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Field Sampling and Flow Injection Strategies for Trace Analysis and Element Speciation

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A thesis submitted in partial fulfilment of the requirements of
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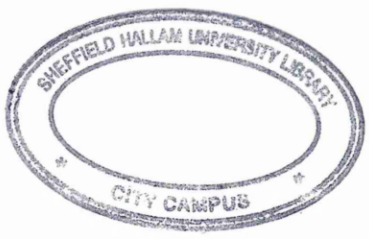
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Abstract

Over the last two decades research has shown that the different forms of trace elements in the environment can cause a variety of health concerns as a result of differences in toxicity. The need to establish efficient, effective and reliable speciation methods has become paramount. A basic aim of this work has been to advance speciation measurement capability for key trace elements (mercury, lead and chromium) by devising an integrated analytical approach that links the sample collection, sample preservation and laboratory measurements in an unified manner.

An introductory chapter first reviews the occurrence of organometallic compounds in the environment and focuses on the identification of the "environmental compartments" where transformations of such species can take place. Speciation studies also assist in understanding the biogeochemical cycling of trace elements. Moreover, a review of the various methodologies used for trace element speciation measurements including hyphenated techniques and/or a variety of chemical/physical pretreatments in combination with flow injection (FI) is discussed.

Chapter 2 describes mercury speciation experiments utilising gas chromatography-microwave induced plasma-atomic emission spectrometry (GC-MIP-AES) and FI. The approach was based on the preconcentration of mercury on sulphydryl cotton and after elution from the microcolumn, separation and quantitation of methyl-, ethyl- and inorganic mercury species. Method development experiments were performed using a derivatisation technique which gave low contamination and allowed rapid analysis of samples. The microcolumn technique was transferred to the field and speciation of mercury in surface waters of the Manchester Ship Canal was undertaken and high methylmercury concentrations (0.052-0.182 $\mu g\ l^{-1}$, as Hg) were detected. In so doing the new approach offered the preservation of the natural speciation state of the water sample directly at the sampling site and during the interval between collection and analysis.

In chapter 3 lead studies are centred on the development of a rapid speciation scheme for neutral and cationic (organic and inorganic) lead species based on activated alumina microcolumn separation in combination with ICP-MS and FI. The approach permitted rapid assessment of the nature of lead contamination in environmental waters. Speciation of lead in surface waters of the Manchester Ship Canal was also undertaken using the field sampling approach in an attempt to confirm a transmethyllation reaction between organolead and inorganic mercury.

A further application for microcolumns, in the context of speciation measurement, is their use as external calibrants and certified reference materials (CRMs) and this is discussed in the penultimate chapter. Key elements were mercury and chromium. After immobilisation of mercury species on SCF microcolumns it was found that recoveries for methyl- and inorganic mercury were quantitative over 4 months in contrast to ethylmercury which was 2 months. Similar studies for chromium species indicated ineffective elution and more vigorous conditions (microwave assisted digestion) for the elution step were used.

A final chapter reviews progress and recommendations are given concerning future research and application for microcolumn field sampling in combination with instrumental analytical techniques.

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Chapter 1: General Introduction

1.1. Environmental Pollution.

Trace elements are introduced in the environment through the contamination of air, water and soils from where they passed to the food chain. Contamination of the environment is due to both natural and human factors [Pontius, 1990]. Natural factors, such as geology or biological growth, cannot be readily controlled and may have a significant impact on the quality of the environment. For example, different elements (such arsenic, barium, cadmium, chromium, lead or mercury) occur naturally in most surface and ground waters from their exposure to element-containing surface rocks. Human factors are usually categorised as two distinct types: point or nonpoint. Point sources derive from industrial activities which may introduce contaminants directly through a regulated discharge (examples are summarised in table 1.1) or accidentally as in the case of a spill or pipe corrosion (e.g. cadmium or lead enter consumer's tap water as a result of galvanised or lead pipes corrosion, respectively).

Trace Element	Industrial Activity
Barium	Oil and gas drilling mud, jet fuels and automobiles plants.
Cadmium	Mining and smelting operations, electroplating and pigment and plasticiser production.
Chromium	Old mining operations, wastes from plating operations and fossil fuel combustion.
Lead	Automobiles and evaporation at petrol plant stations.
Mercury	Fungicides and chemical plants manufacturing chlorine, sodium hydroxide and hydrogen which use a rocking mercury cathode.
Tin	Fungicides and ship paints.

Table 1.1.
Discharge of trace elements to the environment from industrial activities (from [Pontius, 1990]).

Nonpoint sources, in contrast, involve large and diffuse sources of contamination and

they are more complex and difficult to control. For example, application of pesticides, herbicides and fertilisers are key agricultural activities that affect the quality of ground and surface waters [Harrison et al., 1986].

Trace metals in the environment will undergo many transformations from one form to another depending on their physicochemical and biological environment and their intrinsic properties. In recent years studies have been focused on the production of organometallic compounds in the environment from their inorganic form as a result of chemical and/or biological alkylation reactions [Ridley et al., 1977]. The most usual, simple and rapid reaction of production of organometallic compounds is methylation. The formation of methylated compounds in the environment is due to the addition of a methyl group (CH_3) to the inorganic form of the element in the presence of methyldonor molecules [Craig, 1986; Rapsomanikis and Weber, 1986].

The most important methyldonor molecules are methylcobalamin and S-adenosylmethionin. Methylcobalamin is a methylcoenzyme of the vitamin B12, as shown in figure 1.1. The methyl-Co bond in the methylcobalamin is stable but allows the transference of the methyl- group in three different oxidation states: CH_3^-, CH_3^+ and $CH_3\cdot$ (radical). But the transference as CH_3^- and $CH_3\cdot$ is predominant. S-adenosylmethionin (SAM) is an activated form of the methionin, as shown in figure 1.2, and allows the transference of a methyl- group as CH_3^+ to a species that has a pair of free electrons.

According to Ridley et al., 1977 there are three different mechanisms of methylation which depend on the redox potential of the element (E°), as shown in table 1.2.

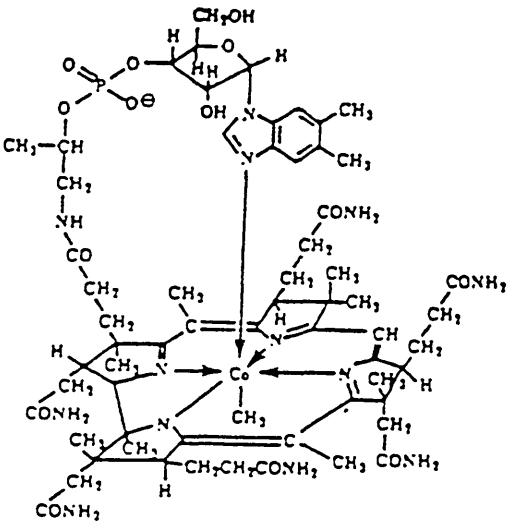

Figure 1.1.
Structure of the methylcobalamin (from [Craig, 1986; Rapsomanikis and Weber, 1986]).

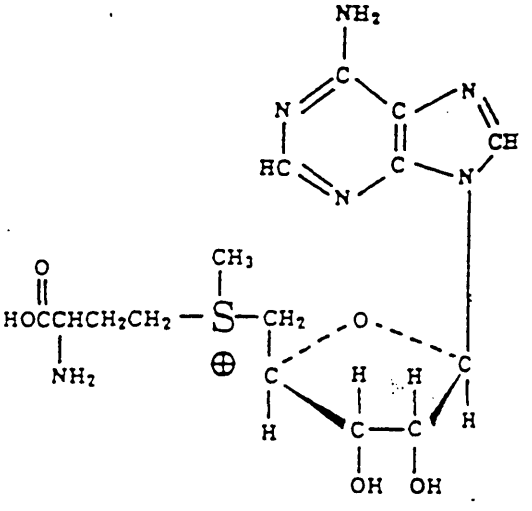

Figure 1.2.
Structure of the molecule S-adenosilmethionin (from [Craig, 1986; Rapsomanikis and Weber, 1986]).

