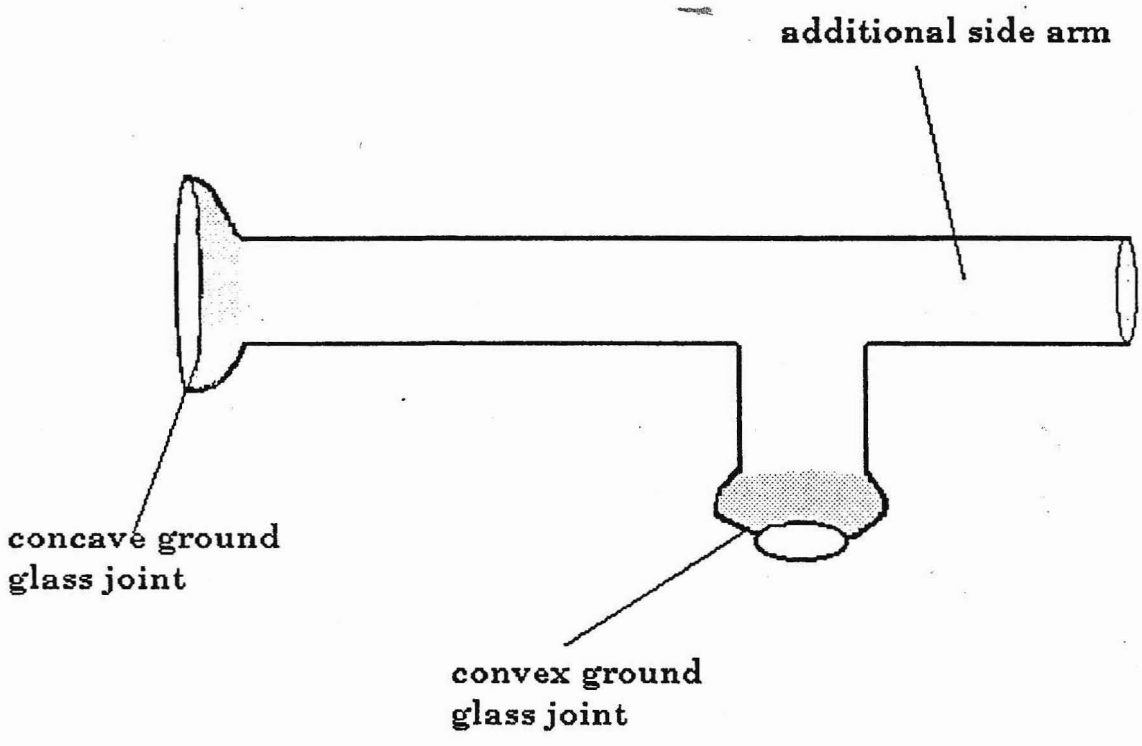


Figure 3.2: The modified elbow join between the torch and the nebuliser in the ICPMS.

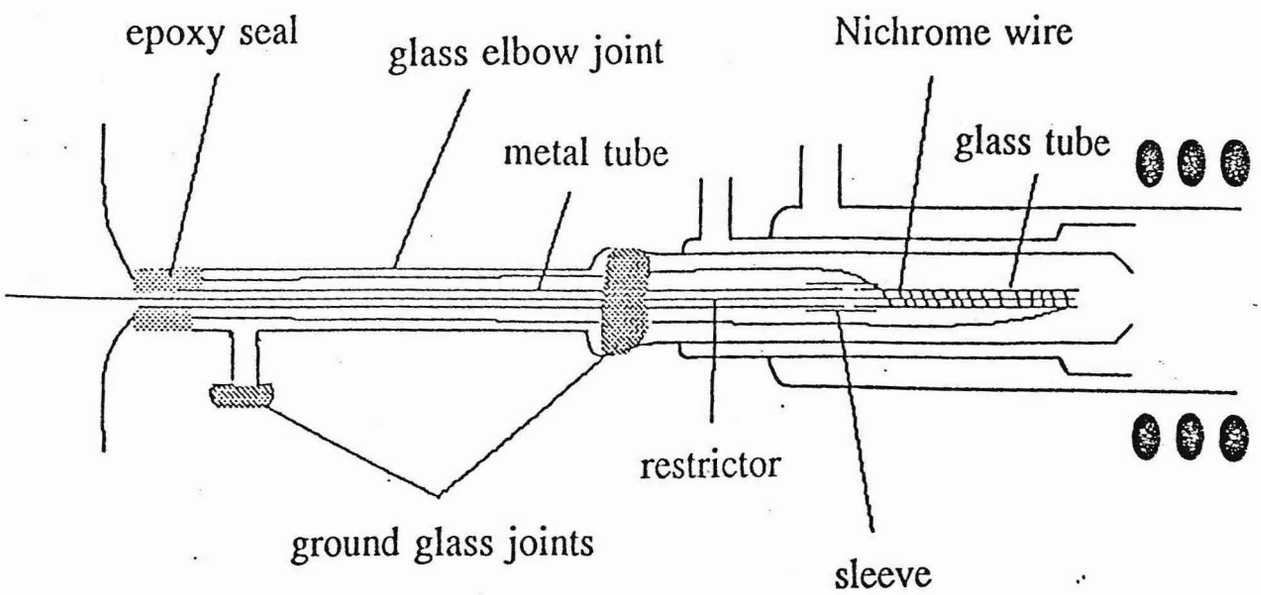


Figure 3.3: A detailed schematic diagram of the SFC-ICPMS interface.

interface so that the end of the restrictor was flush with the plasma end of the melting point tube. The restrictor/transfer line was fixed in place using a nut and vespel ferrule (SGE, Australia). A detailed diagram of the interface is shown in Figure 3.3. The measurements of the interface will depend on make and model of the ICPMS but the design should remain the same. Moreover, some flexibilty is possible as the nebuliser is able to move slightly (although this is not recommended) but the torch should remain fixed. The wires from the filament were attached to the DC power supply and by altering the applied voltage on the filament the temperature could be reasonably controlled.

3.3.3 Temperature - voltage calibration

In order to determine the relationship between the voltage applied to the filament and the temperature of the restrictor the following procedure was used. The torch was removed from the ICPMS and placed on the benchtop and the interface was inserted into the torch. A thermocouple was inserted into the torch next to the filament and the filament was attached to the DC power supply. Starting with an initial applied voltage of 0 V the voltage was increased in 0.5 V increments and the temperature at each successive voltage was measured. At least 45 minutes was left between each increment increase in the voltage and the temperature measurement in order to allow for equilibration of the applied voltage and the temperature.

3.3.4 Evaluation of the Interface Temperature on SFC-ICPMS

To determine the effect of the interface (restrictor) temperature on the analysis the nebuliser (make-up) argon was maintained at 60 ^{o}C. Mixture 1 was injected and the analysis was carried out with the applied voltage on the interface at 6.0 V. After duplicate analyses at this voltage, the

applied voltage on the interface was increased to 6.5 V. At least 30 minutes was allowed for the temperature to equilibrate and mixture 1 was again analysed in duplicate. This procedure was repeated for applied voltages of 7.0, 7.5, 8.0 and 8.5 V. The peak intensities were then graphed as normal x-y plots on a normal scale and on a logarithmic scale. All graphs were drawn using Quattro Pro for Windows© (Borland International, Scotts Valley, USA). This procedure was repeated for standards of triphenylarsine and ferrocene.

3.4 Analysis of Organometallic Compounds

Initially tin mixture 2 was analysed but for reasons to be discussed later all subsequent analyses were carried out on mixture 1. A standard mixture of 1000 μg/g was prepared in dichloromethane and standards of 100, 10, 1, 0.1, 0.01 and 0.001 μg/g were prepared by successive dilution. After focusing the ICPMS on $^{120}Sn^+$ each standard was analysed in duplicate with the make-up argon at 60 ^{0}C and the applied voltage on the restrictor at 8.5 V. The average peak intensity was then plotted against concentration. The same procedure was repeated for triphenylarsine and ferrocene standards with the ICPMS focused on $^{75}As^+$ and $^{56}Fe^+$ respectively ($^{54}Fe^+$ was also used as will be discussed later). All analyses were carried out from the lowest concentration to the highest concentration and a solvent injection was made between each concentration standard. Focusing the instrument was performed using the 10 μg/g standard but after focusing at least three solvent injections were made and a blank run was performed to ensure that there were no memory effects. A duplicate analysis of diethylmercury was also performed with the ICPMS focused on $^{200}Hg^+$. However, as this compound was prepared by derivatisation, it was not pure and the concentration was unknown. Thus, analysis at different concentrations was not performed.

3.5 Effect of the Mobile Phase on the Plasma

To monitor the effect of the mobile phase and the organic solvent on the plasma an injection of solvent was made using the same chromatographic conditions as for the organometallic standards. Duplicate injections were made and the analyses were performed with the ICPMS focused on Ar_2^+, ArO^+, ArN^+ and ArC^+. With the instrument focused on Ar_2^+ the analysis was carried out both with and without a solvent injection.

3.6 Reagents

Tetrabutyltin > 98% purity (Aldrich, Milwaukee, USA) was donated by Mr Bob Stanton, CSIR, Durban, South Africa. Tributyltin chloride (> 90% pure), dibutyltin dichloride (> 97% pure) and tetraphenyltin (> 97% pure) were from Janssen Chimica (Geel, Belgium). Triphenyltin chloride (> 98% pure) and bis(tributyltin)oxide were obtained from Merck (Darmstadt, Germany). Triphenylarsine (> 90% pure) and ferrocene (> 90% pure) were also obtained from Merck (Darmstadt, Germany). Dichloromethane was from Holpro Lovasz (Midrand, South Africa). Bis(1,2-diphenylarsino)ethane and methylmercuric chloride was donated by Dr B. Martincigh, Department of Chemistry and Applied Chemistry, University of Natal, Durban, South Africa. $NaBH_4$ was from Merck (Darmstadt, Germany), magnesium metal turnings were from Saarchem (Krugersdorp, South Africa) as was the 1,2-dibromoethane. Diethyl ether (dried over sodium) was from BDH (Poole, England).

3.7 Derivatisation Procedures

Tributyltin hydride was prepared using the following procedure:
5 µl of tributyltin chloride was added to 100 ml of deionised water in a separating funnel. 10 ml of dichloromethane and 20 ml of 4% (aqueous,

alkaline) $NaBH_4$ was added. The mixture was shaken by hand for at least 5 minutes and the layers were then allowed to separate. The aqueous layer was removed and the organic layer was washed with 20 ml of deionised water. The organic layer was then taken for analysis.
Butyltin trihydride was obtained in the same way. Dibutyltin dihydride was obtained in a similar manner but instead of 5 µl of reagent, 1.3 mg was used. To obtain a mixture of all three compounds, 5 µl each of tributyltin chloride and butyltin trichloride and 1.3 mg of dibutyltin dichloride was added to 100 ml of deionised water and the procedure continued as before. This is a modification of the procedure used by Forster and Howard [42].

Diethylmercury was prepared using the following procedure:
A dry 250 ml round-bottom flask was fitted with a dry condenser suitable for reflux. Filtered 1,2-dibromoethane (10.5 ml) was dissolved in 50 ml of anhydrous ether and about half the solution was placed in the round-bottom flask. The remainder of the solution was kept in a well corked Erlenmeyer flask. Magnesium metal turnings (2.5 g) were added to the solution in the round-bottom flask and a small crystal of iodine was added. After a few minutes the reaction began spontaneously and the solution boiled vigorously. As the boiling subsided approximately 10 ml of the remainder of the ethereal solution was added and this was continued until all of the ethereal solution had been used. After all of the ethereal solution had been added the mixture was warmed on a steam bath for about 15 minutes to ensure completeness of the reaction. Mercuric chloride (about 5 g) was then added to give a saturated solution and the mixture was reheated for about 20 minutes before being cooled in an ice bath. The cool mixture was then poured into a mixture of ice (~ 100 g) and dilute sulphuric acid (~ 60 ml). The diethylmercury was then extracted into dichloromethane and washed five times with deionised water. This procedure is a modification of the procedure given by Mann and Saunders [71]. Although the reaction should be carried out under anhydrous

conditions, the presence of slight traces of water did not appear to hinder the progress of the reaction.

3.8 Sample Collection

Sediment samples were collected from different areas in Durban Bay as shown by the points 1 to 7 marked on the map in Figure 3.4. Two samples were taken from the area adjacent to the mangroves, one sample was taken from the dredge spoil, two samples were taken from the southern sand bar opposite the graving docks, one sample was taken from the yacht mole and one sample was taken from the northern sand bar as close as possible to the main channel. All samples were collected at low tide (11h25) on Thursday 16 December 1993.

The sampling was performed using a cone dredge supplied by the CSIR, Durban, South Africa. A diagram of the cone dredge is shown in Figure 3.5. The cone dredge was attached to a 5 m length of rope and was rinsed with water from the bay. The dredge was then thrown to the extent of the rope before being hauled back in, thus collecting sediment from the bay as it was dragged along. A sample of the sediment was then sealed in a labelled 500 ml glass jar.

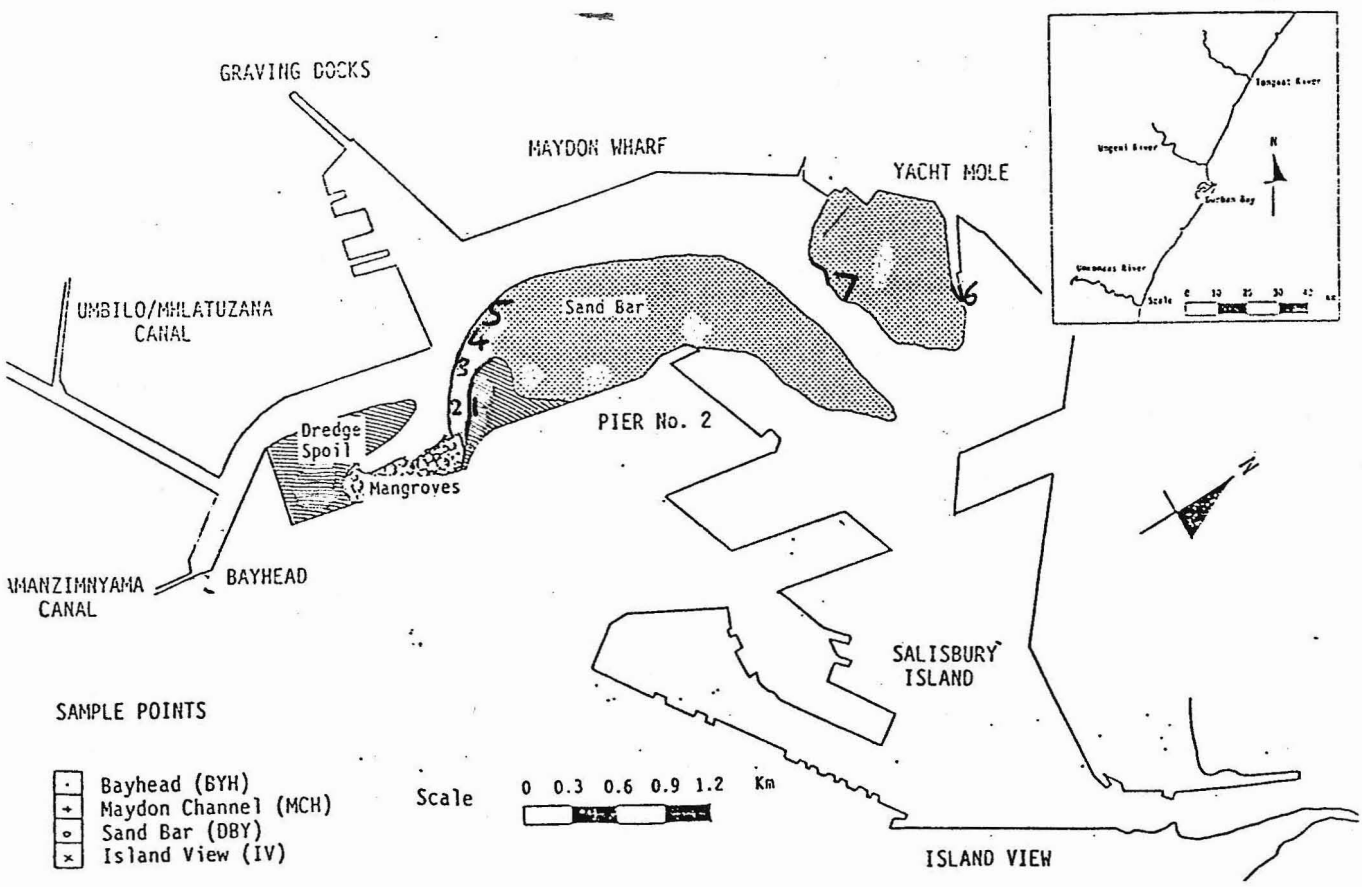


Figure 3.4: Map of Durban bay showing the sites from which samples were taken.

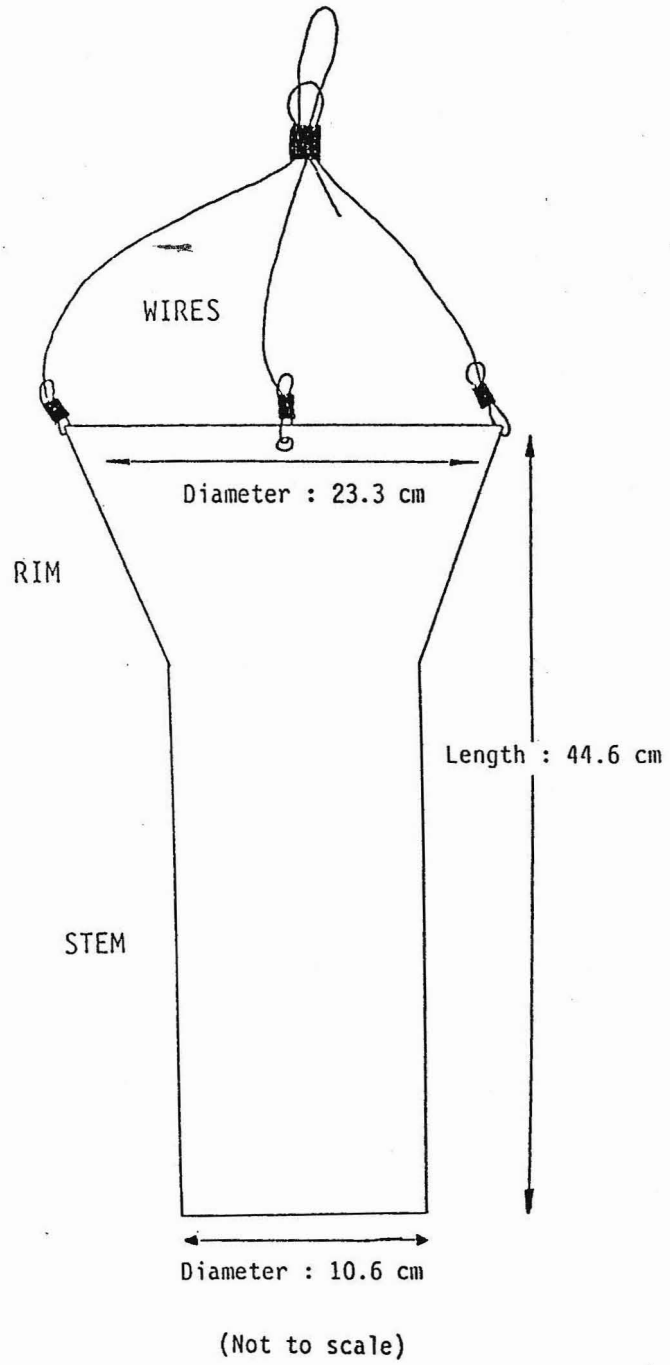


Figure 3.5: A schematic diagram of a cone dredge.

3.9 Sample Preparation and Analysis

A portion of each sample (50 g) was removed from the jar and placed on a watchglass. These 50 g samples were then dried in an oven at 70 ^{0}C for 72 hours. After drying each sample was reweighed and then sifted through a 50 mesh sieve. Each sample was then ground in a mortar and pestle to further reduce particle size. A small portion (about 5 g) of each dried, sifted sample was taken for extraction and analysis. Each sample was placed in the extraction cell and statically extracted with SFC grade CO_2 for 30 minutes. This was followed by a 20 minute dynamic extraction into 10 ml of dichloromethane. This procedure was performed twice on each sample. Extraction was carried out at 75 ^{0}C and 41 MPa. Due to the time limitations of the study it was not possible to attempt soxhlet extraction of the sediment samples. As it was unknown what elements were in the sample, each sample was first analysed by SFC-ICPMS using a survey scan for all elements. The instrument was programmed to do a number of scans throughout the duration of the analysis in an endeavour to detect all elements which were eluting from the SFC. Single-ion scan chromatograms of the most abundant elements were then obtained.

Chapter 4

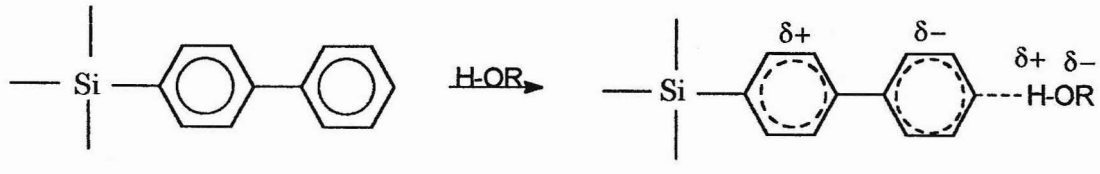
4. 1 SFC Configuration

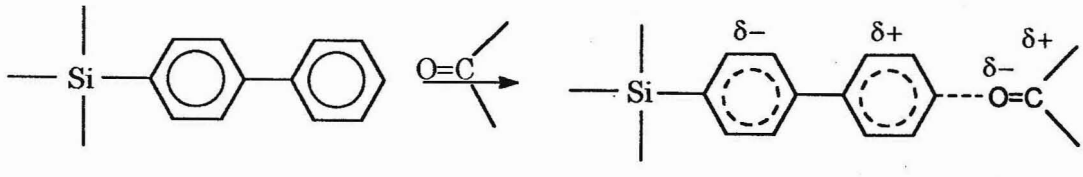
The SFC instrument was set up as close to the ICPMS as possible but in a way similar to that shown in Figure 1.3. The main problem experienced with the operation of the SFC was the contoller software as it was sensitive to power spikes and would terminate the program and exit to DOS at any hint of power fluctuation. This was particularly relevant when the SFC was coupled to the ICPMS as igniting the plasma would invariably cause the SFC software to crash. The only solution to this was to switch off the SFC each time the plasma was ignited. Another major problem initially experienced was the reluctance of the chromatographic software to load from DOS and it usually took several attempts before the software would load. This problem was more noticeable as the study progressed. The checkdisk command (from DOS) was run as was a system testing program. Both programs revealed a number of lost clusters and some bad sectors on the hard drive. After backing up all files, the chromatographic software was reloaded and thereafter the chromatographic software was able to load from DOS without any problem.

A time split injection of 100 ms was chosen as any time greater than this gave excessively large solvent peaks. Near the end of the study the injector rotor became scored and had to be replaced. The PEEK ferrule was easily distorted if overtightened and made column installation difficult. During SFC optimisation, the FID was heated to 350 ^{o}C in order to ensure complete volatilisation of the analytes as they eluted from the restrictor. The hydrogen and air flows were set at the beginning of the study and the settings left unaltered throughout with no significant change in the ability of the detector to ignite being observed. The detector

heater failed near the end of the study and had to be replaced with a new one. The cooler was observed to fill up with water fairly rapidly and excess water had to be removed approximately once a week. Near the end of the study the rupture disk in the syringe pump was blown and the setup had to be changed to temporarily bypass the rupture disk.

The SB-Biphenyl-30 column was chosen as it was decided that this would give the best separation for the wide variety of organometallic compounds which would be analysed. Although the biphenyl group is non-polar it has mobile electrons and thus a dipole can be induced on it. This makes it a good choice for analysing both polar and non-polar organometallic compounds. Non-polar compounds will pass along the non-polar column fairly rapidly and thus elute first. More polar compounds passing along the column will induce a dipole on the biphenyl groups and thus dipole - induced dipole interactions will ensure that the polar compounds are retained longer. Thus, the more polar the compound the longer it will be retained on the column. Another advantage of the biphenyl column is its ability to form an induced dipole with either electron donating or electron withdrawing groups as shown below:





A dramatic increase in peak broadening and tailing was observed as the

study progressed. This is indicative of an increase in column activity and degradation of the column by the organometallic compounds. Similar behaviour was observed on high temperature GC columns by Pretorius *et al.* [4]. A tapered restrictor made in the laboratory was used primarily due to the high cost of frit restrictors. However, recent work by Pinkston *et al.* [72] has shown that for analysis of involatile compounds at high elution pressures frit restrictors perform inadequately. Although the tapered restrictor was adequate its main disadvantage was the difficulty encountered in installing it. During installation the taper had to be cut back to a point at which the desired flow rate was achieved. This was a delicate procedure which more often than not resulted in the flow rate being slightly high. Moreover, the 320 μm tubing which covered the restrictor was prone to coming loose.

4.2 ICPMS Configuration

The ICPMS was set up in the standard way and apart from the interface no changes had to be made to the instrumentation. Although the instrument worked well there were three recurring problems which plagued the study. The first problem was that on occasions the plasma would fail in the middle of an analysis and would not re-ignite. This was due to a fuse in the RF generator which fused quite often. Initially this was a 15 mA fuse but it was found that by replacing it with a 25 mA fuse the problem was alleviated.

The second problem which occurred was the plasma's reluctance to ignite. Although it ignited first time when disconnected from the SFC, when it was connected to the SFC it took several attempts to ignite the plasma. This can be attributed to a change in the flow profile of the make-up gas around the interface. Whereas the initial setup has an unhindered flow of gas, introducing the interface causes a resistance to the normal flow. This

causes eddy currents to form within the flow stream thus making it unstable and causing a slight resistance to the argon flow. Although this occurs in the make-up stream of the gas flows, this instability may extend into the plasma region thus preventing the plasma from igniting. It is possible to speculate on a variety of reasons for this failure in the plasma (including changes in the flow profile of the argon) but further study of this phenomenon is needed.