Redox System	E°(V)	Mechanism
Pb(IV) / Pb(II)	+1,46	Direct substitution (CH_3^-) or transmethylation
Tl(III) / Tl(I)	+1.26	
Se(VI) / Se(IV) in acidic medium	+1.15	
Hg(II) / Hg(0)	+0.854	
Sb(V) / Sb(III)	+0.678	Direct addition (CH_3^+) or radical addition ($CH_3\cdot$)
As(V) / As(III) in acidic medium	+0.662	
Sn(IV) / Sn(II)	+0.154	
Pb(II) / Pb(0)	-0.13	
Ge(IV) / Ge(II)	-0.13	
Sn(II) / Sn(0)	-0.14	
Cr(III) / Cr(II)	-0.41	
As(V) / As(III) in basic medium	-0.67	

Table 1.2.
Redox potential and mechanisms of methylation [from Ridley et al., 1977].

(i) Direct substitution. The CH_3^- is given by methyldonor molecules (such as methylcobalamin) to elements that in their maximum oxidation state have not got a free pair of electrons. For example:

$$CH_3CoB_{12} + Hg^{2+} + H_2O \longrightarrow H_2OCoB_{12} + CH_3Hg^+$$

Chau et al. 1987 proposed a transmethylation reaction in which there is a transfer of CH_3^- from an organometallic compound to the inorganic form of the element. For example:

$$(CH_3)_3Pb^+ + Hg^{2+} \longrightarrow (CH_3)_2Pb^{2+} + CH_3Hg^+$$

$$(CH_3)_3Sn^+ + Hg^{2+} \longrightarrow (CH_3)_2Sn^{2+} + CH_3Hg^+$$

(ii) Radical addition. The $CH_3\cdot$ is given by methyldonor molecules (such as methylcobalamin) to elements with a free electron that can be oxidised to an stable

oxidation state. Fanchiang and Wood, 1981 proposed this mechanism for methylation of tin:

$$CH_3CoB_{12} + Sn(II) \longrightarrow CoB_{12}\cdot + CH_3Sn(III)\cdot$$

$$CH_3Sn(III)\cdot + O_2 \longrightarrow CH_3Sn(IV)$$

$$CH_3Sn(III)\cdot + H_2OCoB_{12}^{+} \longrightarrow CH_3Sn(IV) + H_2O\ CoB_{12}\cdot$$

(iii) Addition of CH_3^+. This mechanism was proposed by Challenger, 1945 and the CH_3^+ is given by methyldonormolecules (such as S-adenosilmethionin) to elements with a low oxidation state to produce a methylated compound with an oxidation state two units higher. Challenger, 1978 proposed this mechanism for methylation of arsenic:

$$CH_3^+ + As(III)(OH)_3 \longrightarrow CH_3As(OH)_3^+$$

$$CH_3As(OH)_3^+ \longrightarrow CH_3As(V)O(OH)_2 + H^+$$

The different forms of trace elements in the environment cause a variety of health concerns as result of differences in toxicity [Berman, 1960; Florence and Baley, 1977]. For instance chromium exits essentially in two oxidation states: +3 and +6. Chromium(III) has been shown to function as an essential element [Mertz, 1967; Mertz, 1969] for maintenance of the normal glucose tolerance factor in mammals. Toxicological hazards are associated with the chromium(VI) form, producing liver and kidney damage, internal haemorrhage, respiratory disorders, skin ulceration and even cancer in humans and animals through inhalation exposure. Another key element is arsenic. Inorganic forms are more toxic than organic forms and arsenic(III) is much more toxic than arsenic(V) [Mertz and Cornatzed, 1971]. Arsenic(III) is believed to exert its toxicity by inhibiting thiol-containing enzymes, whereas arsenate toxicity

results from inhibition of oxidate phosphorilation. For other elements such as mercury, tin and lead, problems are related to both inorganic and organic forms, the latter being more toxic because of their lipid-phase association in biological systems [Craig, 1986]. They target the central nervous system, causing death and / or mental and motor dysfunction [Liebsher and Smith, 1966; Weast, 1972; Van Cleuvenbergen and Adams, 1989].

It is clear that there is a necessity in controlling the trace elements presented in the environment, particularly in natural waters. Authority to establish standards for water systems originated in UK as long ago as 1865. The government of the day had recognised that the problems of pollution created by new industries and their supporting population were severe. Different Pollution Prevention Acts between 1951 and 1974 set limits on the nature of effluents to be discharged to waters because of their toxicity to humans or their adverse effect on the environment. While domestic legislation has been progressively tightened, a new dimension was introduced as a result of the UK joining the European Union (EU). The European legislation programme has established a system of Directives in order to achieve a high level of protection for both environment and for human health. For example, 76/464/EEC (Pollution caused by certain dangerous substances discharged into the aquatic environment) and 80/68/EEC (Protection of ground water against pollution caused by certain dangerous substances) specify a list of contaminants, shown in table 1.2, that occur in drinking water, or are anticipated to occur in drinking water, that pose a health risk. This table 1.2 also give concentrations of those contaminants that represent either a health base no adverse effect level or a level representing a balance between health risks and feasibility of achieving those levels. A comparison of EEC Directives with US primary drinking water regulations can

also be made. It can be observed that similar concentrations for all elements are agreed and the only difference is that in the United States these concentrations are enforceable through the courts. It can also be seen from table 1.2 that EEC Directives and US Regulations available until now are for total element only concentrations. But it is known that trace elements in the environment are present in different chemical forms with different toxicological hazards and hence data on trace element speciation will be required for future legislation.

Inorganic Substances	EEC maximum admissible concentration, mg/l	US maximum contaminant level , mg/l
Arsenic	0.05	0.05
Barium	0.1	1.0
Cadmium	0.005	0.01
Chromium	0.05	0.05
Lead	0.05	0.05
Mercury	0.001	0.002
Selenium	0.01	0.01

Table 1.2.
Comparison of EEC Directives and US primary drinking water regulations (only for inorganic substances) [from Pontius, 1990].

In connection with trace element speciation measurements it is necessary to note that the total concentration of key elements in the environment is at the trace/ultratrace level, as shown in table 1.3, and hence the total element concentration has to be divided into several different chemicals forms (methyl-, ethyl-, ...) which contribute to the total concentration [Pinel et al., 1990]. Measurement of the subcomponents therefore constitutes a considerable analytical challenge.

The various methodologies used for trace element speciation measurements have undergone rapid development in the last decade. In general the methods consist of

coupling of various analytical techniques (hyphenated techniques) or the control of the chemistry of the method (e.g.: separation of individual species based on the use of liquid - liquid or solid - liquid extractions, formation of complex, selective generation of the hydride, ...) to obtain the necessary speciation information.

Element	Sample	Total concentration	%alkylated forms	Chemical forms
Arsenic	**Water (ng ml^{-1})**			
	Lake	60	16	Me
	Sea	1.7 - 1.9	7 - 16	Me
	Organisms (ng g^{-1})			
	Seaweed	33000 - 57600	1 - 8	Me
	Placton	2000 - 3400	22 - 37	Me
Lead	**Air (ng ml^{-1})**	-	0.3 - 22	Me, Et, MeEt
	Water (ng ml^{-1})			
	Rain	130 - 280	<1	Me, Et, MeEt
	Organisms (ng g^{-1})			
	Fish	-	10 - 90	Et
Mercury	**Water (ng ml^{-1})**			
	River	0.013	1	Me
	Sea	0.01 - 0.03	10	Me
	Sediments (ng g^{-1})			
	Unpolluted	200 - 400	0	-
	Polluted	1000 - 20000	1 - 3	Me
	Organisms (ng g^{-1})			
	Fish (river)	360 - 1000	70 - 95	Me
	Fish (sea)	10 - 15000	60 - 90	Me
Tin	**Water (ng l^{-1})**			
	Lake	0.009	53	Me
	Estuary	0.130 - 0.385	16 - 100	Me, Bu
	Sea	0.004	60	Me
	Sediments (ng g^{-1})			
	Unpolluted	830 - 1235	65 - 75	Me
	Polluted	7700 - 25000	3 - 50	Me, Bu
	Organisms (ng g^{-1})			
	Seaweed	250 - 550	20 - 30	Me, Bu
	Fish (sea)	86 - 260	3 - 6	Me
	Oyster (polluted)	3700	97	Bu

Me = Methyl-, Et = Ethyl- and MeEt = Methylethyl-, Bu = Butyl-

Table 1.3.
Relative abundance of organometallic compounds in the environment [from Pinel et al., 1990].