The third problem was with the detector which would often switch off for approximately one minute during a run. This caused a "gap" in the chromatogram during the time in which the detector was inactive. Although this was tolerable (but not acceptable) if it occurred away from any peaks, if it occurred near or during a peak, the result was useless and the analysis had to be repeated. Moreover, this was completely unacceptable for analysis of real samples where the composition was unknown. Although this problem did not occur during survey scan it was prevelant during single-ion scans. Moreover, the problem was greater after the instrument had been running for periods longer than about three hours. Initially it was thought that this problem was caused by a build up of charge within the instrument but this was investigated and no conclusions could be reached. This problem was observed before coupling to SFC and was a problem inherent in the ICPMS instrument. Unfortunately, the cause of this problem could not be determined during the study and hence the problem could not be rectified. Thus, it was tolerated thoughout the study although only results where it was known that "switch off" occurred well away from any peaks were treated as reliable.

4.3 Interface Design

In the initial design of the interface a number of factors had to be considered. These were:

a) The restrictor should be placed as close as possible to the plasma to ensure complete transfer of the analytes into the plasma.

b) The interface should contain as few metallic components as possible. This was considered necessary as it was feared that metal within the torch might cause arcing between the plasma and the metal and thus hinder the analysis.

c) The interface should be designed so that the restrictor could be heated to temperatures similar to those used in the FID so that eluting analytes are completely vapourised.

d) The interface should be designed so that it causes minimum disruption to either the SFC or the ICPMS. This was particularly relevant as other students required use of the ICPMS in normal running mode. The interface designed by Caruso's group required the complete removal of the nebuliser (as shown in Figure 2.1).

It was noted that Caruso's group had used a copper tube to house the restrictor within the torch. This relied on heat transfer along the copper tube to heat the restrictor and hence the temperature could not be controlled. Moreover, the use of a bulky connection to the torch made it necessary to remove the nebuliser and hence disrupt the ICPMS setup and disallowed the ICPMS from being used independantly from the SFC. Careful consideration of the system revealed that a hole in the elbow join between the torch and the nebuliser would allow for a capillary tube to be introduced into the torch. However, this would not allow for the restrictor to be heated.

Thus, it was decided to add a side arm to the elbow join so that it resembled a T-piece. This would allow for the restrictor (contained in some kind of tubing) to be introduced into the torch and the wide bore of the third arm would allow for the introduction of some kind of heating device. This had the added advantage of allowing the argon from the nebuliser to be used as a make-up gas to compliment the low flow rate of mobile phase

from the SFC. This would eliminate the need for an external mechanism being needed to introduce the make-up gas. Another advantage was that the argon could be controlled at any temperature between 0 ^{o}C and 100 ^{o}C by the unit within the ICPMS. Moreover, this caused minimum disruption to the ICPMS and would allow independant use of the ICPMS while still coupled to the SFC. This T-piece is shown in Figure 3.3.

If a metal tube was inserted into the new side arm of the T-piece it could act as a suitable housing and transfer line for the restrictor as it would ensure that heat would be adequately transferred to the capillary transfer line to the restrictor within and it would be maintained at the same temperature as the column. Thus, by heating the make-up (nebuliser) argon to the same temperature as the column the conduction through the metal tube would keep the transfer line at the same temperature as the column. However, if the metal tube was too long and extended too far into the torch it might cause arcing of the plasma. It was found that a modified splitter assembly from SFC provided the characteristics of the tubing needed.

The design so far ensured a well heated transfer line, allowed minimal disruption to the ICPMS and did not have any metal near the plasma. However, if the restrictor was to be as close as possible to the plasma it would protrude from the metal tube and would be unheated, which is contrary to the requirements. Thus, some form of heating filament was needed to heat the restrictor. Nichrome wire was ideal for the filament as it can withstand high temperatures and when tightly wound it would have a high resistance. Thus, by applying a relatively small voltage on the filament the temperature could be altered. Unfortnately the wire could not be wound directly around the restrictor or even a piece of thicker fused silica capillary tubing as they are too brittle for winding the wire. Thus, it was decided to use a piece of capillary glass tubing and it was found that a section of melting point tube was satisfactory as it had the same external

diameter as the metal tube and could be easily joined to it. If the wire was encased in insulation it would minimise the risk of arcing.

With this design all the requirements were satisfied. The restrictor could be placed as close to the plasma as possible and could be effectively heated. There was a minimum of metal near the plasma thus minimising the risk of arcing and the interface had been designed to cause minimal disruption to either instrument.

4.4 Interface Assembly

The interface was assembled according to design but the following points were noted. To insulate the nichrome wire polyimide resin was used as this can withstand high temperatures (400 ^{0}C) and is a good insulator. The wires from the filament could not touch the metal tube at any time as this might have caused a short circuit. Thus, these wires were insulated using the commercial insulator shrinksleeve. To attach the melting point tube to the metal tube a sleeve to cover the join had to be used. This was fashioned from a short section from the tapered end of a Pasteur pipette. The join was sealed with polyimide resin as a commercial adhesive capable of withstanding the high temperatures was not available. The point where the metal tube entered the side arm was sealed with 372 epoxy which is capable of withstanding the moderate temperatures of the make-up gas. This provided a gastight seal thus preventing the make-up argon from leaking out of the system and preventing air and water vapour from entering the system and ultimately extinguishing the plasma.

The column was brought out of the side of the oven into the ICP torch box and attached to the restrictor using a butt connector. Although the column should have been contained in a heated transfer line to prevent liquefaction of the mobile phase, the length of column outside of the oven was kept to a minimum (~ 20 cm) and all analyses were successful. As the

distance outside the oven is short liquefaction of the mobile phase, although undesirable, is less important but must still be minimised. The mobile phase only travels a short distance in its liquid phase and so the diffusion coefficient of the solute in the liquid mobile phase does not decrease low enough to degrade chromatographic efficiency. Thus, as the unheated length of column is increased the mobile phase is liquid for a longer period of time and there will be an increase in peak tailing and even loss of analyte. The major flaw of the interface was the glass sleeve covering the join between the metal tube and the melting point tube. This sleeve was extremely fragile and great care had to be taken in inserting the interface into the torch to prevent this join from breaking. This join was essential in aligning the melting point tube and the metal tube so that the restrictor was correctly aligned with the plasma and did not introduce the sample into the plasma at an angle. This would have caused condensation of analyte on the side of the torch and inefficient transfer of analytes into the plasma.

4.5 A Comparison of the Interface used in this Study with the Interface Developed by Caruso

Although there has been a great deal of research in GC-ICPMS and HPLC-ICPMS, the only work on SFC-ICPMS has been performed by Caruso's group. A comparison of the interface designed for this study with the interface developed by Caruso (shown in Figure 2.1) reveals the advantages and disadvantages of each.

Caruso's interface has the restrictor housed in a copper tube which is inserted as far into the torch as possible. This immediately raises the question of arcing between the copper tube and the plasma although Caruso's group reports that this problem was not observed [15]. The copper tube has the advantage of being streamlined and therefore causing

minimal disruption to the flow profile of the nebuliser gas. The copper tube is held in place using a brass swagelok union which not only increases the amount of metal in the torch box but dramatically increases the bulk of the interface. As there is limited space around the torch and the nebuliser, this large bulk of metal requires that the nebuliser be removed completely and an external source of make-up gas be used. Although this has the advantage of being able to introduce make-up gas heated above 100 °C, it is more difficult to ensure a gas-tight seal around the whole interface. This not only increases the risk of air entering the plasma and interfereing with the analysis but may cause a loss of (expensive) argon and thus increase running costs.

As can be seen in Figure 2.1 it is only the swagelok union and the make-up gas which are heated. Thus, the restrictor is not heated directly but relies on heat transfer along the copper tube and so the temperature of the restrictor is not well controlled. This is unsatisfactory as effective heating of the restrictor is essential for complete volatilisation of the analytes and to effectively combat the Joule-Thompson effect (adiabatic expansion of the mobile phase). Despite these disadvantages this interface has been successfully used and very good detection limits have been obtaind [14, 16-18].

The interface used for this study has already been discussed in detail but it is important to emphasise that it is gastight, does not require the ICP unit to be modified and allows more effective control of the restrictor temperature. However, the glass sleeve joining the metal tube to the melting point tube is fragile and does not withstand much handling. The polyimide insulation around the wire filament can only withstand temperatures below about 400 °C and so the interface temperature can not be taken too high. Moreover, the introduction of wires and a filament causes a change in the argon flow profile. One possible example of this disruption is shown in Figure 4.1 below.

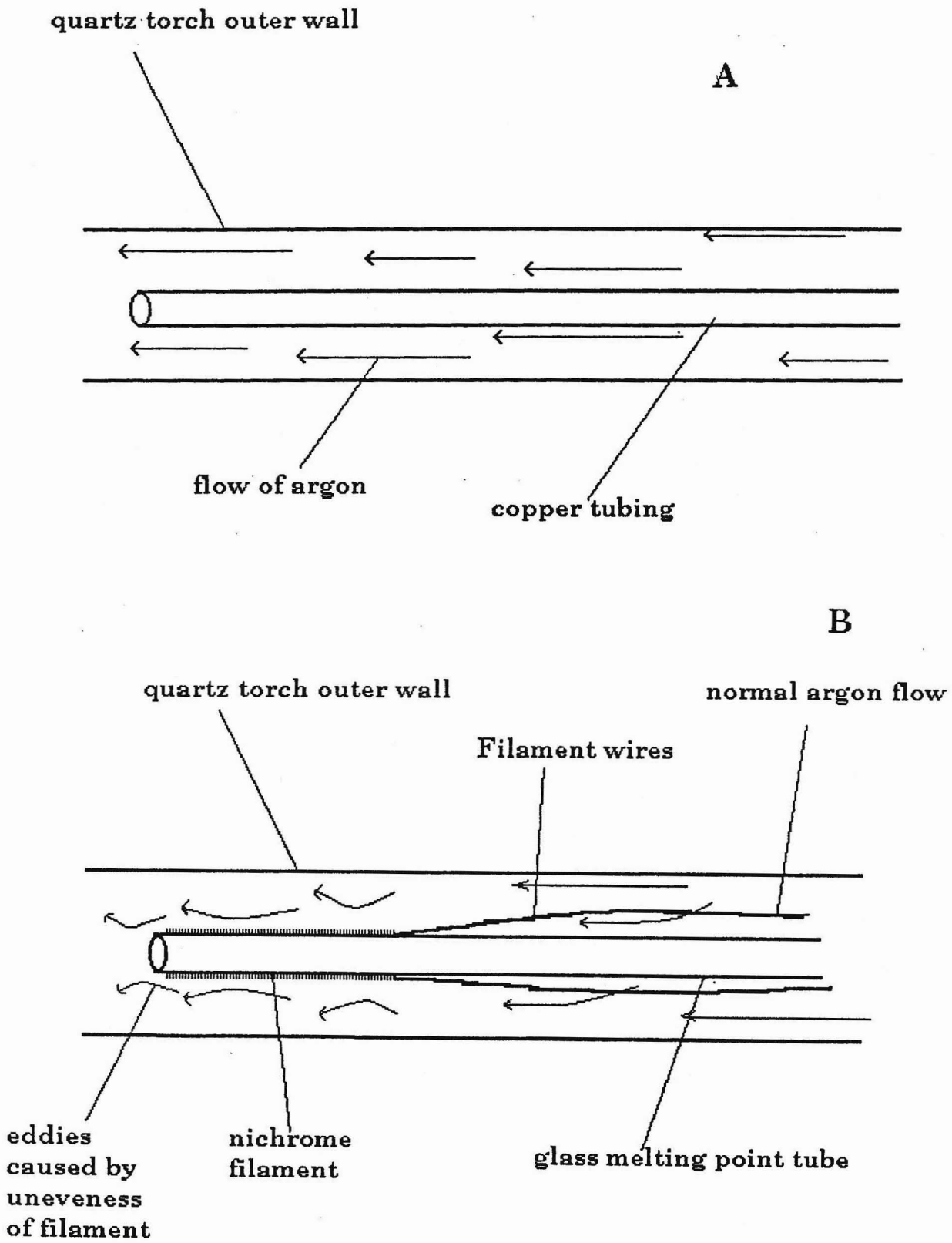


Figure 4.1: A comparison of make-up gas flow profiles in A) Caruso's interface and B) the new interface.

Although the new interface is more than adequate it could still be improved in future studies. Rather than using a filament heater, it should be possible to use a coating of conductive metal paint. This would be more streamlined and may decrease the disruption to the make-up gas flow profile. Morover, it would be better if the various components could be sealed into the interface using a glass seal rather than an epoxy seal. This would minimise the risk of the seal coming loose or breaking when the nut and ferrule is tightened. However, the most important improvement needed would be a more robust join between the melting point tube and the metal tube. It is also necessary to find an accurate method for determining the restrictor temperature as will be discussed in the following section.

4.6 Restrictor Temperature - Applied Voltage Calibration

The procedure used to determine the relationship between the restrictor temperature and the applied voltage on the filament was considered adequate but not as accurate as would be desired. Firstly the thermocouple was too large to fit in the melting point tube next to the restrictor and therefore had to be placed outside the melting point tube next to the filament. This would cause a reading higher than that experienced by the restrictor as the melting point tube would act as an insulator and more heat would dissipate through the polyimide coating than through the glass. Nevertheless, the temperature inside the melting point tube around the restrictor should be high enough to efficiently counteract the Joule-Thompson effect.

A further complication is that the temperature of the make-up argon is 60 ^{o}C. Although this is warm it is still well below the 350 ^{o}C required by the restrictor. Thus, there will be heat loss from the filament to the cooler argon. It is suspected that these two factors combined cause the

temperature of the restrictor under normal operating conditions to be lower than measured using the thermocouple benchtop measurements. From benchtop experiments the applied voltage range of 6 V to 10 V is tentatively considered to give temperatures of about 200 °C to about 320 °C. However, due to fluctuation of results during the experiments and variation of results between different experiments this relationship is only approximate and no accurate calibration can even be considered. Moreover, the voltage was not taken too high as it was feared that this may cause high temperatures which may have degraded the polyimide insulation.

4.7 Optimisation of the Chromatographic Conditions

Before any experiments could be performed using SFC-ICPMS it was necessary to determine the optimum chromatographic conditions for speciation. As these conditions are purely chromatographic and independent of the detector, these experiments were performed using a flame ionisation detector. The FID is ideal as it is a universal detector and was available on the SFC. The first step was to determine which compounds were soluble in the carbon dioxide mobile phase and on increasing the mobile phase density would elute. To do this each compound was injected separately and if no peak was obtained then the initial and final hold times as well as the oven temperature and pressure program were altered in an attempt to find suitable elution conditions. It was found that tetrabutyltin, tributyltin chloride, tetraphenyltin, triphenyltin chloride, ferrocene, triphenylarsine, bis(1,2-diphenylarsino)ethane, tributyltin hydride, dibutyltin dihydride, butyltin trihydride and diethylmercury could be eluted with supercritical carbon dioxide. A chromatogram of a mixture of tetrabutyltin, tributyltin chloride, tetraphenyltin, triphenyltin chloride and bis(1,2-diphenylarsino)ethane is shown in Figure 4.2.

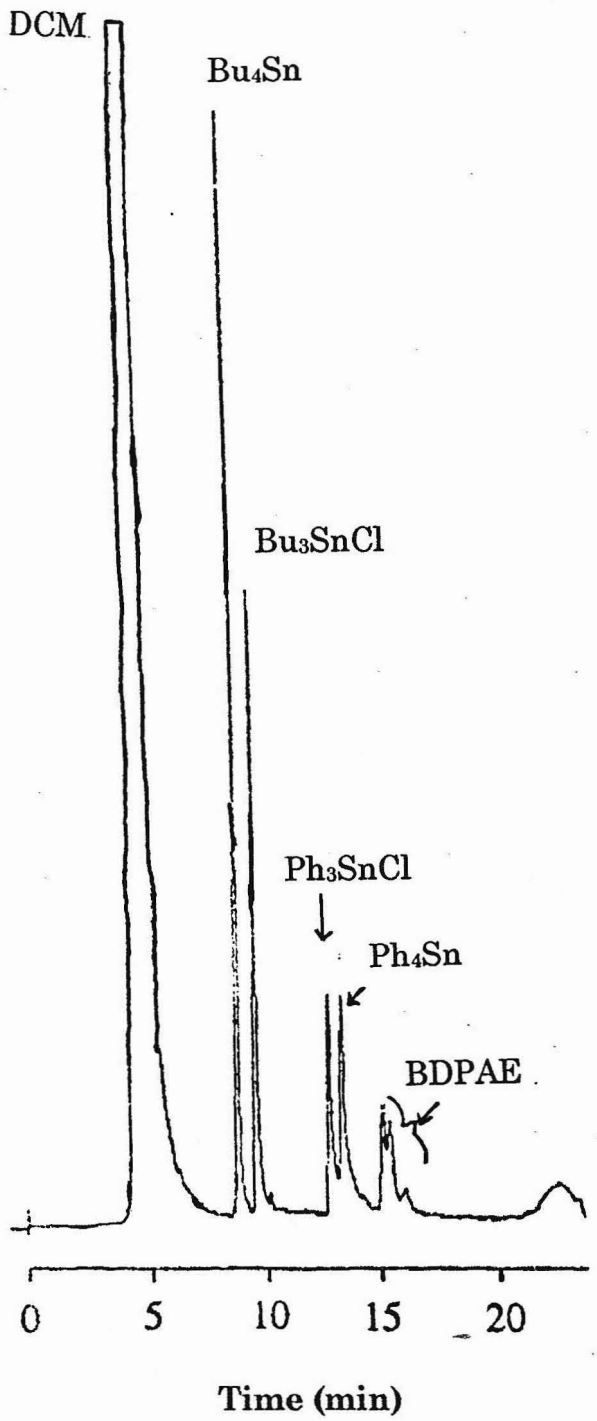


Figure 4.2: Chromatogram showing tetrabutyltin, tributyltin chloride, tetraphenyltin, triphenyltin chloride and bis(1,2-diphenylarsino)ethane. SFC conditions: 10 m SB-Biphenyl-30 column; CO_2 mobile phase programmed from 10 MPa (hold for 1 min) to 40 MPa (hold for 5 min) at 2.5 MPa/min at a constant temperature of 80 ^{0}C. Time split injection of 0.100 s.

Generally, the elution times of compounds will depend on their relative solubilties in the mobile phase and their interaction with the stationary phase. Smaller molecules tend to be more soluble in the mobile phase while higher molecular mass compounds require a higher density mobile phase to remove them from the stationary phase. This is further complicated by the polarity of the compounds. More polar compounds will produce greater dipole-induced dipole interactions on the column and will be retained in the stationary phase longer. Tetrabutyltin is non-polar and relatively small and therefore elutes first from the non-polar biphenyl stationary phase. Tributyltin chloride is smaller but more polar than tetrabutyltin and induces a dipole on the biphenyl stationary phase. Thus, dipole-induced dipole interactions increase its solubility in the stationary phase and it therefore elutes second. Triphenyltin chloride is slightly polar and is a relatively large molecule. It is also able to induce a dipole on the stationary phase and thus decrease its solubility in the CO_2. The dipole-induced dipole interactions cause this compound to elute third. The difference in elution times between tributyltin chloride and triphenyltin chloride is due to the phenyl group being much larger than the butyl group and therefore less mobile. Tetraphenyltin elutes fourth as it is a very large molecule with lower solubility in the mobile phase. Bis(1,2-diphenylarsino)ethane elutes last as it is the largest molecule and has the lowest solubility in the mobile phase.

In the hydride mixture all the compounds are relatively non-polar and were not retained to any great extent. They elute in the order tributyltin hydride, dibutyltin dihydride and butyltin trihydride. Dibutyltin dihydride has a larger peak as the concentration is higher than for the other two compounds. The SFC chromatogram of these compounds is shown in Figure 4.3. A mixture of the chloride and hydride compounds together was not prepared as it was suspected that coelution or poor resolution of some compounds would occur and this would hinder the optimisation of the SFC conditions. Ferrocene eluted successfully at about

9 min as it is a relatively large molecule with limited mobility. The SFC chromatogram of this compound is shown in Figure 4.4. Diethylmercury eluted successfully at about 5 min. The SFC chromatogram of this compound is shown in Figure 4.5.

An attempt was made to analyse dibutyltin dichloride but this was unsuccessful as this compound was too polar for the non-polar CO_2 mobile phase. However, studies by Poole and Oudsema have shown that dibutyltin dichloride can be extracted and eluted using a CO_2 mobile phase modified with formic acid [21]. A similar situation exists with bis(tributyltin)oxide. It was found that methylmercuric chloride did not elute due to its high polarity in comparison to the mobile phase. However, it is suspected that this compound could be eluted using a modified mobile phase. No attempt was made to analyse butyltin trichloride as it was suspected that this compound would be too polar for analysis by SFC (even if a modified mobile phase was used).

In order to optimise the pressure program and the temperature it was necessary to consider a number of factors. Firstly, the peaks should be well resolved to allow accurate identification of the compounds. Secondly, peak tailing should be kept to a minimum as this could affect the resolution. Finally, a compromise had to be reached between resolution and time. If this method is to be considered viable it should ideally have good resolution and short analysis time. Figure 4.6 shows a series of comparative graphs of the pressure program versus peak intensity (calculated on an arbitrary scale). It is clear that the peak intensity increases as the pressure program increases but this does not take into account resolution of the peaks. Figure 4.7 shows a comparison between peak intensity and resolution for triphenyltin chloride. Peak intensity was calculated on an arbitrary scale and resolution was calculated according to the formula:

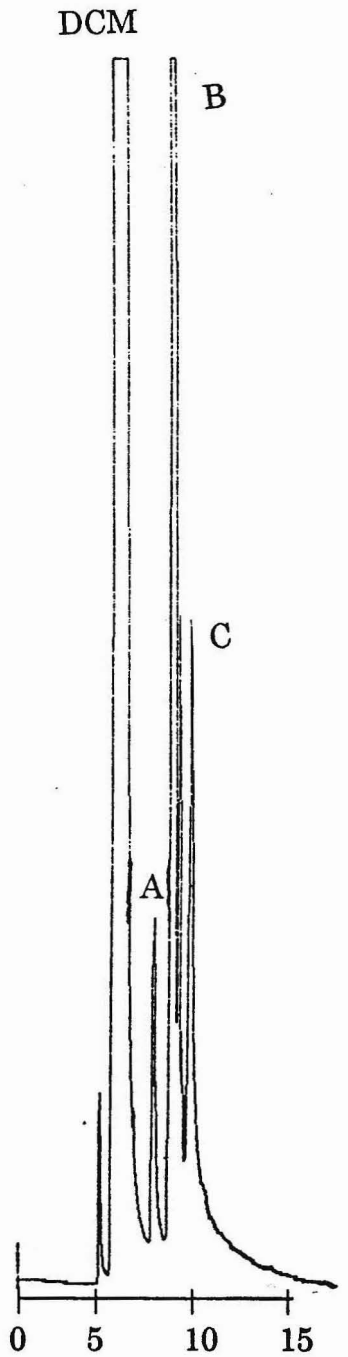


Figure 4.3: Chromatogram showing A) tributyltin hydride, B) dibutyltin dihydride and C) butyltin trihydride. SFC Conditions: 10 m SB-Biphenyl-30 column; CO_2 mobile phase programmed at 2.5 MPa/min from 10 MPa (hold for 1 min) to 40 MPa (hold for 5 min). Constant temperature 80 °C

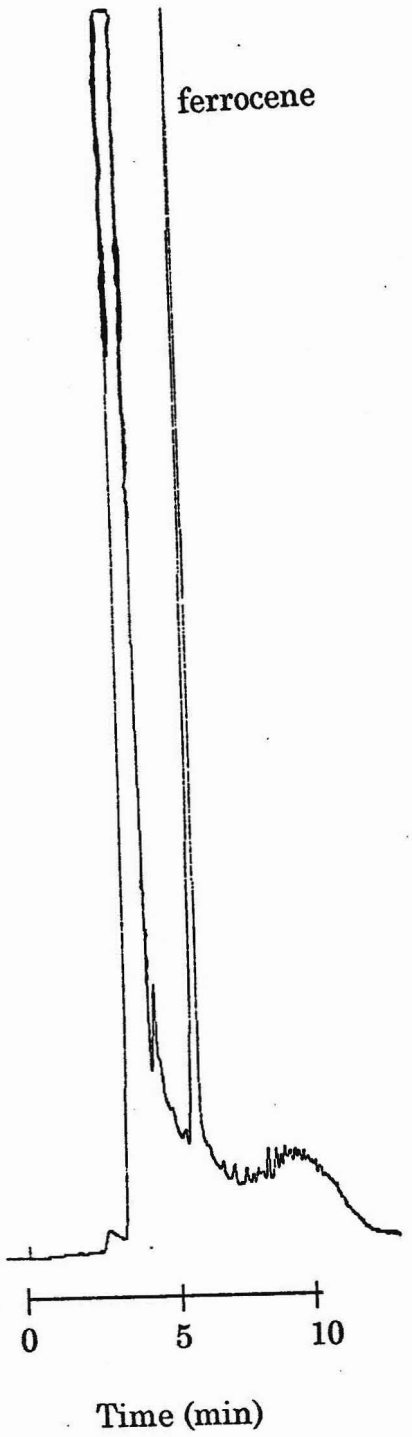


Figure 4.4: SFC-FID chromatogram of ferrocene. SFC conditions: CO_2 mobile phase programmed from 10 MPa (hold for 1 min) to 40 MPa (hold for 5 min) at 2.5 MPa/min and a constant temperature of 80 ^{0}C.

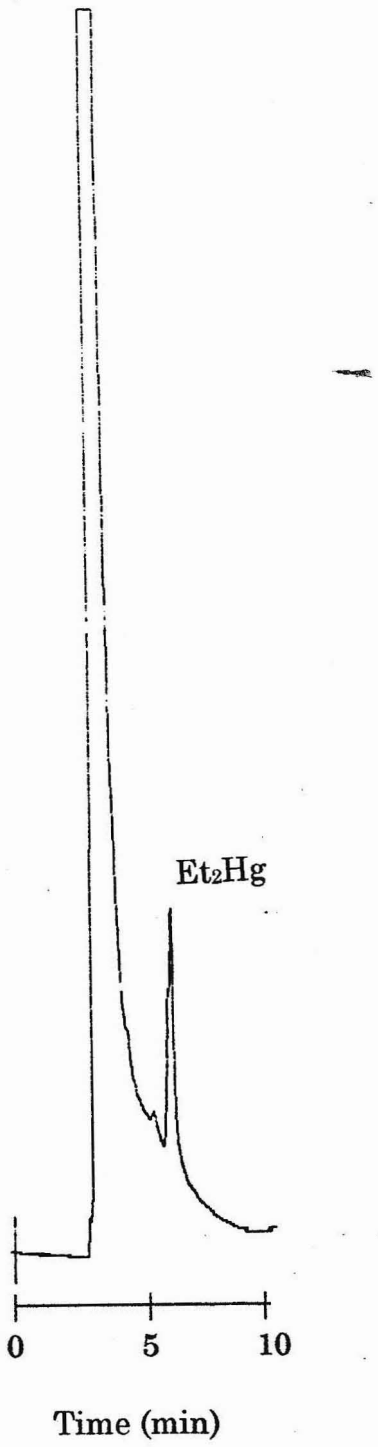


Figure 4.5: SFC-FID chromatogram of diethylmercury. SFC conditions: CO_2 mobile phase programmed from 10 MPa (hold for 1 min) to 40 MPa (hold for 5 min) at 2.5 MPa/min and a constant temperature of 80 ^{0}C.

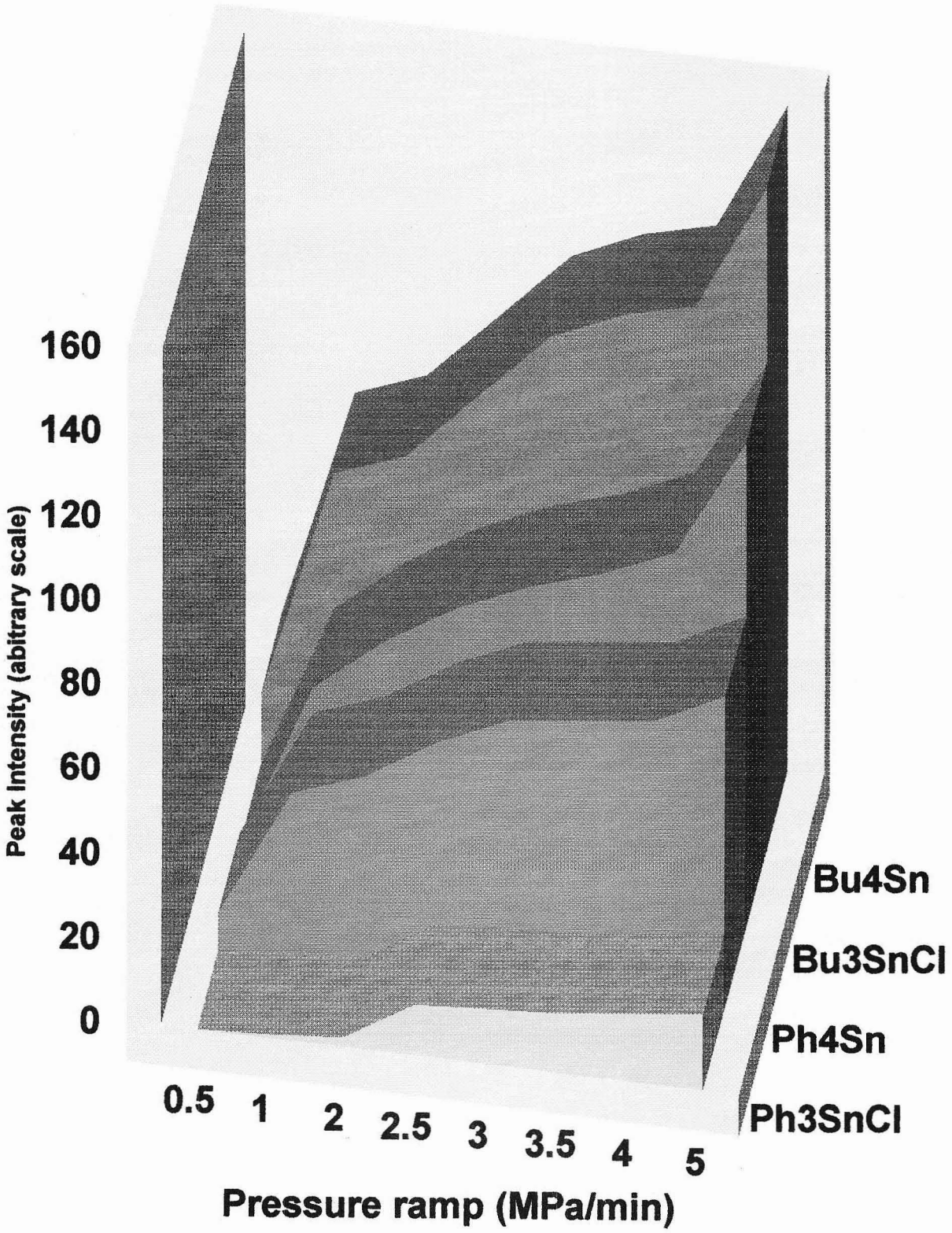


Figure 4.6: The effect of the pressure program on peak intensity as calculated on an arbitrary scale

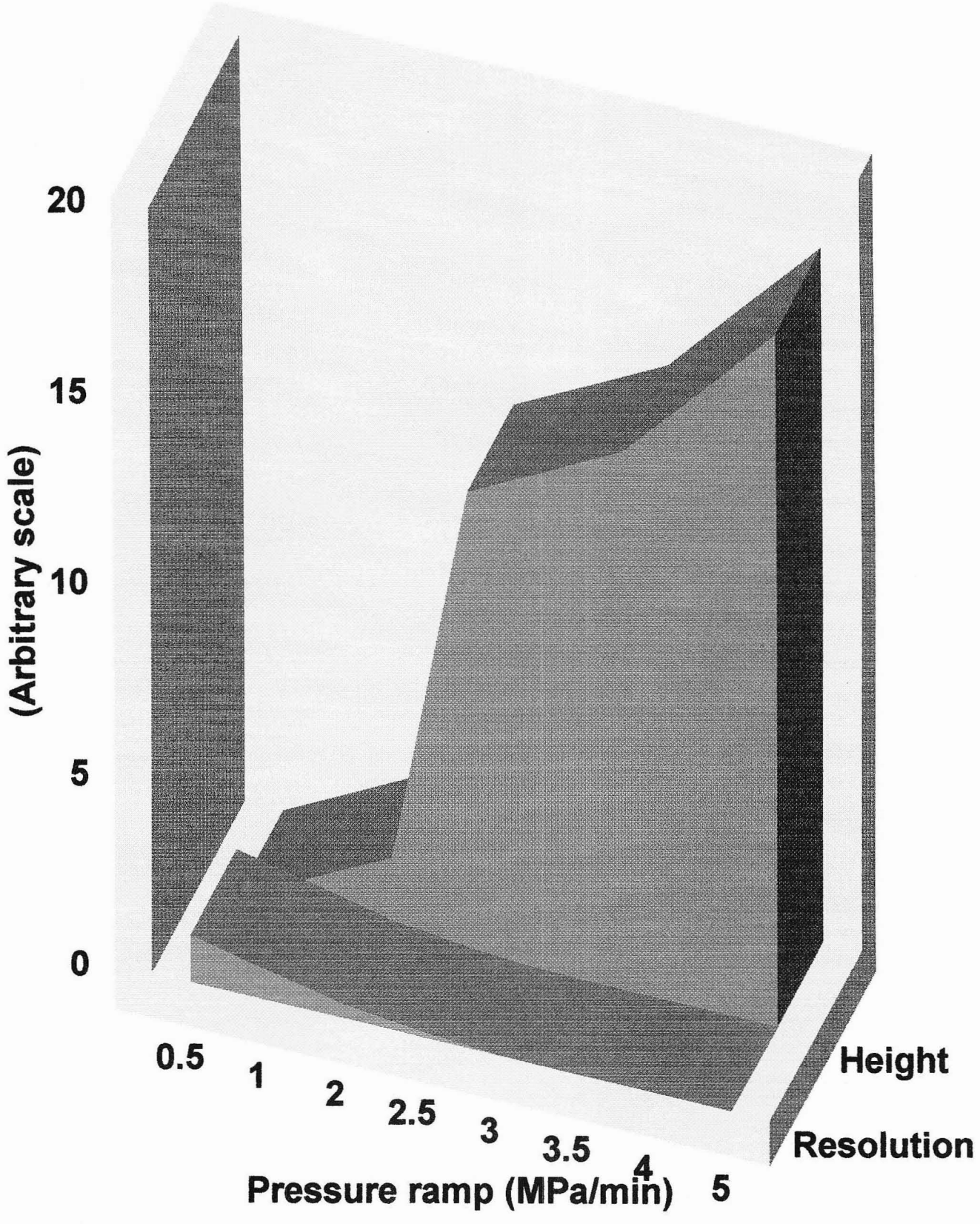


Figure 4.7: A comparison between peak intensity and resolution for Ph3SnCl at different pressure programmes

$$R = \frac{2\Delta t}{W_{b1} + W_{b2}}$$

Although the resolution and the peak intensity are on different scales, the graph still illustrates that on pressure programming the peak intensity increases and the resolution decreases. As efficient separation relies on differences in the solubility, if the solvating power of the mobile phase increases too rapidly separation of the analytes will not occur. A similar trend is observed for all other compounds. Thus, it is necessary to use a program which will give adequate peak intensity and will be well enough resolved to distinguish between peaks. A relatively high peak intensity is necessary for analysis of compounds at low concentrations. If the peak intensity is low for an analyte at high concentration then at low concentration the peak may be not be detected. However, if the peak intensity is high for an analyte at high concentration, then at low concentration the peak intensity should still be relatively high (i.e. visible). Although baseline resolution is ideal this may compromise on peak intensity and it may be necessary to choose a higher pressure program with lower resolution.

Figure 4.7 indicates that there is little or no resolution between tetraphenyltin and triphenyltin chloride for a pressure program above 2.5 MPa/min. However, for tetrabutyltin and tributyltin chloride resolution is 1.36 at 2.5 MPa/min, 1.31 at 3.0 MPa/min and 1.25 at 3.5 MPa/min. As FID is less sensitive than ICPMS as a detector, it was decided that the tetraphenyltin and triphenyltin chloride peaks should still be detected when SFC was coupled to ICPMS. Thus, it was decided to consider 2.5, 3.0 and 3.5 MPa/min as possible pressure programs.

The effect of the pressure program was studied at a constant temperature

of 60 °C. This ensured that density changes in the CO_2 were only related to pressure changes. To optimise the separation temperature it was necessary to use a temperature which was above the critical temperature of the mobile phase but low enough for the analysis of involatile compounds. This effectively limited the temperature to a range between 40 and 90 °C. Using a pressure program of 3 MPa/min chromatograms were obtained at 50, 70, 75 and 80 °C. The density of the mobile phase at different temperatures and pressures is given in Appendix B. A slight trend for increasing peak intensity with decreasing temperature was observed for the butyltin compounds but a decrease in peak intensity was observed for the phenyltin compounds. Thus, it was necessary to choose an intermediate temperature which would not decrease the peak intensity of the butyltin species but which would increase the peak intensities of the involatile phenyltin species. Thus, it was decided to use a temperature of 75 °C and a pressure program of 3 MPa/min. This would give reasonably well resolved peaks with a fairly high intensity.

Although the chromatographic conditions were optimised using a 10 m column, due to column activation the remainder of the study had to be carried out using a 2 m column. The use of a 2 m column did not show significant decrease in resolution and so the chosen pressure program and temperature were not altered to compensate for the shorter column. Moreover, for standards containing only one compound it was possible to increase the pressure ramp as resolution of peaks was not a relevant factor.

4.8 Focusing the ICPMS

Caruso *et al.* analysed all compounds with the ICPMS tuned on Ar_2^+ (m/z = 80) [18]. The instrument was tuned by observing the effect of different, isobaric mobile phase pressures on the signal at this mass to charge ratio and then tuning the instrument to give the maximum signal at each

pressure. This procedure is suitable if a number of different elements are to be studied in the same sample using a survey scan or, if the software is available, time resolved acquisition. However, m/z = 80 is relatively low and is not suitable for higher mass elements such as tin (m/z = 120), mercury (m/z = 200) or lead (m/z = 207). This is particularly relevant for single-ion monitoring where only one element is being considered. One possible method of overcoming this is to tune the instrument on a higher mass to charge ratio. This was the solution for Pretorius *et al.* [4] who tuned the instrument on mercury vapour. However, although this is more desirable than using a low mass to charge ratio, it is still not ideal. Both methods are particularly susceptible to signal drift and may not be accurate enough for real samples. Thus, for this study it was decided to tune the instrument on the element of interest. This would optimise the signal for each ion under consideration and hence increase the detection limit.

To obtain the optimum lens settings for the element of interest, it was first necessary to tune the lenses on a known standard used specifically for optimising ICPMS conditions. This was commonly a 1000 μg/ml solution of indium in a 1% nitric acid solution. Thus, to tune the instrument on indium the ICPMS was normally disconnected from the SFC (although the interface designed for this study allows a nebulised sample to be introduced through the interface) and the lens settings were returned to their preset values. These settings were standard conditions from which the instrument could be optimised for any particular analysis and they are shown in Table 3.2. The indium solution was then introduced through the normal ICPMS nebulisation system and the lens settings were altered until the maximum indium signal was obtained.

The order in which the lenses were tuned was important. The multiplier, cone bias, extraction 2, pre-filter and differential curvature lenses had been optimised and it was best to leave them unaltered as shown in Table

3.4. The lenses are then optimised in the order L3, L1, extraction 1, L2, L4, collector, front plate and finally pole bias. The lenses are tuned in this order as a change in one setting can cause a change in another. Thus, L3 and L1 are focussed using an iterative procedure. If these lenses were focussed after the others it would cause the other lens settings to change and thus L3 and L1 must be focussed first. Extraction 1, L2 and L4 are then focussed as their settings may influence the collector, front plate and pole bias. The collector, front plate and pole bias settings should not affect the other lenses and so they can be focussed last.

Once the the instrument has been standardised on indium it can be focussed on the element interest. This is done as previously described. The initial run will show a significant increase in the baseline. After each successive run the increase in the baseline will be less significant until a point is reached where the increase in the baseline will be unnoticeable or insignificant. The first, and final focussing runs on $^{120}Sn^{+}$ for a mixture of 1000 μg/g each of tetrabutyltin and tributyltin chloride is shown in Figure 4.8. There is a dramatic increase in the baseline for the first run but the final run (the fourth) shows no increase at all. Thus, once the lenses have been focussed on the element of interest the relevant analysis of that element can be performed.

When a new element is to be studied it is necessary to refocus the lenses on the new element in the same way. Two focussing runs on $^{75}As^{+}$ are shown in Figure 4.9. However, even if the element to be analysed remains unchanged (and so theoretically the lens settings should remain unaltered) the ICPMS is susceptible to signal drift and the settings should be checked each day before the analyses are begun. It is not necessary to restandardise the instrument on indium as this would become tedious and a hindrance to the study. However, it is necessary to do a sacrificial run to check that no increase in the baseline can be obtained.

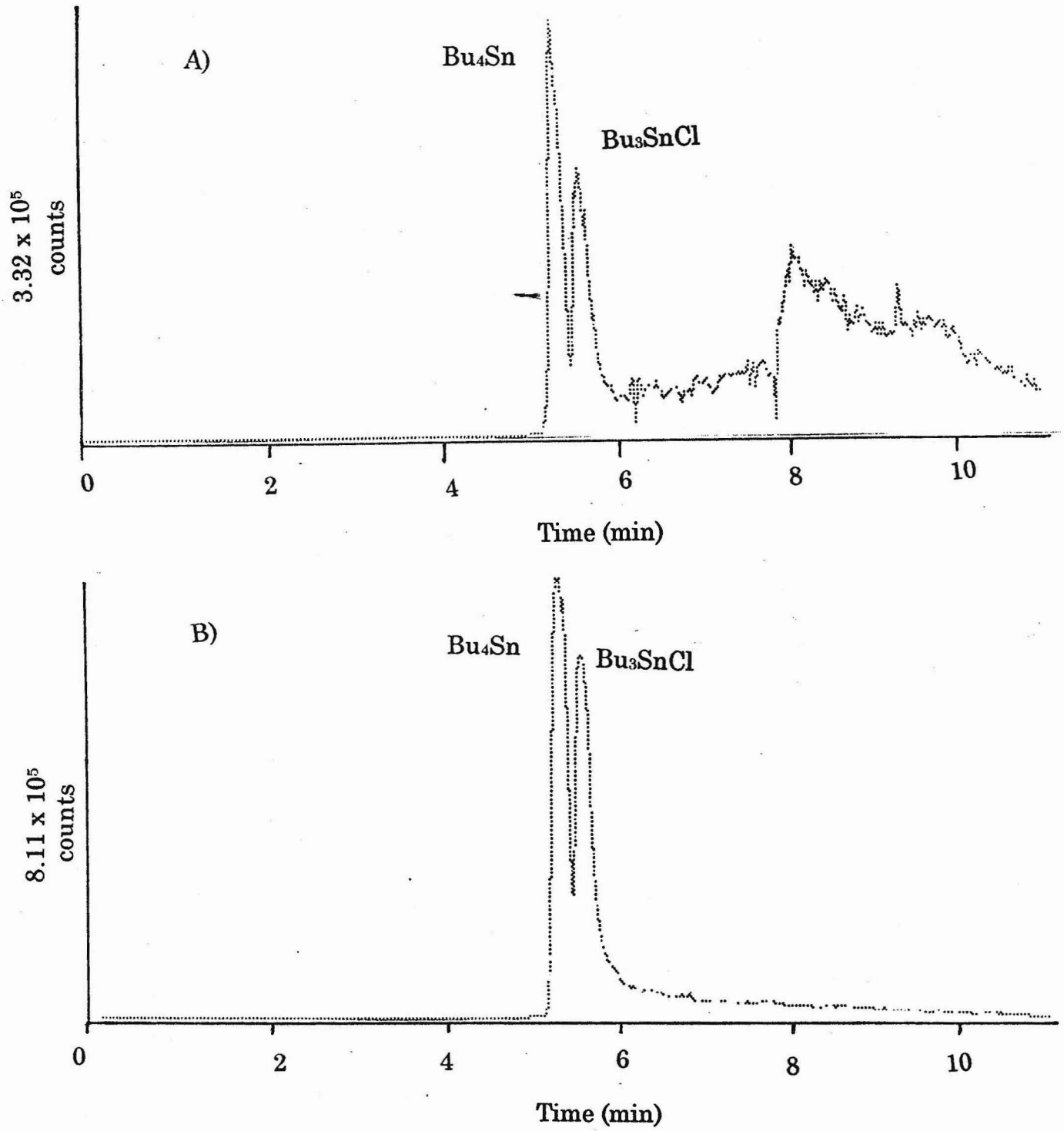


Figure 4.8: Chromatograms obtained for focussing the ICPMS on $^{120}Sn^{+}$. A) The first run and B) the final run. SFC conditions: CO_2 mobile phase programmed from 12 MPa/min (hold for 30 s) to 40 MPa/min (hold for 1 min) at 3 MPa/min; constant temperature 75 ^{0}C. Time split injection 0.100 s. ICPMS conditions: 2047 channels, dwell time 300 000 μs.

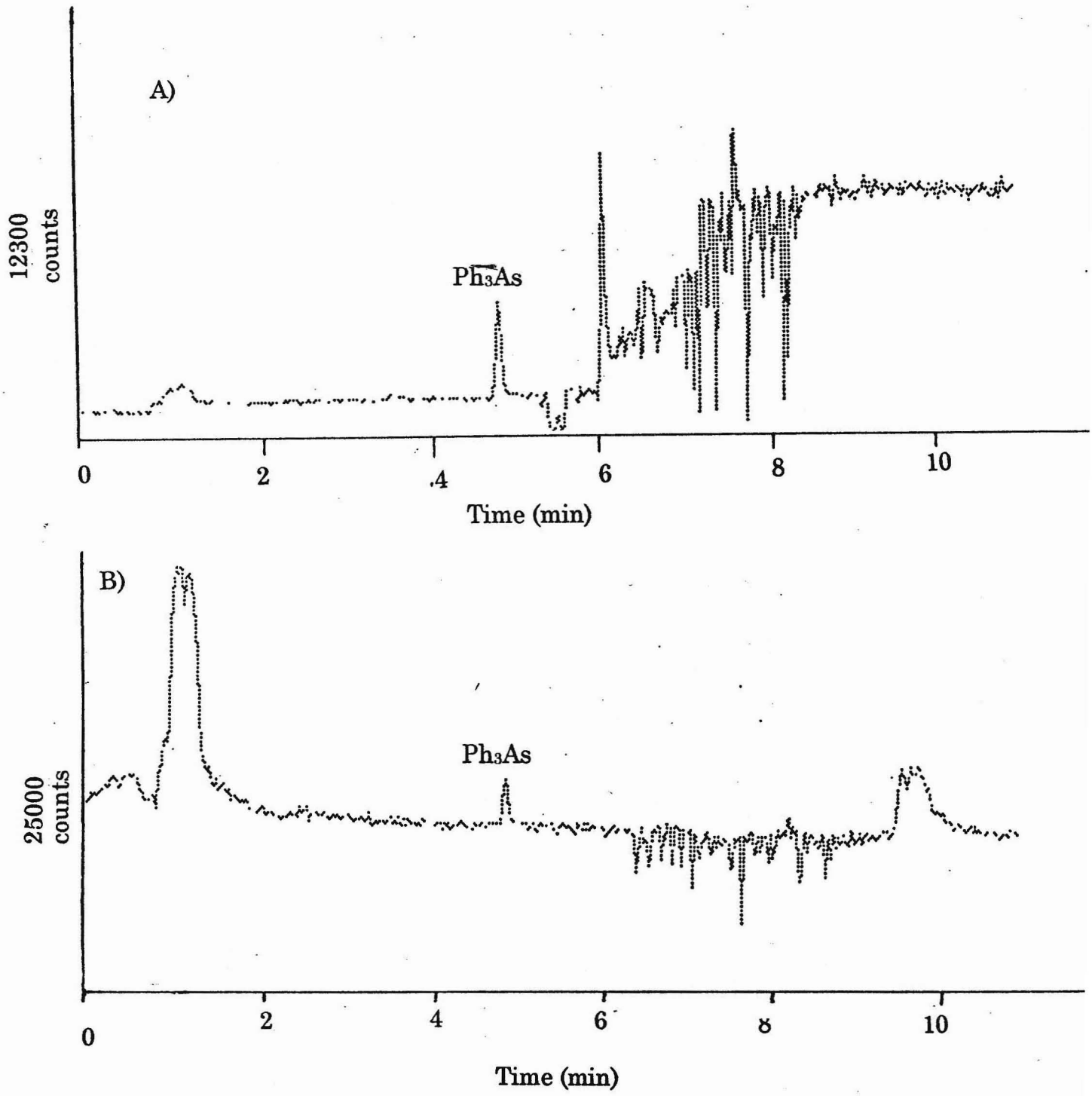


Figure 4.9: Chromatograms showing the focussing on $^{75}As^{+}$. A) The first run and B) the second run. SFC conditions: CO_2 programmed from 12 MPa (hold for 30 s) to 40 MPa (hold for 1 min) at 3.5 MPa/min; constant temperature 75 ^{0}C. Time split injection 0.100 s. ICPMS conditions: 2047 channels, dwell time 300 000 µs.

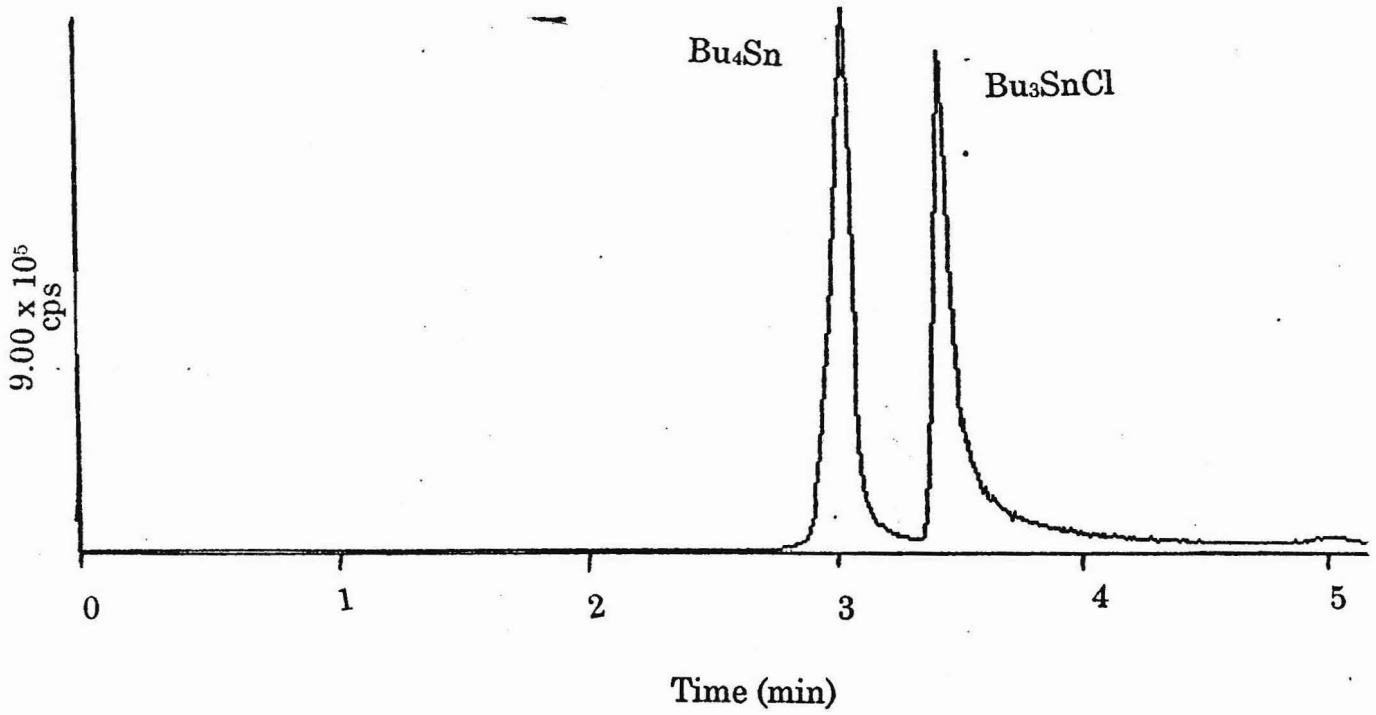


Figure 4.10: Chromatogram of $^{120}Sn^{+}$ for 10 µg/g tetrabutyltin and tributyltin chloride. SFC conditions: CO_2 mobile phase programmed from 12 MPa (hold for 30 s) to 2.4 MPa (hold for 1 min) at 3 MPa/min; constant temperature 75 °C. Time split injection 0.100 s. ICPMS conditions: 2047 channels, dwell time 200 000 µs.