1.2. Hyphenated Techniques.

Various hyphenated techniques have been developed in the last decade. They are dependent on a suitable interface being devised. The most widely used methods involve the coupling of chromatography (gas or liquid) with atomic spectrometry (absorption, emission or fluorescence) which offers high separation ability combined with high sensitivity and selectivity.

The theoretical principles governing these two techniques (chromatography and atomic spectrometry) are completely different. In both gas and liquid chromatography the distribution of solutes between the stationary and mobile phase is an equilibrium process and so solutes are resolved according to their differing affinities towards the two phases, which results in differential migration rates through the system. Solutes exhibiting a preference for the mobile phase are eluted relatively quickly while those with a high affinity for the stationary phase are retarded. Two main theories have been developed to describe the transfer of solutes through a column. Plate theory explains chromatography in terms of multi-step equilibrium. Rate theory considers solute transport in terms of kinetics and hydrodynamics. The latter theory accounts for zone spreading as the manifestation of diffusion and non-equilibrium mechanisms.

Qualitative analysis of solutes is based on their migration rates in an operationally defined system. The migration rate of a solute is characterised either by the retention time, t_R (the total time elapsed from the injection of a sample into the system until detection of the maximum of the elution peak of the solute), or by the retention volume, V_R (the total volume of eluent that flows through the system during the time t_R). Thus

$V_R = t_R F_C$ where F_C is the volume flow-rate of the mobile phase, usually expressed in ml min^{-1}, at the column temperature.

Atomic spectroscopy involves several techniques and the common factor in these techniques is that quantification is realised by measuring the amount of energy associated with electronic transitions in atoms or ions. Such transitions are governed by a set of selection rules. The nature of the electronic transitions considered defines the specific technique: absorption, emission and fluorescence. *Absorption* occurs when the energy from external radiation promotes an electron to a higher energy orbital. The external radiation must correspond exactly to the transition energy needed for absorption to occur. Radiation is absorbed by electrons during excitation to higher energy states. Beer's law relates the energy absorbed to analyte concentration, thereby enabling quantification in atomic absorption spectrometry (AAS). *Emission* is the release of energy that occurs after electrons have been excited to a higher energy level. The sample must first be atomised. Then, the atoms must also be excited, since emission only takes place from excited, higher energy levels. Excitation energy can come from a hot environment (candle flame or plasma), or from bombardment by fast moving charged particles, as in fluorescent light. Once a sufficient number of excited atoms is created, they begin to emit light as they return to the "ground" state. The emitted light is then dispersed into the various wavelengths and measured. This process is shown in these equations:

$$A + \text{energy} \longrightarrow A^*$$

$$A^* \longrightarrow A + \text{photon}$$

(where A is the atom at ground state, and A* is the excited atom)

Fluorescence is absorption followed by emission, but with a reduction in energy. Sometimes when an atom is excited, it relaxes to slightly lower energy levels before fluorescencing and returning to the ground state. This cause emission at longer wavelengths than the exciting radiation. Atomic fluorescence radiation is emitted isotropically by the atomic vapour and can be observed from all directions around the atom cell. An atomic fluorescence spectrum consists typically of only a few lines, the relatively intensity of which depends on the type of atomiser and light source.

Gas Chromatography (GC).

GC is applied to those compounds that can be volatilised either directly or after derivatisation (formation of hydrides or alkylation). Traditional detectors such as electron-capture have been used for speciation of organic species of lead [Forsyth and Marshall, 1983], mercury [Filipelli, 1987], selenium [Uchida et al., 1980] and arsenic [Daughtrey et al., 1975; Andreae, 1977] but the use of extremely pure solvents is necessary, together with tedious cleaning procedures, to avoid co-elution of electron-capturing species with the organic compounds. GC in combination with atomic spectrometry can avoid extensive cleaning and offers high sensitivity and selectivity.

The GC-AAS coupling was firstly described by Kolb et al., 1966 for the analysis of organolead compounds in petrol in 1966. The eluent from the chromatographic column was introduced into the nebuliser of an unmodified air-acetylene flame AAS. In 1983, it was used for speciation of methyllead species in environmental samples [Chau et al., 1983]. However, the application of this coupling to environmental samples yielded low sensitivity as a result of the short residence time of the atoms in the flame, dilution effects with the fuel and the low temperature of the flame. Enhanced sensitivity

can be obtained with systems employing a silica furnace detector which has been either electrothermally heated [Radziuk et al., 1979] or suspended in an air-acetylene flame above the burner head and in the path of the light-beam [Chau et al., 1976]. These systems have been used for speciation of organic compounds of lead [Chau et al., 1976; Chakraborti et al., 1984; Radojecvic et al., 1986], selenium [Chau et al., 1975; Van Loon, 1979; Jiang et al., 1982], tin [Chau et al., 1982; Maguire and Tkacz, 1983; Donard and Weber, 1985], antimony [Andreae et al., 1981] and germanium [Andreae and Froelich, 1981].

In the GC-AES coupling, the first detector used was flame photometry for the analysis of organotin compounds [Waldock et al., 1989; Tolosa et al., 1991]. Atomic emission spectrometry is not as selective as AAS but the different plasmas used as atomisation / excitation sources (inductively coupled plasma (ICP), direct current plasma (DCP) and microwave induced plasma (MIP)) offer very high excitation temperatures giving very intense emission lines and low detection limits. The coupling of GC-ICP-AES has given relatively low sensitivity (above μg level) due to the dilution effects with the reagent gases [Hill et al., 1993]. This coupling has been applied to the speciation of organolead compounds [Ibrahim et al., 1985] in 1985. Enhanced sensitivity was obtained by the GC-DCP-AES coupling [Krull et al., 1989]. However, GC-MIP-AES, which is commercially available, is the most commonly used because of its sensitivity and selectivity [Uden, 1991; Lobinski and Adams, 1993]. It has been effectively used for the analysis of diverse environmental samples allowing multielemental analysis and speciation [Harrison and Rapsomanikis, 1989; Lobinski et al., 1994].

The GC-AFS coupling has similar selectivity to GC-AAS and a simple interface. However, a limitation is the low stability of the light source (EDL lamps). The GC-AFS system was used in 1979 for speciation of lead [Radziuk et al., 1979; Van Loon, 1979]. More recently GC-ICP-MS has been developed and offers sensitivity increases of over two orders of magnitude relative to GC-ICP-AES [Hill et al., 1993]. In contrast to GC-MIP-MS there is not any increment in sensitivity related to GC-MIP-AES [Suyani et al., 1989]. The main limitation of GC-ICP-MS for the analysis of organometallic compounds is the high cost.

Liquid chromatography (High Performance Liquid Chromatography).

High Performance Liquid Cromatography (HPLC) is of value for determining non volatile compounds which are not amenable to GC unless derivatisation procedures are used. Thus HPLC avoids the needs for derivatisation which results in a reduction of the time of preparation of the samples and potential losses and transformation of the species during the derivatisation step. Moreover, different parameters such as mobile and stationary phases can be optimised in order to obtain better separation of the different species. However, the coupling to atomic spectroscopy is still underdeveloped, due to the difficulty of developing a suitable interface, with limits of detection inferior to those of the GC technique.

The use HPLC with flame AAS was also demonstrated. It is known that the flow rate in HPLC is 0.5 - 4 ml min^{-1} which is compatible with nebuliser flow rate and the use of a tube interface was proposed. However, the major difficulty was related to the optimum flow rate which is higher in the nebuliser than in the HPLC column. In order to solve this problem an additional carrier / solvent stream at the end of the HPLC column was

proposed; this did however result in a decrease in sensitivity due to dilution of the sample [Yoza and Obashi, 1973]. Different interfaces were developed [Slavin and Smith, 1979; Ebdon et al., 1985; Gustavsson and Nygren, 1987] but their application to the speciation of organometallic compounds in the environment is still limited due to low sensitivity. The effluent from the HPLC column was also introduced into the nebuliser of an FAAS instrument [Gustavsson and Nygren, 1987; Nygren et al., 1989]. In this way the limits of detection for ionic alkyllead compounds are in the order of 1 - 2 ng.