Thus, by focussing on the element of interest it is possible to maximise the signal at a particular mass to charge ratio rather than at a mass to charge ratio remote from the element of interest. Evidence of this is seen in the difference in the peak intensities obtained in this study and those obtained in Caruso's study. In Caruso's study the peak intensity for 10 μg/g of tetrabutyltin was in the order of 50 000 counts per second [18]. In this study the intensity is in the order of 900 000 counts per second as shown in Figure 4.10. The increase in signal intensity can also be attributed to optimisation of the chromatographic conditions before coupling to ICPMS.

Chapter 5

5.1 The effect of the Mobile Phase on the Plasma

Before any analysis can be carried out on organometallic compounds it is necessary to study the effect of the mobile phase on the plasma. If the CO_2 affects the plasma in any way this may be a source of spectral interference which decreases the sensitivity of the instrument to certain elements. The most common ion formed in the plasma is the argon dimer, Ar_2^+. As argon is the plasma gas this cation should be abundant and should have a high intensity baseline. This is confirmed in Figure 5.1 (a) which shows the baseline obtained with the lenses tuned at m/z 80 (Ar_2^+) and with no mobile phase flowing. Clearly there is no change in the plasma with time and only a slight negative drift is observed. If mobile phase is flowing at normal analysis conditions there is a slight but distinct drop in the baseline approximately half way through the analysis. This can be attributed to a slight quenching of the plasma as the flow of CO_2 increases. If dichloromethane solvent is injected there is a small negative peak at about 1½ minutes. The solvent contains significant amounts of chloride which causes the formation of $ArCl^+$ instead of the dimer. As this chloride is only present when the solvent elutes $ArCl^+$ formation occurs for a limited period and so a negative peak in the Ar_2^+ spectrum is observed. These trends are illustrated in Figure 5.1 (b). From these results it can be seen that the mobile phase does not interfere significantly with the plasma and so there should be no background spectral interferences caused by changes in the plasma due to the mobile phase.

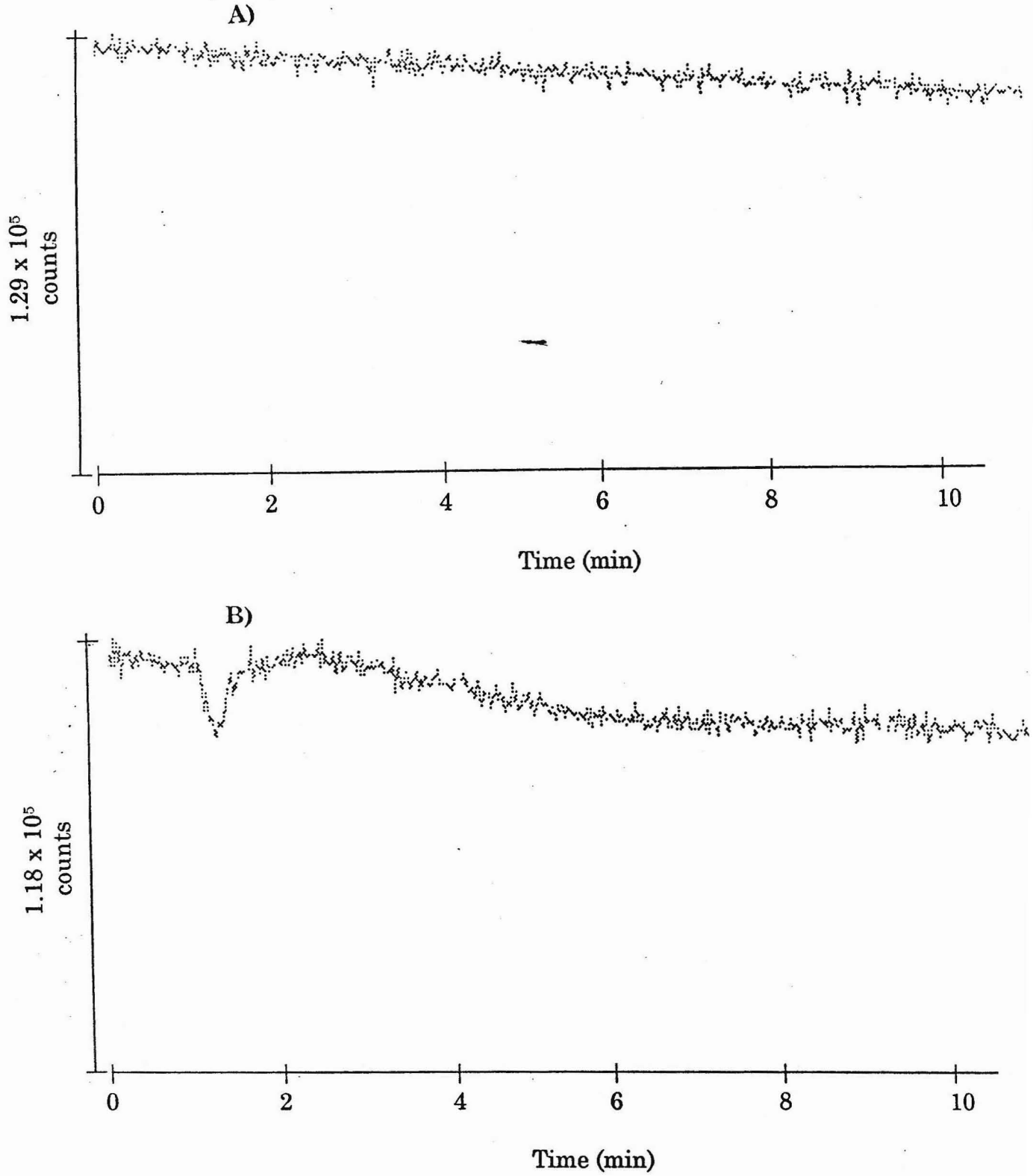


Figure 5.1: Chromatograms with the ICPMS tuned on Ar_2^+ with a) no mobile phase flowing and b) a dichloromethane injection. SFC conditions for b): CO_2 mobile phase programmed from 12 MPa (hold for 30 s) to 40 MPa (hold for 1 min) at 3 MPa/min; constant temperature of 75 ^{0}C and a time split injection of 0.100 s.

These results are in direct contrast with those obtained by Caruso [18] who observed three distinct regions (zone A - before solvent elution, zone B - solvent elution, and zone C - after solvent elution). During the initial hold and before the solvent (dichloromethane) eluted the results correspond. In zone B, the period of solvent elution, the results are similar but the negative peak obtained by Caruso's group is significantly larger than obtained in this investigation. The greatest difference between results occurs after the solvent has eluted (that is in zone C).

In zone C, after the solvent had eluted, Caruso found a significant drop in the baseline (approximately 8000 counts per second) which indicated that the plasma was sensitive to the flow of the mobile phase. However, Caruso's study made use of an extremely high pressure ramp of 15 MPa/min while this study made use of a much lower pressure ramp. The difference in the results can thus be ascribed to studies of pressure and time respectively. The results obtained by Caruso indicate that the plasma is sensitive to pressure (and hence flow) changes in the mobile phase. However, the results obtained in this study indicate that, provided the pressure ramp (and hence change in flow rate) is low enough, there will be no significant change in the plasma with time and so the mobile phase can be regarded as having no effect on the plasma. The results in this study are more relevant as typical SFC uses lower pressure ramps.

As the mobile phase introduces carbon into the plasma it is possible that another interfering ion, ArC^+, could be formed. This would interfere with the analysis of $^{52}Cr^+$ which could be a problem for the analysis of chromium based metalloporphyrins. Thus, the lenses were focused at m/z 52 and dichloromethane solvent was injected. As shown in Figure 5.2 there is no significant change in the baseline and the intensity is relatively low. The gap in the baseline is due to detector switch-off as mentioned earlier. Thus, the introduction of carbon from the mobile phase does not significantly affect the plasma. This is in contrast to the results obtained by Caruso [18] who found a significant increase in the baseline

(about 55 000 counts per second) during the analysis. The difference in results can be ascribed to changes with pressure and time. At the high pressure ramp used by Caruso there is a rapid increase in the rate at which carbon is introduced into the plasma and hence a corresponding increase in the formation of ArC^+. However, at a lower pressure ramp the increase in the rate at which carbon is introduced into the plasma is low enough for the rate of formation and dissipation of ArC^+ to be constant. Thus, there is no change in the plasma with time and little interference caused by the formation of ArC^+ due to the introduction of carbon from the mobile phase.

The mobile phase contains twice as much oxygen as carbon and hence it would be expected that there is greater interference from ArO^+ than from ArC^+. This is confirmed in Figure 5.3 which shows that the intensity of the baseline for ArO^+ is over 160 times greater than that of ArC^+. This is due to reaction of the argon with the oxygen from the mobile phase and atmospheric oxygen. This interference at m/z 56 is significant as it hinders the analysis of iron porphyrins and organoiron compounds such as ferrocene (it will be shown later that this is not the only difficulty in analysing iron). There is a slight decrease in the baseline at the beginning of the analysis but this levels out after about 2 minutes. This should not interfere with the analysis but the high intensity of the baseline will be significant. Thus, ArO^+ will interfere with the analysis of $^{56}Fe^+$. As Caruso did not study ArO^+ there can be no comparison of the formation of this ion with pressure and time. However, it would be reasonable to assume that at a high pressure ramp the rate of introduction of oxygen into the plasma would cause an increase in ArO^+ formation and an increase in the baseline with increasing pressure would be observed.

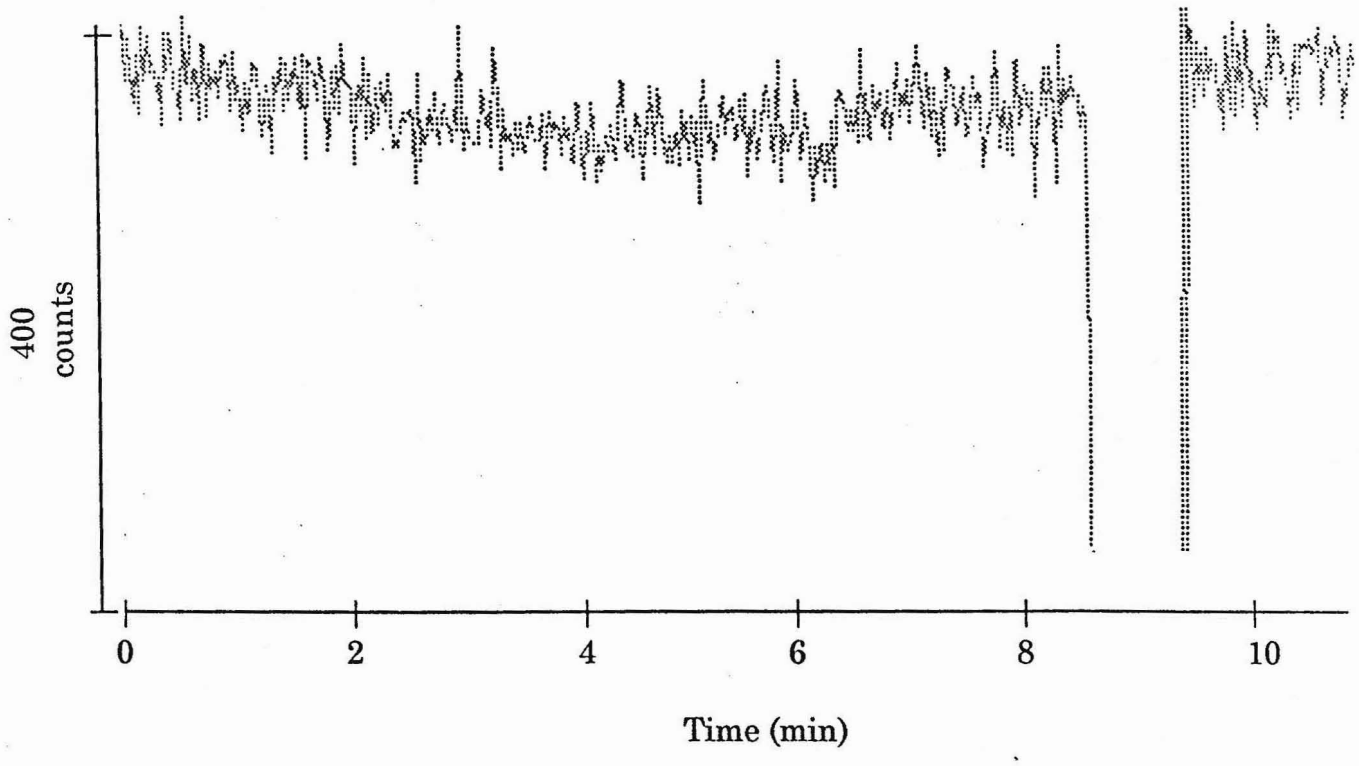


Figure 5.2: Chromatogram obtained at m/z 52 (ArC^+). SFC conditions: CO_2 mobile phase programmed at 3 MPa/min from 12 MPa (hold for 30 s) to 40 MPa (hold for 1 min) at 75 °C. Time split injection of 0.100 s.

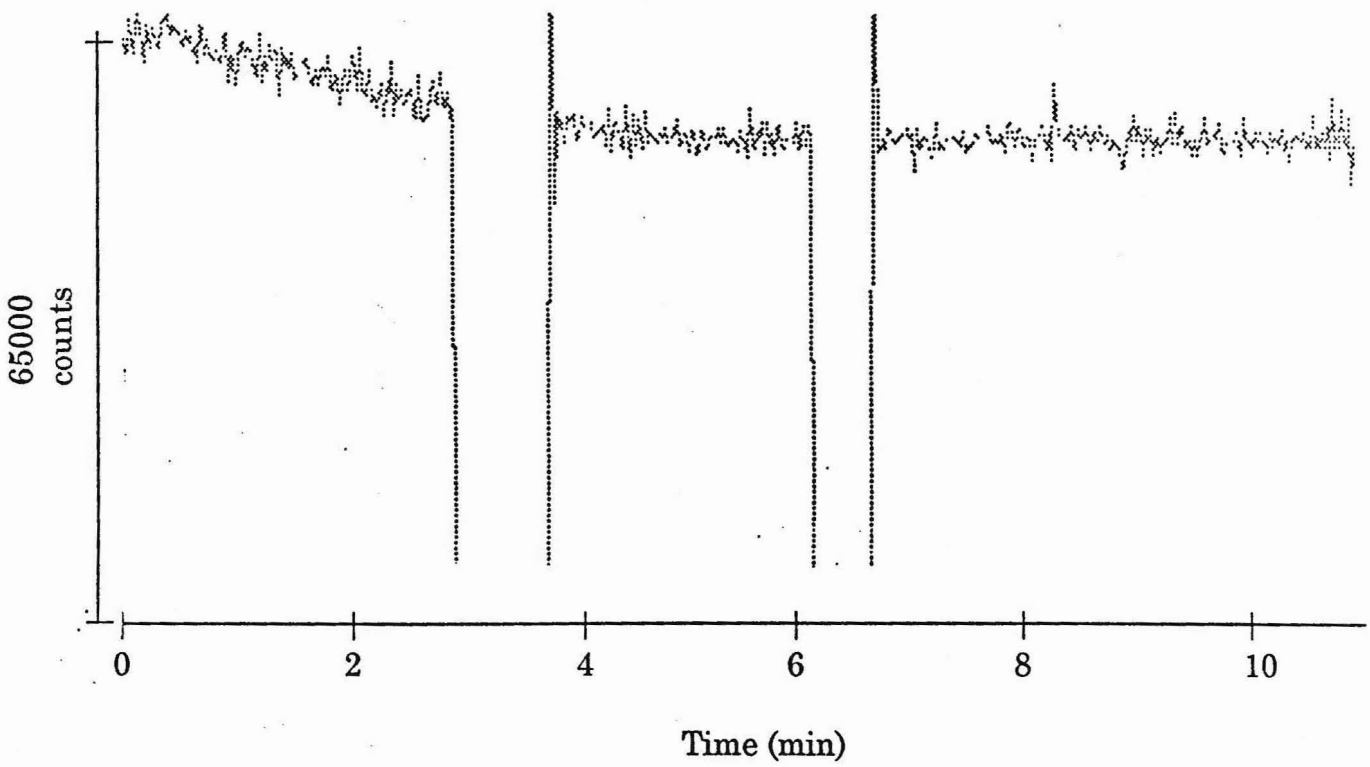


Figure 5.3: Chromatogram obtained at m/z 56 (ArO^+). SFC conditions: CO_2 mobile phase programmed from 12 MPa (hold for 30 s) to 40 MPa (hold for 1 min) at 3 MPa/min. Constant temperature of 75 ^{0}C and a time split injection of 0.100 s.

Thus, from the results presented above it is clear that the plasma is not significantly affected over time by the mobile phase. Although Ar_2^+ has a high intensity baseline it does not correspond to a mass to charge ratio relevant in the analysis of organometallic compounds. Moreover, there is little change in the baseline indicating that the mobile phase does not quench the plasma. The formation of $ArCl^+$ when solvent is injected should not cause a problem. Although $ArCl^+$ has the same mass to charge ratio as arsenic, it should only occur as a peak when the solvent elutes and should not increase the intensity of the baseline. ArC^+ does not interfere with the plasma in any way and the intensity of the baseline remains relatively low. The greatest interference caused by the flow of the mobile phase is from ArO^+ which is formed by reaction of the argon with both atmospheric oxygen and oxygen introduced by the mobile phase. The results in this study indicate changes in the plasma with time whereas results obtained by Caruso indicate changes in the plasma with rapid changes in pressure.

5.2 Organotin Compounds

5.2.1 The Effect of Analyte Concentration

The first step in building up an overall impression of the SFC-ICPMS technique was the investigation of analyte concentration on the detector signal. Although there are 37 isotopes of tin only 10 of them occur naturally [73] with ^{120}Sn being the most abundant. As there are no interferences at this mass to charge ratio $^{120}Sn^+$ was chosen as the monitoring ion. Initially a mixture of tetrabutyltin, tributyltin chloride, triphenyltin chloride and tetraphenyltin was analysed. However, irrespective of the concentration or sample composition, a large peak was obtained for triphenyltin chloride. This indicated a significant memory effect within the SFC and as no peak was obtained under normal analysis conditions but with the injection stage eliminated, it was reasonable to assume that this memory effect was caused by strong absorption within

the injector. This memory effect severely hampered the study of triphenyltin chloride and tetraphenyltin and thus it was decided to use only a mixture of tetrabutyltin and tributyltin chloride for the investigation of analyte concentration. This memory effect was noted throughout the study of tin compounds. Before the investigation proceeded the mobile phase was passed through the column at 40 MPa and 75 ^{0}C for 2 hours. Thereafter three injections of dichloromethane solvent were made and finally a chromatogram was obtained to determine whether any interfering peaks were present. Although there was a high intensity triphenyltin chloride peak no other peaks were present. As triphenyltin chloride elutes much later than tetrabutyltin and tributyltin chloride, this was not considered to hinder the analysis. Thus, the tetrabutyltin/tributyltin chloride mixture was analysed starting from the lowest concentration of 0.001 μg/g to the highest of 100 μg/g.

The trend observed is graphed in Figure 5.4. At low concentrations the trends for tetrabutyltin and tributyltin chloride are different but at higher concentrations the graphs converge. Tetrabutyltin has a practical detection limit (from the graph) of 0.001 μg/g (1 ppb) which corresponds to 0.07 pg as tin. However, at this concentration the peak intensity is still relatively high and it is reasonable to assume that the limit of detection is at about 0.035 μg/g (0.5 ppb). Tributyltin chloride has a practical detection limit of 0.1 μg/g (100 ppb) which corresponds to 6.7 pg as tin. The difference in these practical detection limits can be explained in terms of the peak shape for each compound.

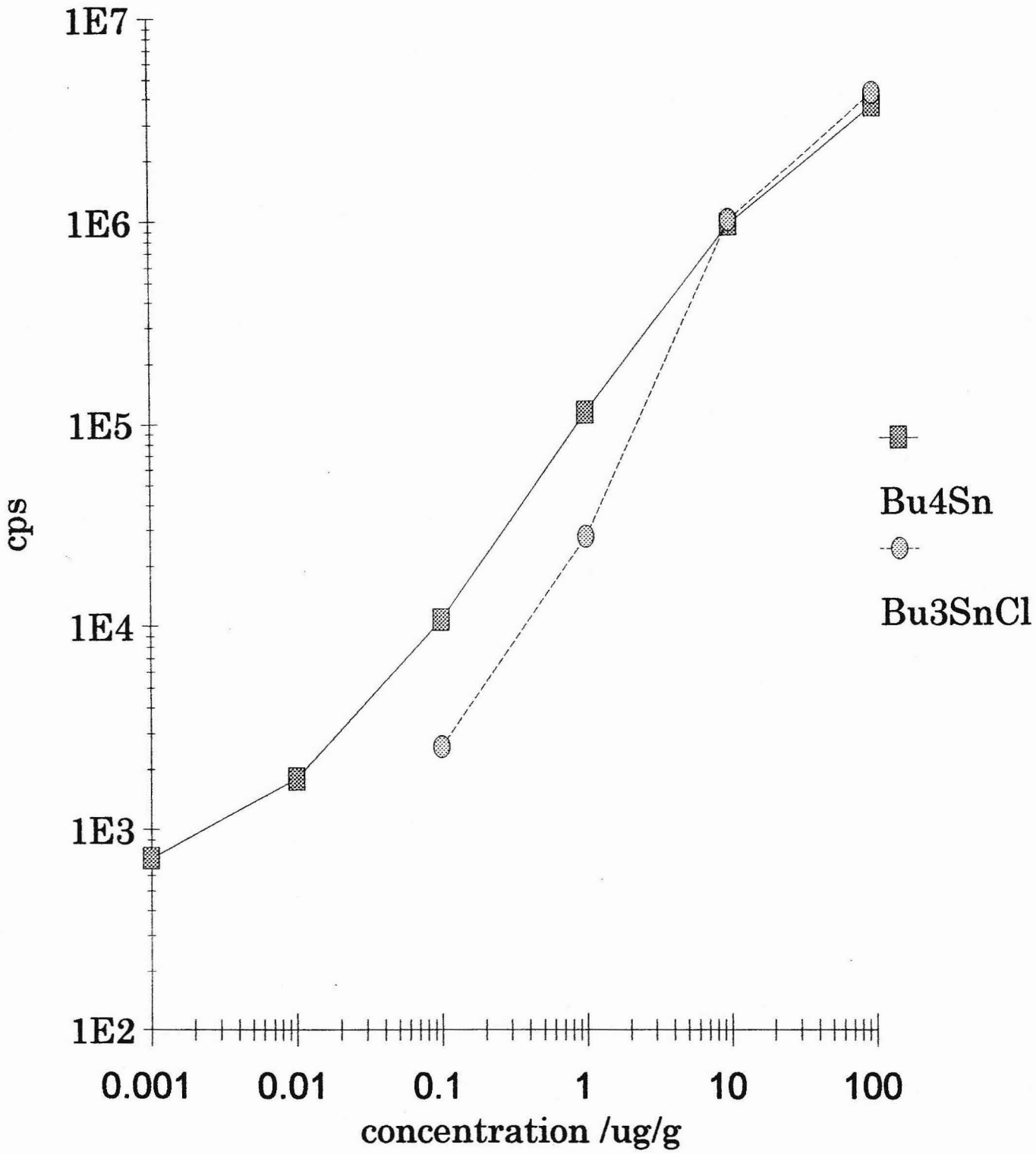


Figure 5.4: Acomparison of the general trends of analyte concentration and peak intensity for tetrabutyltin and tributyltin chloride.

Tetrabutyltin is non-polar and hence has a symmetrical peak with no peak tailing. Thus, even at low concentrations the peak is well defined with a high intensity. Tributyltin chloride is more polar and will be adsorbed on any active site in the column. This, together with its fairly low stationary phase compatibility, results in significant peak tailing. This trend in peak shape is shown in Figure 5.5. Thus, at low concentrations the peak tailing on the tributyltin chloride peak is so great that the peak is invisible. As the concentration increases, although the amount of peak tailing remains the same, in comparison to the amount of compound moving along the column it becomes less significant. As the concentration of both compounds increases above 10 μg/g (10 ppm) the column or detector is overloaded and there is a deterioration in peak shape. As tetrabutyltin is more volatile and less polar than tributyltin chloride the intensity is greater and so if the column or the detector is overloaded tetrabutyltin will show the greatest deterioration in peak shape.

The theoretical detection limits for these compounds (calculated as picograms tin) are calculated as:

Theoretical detection limit (TDL) = 3σ

where $$\sigma = \text{uncertainty} \times \frac{1}{\text{sensitivity}}$$

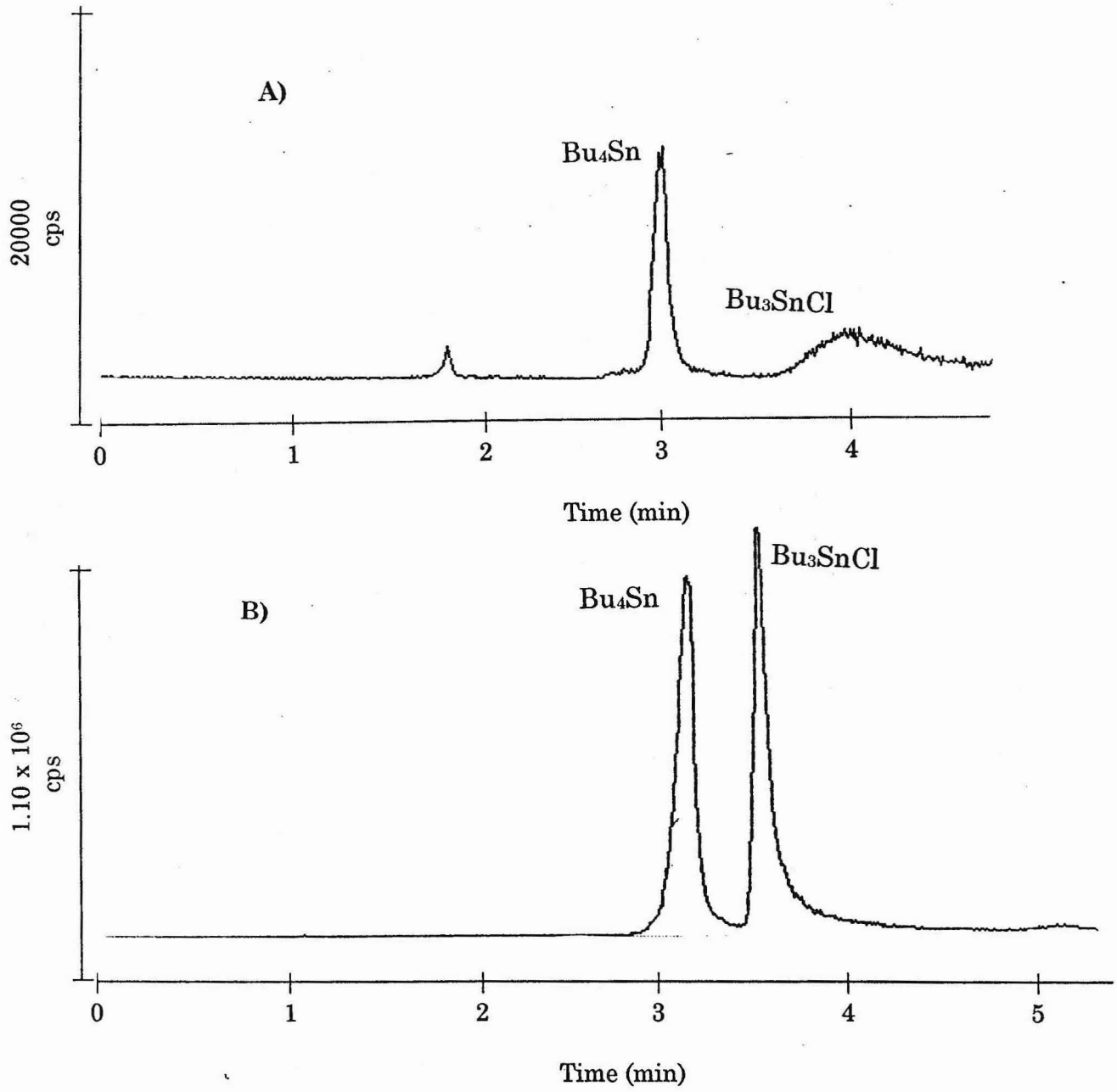


Figure 5.5: Chromatograms for $^{120}Sn^+$ to illustrate the difference in peak shape for tetrabutyltin and tributyltin chloride at A) 0.1 μg/g and B) 10 μg/g. SFC conditions: CO_2 mobile phase programmed at 3 MPa/min from 12 MPa (hold for 30 s) to 24 MPa (hold for 1 min). Constant temperature 75 °C, time split injection 0.100 s.

Thus, for triplicate injections of the 10 μg/g mixture a 0.100 s injection time corresponds to an injection of 67 nl and for a 10 μg/g solution the injection will be 670 pg. The standard deviation of the baseline is 27 cps and for tetrabutyltin the sensitivity is 2287 cps/pg. Thus, for tetrabutyltin the theoretical detection limit is 0.035 pg as tin and for tributyltin chloride the theoretical detection limit is 0.025 pg as tin. The theoretical detection limit and the practical detection limit for tetrabutyltin are similar and in the sub-picogram range. However, for tributyltin chloride the practical detection limit is almost 300 times higher than the calculated detection limit. This is due to the large amount of peak tailing which occurs for tributyltin chloride. Thus, under present conditions the method is not suitable for trace analysis of tributyltin chloride (and other polar compounds). However, if a modifier were added to the mobile phase, provided it had no significant effect on the plasma, it should be possible to bring the practical detection limit of tributyltin chloride in line with the calculated detection limit.

The shape of the graphs in Figure 5.4 can be explained in terms of the detector's ability to "see" the compounds of interest. For tetrabutyltin there is a slight increase in intensity between 0.001 μg/g and 0.01 μg/g but thereafter there is a relatively sharp increase in intensity to an analyte concentration of 10 μg/g. Above 10 μg/g the peak intensity again begins to level off with increasing analyte concentration. At low concentrations the detector is able to detect the $^{120}Sn^{+}$ ion but any increase in concentration gives a small increment increase in signal. However, at higher concentrations, each increment increase in the concentration is significantly easier for the detector to "see" and so there is a large increase in peak intensity. At high concentrations the detector becomes overloaded and each increment increase in concentration will overload the detector further and the increase in ion concentration becomes harder to see. Thus, at high concentration the graph begins to level out. A similar argument explains the graph shape for tributyltin chloride. The gap between the two

graphs at lower concentrations is due to the peak tailing (and hence lower intensity) of tributyltin chloride. The detector is linear over three orders of magnitude from 0.1 to 10 μg/g (100 ppb to 10 ppm) for tetrabutyltin and the slope for this linear range on a log-log graph is 0.970 and for tributyltin chloride it is 1.29.

Thus, for a non-polar tin compound (tetrabutyltin) the method shows low detection limits with a linear range over 3 orders of magnitude. For the more polar tributyltin chloride the practical detection limit is far higher than the calculated value. This discrepancy arises from the greater adsorption of the polar compound onto the active sites on the column. This causes significant peak tailing which lowers the intensity of the peak and even eliminates the peak at low concentrations. The theoretical detection limit is calculated from the concentration which gives the best peak shape. The calculation assumes that the peak shape is ideal and will not change as concentration is varied. Thus, the calculated value gives a much lower detection limit than is practically possible. In order to lower the practical detection limit it would be necessary to add a modifier to the mobile phase in order to minimise the tributyltin chloride peak tailing. However, it would be necessary to ensure that the modifier used did not interfere with plasma.

5.2.2 The Effect of the Applied Voltage

To study the effect of the restrictor temperature on the peak intensity for organotin compounds a 10 μg/g solution of tetrabutyltin and tributyltin chloride was chosen. This concentration resulted in an injection of 67 ng onto the column and gave the best peak shape for both compounds with minimal peak tailing and good resolution. As stated earlier it is not possible to accurately assign a temperature to a particular applied voltage on the interface. Thus, the study was carried out in terms of the applied

voltage on the interface rather than the temperature on the restrictor. From the bench top experiments it was found that an applied voltage of 6.5 V corresponded to a temperature of about 200 ^{0}C and 9.0 V corresponded to about 320 ^{0}C (Appendix E). Thus, the range from 6.5 V to 8.5 V was chosen to study the effects of temperature. The temperature was increased in 0.5 V increments and enough time was allowed before injecting the tin mixture to ensure that thermal equilibrium had been reached. Plots of peak intensity versus applied voltage on the interface are shown in Figure 5.6. Typical results for duplicate analyses are shown in Appendix D. The peak intensity increases as the applied voltage on the interface (and the temperature of the restrictor) is increased. This is explained in terms of the Joule-Thompson effect. At lower temperatures there is adiabatic expansion of the mobile phase as it exits the restrictor. Thus, the restrictor is cooled and the analytes condense in and around the restrictor and on the cool walls of the torch and there is incomplete introduction of the analyte into the plasma. However, at higher temperatures the Joule-Thompson effect is minimised and the analytes are more efficiently transferred to the plasma. Thus, there is an increase in the peak intensity as the adiabatic expansion of the mobile phase becomes less significant. Tetrabutyltin is more volatile than tributyltin chloride (see Appendix C) and is less likely to condense in the restrictor or the torch. As the study was carried out from lower to higher temperatures it is likely that at the lower temperatures condensation occurred around the restrictor. As the temperature increased, the condensed analyte was volatilised and introduced into the plasma with each new injection. This resulted in a non-linear plot which is particularly noticeable for the less volatile tributyltin chloride. This also explains the difference in peak intensities between the two compounds. As tributyltin chloride is less volatile, when the temperature is increased, more of the analyte condenses around the restrictor at lower temperatures and there is more analyte to be volatilised at higher temperatures. Above 7.5 V the increase in peak intensity as temperature increases is linear as would be expected.

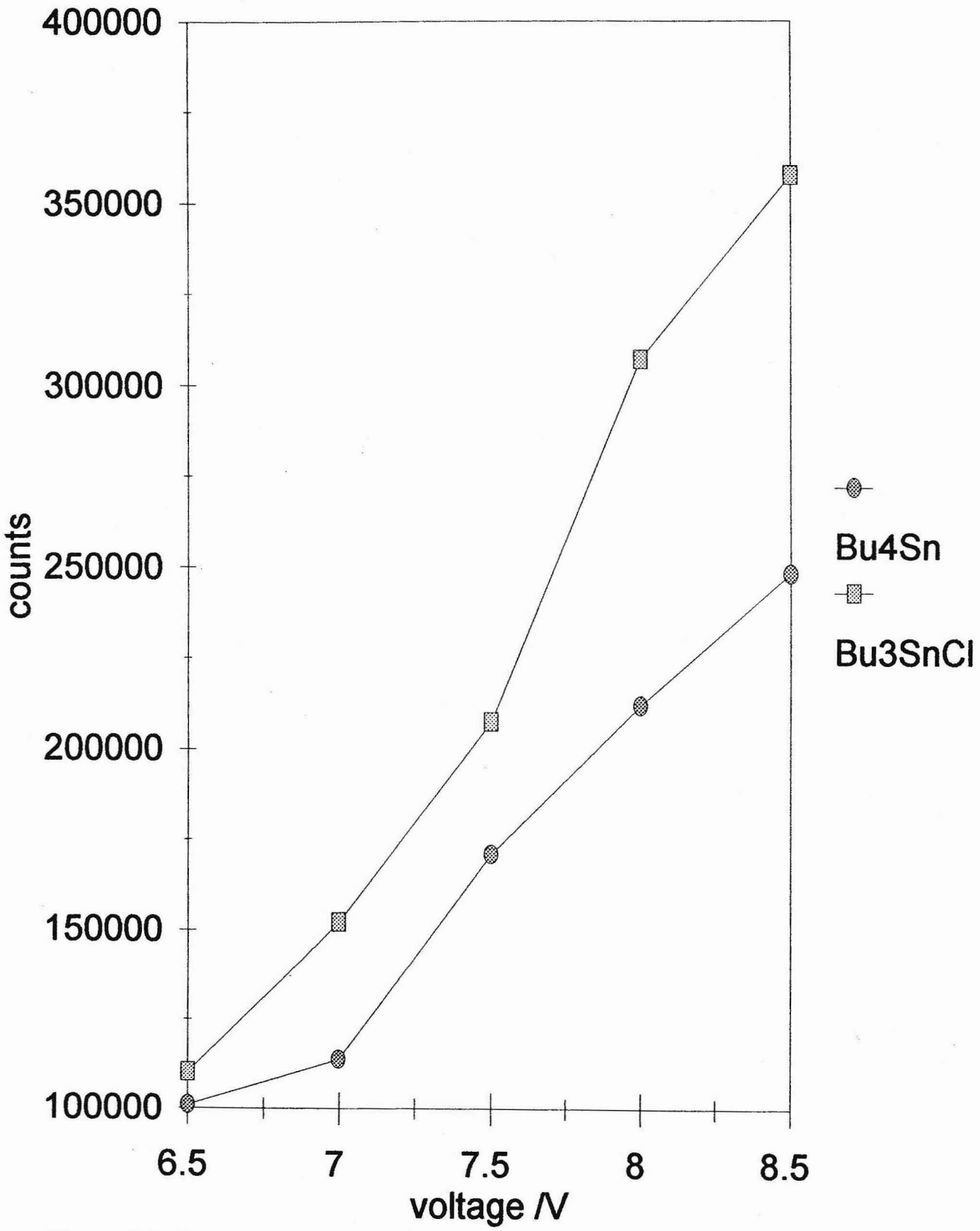


Figure 5.6: The effect of the Applied voltage on the interface on tetrabutyltin and tributyltin chloride.

In retrospect, if the study had considered the higher temperatures first then the graph would have been different. The difference in the intensity between the two compounds can also be ascribed to volatility. It is also important to note that neither compound has yet reached a plateau where there is no increase in peak intensity with increasing voltage. This would indicate complete volatilisation and transfer of analytes to the plasma. Thus, by improving the interface and the temperature calibration procedure it should be possible to optimise the temperature on the restrictor for more efficient analyte transfer to the plasma and so increase the sensitivity of the detector and detection limits even further. A brief discussion of ways to improve the interface is given in section 4.5.

Chapter 6

6.1 Organoarsenic compounds

6.1.1 Interferences

Although arsenic has 21 isotopes only one occurs naturally [73] at m/z 75. This is a problem in ICPMS as there are a number of interfering ions which also occur at this mass to charge ratio. The most significant interference is $^{40}Ar^{35}Cl^+$. As demonstrated in the investigation of the effect of the mobile phase on the plasma, chloride in the organic solvent will yield $^{40}Ar^{35}Cl^+$. Provided the chloride exists only in the solvent this should not cause a problem as the $^{40}Ar^{35}Cl^+$ should only be seen as a peak when the solvent elutes. However, if the analysis is performed in a chloride rich atmosphere the $^{40}Ar^{35}Cl^+$ will be formed at a constant rate throughout the analysis thus increasing the baseline and lowering the instrument's sensitivity to arsenic. Although the absence or presence of chloride or chlorine in the atmosphere should be noted before the analysis is carried out, this is not obvious to the new or inexperienced researcher. Moreover, if a calibration were to be performed one would have to take into account the instrument drift of the ICPMS. As this can be quite significant, a calibration at each concentration of analyte is necessary before calibration over several concentrations can be performed. As the chloride or chlorine content of the atmosphere may change during this calibration procedure, this would result in further uncertainty. Thus, although these factors indicate that accurate calibration is not possible, if one assumed ideal conditions with no instrument drift, a constant level of noise and constant atmospheric chloride concentration, then a calibration taking into account the atmospheric chloride and chlorine would be possible.

The second interference which is experienced by arsenic is that of $^{59}Ni^{16}O^+$

[47]. The sampling cones within the ICP-MS interface are made of nickel which reacts with oxygen in the plasma. Although this oxygen is normally atmospheric oxygen, with SFC-ICPMS there is additional oxygen being added by the CO_2 mobile phase. Thus, it is reasonable to assume that for SFC-ICPMS $^{59}Ni^{16}O^+$ is even more relevant than usual. However, in stand alone ICPMS $^{59}Ni^{16}O^+$ interference should remain a constant interference as it has a fixed origin, that is the sampling cone and the atmospheric oxygen. Thus it should be possible to treat $^{59}Ni^{16}O^+$ interference as a systematic experimental error. Nevertheless, as this interference affects the intensity of the baseline it will still significantly affect the detection limits of arsenic in SFC-ICPMS as programming the CO_2 mobile phase will change the amount of oxygen entering the plasma. Thus, this can no longer be treated as systematic error.

In this study it was found that the baseline intensity when monitoring arsenic was high (~ 35 000 counts per second). This is due to the $^{59}Ni^{16}O^+$ interference rather than the $^{40}Ar^{35}Cl^+$ interference as the instrument was housed in a temperature and humidity controlled room. To a large extent this would remove any chloride which may be associated with the humidity in the air and provide a relatively chloride deficient atmosphere. Thus, the high baseline intensity must be largely due to $^{59}Ni^{16}O^+$. However, the elution solvent (dichloromethane) contained high concentrations of chlorine and solvent peaks were observed due to the formation of $^{40}Ar^{35}Cl^+$ as shown in Figure 6.1. As the solvent eluted early in the analysis this did not interfere with the arsenic peaks and $^{40}Ar^{35}Cl^+$ interference was not considered as significant as $^{59}Ni^{16}O^+$ interference.

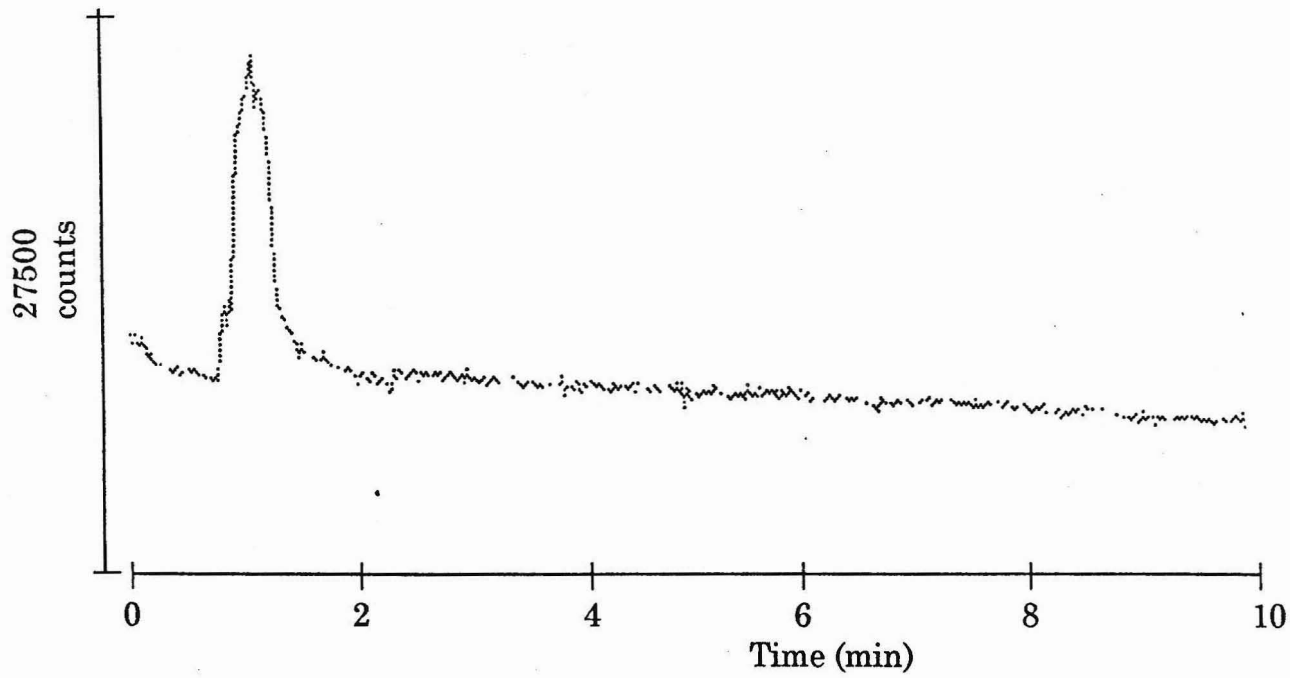


Figure 6.1: Chromatogram showing $^{40}Ar^{35}Cl^{+}$ interference as dichloromethane elutes. SFC conditions: CO_2 mobile phase programmed at 3.5 MPa/min from 12 MPa (hold for 30 s) to 40 MPa (hold for 1 min). Constant temperature 75 ^{0}C, time split injection 0.100 s.

6.1.2 The Effect of Analyte Concentration

To study the effect of analyte concentration on peak intensity for arsenic compounds, triphenylarsine was chosen as the analyte of interest. The choice of arsenic analyte was prompted by two main factors. Firstly, bis(1,2-diphenylarsino)ethane gave inconsistent results during the optimisation experiments using the FID as detector and it was decided that this could give incorrect results for the study of SFC-ICPMS. The second reason for using triphenylarsine is its greater applicability to environmental analysis. Triphenylarsine should have characteristics similar to those of trimethylarsine (Gosio gas) which is toxic. Although the phenyl ligand is more bulky and so less mobile along the biphenyl column, the triphenylarsine molecule has a similar structure to trimethylarsine and, apart from retention time, should have similar chromatographic properties. Thus, triphenylarsine was used instead of bis(1,2-diphenylarsino)ethane.

The ICPMS was focused on $^{75}As^{+}$ and, as with tin, the necessary checks were made to ensure that there was no triphenylarsine remaining on the column. The analyte was then analysed at varying concentrations starting at the lowest concentration of 0.001 μg/g (1 ppb) and ending at the highest concentration of 10 μg/g (10 ppm). Below 0.1 μg/g (100 ppb) it was not possible to see the analyte due to the high baseline (~ 40 000 cps). As discussed previously this high baseline is due to $^{59}Ni^{16}O^{+}$ formed on the sampling cone. This is a systematic error which can not be corrected and so the practical detection limit for arsenic is 0.1 μg/g (100 ppb). Although the addition of modifiers to the mobile phase may provide slight improvement in the results the detection limit would not be lowered unless a different material was used in the construction of the sampling cones.

Between 0.1 μg/g (100 ppb) and 1 μg/g (1 ppm) there is only a slight

improvement in peak intensity with the increase in concentration. This is again due to the instruments ability to "see" the element of interest. This argument is even more relevant for arsenic than it is for tin as the detector must now distinguish between the arsenic and the large amount of background interference. Thus, between 0.1 and 1 μg/g there is still too much interference and so there is only a small increase in the intensity of the arsenic peak. However, above concentrations of 1 μg/g the arsenic predominates and the interferences are less relevant. Thus, the increase in peak intensity with increasing analyte concentration is much larger (~ 150 000 counts as opposed to ~ 25 000 counts for the 0.1 to 1 μg/g increase). This trend is shown in Figure 6.2 below.

Above 10 μg/g the detector and the column were overloaded and so any concentrations above 10 μg/g were not considered. Due to the background interference only three points could be obtained and so it was not possible to find a linear range for ICPMS detection of arsenic. The sensitivity of the ICPMS to arsenic is 2460 cps/pg. Thus, calculating the detection limit for a 10 μg/g triphenylarsine solution the theoretical detection limit is 3.4 pg as arsenic. When compared to the theoretical detection limits of tin this is very high as is the uncertainty of the 2800 cps. However, it is unlikely that this could be improved as there is such a high background interference. The practical detection limit corresponds to 6.7 pg which is double the theoretical detection limit. This is due to the increment increase in concentration studied. If a concentration of 0.05 μg/g (50 ppb) had been analysed it is reasonable to assume that the theoretical and the practical detection limits would have been similar. Chromatograms of triphenylarsine at concentrations of 0.1 μg/g (100 ppb) and 10 μg/g (10 ppm) are shown in Figure 6.3.

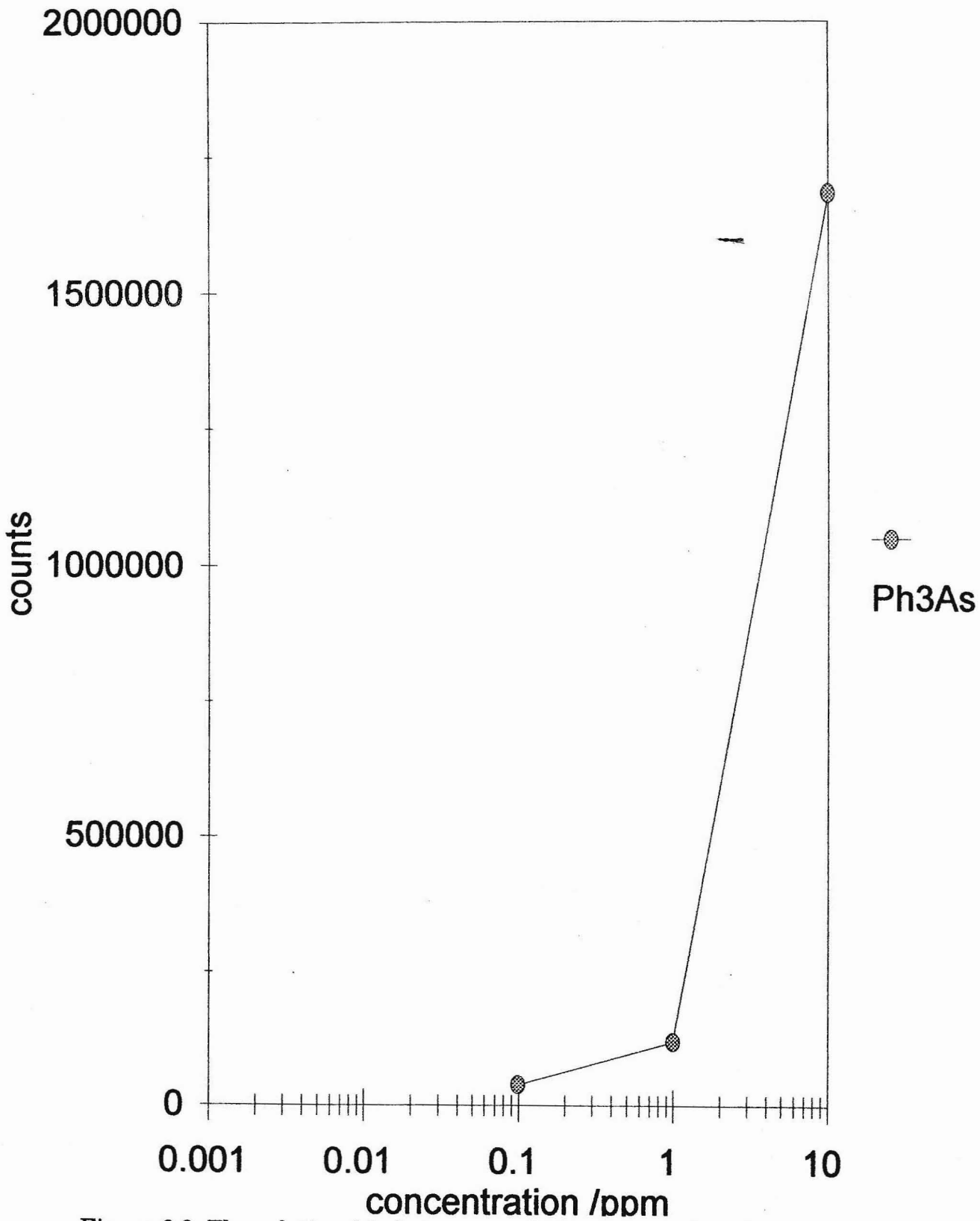


Figure 6.2: The relationship between peak intensity and analyte concentration for 75As+ in triphenylarsine.