The HPLC interfaced to graphite furnace atomic absorption spectroscopy (GFAAS) does not suffer from this disadvantage but the analysis may be exceedingly slow because the GFAAS instrument accepts only discrete liquid droplets, at a rate of a single drop every two minutes. In order to solve this problem the effluent from the HPLC was stored in an autosampler previous to its introduction into the GFAAS. This method was applied to speciation of organic compounds of tin [Brinckman et al., 1977; Vickrey et al., 1979], lead [Vickrey et al., 1979; Koizumi et al.,1979] and arsenic [Brinckman et al., 1980]. The limits of detection for these applications were in the range of 0.5 - 100 ng as organometallic form. Nygren et al., 1988 proposed an improvement in the HPLC-GFAAS interface which consisted of a specially designed silica furnace detector cell that was electrothermally heated. This technique was applied to the speciation of di- and tributyltin compounds, however the coupling of HPLC-GFAAS is not recommended due to the discontinuity of GFAAS and the problem caused by the solvents used in HPLC.

The coupling of HPLC with AAS has also utilised post column vapour generation to

improve detection capability. The species were separated in the HPLC column and the effluent was continuously treated with sodium borohydride to form the hydride prior to passage through a gas/solvent separation device and introduction into the AAS. In this way the interferences were minimised. The technique was used for speciation of mercury species [Holak, 1982; Munaf et al., 1990]. Limits of detection were in the range 0.1 - 0.6 ng.

Coupling of HPLC to microwave induced plasma (MIP), direct current plasma (DCP) or inductively coupled plasma (ICP)-AES present different problems. The main limitation in the coupling of HPLC-MIP-AES is that the liquid effluent used in HPLC extinguishes the plasma. Application of new capillary columns, which operate at flow rates of a few $\mu l\ min^{-1}$, to the determination of trace elements to environmental samples, is limited due to their low capacity. The HPLC-DCP-AES system is robust and stable and exhibits a high tolerance for organic solvents [Krull, 1983]. The main limitation is the high detection limits (in the range of 100 $\mu g\ l^{-1}$) for the analysis of trace elements in environmental samples. The HPLC-ICP-AES also presents problems because the plasma exhibits low tolerance for organic solvents using conventional nebulisers. This problem can be solved by using direct injection nebulisers (DIN) or ultrasonic nebulisers. More details are given in the coupling of HPLC and ICP-MS. It has also proved necessary to implement post column vapour generation to improve detection capability. By using this system Colon and Barry, 1991 obtained limits of detection of a few nanograms.

The use of HPLC with ICP-MS has been reviewed a number of times [Hill et al., 1993; Seubert, 1994; Uden, 1995]. Several problems have been found in the coupling of

HPLC and ICP-MS which are related to the use of organic solvents used in the mobile phase to achieve the separation. The presence of organic solvents has a number of detrimental effects in ICP-MS: (i) organic solvents have a higher vapour pressure which leads to an increased solvent loading of the plasma. An increased solvent load reduces the stability of the plasma as it adversely affects the electron number density, ionisation and excitation temperatures of the plasma [Blades, 1985]. (ii) Organic solvents depress the sensitivity of the instrument and the deposition of unburned carbon on the sampler and skimmer cones can cause blocking [Hutton, 1986]. These problems can be overcome to an extent by adding a small amount of oxygen to the nebuliser gas flow and operating the plasma at higher powers. Both problems have been reported to be solved by using a direct injection nebuliser (DIN) [Wiederin et al., 1991; Shum et al., 1992a; Shum et al., 1992b] which allows the direct injection of eluents containing up to 85% of methanol into the plasma. Another approach is to produce desolvated aerosol, followed by removal of the solvent, to introduce a dry analyte particle into the plasma by using devices such as thermospray or ultrasonic nebuliser with a membrane desolvator [Montaser et al., 1991; Botto and Zhu, 1994] or a condenser [Vermeiren et al., 1988; Botto, 1993]. Alternatively the aerosol can be heated after nebulisation, either in the spray chamber or in a separate chamber followed by condensing the vapour [Hill et al., 1992a; Eastgate et al., 1993; Luo and Berndt, 1994; Albes et al., 1994; Pin et al., 1995] or membrane desolvation [Hill et al., 1992b; Gustavsson, 1988; Bäcktröm et al., 1989].

1.3. Control of Chemistry.

Trace element speciation can be performed using a variety of chemical/physical pre-

treatment operations including ion exchange, solvent extraction (liquid - liquid or solid - liquid extractions), formation of complexes and selective generation of the hydride prior to instrumental determination of species. These operations can be performed in a rapid and convenient way using flow injection (FI) techniques in combination with sensitive detection systems. Most systems have been concerned with the quantification of different oxidation states of an element. Pacey and Bubnis, 1984 were the first to recognise the potential of FI as a tool for trace element speciation. They developed a FI system to allow rapid sequential determination of iron(II) and iron(III). The FI system, shown in figure 1.3, contained a microcolumn of anion exchange resin which displayed an affinity for anionic ferric species (e.g. $FeCl_4^-$) in contrast to that for ferrous species (e.g. $FeCl_2$). In operation, HCl (6 M) was pumped (P1) through the microcolumn and sample was injected (V_i) into the flowing stream. The iron(III) species, as $FeCl_4^-$, were retained by the resin whereas the iron (II) species, as $FeCl_2$, was unretained and passed directly to the flame AAS. Valve (Vs) was then switched to allow passage of H_2O through the microcolumn and the abrupt change in pH effected elution of iron(III), as $FeCl_3$. In this way the rapid sequential determination of iron(II) and iron(III) was possible.

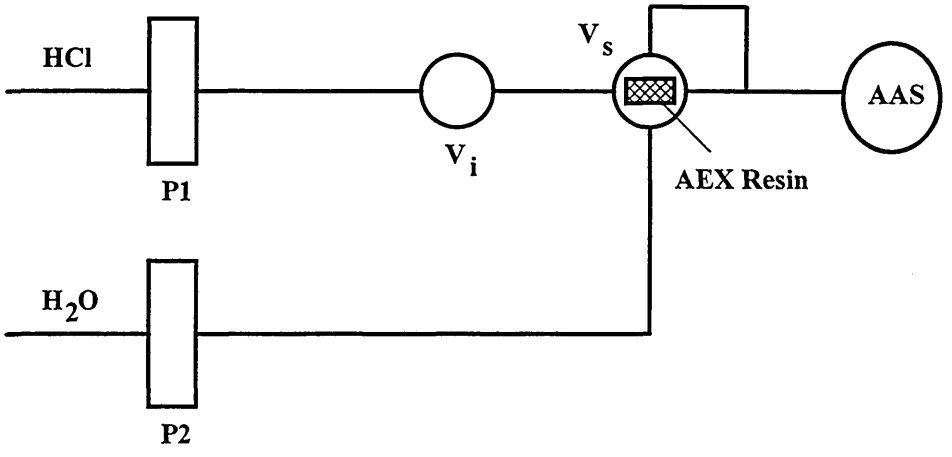

Figure 1.3.
Flow injection (FI) system for iron speciation (from ref. [Pacey and Bubnis, 1984]).

Since then, flow injection (FI) has been found very useful in speciation studies and different FI configurations have been used for different key elements such as iron, chromium, selenium, mercury and tin. Lynch [Lynch et al., 1984a; Lynch et al. 1984b] described two FI systems for the determination of iron(II) and total iron in mineral process liquids. The first approach [Lynch et al., 1984a] is shown in figure 1.4 and employs the spectrophotometric reaction of the iron(II) with 1,10-phenantroline and AAS (λ = 508 nm) to determine total iron, allowing iron(III) concentration to be calculated by difference. This difference method become less applicable as the iron(II) to iron(III) ratio increases and, with mineral process liquors, a ratio of 10:1 was considered to be the limit.