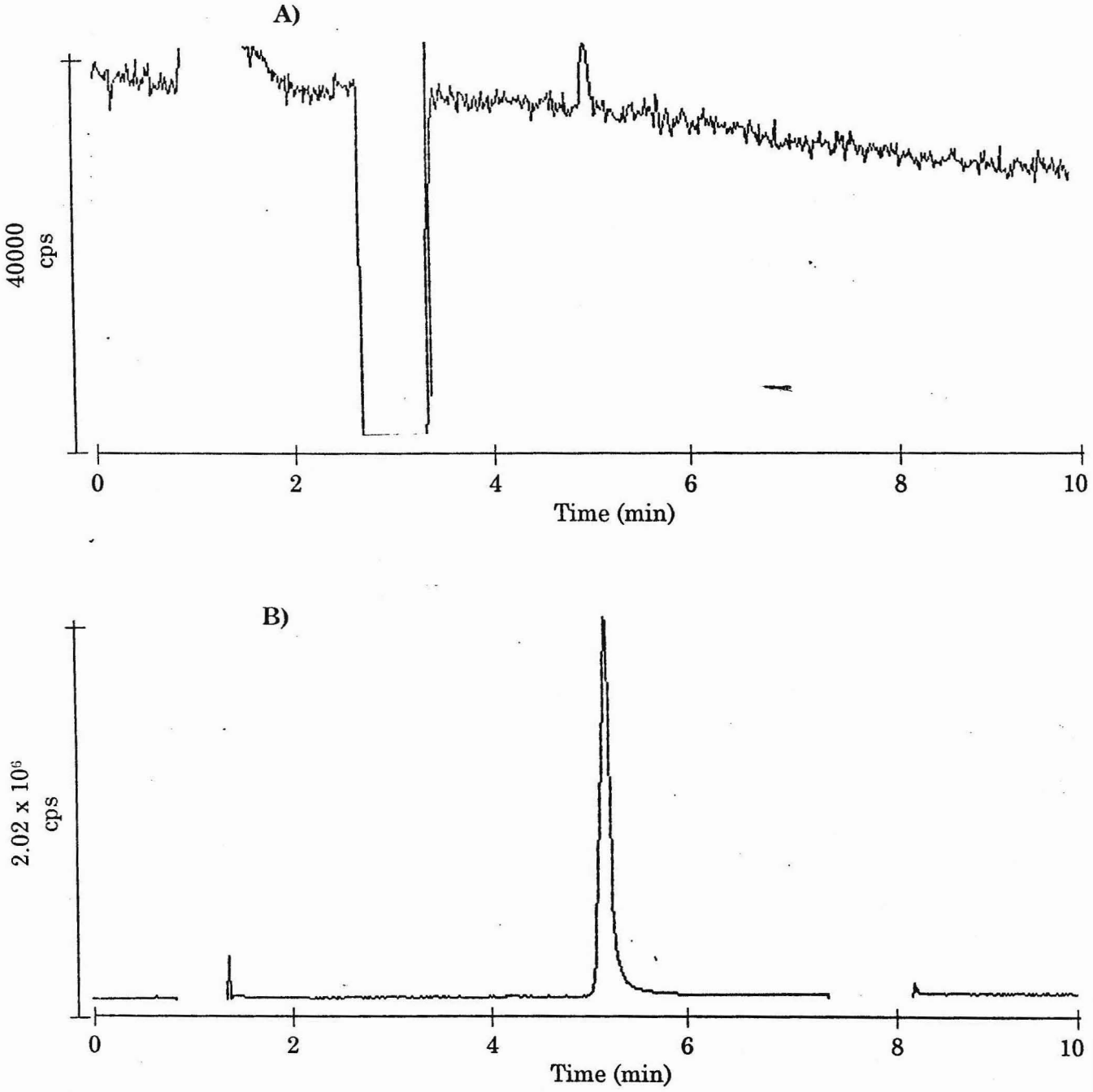


Figure 6.3: Single-ion chromatograms of $^{75}As^{+}$ in triphenylarsine at A) 0.1 μg/g and B) 10 μg/g. SFC conditions: CO_2 mobile phase programmed from 12 MPa (hold for 30 s) to 40 MPa (hold for 1 min) at 3.5 MPa/min and a constant temperature of 75 ^{0}C, time split injection 0.100 s.

6.1.3 The Effect of Applied Voltage

The effect of the applied voltage on the interface (and hence the restrictor temperature) was studied in the same way as with the tin compounds with the exception that a 1 μg/g solution was used. As would be expected there is a general increase in peak intensity with increasing voltage (and temperature). This is similar to tin with the increasing temperature being more able to combat the Joule-Thompson effect and thus increase the amount of analyte entering the plasma at higher voltages (temperatures). However, as demonstrated in Figure 6.4, the shape of the graph is somewhat different to that obtained for the tin compounds.

The first noticeable difference between tin and arsenic is the amount by which the intensity increases (the increase in peak intensity over the same temperature range is over 4 times higher for tin than for arsenic). However, the most noticeable difference is the shape of the graph. The lower increase in peak intensity for triphenylarsine can be explained by the lower concentration used. In investigating triphenylarsine a 1 μg/g solution was used whereas for the tin compounds a 10 μg/g solution was used. Moreover, as the tin compounds are less volatile than triphenylarsine they are more prone to condensation caused by the Joule-Thompson effect. Thus, at higher temperatures there is a greater amount of condensed analyte which is volatilised from in and around the restrictor. This, together with the higher concentration of eluting analyte, causes the increase in peak intensity for the tin compounds to be much larger than for triphenylarsine. As triphenylarsine is more volatile than the tin compounds it will not condense in and around the restrictor as much and they will elute at a more constant rate. However, the involatile tin compounds will elute less at lower temperatures as they condense around the restrictor, and more at high temperatures as the condensed analyte is volatilised.

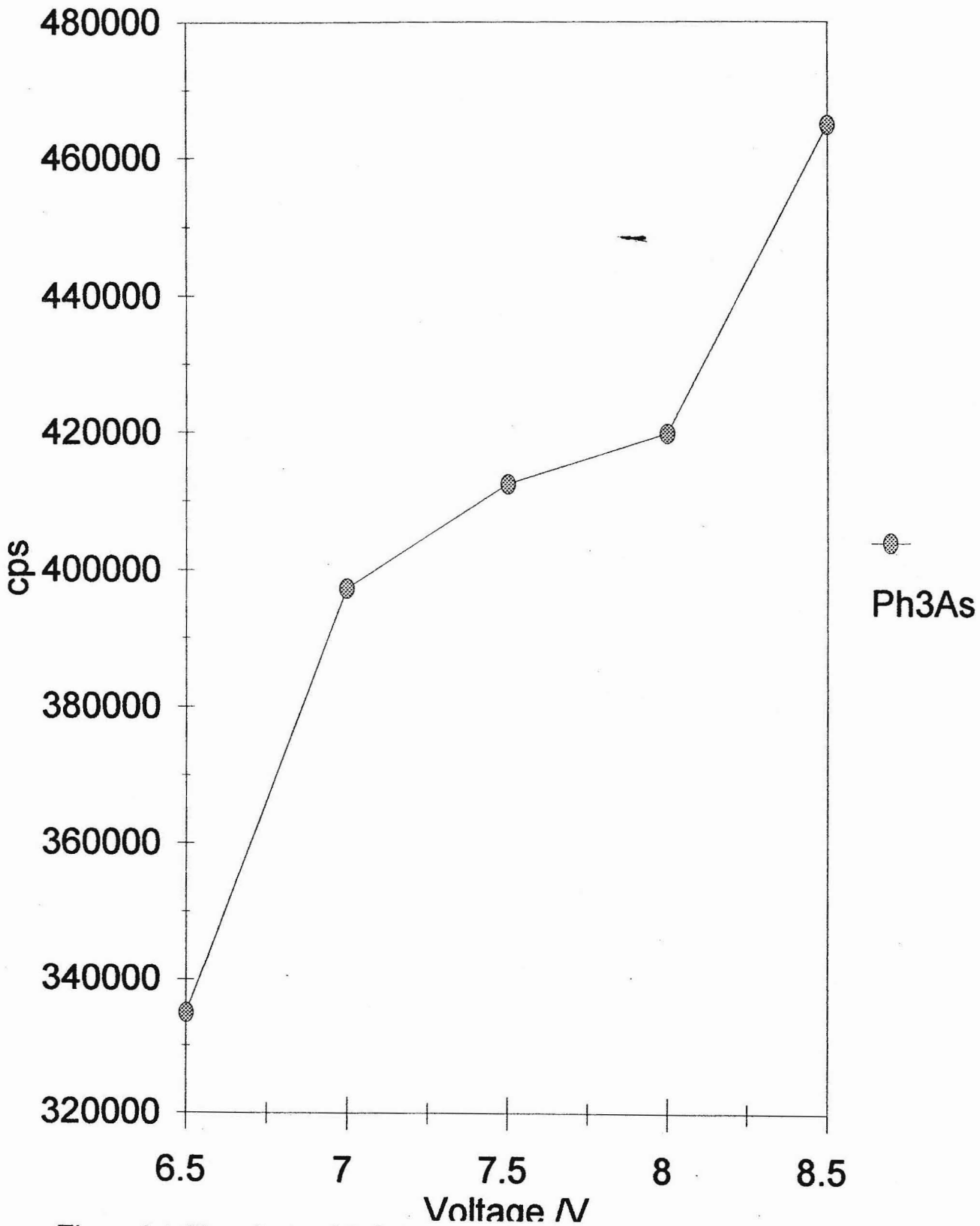


Figure 6.4: The relationship between peak intensity and applied voltage on the interface for 75As+ in triphenylarsine.

The plateau in the curve between 7.0 and 8.0 V suggests that the optimum interface temperature for triphenylarsine has been reached. This would mean that further increase in the interface temperature to counteract the Joule-Thompson effect would be unnecessary. However, above 8.0 V there is again a rise in peak intensity as the restrictor temperature is increased. Assuming that restrictor temperature is close to the optimum temperature (as suggested by the plateau region) then the increase in peak intensity above an applied voltage of 8.0 V could be due to the elution of triphenylarsine which was strongly adsorbed in the restrictor at lower interface voltages (and restrictor temperatures). This is especially relevant considering that the study was carried out from low to high temperatures. If this is true then the curve should return to a plateau above 8.5 V. However, it was not possible to test this assumption at a higher voltage as it was feared that higher voltages may cause unacceptably high temperatures as discussed earlier.

6.2 Organoiron Compounds

6.2.1 Interferences

Iron is difficult to analyse using an ICPMS detector as there are a number of interferences with the same mass to charge ratio as the most common iron isotopes. Although there are 16 isotopes of iron only 4 occur naturally [73] with the most abundant being ^{56}Fe and ^{54}Fe. However, $^{56}Fe^+$ is susceptible to interference from $^{40}Ar^{16}O^+$ while $^{54}Fe^+$ is susceptible to interference from $^{40}Ar^{14}N^+$. These are the most abundant isotopes of iron and are the most suitable for analysis. The other isotopes are ^{57}Fe and ^{58}Fe with natural abundances of 2.20% and 0.28% respectively which were considered to be too low to provide accurate estimates of the detection limits. Although $^{40}Ar^{16}O^+$ is not usually a major problem in ICPMS, with SFC-ICPMS it is more relevant as there is a large amount of oxygen being

introduced by the mobile phase. This causes an increase in the baseline intensity as described earlier and hence $^{56}Fe^{+}$ is difficult to analyse at low concentrations. Nitrogen is the most abundant component of air and so $^{40}Ar^{14}N^{+}$ is always a problem for the analysis of $^{54}Fe^{+}$ (even in stand alone ICPMS analyses).

These interferences are constant and could be considered as systematic errors during the analysis. However, when focusing the ICPMS on one of the iron isotopes not only is the iron signal enhanced but the background intereferences at m/z 56 and m/z 54 are also enhanced. Thus, by attempting to focus on the most abundant iron isotopes one is effectively decreasing the iron signal by increasing the baseline intensity through increasing the interference signal.

6.2.3 The Effect of Analyte Concentration

An attempt was made to study the effect of analyte concentration on peak intensity. As it was known that interferences occurred for both of $^{54}Fe^{+}$ and $^{56}Fe^{+}$ both isotopes were studied in an attempt to use the isotope with the least interference. For both isotopes solutions of 1, 10 and 100 μg/g of ferrocene were studied. No results were obtained for $^{56}Fe^{+}$ as the $^{40}Ar^{16}O^{+}$ interference was too great. It was found that the $^{40}Ar^{14}N^{+}$ baseline for $^{54}Fe^{+}$ was almost 20 times lower and so ^{54}Fe was the isotope studied. Nevertheless, duplicate injections of 1 and 10 μg/g of ferrocene showed no result and 100 μg/g of ferrocene showed only a small peak as illustrated in Figure 6.5. Thus, it was not possible to establish a relationship between peak intensity and concentration or applied voltage on the interface for iron. The only possible conclusion for the analysis of iron using SFC-ICPMS is that the practical detection limit is 100 μg/g (100 ppm).

Later analyses (on real samples) using a survey scan revealed small peaks

at m/z 94 and m/z 96. Although these would normally correspond to molybdenum, a possibility proposed by the VG PlasmaQuad software was $^{40}Ar^{54}Fe^{+}$ and $^{40}Ar^{56}Fe^{+}$. Thus, it was possible that any iron eluting was reacting with the argon in the plasma and thus would not be detected as iron. To investigate this possibility single-ion chromatograms were obtained for ferrocene with the ICPMS focused on m/z 94 and m/z 96. However, these gave negative results and this option can be discounted.

The reason that $^{56}Fe^{+}$ could not be analysed was due to the interference of the $^{40}Ar^{16}O^{+}$ which gave a very high baseline (~ 129 000 counts). This is unfortunate as ^{56}Fe is the most abundant isotope and would be ideal for studies of analyte concentration. As ^{57}Fe and ^{58}Fe are the least abundant isotopes they would not be suitable for study of analytes at low concentration (especially when taking into account that they are also prone to interference but on a lesser scale). Thus, the only possible choice was $^{54}Fe^{+}$. However, this has a relatively low abundance of 5.8%. This, coupled with the $^{40}Ar^{14}N^{+}$ interference ensures that low concentrations of iron can't be detected. Although it should be possible to increase the ferrocene concentration above 100 μg/g and obtain a high concentration dependence of peak intensity on analyte concentration, this would flood the detector with interfering ions and the ion signal would be degraded. Thus, SFC-ICPMS is not suitable for the analysis of iron containing compounds. However, it is still possible to analyse $^{56}Fe^{+}$ at low concentrations using capillary GC-ICPMS as described by Kim *et al.* [74]. As there is no oxygen being introduced into the plasma by the mobile phase, it is possible to monitor $^{56}Fe^{+}$ with little difficulty provided that the iron compound has been dissolved in an oxygen deficient solvent. However, GC-ICPMS is only suitable for volatile iron organometallic compounds and less volatile compounds require derivatisation to a more volatile species before analysis.

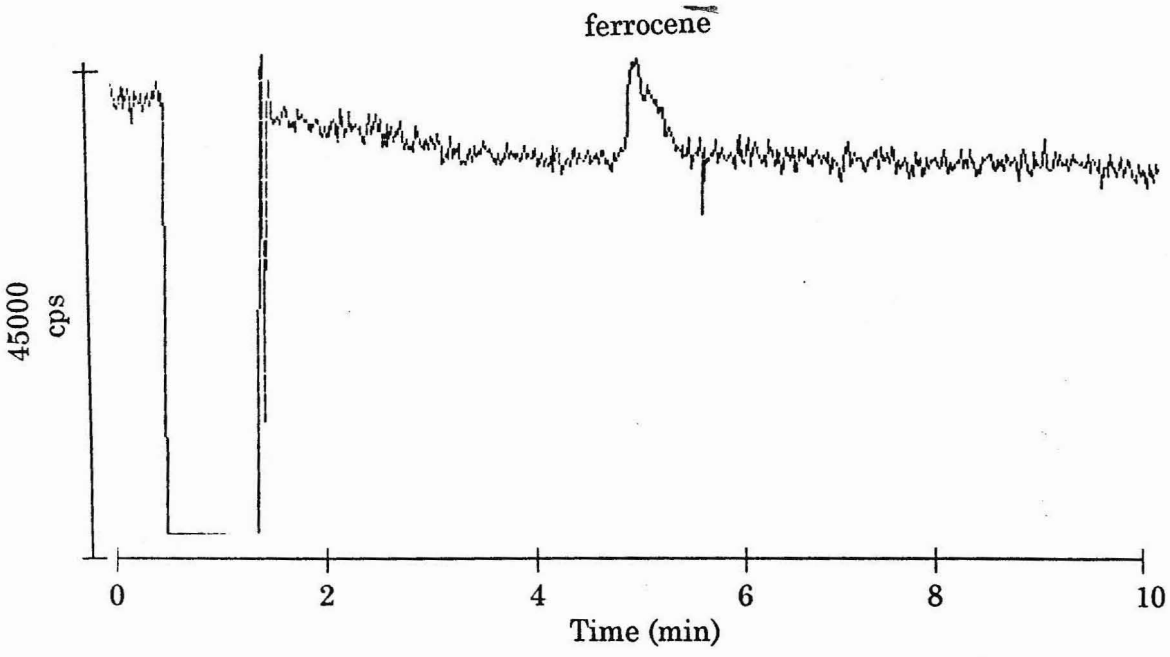


Figure 6.5: Single-ion chromatogram of $^{54}Fe^{+}$ in a 100 μg/g solution of ferrocene. SFC conditions: CO_2 mobile phase programmed from 12 MPa (hold for 30 s) to 40 MPa (hold for 1 min) at 3.5 MPa/min at a constant temperature of 75 ^{0}C, time split injection 0.100 s.

6.3 Other Organometallic Compounds

Although tetrabutyltin, tributyltin chloride, triphenylarsine and ferrocene were the main subjects of the study, other organometallic compounds were considered. These were diethylmercury and triphenylbismuth. Diethylmercury had to be synthesized from mercuric chloride as most suppliers have discontinued the manufacture of dimethylmercury and diethylmercury. As the diethylmercury was manufactured in the laboratory using the Grignard reaction it was not possible to determine the exact concentration. Thus, no attempt was made to investigate relationships between peak intensity and analyte concentration or between peak intensity and the applied voltage on the interface. Nevertheless, the ICPMS was focused on $^{202}Hg^{+}$ and a chromatogram was obtained. This is shown in Figure 6.6. As mercury is a heavy metal it is safe from the interferences which hinder the lower mass metals. Thus, if standards of different known concentrations are prepared it should be possible determine accurate detection limits for diethylmercury (or dimethylmercury). It is reasonable to assume that these detection limits will be very low as there are no interferences or other factors which should hinder the analysis. This is confirmed by a 3 pg detection limit obtained by Carey *et al.* [16].

Near the end of the study an attempt was made to analyse $^{209}Bi^{+}$ in triphenylbismuth but no results could be obtained due to instrumental failure of the ICPMS. However, as there are no interferences at m/z 209 future researchers should be able to analyse bismuth in the same way as arsenic and tin. In general any non-polar and slightly polar compounds of heavy metals such as bismuth, antimony and mercury could be studied in

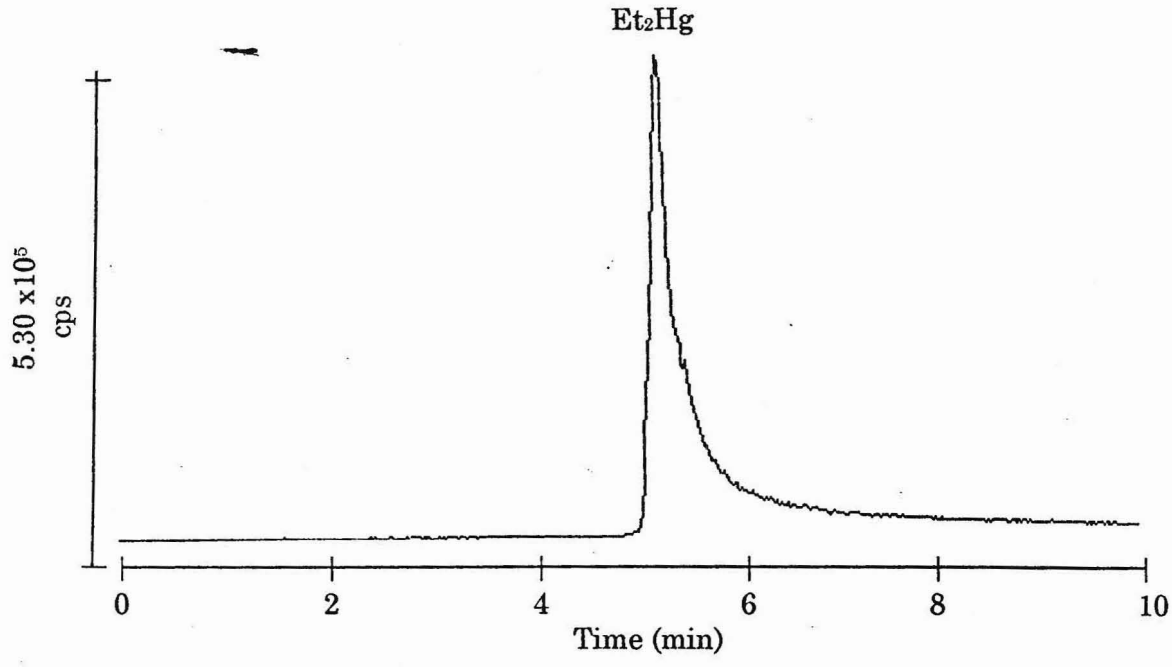


Figure 6.6: Chromatogram of $^{202}Hg^{+}$ in diethylmercury. SFC conditions: CO_2 mobile phase programmed from 12 MPa (hold for 30 s) to 40 MPa (hold for 1 min) at 3.5 MPa/min and a constant temperature of 75 ^{0}C, time split injection 0.100 s.

the future. Lead has already been studied by Caruso's group with good results [16-18]. An attempt was made to analyse Nickel, Vanadium and Tungsten porphyrins supplied by Warren Pretorius from the University of Plymouth. However, these compounds proved too polar for analysis by SFC using an unmodified mobile phase. If a modifier were added to the mobile phase it may be possible to analyse these compounds in the same manner as the other organometallic compounds studied.

Chapter 7

7.1 Supercritical Fluid Extraction

Although SFE was not the primary aim of this study, an investigation of the supercritical fluid extraction of some tin compounds was carried out to determine the best conditions for the extraction of organometallic compounds from real samples. Topsoil samples were spiked with tetrabutyltin and tributyltin chloride and the sample was extracted using both dynamic and static extractions over a range of different pressures. It was found that the dynamic extractions gave no results at all neither did a series of short (~ 30 min) static extractions repeated on the same sample at high pressure (41 MPa). However, if the organotin compounds were placed directly in the extraction cell and extracted, both compounds were extracted without difficulty. An SFC-FID chromatogram of the extract is shown in Figure 7.1.

The results show that the tin compounds are soluble in the CO_2 but can not be desorbed from the topsoil. Thus, it is reasonable to assume that the soil matrix is the dominant factor. Either the matrix prevents efficient diffusion of the supercritical solvent into the topsoil or the organometallic compounds are too strongly adsorbed to be solvated under the conditions used. Higher extraction pressures could not be used due to the limitations of the instrument being used. However, if the pressure could have been raised to above 60 MPa and if a modifier could be introduced to the mobile phase it may be possible to extract the organotin compounds from the soil matrix. These are possible topics which could be examined in future studies. Similar results to those of organotin compounds were obtained for bis(1,2-diphenylarsino)ethane. A chromatogram of the bis(1,2-diphenylarsino)ethane extract is shown in Figure 7.2. Although no positive results could be obtained with spiked topsoil samples it was

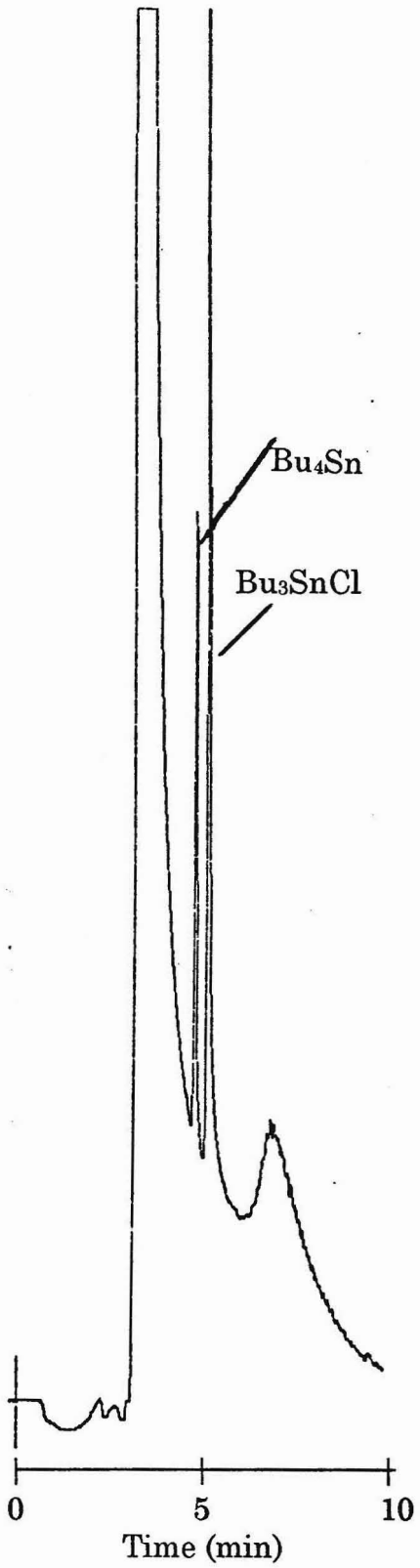


Figure 7.1: SFC-FID chromatogram of a tetrabutyltin/tributyltin chloride extract. SFE conditions: static CO_2 extraction for 30 min followed by 20 min dynamic extraction. SFC conditions: CO_2 mobile phase programmed at 3 MPa/min from 12 MPa (hold for 30 s) to 40 MPa (hold for 1 min); constant temperature at 75 °C.