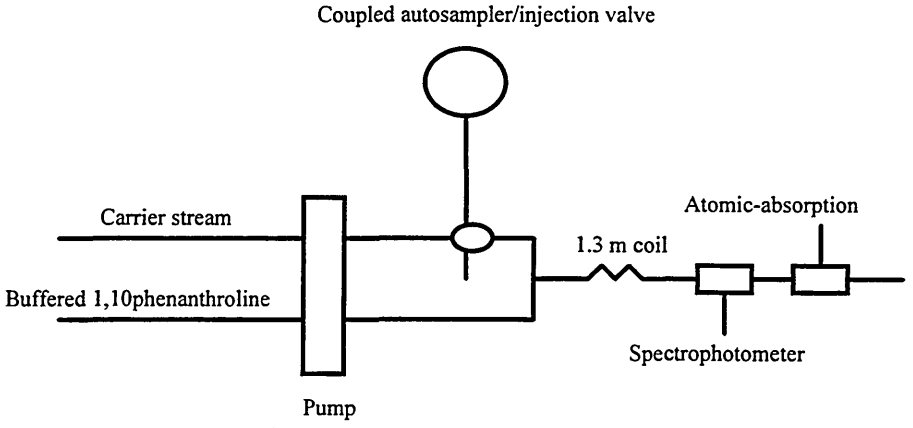

Figure 1.4.
Flow injection (FI) system for the determination of iron(II) and total iron (from ref. [Lynch et al., 1984a]).

An alternative approach was also described by Lynch et al., 1984b for the determination of iron(II) and iron(III) that is applicable to a wide range of sample mixtures of interest to the mineral processing. The system is shown in figure 1.5 and consists of two parallel

colorimetric streams, one for iron(II) and other for iron(III), with sample injection into each stream by coupled, synchronised injection valves.

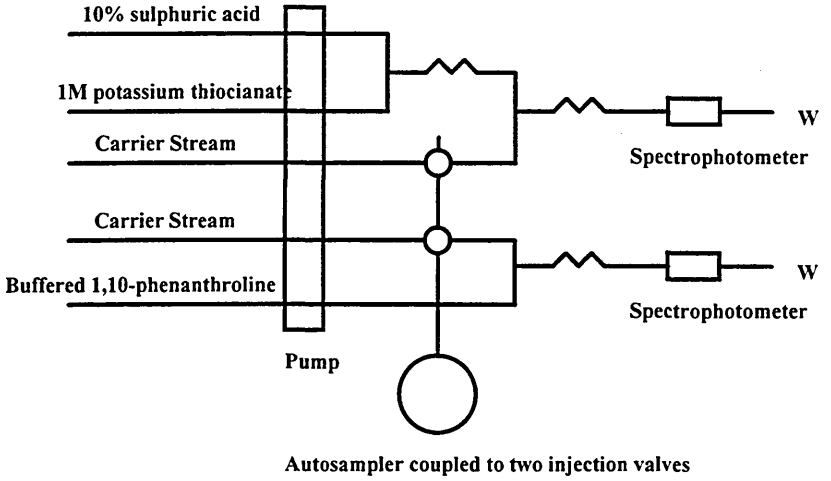

Figure 1.5.
Flow injection (FI) system for the determination of iron(II) and iron(III) (from ref. [Lynch et al. 1984b]).

The colorimetric reaction for the determination of iron (II) is with 1,10-phenanthroline, as in the first system, whereas in the determination of iron(III) the thiocyanate ion is used to form a coloured complex. The concentration of each component is therefore determined independently and not by difference. The system has also been employed for the analysis of hydrochloric acid extracts of geological core samples. The detection limit is 0.1 mg l^{-1}.

A rapid on-line speciation approach for chromium was proposed by Cox et al., 1985 and was based on the use of FI and ICP-AES. The FI system, illustrated in figure 1.6, utilised a microcolumn of activated alumina to effect chromium(III) and chromium(VI) species separation.

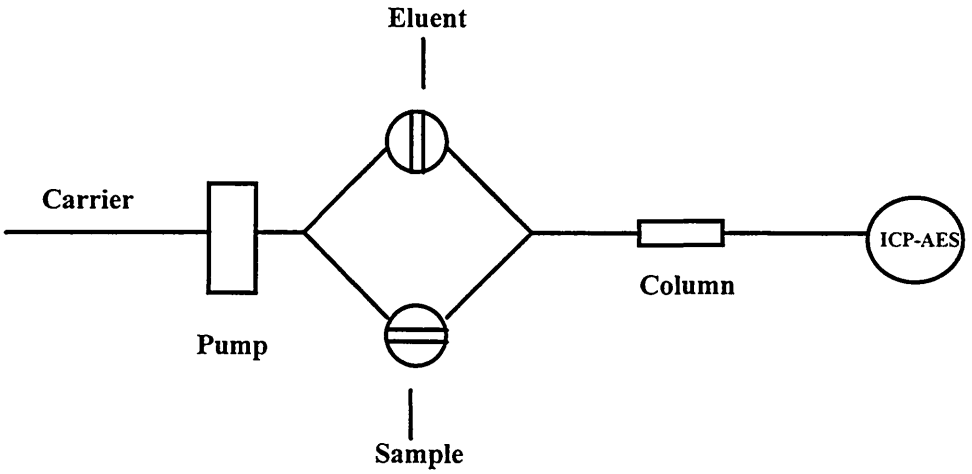

Figure 1.6.
Flow injection (FI) manifold for chromium speciation (from ref. [Cox et al., 1985]).

Under acidic conditions activated alumina displays an affinity for anionic species in contrast to that for chromium(III). Thus on injection of samples containing the two chromium species, chromium(VI) was retained on the column whereas chromium(III) was unretained and passed directly to the ICP. A subsequent injection of ammonium hydroxide was then used to elute chromium(VI) enabling time-resolved emission signals for the two species to be monitored. Activated alumina displays amphoteric properties and a novel aspect of this work is that by utilising a basic carrier stream (0.02 M NH_4OH) and nitric acid as eluent the order sequence for the two species is reversed, i.e. chromium(III) undergoes deposition (chromium(VI) unretained)/elution [Cox et al., 1985]. Speciation data for certified reference materials (total chromium concentration specified) and rivers, using both acidic and basic alumina was reported.

A spectrophotometric method for the determination of selenium(IV) and selenium(VI) as selenite - selenate was developed with the aid of a simple FI system, shown in figure 1.6, by Linares et al., 1986.

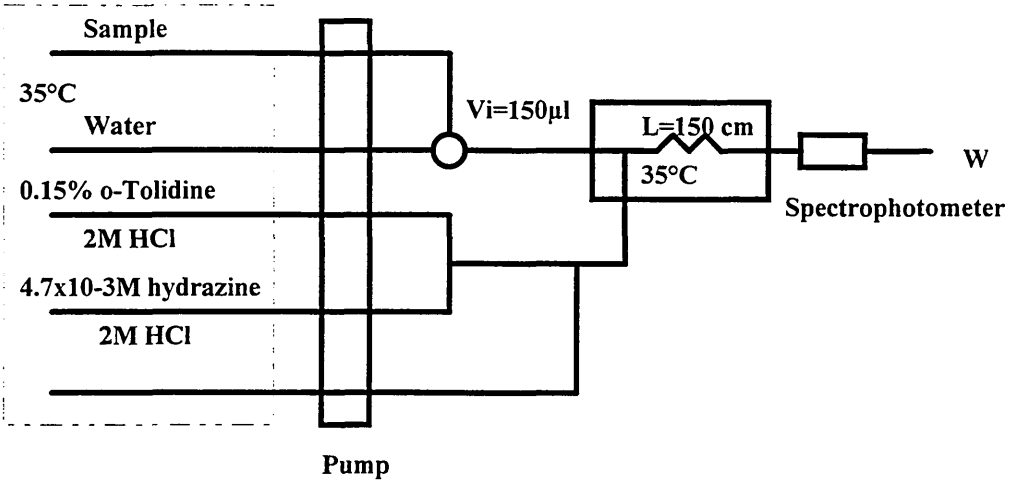

Figure 1.6.
Flow injection (FI) system used for selenium speciation (from ref. [Linares et al., 1986]).