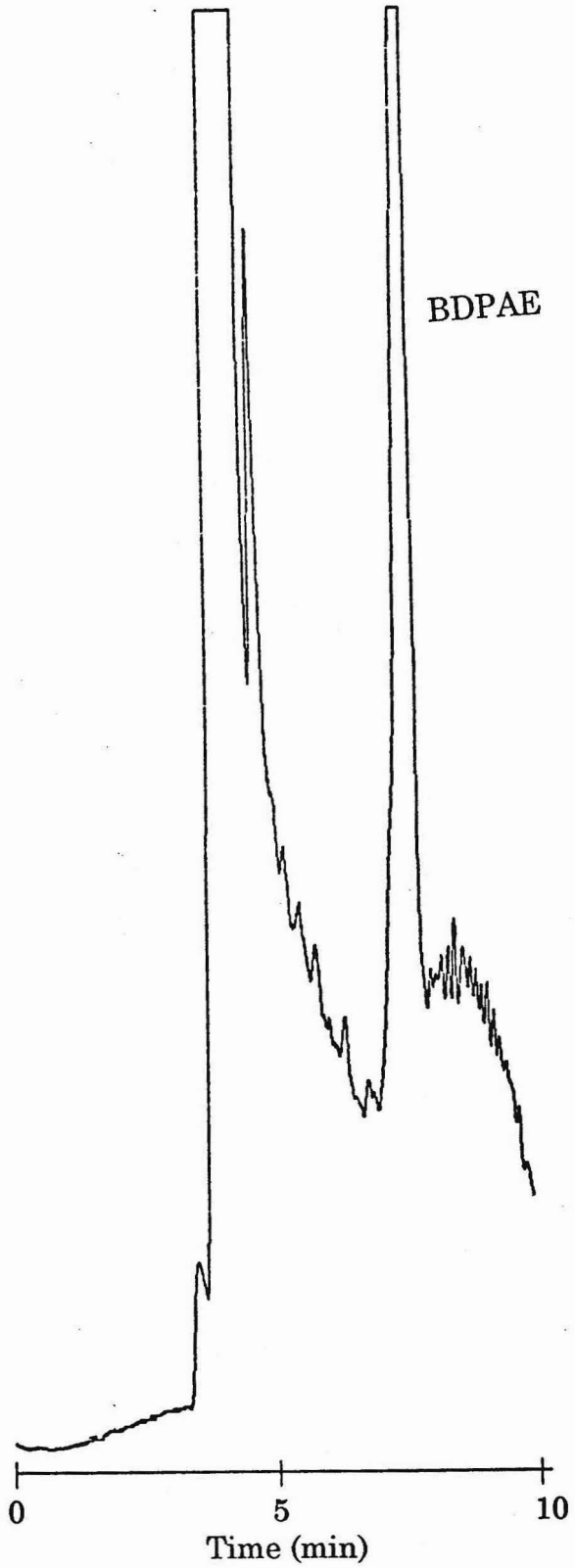


Figure 7.2: SFC-FID chromatogram of bis(1,2-diphenylarsino)ethane extract. SFE conditions: CO_2 static extraction at 41 MPa for 30 min followed by 20 min dynamic extraction. SFC conditions: CO_2 mobile phase programmed at 3 MPa/min from 12 MPa (hold for 30 s) to 40 MPa (hold for 1 min); constant temperature 75 °C.

decided to attempt extracting sediment samples from Durban Bay using a pressure of 41 MPa and a temperature of 70 ^{0}C.

7.2 Analysis of Real Samples

7.2.1 Selection of Sampling Points

As this study is concerned with organometallic compounds which are toxic or general pollutants, it was decided to take samples from areas of the bay which were either ecologically sensitive or were likely to have a high concentration of organometallic pollutants. The sampling points are shown on the map in Figure 3.4. The first three sampling points were on the dredge spoil next to the mangroves. The mangroves are particularly sensitive ecosystems with many sensitive species of fish (such as mudskippers) and crustaceans (such as the mangrove crabs). The crustaceans are particularly prone to organometallic pollution as they accumulate these compounds in their shells. The dredge spoil could contain large concentrations of organometallic compounds which are leached from the anti-fouling paints on ships. These compounds are largely insoluble in water and would therefore collect in the sediment. As this sediment is dredged and dumped near the mangroves it could increase the risk to the sensitive mangrove ecosystem. Sampling points 4 and 5 were on the southern sand bar near the bayhead. This area would be prone to accumulation of organometallic compounds which leach from the paints on small craft which pass this area on their way to and from the yacht clubs at the bayhead. Moreover, it could accumulate pollutants which drain into the bay from the Aminzimnyama and uMhlatuzana canals.

The sixth sampling point was at the yacht mole. This is the main anchorage for most of the small pleasure craft in the Natal South Coast

region. As many of the paints used on these craft contain some kind of organometallic compound it is possible that leachates could concentrate the organometallic compounds in the sediment. Moreover, there could be large concentrations of organolead compounds which land in the bay from the fuel of these boats. The final sampling point was on the northern sand bar. This is as close to the center of the bay as possible and is on the edge of the Maydon channel. Thus, it would be expected to collect any pollution arising from the ships passing through the channel to the Maydon wharf and the graving docks.

The appearance of the sampling areas was noted as this could give an idea of what could be expected in the sample. Point 1 was taken from a small, shallow pool of water approximately 20 m north of the mangroves. The sediment was fairly light in colour and was very coarse. There were large numbers of shells in the area but mangrove crabs were abundant. However, there were no other species, such as mudskippers, present. Point 2 was at the edge of the mangroves where the estuary enters the bay. The appearance of this sampling point was similar to the first. Thus, it is possible that some pollution may have affected the more sensitive species but not yet reached fatal levels in the crustaceans. Point 3 was a pool of very soft sediment on the edge of the sand bar. No shells were present and there was no evidence of any marine life. The sediment was dark in colour and was quite fine in texture. Point 4 was on the edge of the sand bar near the uMhlatuzana canal. There were a number of shells in the area and the sediment was fine and light in colour. Point 5 was approximately 5 m off the sand bar near the buoy marking the channel. There was no evidence of marine life and an absence of shells. The sediment was ultra fine and light in colour. Point 6 was in the yacht mole about 2 m from the yachts. The sediment was very fine and very light in colour. There was a lot of plastic and metal pollution in the area. Point 7 was taken from the Maydon channel about 5 m from the northern sand bar. The sediment was very dark in colour and very fine. There was an abundance of metal and

plastic pollution and there was evidence of oil on the sediment. All samples were collected at low tide as this gave the best accessibility to the sampling points. At high tide many of these points are under water and a boat would be needed to reach them.

7.2.2 Sample Preparation

All samples were dried slowly at a moderate (70 ^{0}C) temperature to avoid evaporating any volatile compounds. After drying the samples were reweighed and it was found that the loss of moisture was virtually identical for all samples. Thus, by mass it was calculated that the sediment moisture content was 20 % ± 1% for all samples. The dried samples were then sifted to obtain a small, uniform particle size and the sifted samples were then ground further with a mortar and pestle to decrease the particles to a size which it was hoped would be more suitable for extraction.

Each sample was extracted twice using two static extractions at 41MPa and 75 ^{0}C for 30 minutes each followed by a 20 minute dynamic extraction into dichloromethane. This solution was then made up to 10 ml. All extracted samples were stored at 4 ^{0}C until they were analysed.

7.2.3 Sediment Analysis

As it was not known what compounds were present in the samples single-ion monitoring would have been tedious and possibly would not give any results. Thus, it was decided to use a survey scan on each sample. Each sample was injected and analysed on the SFC and a survey scan was set up to scan from m/z 45 to m/z 210 repeatedly throughout the analysis. Thus, for each injection the eluent from the SFC was scanned at least six times with time. Thereafter, each scan was analysed by the computer to

determine all possible ions for each peak. The most likely ions were then analysed using single-ion monitoring while less likely ions were ignored. Although this method was not ideal it was capable of showing the most abundant ions eluting from the sample. It was then possible to focus on these ions and use single-ion monitoring to determine whether they existed in any significant concentration. Survey scans of the sample taken from point 3 are shown in Figure 7.3.

It is important to note for Figure 7.3 that this represents just one scan. The overall analysis consists of at least six such scans. Each scan was broken into four sections as this provides a better scale for printing the result. In the low mass region the only signals are from the expected interfering ions. At m/z 115 there is a high indium signal but this can be disregarded as indium solutions are used to calibrate the ICPMS and this signal was considered the result of indium impurities remaining in the instrument. Tin is also in evidence but a single-ion scan of $^{120}Sn^{+}$ showed a peak at the same retention time as triphenyltin chloride. Thus, this was not considered to be present in the sample but rather the memory effect of this compound as discussed earlier. In the high mass region mercury and lead are apparent. However, single-ion scans on these metals gave no peaks and it is possible that they occur in the extract in concentrations too low for detection by the ICPMS.

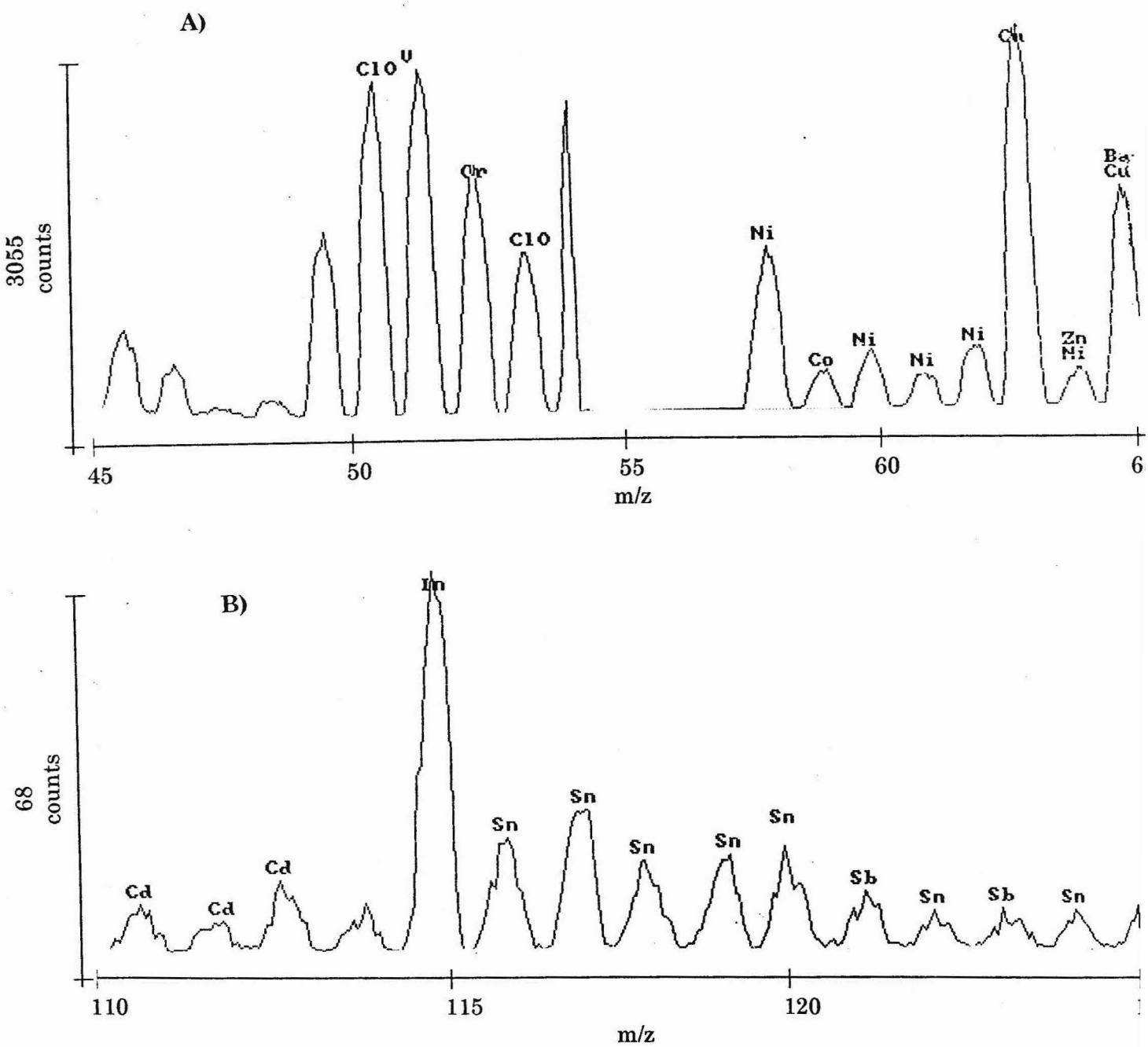


Figure 7.3: Survey scans of the extracted sample taken at point 3 on the dredge spoil for A) m/z 45 - m/z 65 and B) m/z 110 - m/z 125. SFC conditions: CO_2 mobile phase programmed from 12 MPa (hold for 30 s) to 40 MPa (hold for 1 min) at 3 MPa/min and constant temperature of 75 ^{0}C, time split injection 0.100 s.

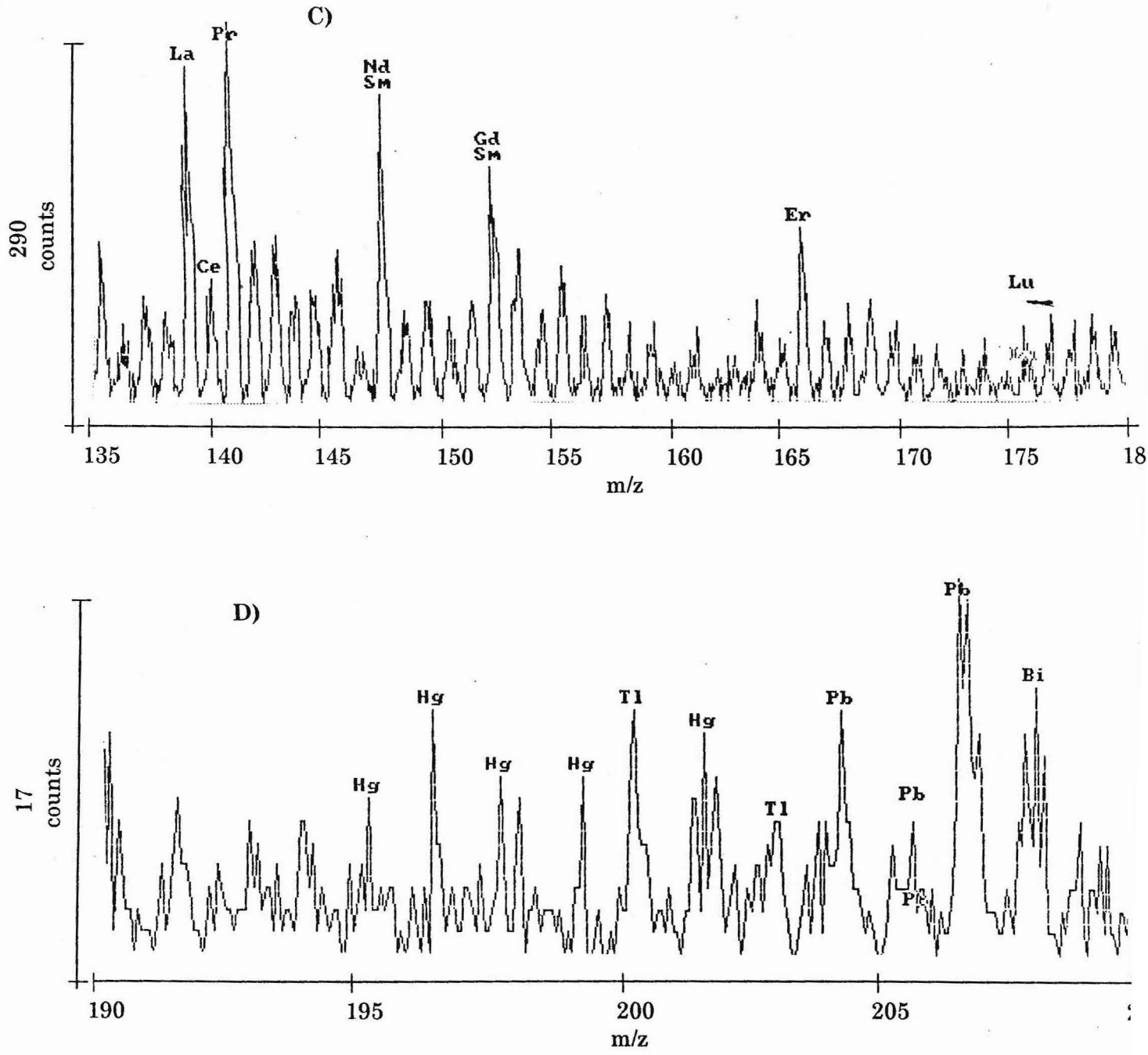


Figure 7.3(contd.): Survey Scans of the extracted sample taken from the dredge spoil for C) m/z 135 - m/z 180 and D) m/z 190 - m/z 210. SFC conditions as before.

Although these results indicate that no organometallic compounds are present in the extracted samples it is not necessarily true for the sample as a whole. Solvent extraction and GC-AES work by the CSIR has shown significant organometallic pollution in Durban Bay [75]. Thus, it would appear that the extraction method used was insufficient for these samples. As discussed earlier it is possible that higher extraction pressures and a mobile phase modifier could extract these samples. However, the optimisation of SFE conditions for the extraction of organometallic compounds from real samples was not the primary aim of this study and should be the basis of a study on its own. Future studies should concentrate on optimising the pressures and temperatures for extraction of organometallic compounds. Studies should also concentrate on extraction efficiency using pure and modified mobile phase and if necessary investigate various chelating agents which may aid the extraction of more polar analytes. Nevertheless, if organometallic pollutants could be easily and accurately extracted from real samples, SFC-ICPMS is a powerful method for speciating these compounds and determining their concentrations at trace levels.

Chapter 8

The mettle of any new or developing method will only be proven with use and recommendation over time. However, when developing a new method it is still possible to evaluate the method in terms of its ability to analyse compounds accurately and precisely at low concentrations and assess the method in terms of its cost, ease of use and general configuration. The results of this initial evaluation are an important indicator as to whether a method should be developed further or whether it is likely to prove a failure. SFC-ICPMS is a method which is developing for the speciation and detection of organometallic compounds at trace levels. Thus, in order to assess its longevity it is important to critically examine the range of organometallic compounds which can be speciated, the detection limits for these compounds, the effect of the SFC mobile phase on the plasma, the ease of use of the method and the cost of maintaining the instruments involved. This study was a preliminary investigation of some of these factors and included the development of a new interface for coupling SFC to ICPMS.

The best way of evaluating this hyphenated technique is to first consider each instrument separately before proceeding with an evaluation of the hyphenated technique. SFC is a fast developing method and is becoming more readily available. As the mobile phase decompresses to a gas it can be used with a large variety of detectors such as FID, FPD, UV and ICPMS. Thus, it is possible to optimise chromatographic conditions using one detector before coupling the instrument to another. Moreover, as the mobile phase decompresses to a gas there is no organic solvent which must be handled and disposed of and the sample is usually aspirated in the detector (apart from the UV detector) so there is usually no effluent from the SFC at all. Although this study was limited to non-polar and slightly polar compounds by the non-polar CO_2 mobile phase, Poole *et al* have

shown that a modified mobile phase can extend the use of SFC to the analysis of polar compounds as well. Thus, SFC is suitable for speciating organometallic compounds which range in polarity from non-polar to polar. However, capillary SFC requires skill in its operation, especially with the installation of columns and restrictors and in the manufacture of the restrictors. Thus, a well trained, experienced operator is preferable in order to minimise the amount of time taken for configuring the instrument and maintaining it. As many of the additional items such as ferrules and butt connectors are costly to replace, an inexperienced operator may, through no fault of their own, cause unnecessary wastage if these items. Moreover, this study has found that there is an increase in column activity over a relatively short period of time (~ 1 year) thus indicating that organometallic compounds can degrade the column. This adds to the cost of maintaining the SFC as columns would need to be replaced more frequently.

ICPMS is a metal selective detector which offers the possibility of isotope dilution analysis. It also has sub-picogram detection limits for most metals and thus it is a powerful method for the analysis of trace metals. However, the cost of these instruments is very high and it is essential to have a well trained, experienced operator to maintain and operate the ICPMS. An inexperienced operator can dramatically increase the time taken for setting up and performing the analysis and if something goes wrong with the instrument an inexperienced operator can take an unacceptably long time to diagnose and rectify the fault. Maintaining the ICPMS is also costly and requires a great deal of dedication as it is recommended that certain parts of the instrument be dismantled and cleaned on a regular basis.

When optimising the chromatographic conditions it is necessary to decide on what factors are most important for a particular analysis. Resolution may be more important than time and at trace levels peak intensity is

extremely important. Thus, it is necessary to use iterative studies of pressure and temperature to find the best compromise between resolution, peak intensity and time. It was found that a higher pressure program decreased the resolution but increased peak intensity while a lower pressure ramp gave good resolution but gave unacceptably low peak intensity. Moreover, Caruso's studies showed that a high pressure ramp could significantly affect the plasma. Thus, it was decided to use a moderate pressure ramp of 3 to 3.5 MPa/min. It was also necessary to use an intermediate temperature of 75 ^{0}C as high temperatures would affect thermally labile compounds while lower temperatures could affect the involatile compounds.

When coupling SFC to ICPMS it is necessary to consider a number of factors. The restrictor must be efficiently heated in order to minimise the Joule-Thompson effect. In Caruso's study this was done by enclosing the restrictor in a copper tube, the end of which was heated, and relying on heat transfer along the tube to heat the restrictor. Although this works it does not give direct control of the restrictor temperature. The interface developed for this study had the restrictor enclosed in a glass tube wound with a nichrome filament heater. By changing the applied voltage on the filament it was possible to control the temperature of the restrictor. This is a more effective method of controlling temperature but measuring the temperature of the restrictor proved difficult and only an approximate temperature/voltage relationship could be used. The interface designed for this study was a modification of the elbow join between the nebuliser and the ICP torch. Thus, it was more compact and easier to install than that developed by Caruso but is more fragile. Thus, both interfaces have both merits and drawbacks and work still needs to be done on finding the "ideal" interface for coupling SFC to ICPMS. For now it is a matter of user preference whether to use the more compact interface used in this study or the more robust interface designed by Caruso's group.

If SFC-ICPMS is to be used successfully then it should be free of spectral interference caused by changes in the plasma resulting from the introduction of the mobile phase. The expected interferences of Ar_2^+, $^{40}Ar^{16}O^+$ and $^{40}Ar^{12}C^+$ were investigated by observing changes in the baseline with time under normal analysis conditions. As expected there was little change in the baseline with time for Ar_2^+ but the baseline intensity was high. Moreover, an injection of a chlorinated solvent resulted in a negative peak as the solvent eluted. Although Ar_2^+ is not a significant interference, the $^{40}Ar^{35}Cl^+$ generated by the chlorinated solvent could interfere with $^{75}As^+$ analyses. Monitoring $^{40}Ar^{12}C^+$ showed no significant change in the baseline with time and the baseline intensity was relatively low. Thus, this may be a small interference for $^{52}Cr^+$ but it is not as significant as other interferences. $^{40}Ar^{16}O^+$ had a relatively high baseline but showed no significant change with time. This is a significant interference for $^{56}Fe^+$. The absence of any significant change in the baseline over time for all of these ions indicates that there is no change in the plasma as the mobile phase is introduced. These results differ to those obtained by Caruso *et al* but they show how the mobile phase may affect the plasma with time whereas Caruso's results show how the mobile phase affects the plasma with rapid increase in pressure. Thus, under normal analysis conditions the analysis will not be affected by changes in the plasma caused by introduction of a CO_2 mobile phase.

Possibly the most important step in the analysis at trace levels is focusing the ICPMS on the element of interest. A few sacrificial runs of a standard solution before the analysis are used to focus the lenses in the ICP-MS interface on the ion to be analysed. This optimises the number of ions entering the detector and thus increases the output signal. Thus, it is possible to dramatically increase the signal for a particular ion and thus improve the detection limits. Although previous studies have focused the instrument on Ar_2^+ or Hg vapour in an attempt to obtain optimum signals for a variety of elements, it is better to consider one element at a time and

to focus the instrument on a standard of that element.

The first group of compounds considered in this study were the organotin compounds. Initially tetrabutyltin, tributyltin chloride, tetraphenyltin and triphenyltin chloride were considered. However, triphenyltin chloride was strongly adsorbed in the injector and caused significant interference at low concentrations. Thus, it was decided to concentrate on the two butyltin compounds. Tetrabutyltin and tributyltin chloride were analysed and the detection limit for each compound was calculated. The calculated detection limits for a 10 μg/g solution for both compounds were in the sub-picogram range with the limit of tetrabutyltin being 0.035 pg as tin and the limit of tributyltin chloride being 0.025 pg as tin. The practical detection limit for tetrabutyltin was similar to the calculated detection limit while the practical detection limit for tributyltin chloride was only 6.7 pg as tin. If a modifier were added to the mobile phase it should be possible to decrease the peak tailing and hence improve the practical detection limit. At high concentrations both compounds overload the column or detector and there is degradation of the peak shape.