The use of a molecular optical technique in conjunction with FI technique for the determination of selenium has the disadvantage that most of the reactions involving selenium are very slow. A Landlolt process in which Se(IV) catalyses the reaction between chlorate and hydrazine in a hydrochloric medium was chosen for this study. The steps involved in this reaction are:

$$ClO_3^- + 5\ Cl^- + 6\ H^+ \rightleftharpoons 3\ Cl_2 + 3\ H_2O \quad \text{(slow)} \quad (1)$$

$$2\ Cl_2 + (N_2H_6)^{2+} \rightleftharpoons N_2 + 4\ Cl^- + 6\ H^+ \quad \text{(fast)} \quad (2)$$

The presence of hydrazine in the medium slows down step (1) which is fairly fast in its absence or when the medium is very acidic. Se(IV) acts as a catalyst for this first step, the development of which is shown by the coloured oxidation product of o-tolidine ($\lambda_{max.} = 440$ nm).

In the case of mercury speciation, the FI system used for the determination and speciation of methyl- and inorganic mercury [Wei and McLeod, 1992] is shown in figure 1.7. Species separation was achieved using a microcolumn of sulphydryl cotton which has a relatively high affinity for methylmercury. Determination of mercury was attained using the cold vapour-atomic fluorescence technique and with reagent streams of bromide/bromate and tin chloride serving to oxidise methylmercury and generate elemental mercury, respectively [Wei and McLeod, 1992]. The analytical procedure consisted of sample injection and the deposition of methylmercury on the column. Inorganic mercury was unretained and was transported to the detector. An injection of HCl then served to elute methylmercury and enable rapid sequential monitoring of species. In addition to providing an elegant means for species separation the microcolumn technique provides a powerful method for on line preconcentration. In the sampling stage relatively large volumes (e.g. 10 ml) of sample can be passed through the microcolumn and analyte is subsequently eluted by injection of a small volume of eluent (0.5 ml). The FI-CV-AFS technique affords a very high detection capability and natural concentration levels of mercury (ng l^{-1}) in waters may be quantified.

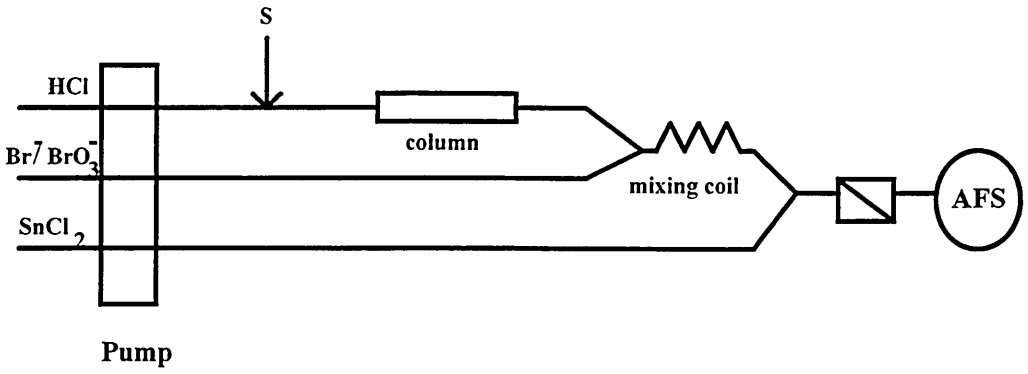

Figure 1.7.
Flow injection system for methyl- and inorganic mercury speciation (from ref.[Wei and McLeod, 1992]).

Finally, Szpunar-Lobinska et al., 1993 proposed a FI system, shown in figure 1.8, for on-line integrated preconcentration/derivatisation of ionic butyltin and phenyltin species. Four principal operations can be distinguished in each cycle: preconcentration, derivatisation, elution and washing. *Preconcentration.* A 10-50 ml sample (S) was drawn through a C_{18} microcolumn (C) fitted in the injection loop of the Rheodyne six-way valve (V_1) by pumping at a flow-rate of 2.0 ml min^{-1}. The injection loop of the valve V_2 was filled with the $NaBEt_4$ solution (R). *Derivatisation.* The column was washed with 5 ml of citrate-ammonia buffer solution (B) (pH 9.0) and then the V2 valve was switched allowing the derivatising agent to enter the column. Then the column was dried by pumping air. *Elution.* The derivatised organotin species were eluted from the column with 250 µl of methanol (E) by means of an all glass syringe. A 5 µl volume of tetrabutyltin (TeBT) in methanol was added as an internal standard. *Washing.* The sample-delivery channel of the manifold and the column were washed with 5 ml of methanol.

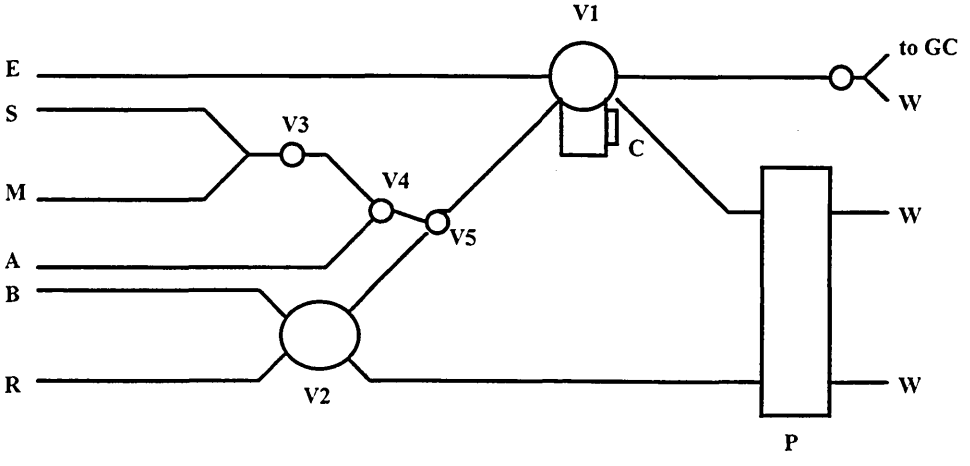

Figure 1.8.
Flow injection (FI) used in on-line preconcentration-derivatisation of organotin compounds (from ref. [Szpunar-Lobinska et al., 1993]).

It can be concluded that the key to development of reliable speciation schemes relies on the availability of sensitive and selective measurement schemes. However it is also clear that the need to be able to maintain and preserve the natural speciation state of the sample if there is a significant time interval between sampling and analysis or if the sample is subjected to significant sample processing (e.g. derivatisation) prior to instrumental detection. Very little work has been done in this area and thus many speciation measurements on real samples must be viewed with some caution until it can be shown that the sample preservation schemes are valid.

For trace element speciation measurements, the sampling, filtration and storage of natural water samples are the most critical stages in the whole analysis procedure (as is the case for total element determination) and analyses have to be performed as soon as possible after collection. Otherwise, storage at 4°C appears to be the best alternative. Florence and Baley, 1980 have given guidance and recommendations for speciation measurements in natural water samples such as the type of sampling bottle and method of cleaning and pH of storage, avoiding freezing of water samples, etc. Ideally, the chemical composition of the water sample being analysed should be measured in situ. In this way the chemical speciation is subjected to less interferences by external influences. In situ analysis has the further advantage of instantaneous results. Thus, if anything unusual is found at a particular location further investigations and clarifications can be performed immediately. Various in situ methods have been developed by using specified sensors detection, such as pH and dissolved oxygen. However, few in situ methods for the determination and speciation of trace elements have been attempted, because the most widely used methods generally utilise sophisticated laboratory instrumentation. A system for complete in situ analysis of trace elements presents many

problems, especially expense. Thus there is an opportunity to devise and develop new techniques for sampling, storage and preservations of water samples such as field sampling techniques. Paulsen at al., 1974 described the criteria for developing field sampling techniques and they are summarised as follow: (i) the system must be sampled in a manner that renders it physically and chemically inert; (ii) the sample must be in a form to be analysed, preferably with one step, with no chemical preservative and preparative steps involved; (iii) the analysis method must be one that is potentially applicable for shipboard or field use in order to get rapid analytical results; (iv) the analytical method must provide high quality resolution for simultaneous identification of different element species; (v) samples must occupy a small space for convenient storage.

Some authors [Eisner et al., 1967; Bauman et al., 1967; Eisner and Mark, 1969; Hughes et al., 1971; Vernon and Eccles, 1973; Kohata et al., 1991; Kolotyrkina et al., 1991; Cox and McLeod, 1992; Wei et al., 1994], proposed a microcolumn field sampling technique which used ion exchange materials for direct sample of trace elements in natural waters. This sampling technique can be used on line with different analytical techniques. For example, Kolotyrkina et al., 1991 proposed to use an ion exchange microcolumn for sampling and preconcentrating trace manganese. A shipboard FI method with a spectrophotometer allowed the determination of manganese in the concentration range 10 ng l^{-1} - 20 μg l^{-1} in deep sea water samples. The microcolumn technique has also been used off line with different analytical techniques for chromium [Cox and McLeod, 1992] and mercury [Wei et al., 1994] speciation. In the case of chromium, acidic or basic alumina microcolumns were used to retain chromium (VI) or chromium(III), respectively. Microcolumn field sampling was performed at two sampling sites on the

river Don and the river Rother (South Yorkshire area). The results obtained by quantitation with ICP-AES performed over an one month period were reported (8.5 - 19.4 μg l^{-1} and 3.1 - 4.5 μg l^{-1} for chromium(III) and chromium(VI) respectively) together with data for total chromium (13.4 - 22.0 μg l^{-1}). In the case of mercury, sulphydryl cotton (SCF) microcolumns were used to retain organomercury species. Microcolumn field sampling was performed in the Manchester Ship Canal. The results obtained by quantitation with cold vapour-atomic fluorescence spectroscopy (CV-AFS) revealed significant concentrations of mercury at all sampling stations (0.004 - 0.058 μg l^{-1} as Hg and 0.045 - 0.530 μg l^{-1} as Hg for organo- and inorganic mercury respectively), confirming the extensive contamination of the water course. Microcolumn field sampling differs from the traditional sampling procedures in that it may allow the preservation of the different analytes present at trace levels, ng l^{-1}, in natural waters until analysis is performed in the laboratory, avoiding the risk of contamination or analyte loss. The procedure is shown in figure 1.9.

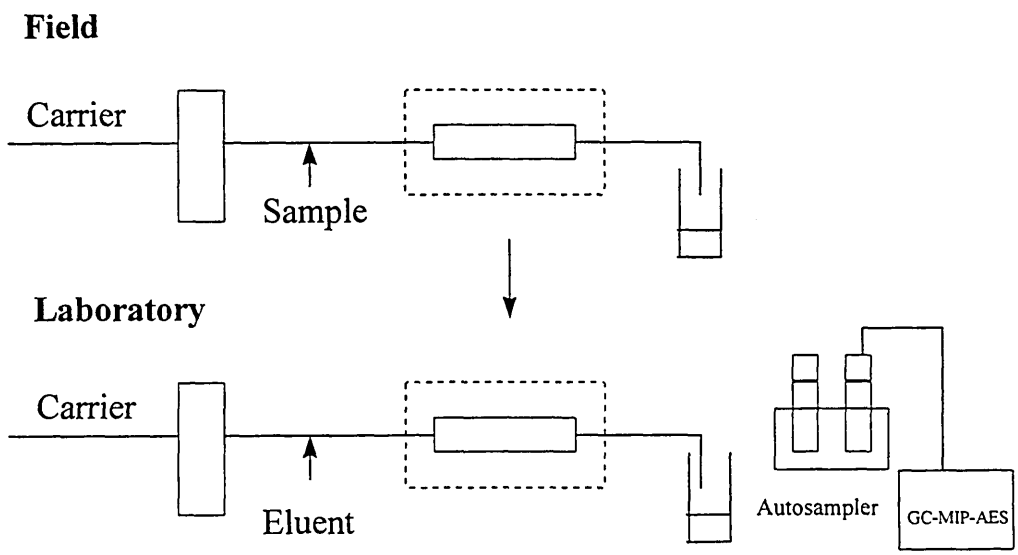

Figure 1.9.
Microcolumn field sampling procedure.

As can be seen instead of undertaking sample processing in the laboratory samples are processed immediately on sample collection in an attempt to stabilise/immobilise species of interest. Microcolumns with retained analytes are then returned to the laboratory and inserted into the FI system for elution/final measurement. In this way it is hoped to avoid the problems associated with maintaining species integrity during the time delay between sampling and laboratory analysis.

A further potential application of microcolumn is their use as external calibrants and certified reference materials (CRMs). Given the interest and increasing acceptance of methodology based on on-line FI procedures there will be an increasing demand for CRMs in microcolumn format. Microcolumns with retained analytes could offer the ability to recover species upon elution (by inserting the microcolumn into a FI system) with a verified, certified recovery after a long term period. In this way it is hoped to avoid the problems associated with maintaining species integrity in aqueous solution.

1.4. Aim of the Research.

The aim of the research described in this thesis is to advance speciation measurement capability by exploiting novel pre-treatment chemistry and instrumentation. For example, the use of microcolumns in flow injection systems may allow the development of highly sensitive and reliable speciation schemes because of the opportunities offered by the integration of the different stages in the speciation measurement, as shown in figure 1.10.

For instance, microcolumns offer the ability to maintain and preserve, in situ, the natural

speciation state of the sample until the analysis is performed. In this aspect microcolumns can be used as a new sampling tool. Also microcolumns allow on-line sample preconcentration/separation of the analytes in a rapid and convenient way. Microcolumns also can be combined (on- or off-line) with sensitive measurement techniques such as GC-MIP-AES or ICP-MS using different flow injection configurations.

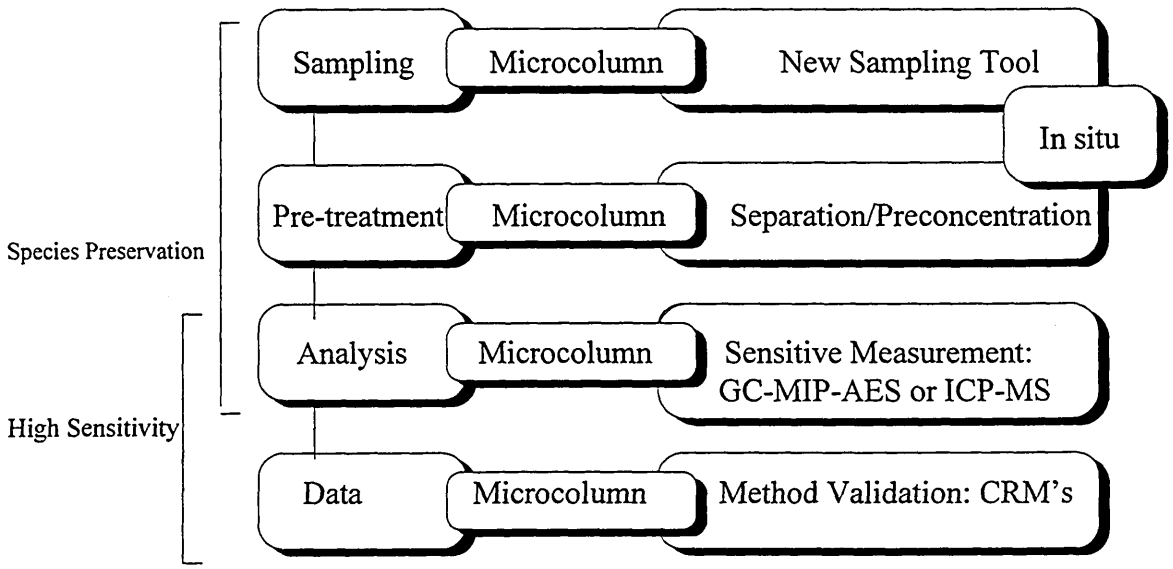

Figure 1.10.
Stages in speciation measurements using microcolumns.

To test the above, experiments will focus on the determination and speciation of mercury in waters. Flow injection systems utilising microcolumns of sulphydryl cotton will be combined to effect microcolumn field sampling/preconcentration prior to determination using GC-MIP-AES. It is planned to produce new speciation data for mercury in the Manchester Ship Canal, a watercourse contaminated with mercury.