The increase in the restrictor temperature also causes an increase in peak intensity for these compounds. This is due to the increased counteraction of the Joule-Thompson effect as the restrictor is heated. The adiabatic expansion of the mobile phase is minimised at high temperatures and so the analytes are more efficiently transferred to the plasma. Differences in the volatility between tetrabutyltin and tributyltin chloride resulted in the differences between the peak intensities of these compounds at different temperatures.

The organoarsenic compound triphenylarsine was also studied extensively and it was found that it generally suffered from interference by $^{59}Ni^{16}O^{+}$ which caused an increase in the baseline intensity. This resulted in high practical and calculated detection limits which could not be improved

without eliminating the spectral interference. Moreover, chlorinated solvents elute as $^{40}Ar^{35}Cl^{+}$ peaks. However, provided these peaks elute long before the analyte they should not cause any increased interference. Triphenylarsine was studied at concentrations ranging from 0.001 μg/g to 10 μg/g but due to the high detection limit no linear range could be determined. As this is a non-polar compound there was no problem with peak tailing.

Increase in the restrictor temperature resulted in an increase in the peak intensity of triphenylarsine until a plateau was reached. This suggests that the optimum restrictor temperature for this compound had been reached but a slight increase in peak intensity after the plateau suggests that this is not the case. This latter increase in peak intensity could have been desorption of analyte from around the restrictor but this could not be investigated at higher temperatures as it was feared that higher temperatures could have degraded the polyimide insulation on the heating filament.

Other compounds considered were ferrocene and diethylmercury. Ferrocene was difficult to analyse as $^{56}Fe^{+}$ suffers from $^{40}Ar^{16}O^{+}$ interference while $^{54}Fe^{+}$
suffers from $^{40}Ar^{14}N^{+}$ interference. ^{57}Fe and ^{58}Fe are not abundant enough to be useful in trace level analysis. It was found that ferrocene could not be analysed below 100 μg/g as the background interference was too high. Thus, it was not possible to determine a relationship between concentration and peak intensity or applied voltage on the interface and peak intensity. As the ICPMS detects a specific isotope of an element and does not distinguish between compounds this problem will occur with all organoiron compounds. Diethylmercury was synthesised in the laboratory using the Grignard reaction and the exact concentration could not be determined. Thus, no attempt was made to find relationships between concentration or applied voltage on the interface and peak intensity for

this compound. However, as diethylmercury is non-polar and does not suffer from spectral interferences, it should be possible to determine the relevant relationships for this compound.

Thus, SFC-ICPMS is suitable for the analysis of non-polar and slightly polar alkyltin compounds at trace levels. If a modifier is added to the mobile phase it should be possible to extend the scope of the method to more polar alkyltin compounds. Phenyltin compounds suffer from peak tailing and in this study resulted in severe memory effects. Thus, this method is not as suitable for phenyltin compounds. Non-polar organoarsenic compounds can be analysed using SFC-ICPMS but not at trace levels as $^{59}Ni^{16}O^{+}$ causes significant spectral interference. The method is unsuitable for organoiron compounds as the spectral interference from $^{40}Ar^{16}O^{+}$ and $^{40}Ar^{14}N^{+}$ is too great. Although further investigation is necessary this method should also be suitable for the analysis of dialkylmercury compounds at trace levels.

Supercritical fluid extraction of organometallic compounds was investigated briefly and it was found that extraction of spiked topsoil samples was difficult. Nevertheless, this extraction method was attempted on real samples taken from Durban Bay. During optimisation experiments it was found that a series of short static extractions each followed by a short dynamic extraction gave the best results. The SFC-ICPMS results of these real samples were obtained using two detection methods: survey scans of all elements present in the sample and a single-ion scan of the most abundant elements. All survey scans showed a very low abundance of most elements and single-ion scans of the most abundant elements gave no results. It is unlikely that this is due to a lack of organometals in the Bay but rather it is due to the inability to obtain high enough pressure in the extraction. Moreover, a mobile phase modifier during the extraction should also improve the extraction efficiency.

Selection of the sampling sites when developing a method is important as they should be areas which are ecologically sensitive to the compounds of interest and should be expected to contain measurable amounts of these compounds. The sensitivity of the area can provide valuable information on the effects of these compounds and the appearance of the area can provide a clue as to the extent of the pollution in the area. However, it is also important to choose an area where it is likely that the compounds of interest, although at trace levels, are still measurable without difficulty. As the method is still under development it still needs to be optimised for use on real samples.

Although these results have shown the suitability of SFC-ICPMS for the speciation and detection of some organometallic compounds at trace levels, there still needs to be extensive research into the development of this method. The result of this study and those of Caruso's group indicate that this method could eventually be a suitable alternative for GC and HPLC analysis of organometallic compounds. SFC-ICPMS can be better controlled in its selectivity and it eliminates the need for extensive sample handling and treatment before analysis. However, although the interfaces so far developed for coupling SFC to ICPMS are adequate they still need to be refined further. Specifically, an effective means of controlling and measuring the restrictor temperature is essential. It would also be desirable to build up a database of organometallic compounds and their suitability for analysis by SFC-ICPMS. Thus, although this method may eventually become popular for the speciation of organometallic compounds, at present the prohibitive cost of maintaining these instruments and the relative lack of skilled operators suggest that SFC-ICPMS will have a limited but useful applicability in environmental analysis of real samples containing trace levels of organometals.

Appendix A

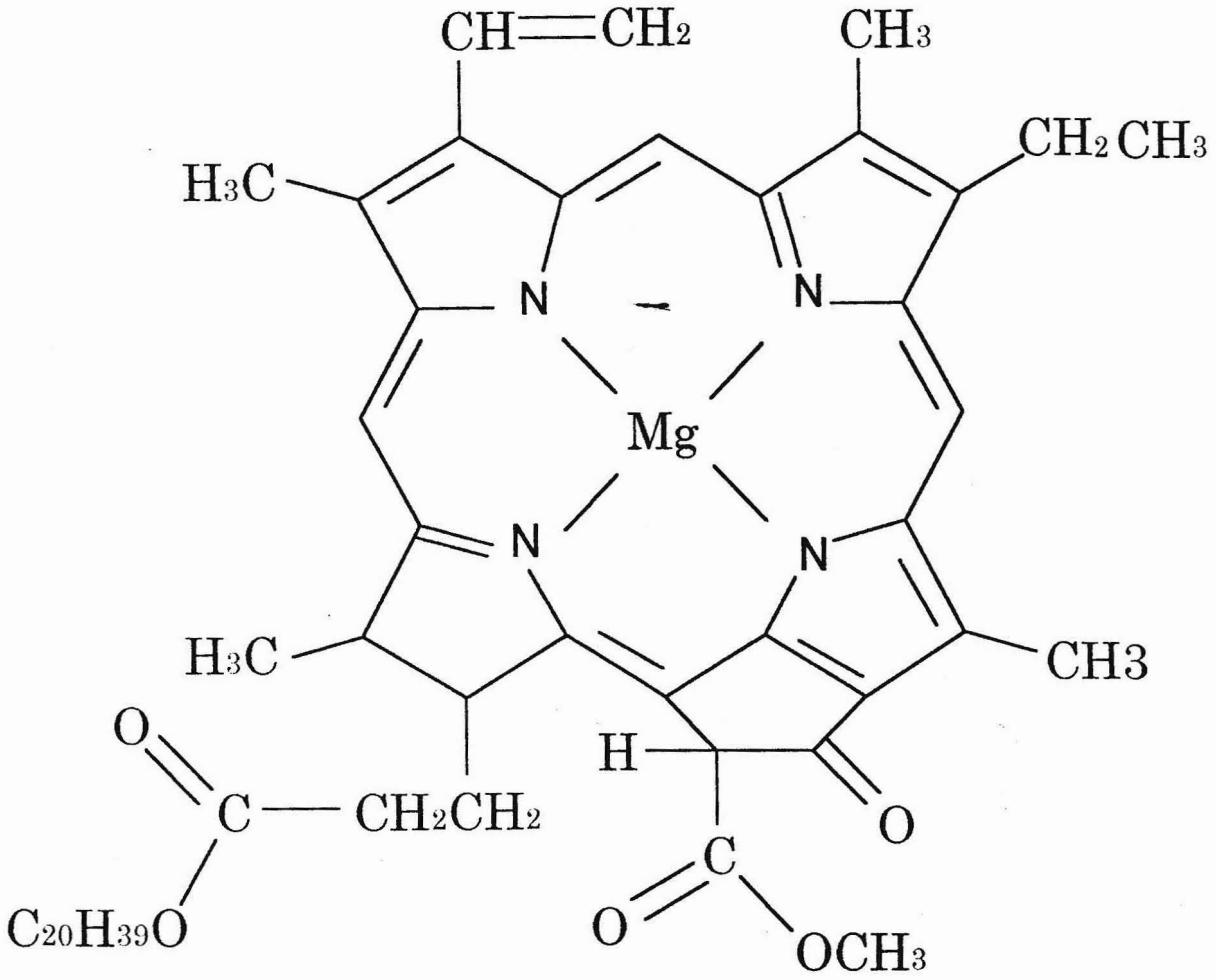


Figure A: Structural diagram of the *chlorophyll a* molecule.

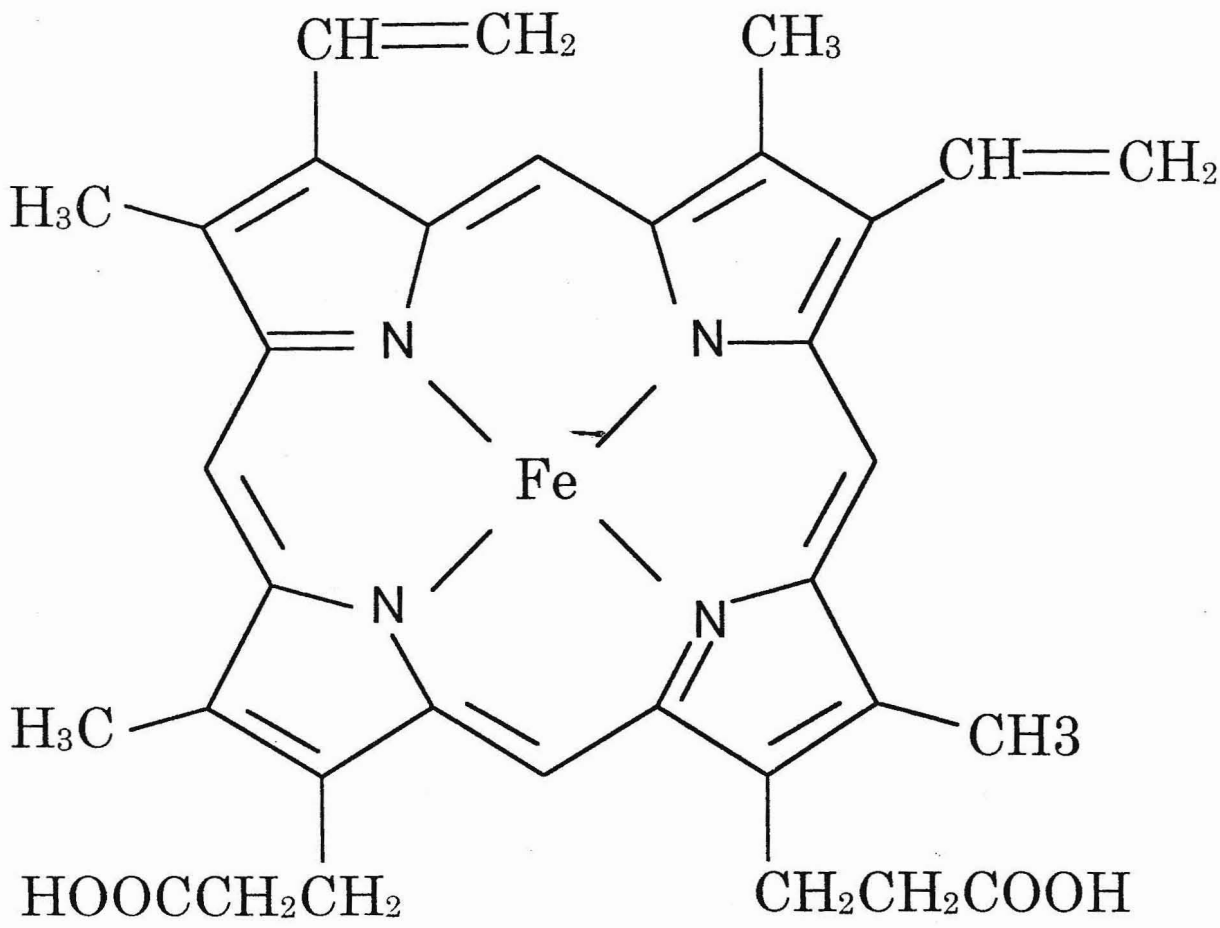


Figure B: Structural diagram of a *haemoglobin* (*ferrohemoglobin*) molecule.

Appendix B

Table A: CO_2 density at different pressures and temperatures (calculated using the SFC software).

Pressure /MPa	Temperature /^{0}C	Density /g cm^{-1}
3	50	0.0571
3	70	0.0522
3	75	0.0511
3	80	0.0501
12	50	0.5985
12	70	0.3560
12	75	0.3264
12	80	0.3057
40	50	0.9264
40	70	0.8579
40	75	0.8403
40	80	0.8234

Appendix C

Table A: Various organometallic compounds and their boiling points at different purities.

Compound	Purity /%	Boiling point /^{0}C
Ph_4Sn	97	> 420
Bu_2SnCl_2	97	135 /10 mmHg
$BuSnCl_3$	95	93 /10 mmHg
Bu_3SnCl	96	171-173 /25 mmHg
$Bu_2Sn(OMe)_2$	98	136-139
Bu_3SnOMe	97	97 /0.06 mmHg
$(Bu_3Sn)_2O$	96	180 /2 mmHg
Me_2Hg	97	92 (flash 20)
MeHgCl	98	170-173
Ph_3SnCl	98	240

Appendix D

Table I: Results of peak intensity and applied voltage for tetrabutyltin.

Applied voltage /V	Intensity: first run /counts	Intensity: second run /counts	Average intensity /counts
6.5	103 205	99 000	101 103
7.0	121 988	105 997	113 993
7.5	158 517	183 808	171 163
8.0	211 893	213 597	212 745
8.5	232 492	263 856	248 174

Table II: Results of peak intensity and applied voltage for tributyltin chloride.

Applied voltage /V	Intensity: first run /counts	Intensity: second run /counts	Average intensity /counts
6.5	109 840	110 786	110 313
7.0	136 664	168 189	152 427
7.5	188 648	226 591	207 620
8.0	305 100	307 028	306 064
8.5	327 814	387 707	357 760

Appendix E

Table I: Data obtained for the applied voltage-temperature calibration (over an average of 3 runs).

Applied Voltage /V	Temperature /^{o}C
0	23 (ambient)
0.5	39 (± 2)
1.0	58(± 2)
1.5	73(± 4)
2.0	92(± 3)
2.5	106(± 7)
3.0	129(± 5)
3.5	138(± 6)
4.0	149(± 6)
4.5	165(± 8)
5.0	187(± 7)
5.5	205(± 4)
6.0	221(± 10)
6.5	234(± 6)
7.0	256(± 12)
7.5	270(± 12)
8.0	278(± 15)
8.5	303(± 14)
9.0	310(± 17)
9.5	320(± 19)

References

[1] G. E. Coates, M. L. H. Green, P. Powell and K. Wade, *Principles of Organometallic Chemistry*, Chapman and Hall, London, 1979, Ch 1, pp 1-13

[2] R. Lobinski and F. C. Adams, *TRAC*, **12** (1993) pp 41-49

[3] W. Pretorius, M. Foulkes, L. Ebdon and S. Rowland, *J. High Resolut. Chromatogr.*, **16** (1993) pp 157-160

[4] W. G. Pretorius, L. Ebdon and S. J. Rowland, *Development of a High Temperature Gas Chromatography-Inductively Coupled Plasma-Mass Spectrometry Interface for the determination of metalloporphyrins*, submitted for publication

[5] M. Ceulemans, C. Witte, J. Szpunar-Lobinska, R. Lobinski and F. C. Adams, *Third International Symposium on Hyphenated Techniques in Chromatography - Book of Abstracts*, University of Antwerp, Antwerp, Belgium, 22-25 February 1994, **C15**

[6] D. Murray, *Internet communication - Sci. Chem newsgroup*, 24 March 1993

[7] U. Schmidt and F. Huber, *Nature*, **259** (1976) pp 157-158

[8] J. A. J. Thompson and J. A. Crerar, *Marine Pollut. Bull.*, **11** (1980) pp 251-253

[9] R. S. Braman and C. C. Foreback, *Science*, **182** (1973) pp1247-1249

[10] D. Bromage, *Internet communication - Sci. Chem.*, 13 August 1993

[11] Software Toolworks, *Multimedia World Atlas*, IBM PC compatible CD-ROM

[12] R. B. Clark, *Marine Pollution*, 2nd edition, Clarendon Press, Oxford, 1989, pp 66-105

[13] J. R. Ashby and P. J. Craig, *Organometallic Compounds in the Environment*, in *Pollution: Causes, Effects and Control*, ed. R. M. Harrison, 2nd edition, Royal Society of Chemistry, London, Ch16, pp 309-342

[14] J. M. Carey and J. A. Caruso, *TRAC*, **11** (1992) pp 287-293

[15] J. A. Caruso, Personal Communication, April 1993

[16] J. M. Carey, N. P. Vela and J. A. Caruso, *J. Anal. At. Spectrom.*, **7** (1992) pp 1173-1181

[17] W-L. Shen, N. P. Vela, B. S. Sheppard and J. A. Caruso, *Anal. Chem.*, **63** (1991) pp 1491-1496

[18] N. P. Vela and J. A. Caruso, *J. Anal. At. Spectrom.*, **7** (1992) pp 971-977

[19] E. Blake, M. W. Raynor and D. Cornell, *J. Chromatogr.*, **683** (1994) p 223

[20] S. B. Hawthorne, *Anal. Chem.*, **62** (1990) pp 633A-642A

[21] C. F. Poole, J. W. Oudsema and K. G. Miller, *Proceedings of the 5th International Symposium on Supercritical Fluid Chromatography and Extraction*, Baltimore, Maryland, USA, January 11-14, 1994, p 18

[22] J. Dachs and J. M. Bayona, *J. Chromatogr.*, **636** (1993) pp 277-283

[23] M. Ashraf-Khorassani, J. W. Hellgeth and L. T. Taylor, *Anal. Chem.*, **59** (1987) pp 2077-2081

[24] S. R. Almquist, L. Nyholm and K. E. Markides, *poster SFC 23, 14th International Symposium on Capillary Chromatography*, Riva del Garda, Italy, September, 1992

[25] M. A. Morrissey and H. H. Hill, Jr, *J. High Resolut. Chromatogr.*, **11** (1988) pp 375-379

[26] K. Jinno, K. Yamamoto, J. C. Fetzer and W. R. Biggs, *J. Microcol. Sep.*, **4** (1992) pp 187-190

[27] D. E. Raynie, S. M. Fields, N. M. Djordevic, K. E. Markides and M. L. Lee, *J. High Resolut. Chromatogr.*, **12** (1989) pp 51-52

[28] D. Pyo and D. Ju, *Analyst*, **118** (1993) pp 253-255

[29] J. J. Langenfeld, S. B. Hawthorne, D. J. Miller and J. Tehrani, *Anal. Chem.*, **64** (1992) pp 2263-2266

[30] S. Mitra and N. K. Wilson, *J. Chromatogr. Sci.*, **29** (1991) pp 305-309

[31] S. H. Page, H. Yun, M. L. Lee and S. R. Coates, *Anal. Chem.*, **65** (1993) pp 1493-1495

[32] J. Via and L. T. Taylor, *3rd International Symposium on Hyphenated Techniques in Chromatography*, University of Antwerp, Antwerp, Belgium, 22-25 February, 1994, A17

[33] W. Baeyens, *TRAC*, **11** (1992) pp 245-254

[34] R. Lobinski and F. C. Adams, *J. Anal. At. Spectrom.*, **7** (1992) pp 987-992

[35] R. Lobinski and F. C. Adams, *Anal. Chim. Acta*, **262** (1992) pp 285-297

[36] Y. K. Chau, P. T. S. Wong, G. A. Bengert and J. L. Dunn, *Anal. Chem.*, **56** (1984) pp 271-274

[37] R. M. Harrison and C. N. Hewitt, *Intern. J. Environ. Anal. Chem.*, **21** (1985) pp 89-104

[38] Y. Liu, V. Lopez-Avila, M. Alcaraz and W. F. Beckert, *J. High Resolut. Chromatogr.*, **16** (1993) pp 106-112

[39] J-A. A. Jackson, W. R. Blair, F. E. Brinckman and W. P. Iverson, *Environ. Sci. Technol.*, **16** (1982) pp 110-119

[40] Y. K. Chau, S. Zhang and R. J. Maguire, *Analyst*, **117** (1992) pp 1161-1164

[41] C. L. Matthias, J. M. Bellama, G. J. Olson and F. E. Brinckman,

Environ. Sci. Technol., **20** (1986) pp 609-615

[42] R. C. Forster and A. G. Howard, *Anal. Proc.*, **26** (1989) pp 34-36

[43] K. E. Laintz, G. M. Shieh and C. M. Wai, *J. Chromatogr. Sci.*, **30** (1992) pp 120-123

[44] D. R. Gere, C. R. Knipe, P. Castelli, J. Hedrick, L. G. Randall Frank, H. Schulenberg-Schell, R. Schuster, L. Doherty, J. Orolin and H. B. Lee, *J. Chromatogr. Sci.*, **31**(1993) pp 246-258

[45] A. L. Gray , *Analyst*, **100** (1975) pp 289-299

[46] R. S. Houk, *Anal. Chem.*, **58** (1986) pp 97A-105A

[47] E. H. Evans and J. J. Giglio, *J. Anal. At. Spectrom.*, 8 (1993) pp 1-18

[48] R. Lobinski and F. C. Adams, *Analusis*, **20** (1992) pp M28-M31

[49] R. Lobinski and F. C. Adams, *3rd International Symposium on Hyphenated Techniques in Chromatography*, University of Antwerp, Antwerp, Belgium, 22-25 February 1994, B12

[50] Y. K. Chau, P. T. S. Wong, G. A. Bengert and O. Kramer, *Anal. Chem.*, **51** (1979) pp 186-188

[51] Y. Liu and V. Lopez - Avila, *J. High Resolut. Chromatogr.*, **16** (1993) pp 717-720

[52] S. J. Hill, *Anal. Proc.*, **29** (1992) pp 399-401

[53] S. C. K. Shum, H-M. Pang and R. S. Houk, *Anal. Chem.*, **64** (1992) pp 2444-2450

[54] G. R. Peters and D. Beauchemin, *J. Anal. At. Spectrom.*, **7** (1992) pp 965-969

[55] G. R. Peters and D. Beauchemin, *Anal. Chem.*, **65** (1993) pp 97-103

[56] W. Pretorius, M. Foulkes, L. Ebdon and S. Rowland, *J. High Resolut. Chromatogr.*, **16** (1993) pp 157-160

[57] L. Ebdon, *3rd International Symposium on Hyphenated Techniques in Chromatography*, University of Antwerp, Antwerp, Belgium, 22-25 February 1994, B03

[58] P. A. Steenkamp and P. P. Coetzee, *S. Afr. J. Chem.*, **47** (1994) pp 29-32

[59] M. W. Raynor, K. D. Bartle, I. L. Davies, A. A. Clifford and A. Williams, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, **11** (1988) pp289-291

[60] S. Ashraf, K. D. Bartle, A. A. Clifford, R. Moulder, M. W. Raynor and G. F. Shilstone, *Analyst*, **117** (1992) pp 1697-1700

[61] M. Kane, J. R. Dean, S. M. Hitchen, W. R. Tomlinson, R. L. Tranter and C. J. Dowle, *Analyst*, **118** (1993) pp 1261-1264

[62] J. L. Snyder, R. L. Grob, M. E. McNally and T. S. Oostdyk, *J.*

Chromatogr. Sci., **31** (1993) pp 183-191

[63] J. W. Oudsema and C. F. Poole, *Fresenius J. Anal. Chem.*, **344** (1992) pp 426-434

[64] D. J. Miller, S. B. Hawthorne and M. E. P. McNally, *Anal. Chem.*, **65** (1993) pp 1038-1042

[65] J. J. Langenfeld, M. D. Burford, S. B. Hawthorne and D. J. Miller, *J. Chromatogr.*, **594** (1992) pp 297-307

[66] J. J. Langenfeld, S. B. Hawthorne, D. J. Miller and J. Pawliszyn, *Anal. Chem.*, **65** (1993) pp 338-344

[67] S. B. Hawthorne, J. J. Langenfeld, D. J. Miller and M. D. Burford, *Anal. Chem.*, **64** (1992) pp 1614-1622

[68] S. B. Hawthorne, D. J. Miller, D. E. Nivens and D. C. White, *Anal. Chem.*, **64** (1992) pp 405-412

[69] S. B. Hawthorne, D. J. Miller, M. D. Burford, J. J. Langenfeld, S. Eckert-Tilotta and P. K. Louie, *J. Chromatogr.*, in press

[70] N. S. Simon, personal communication

[71] F. G. Mann and B. C. Saunders, *Practical Organic Chemistry*, 4th edition, Longman Scientific, Essex, pp 280-285

[72] J. D. Pinkston and R. T. Hentschel, *J. High Resolut. Chromatogr.*, **16** (1993) pp 269-272

[73] *Interactive Periodic Table 1.0*, IBM compatible CD-ROM for

Microsoft Windows™, Attica Cybernetics, 1993

[74] A. Kim, S. Hill, L. Ebdon and S. Rowland, *J. High Resolut. Chromatogr.*, **15** (1992) pp 665-668

[75] B. Stanton, *Council for Scientific and Industrial Research*, Durban, personal discussions and communications.

[76] E. Blake, M. W. Raynor and D. Cornell, *American Laboratory*, **26** (1994) pp 48-50

[77] E. Blake, M. W. Raynor and D. Cornell, *J. Chromatogr.*, **683** (1994) p 223

[78] E. Blake, M. W. Raynor and D. Cornell, *J. High Resolut. Chromatogr.*, **18** (1995) p 33