Other experiments will focus on the determination and speciation of lead in natural waters. Automated flow injection methodology based on activated alumina separation will be developed in combination with ICP-MS to provide a new speciation capability. Again, it is planned to produce new speciation data for lead in the Manchester Ship Canal and attempt to correlate data for organolead and methylmercury in the canal water.

Another aspect of the work concerns the development of a new class of CRM for mercury and chromium analysis/speciation. A basic aim of the research has been to study time stability data of mercury and chromium species immobilised on the solid support instead of in aqueous solution. This work is vital if methodology based on microcolumn field sampling is to become accepted by the analytical community.

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Chapter 2: Mercury Speciation

2.1. Introduction.

As a result of natural sources and man's activities (such as mercury fungicides, mercury cell chloroalkali processes and mercury impregnated seed dressings) inorganic mercury is transported into the atmosphere from where it passed to the aquatic environment by wet deposition [Lindberg et al., 1987]. The presence of inorganic mercury in the aquatic environment is critical because it is efficiently transformed into its most toxic form (methylmercury) [Craig, 1986]. The methylation of inorganic mercury in aquatic systems has been reviewed by Gilmour and Henry, 1991. In summary it can be said that inorganic mercury may be methylated both chemically and biologically and possibly through a mixed process. Chemical methylation of mercury may occur in the presence of tin and lead alkyls (such as trimethyllead [Jewett and Brinckman, 1974]). Biologically, inorganic mercury can be methylated by bacteria and higher organisms. Then, the methylmercury obtained is absorbed in biological systems such as fish and shellfish from where it is passed to man.

The most disastrous case of environmental poisoning to date and perhaps the most illustrative of the complicated ecological pathways of mercury occurred 40 years ago in the Japanese fishing port of Minamata [Smith and Smith, 1975]. A chemical company constructed a plastic plant which used organomercurials as catalyst and each year a small quantity of methylmercury chloride was discharged into the Minamata river and adjacent Yatsushiro Bay. In 1953, a strange illness affected the local people. This came to be known as Minamata disease. By 1963 it had been established that the disease was caused by mercury in the staple food of the community, fish taken from the Yatsushiro Bay. By 1975, it was estimated that as many as 10,000 persons had been affected by

Minamata disease. In all there were some 703 seriously and permanently maimed and more than 100 died, in addition to the birth of numerous babies with congenital defects.

In order to control industrial discharges of mercury into the aquatic environment, the Japanese government established regulations. It is necessary to remark that regulations specified are for both total mercury and methylmercury concentrations. The European Commission which sets the legislative program for the European Union (EU), has also recognized the potential global impact of mercury pollution. They have established two relevant Directives: 82/176/EEC and 84/156/EEC on discharge of mercury by the chloroalkali industry and other sectors, respectively. Generally, the Directives establish emissions standards for effluent quality from specified processes or industries and/or quality objectives. The term "environmental quality objective" in these Directives accords more closely with the meaning of term "environmental quality standard" (EQS) as commonly used in the UK, that is the concentration of a substance in the receiving water which must not be exceeded if the water is to be suitable for a particular purpose or use, or to achieve a certain level of protection for aquatic life. EQSs are set, based on toxicity, persistence and bioaccumulation, for a number of dangerous substances giving rise to the Dangerous Substances Directive (76/464/EEC). This Directive has set standards for List I Substances which have been identified as posing a threat to the aquatic environment on the basis of their toxicity, persistence and bioaccumulative properties. Mercury is included in List I and the EQS for mercury, as dissolved metal in the aquatic environment, is 0.3 $\mu g\ l^{-1}$. It is necessary to remark that these Directives are for total mercury only. But as already mentioned for natural waters mercury may be present both in organic and inorganic forms and hence data on mercury speciation is necessary. The different fractions that make up total lead are shown in figure 2.1. It has

also been reported that many mercury compounds (mercury complexes with humic substances) are associated with particulate matter (which is retained in a 0.45 μm filter, as shown in figure 2.1) in water samples. They are derived from soils and sediments, where they form through the chemical and biologically mediated breakdown of biological tissue [Boggs et al., 1985].

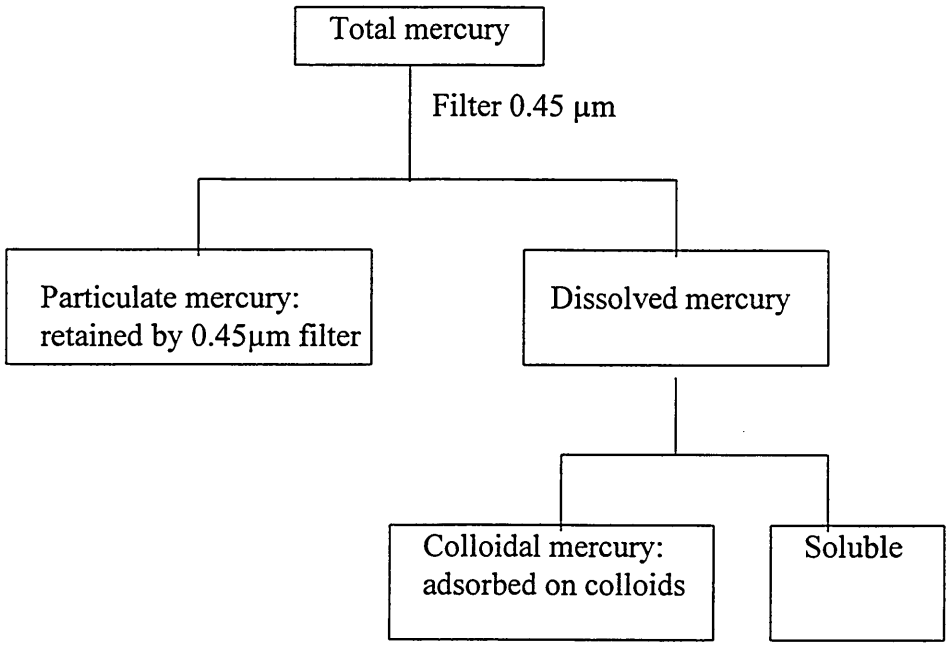


Figure 2.1.
Different fractions that make up total mercury concentration in water samples.

Numerous hyphenated techniques have been developed for the determination and speciation of mercury in environmental samples. This is due to the fact that mercury speciation clearly must involve separation followed directly by specific determination and quantification of each individual mercury species. The hyphenated techniques used for mercury speciation can be classified according to the separation step which involves chromatographic or non-chromatographic separations. Some examples of separations using non-chromatographic separation techniques include: selective chemical

reductants/pretreatments with cold vapour-atomic absorption spectrometry/atomic emission spectrometry (CV-AAS/AES) [Oda and Ingle, 1981; Birnie, 1988; Wei and McLeod, 1992], solvent extraction and ICP-MS [Beauchemin et al., 1988] or electrochemical separation and AAS [Filipelli, 1987]. However the use of chromatographic separations, such as GC or HPLC, with sensitive detection/ quantitation systems have been presented as the most useful tool for studying the speciation of mercury. The use of GC with AAS or AES has been reviewed a number of times [Harrison and Rapsomanikis, 1989; Chau, 1992]. Recently, the use of GC with AFS [Alli et al., 1994] or ICP-MS [Prange and Jantzen, 1995] has also been proposed.

One common characteristic of hyphenated methods based on capillary GC is that pre-derivatisation of the analytes is required. This is essential due to thermal instabilities of mercury species in the inlet system and/or in the GC column. The most common derivatisation procedures used are aqueous phase ethylation using sodium tetraethylborate [$NaB(CH_2CH_3)_4$] [Fishers et al., 1991; Filipelli et al., 1992] and Grignard alkylation using propylation (propylmagnesium chloride) or butylation (n-butylmagnesium chloride). Grignard alkylation must be preceded by extraction of the organomercury species into an organic solvent, which is usually done with complexing agents like tropolone or dithiocarbamates [Bulska et al., 1991; Emteborg et al., 1993]. Another common characteristic of the use of GC detection system is that some form of preconcentration step is necessary due to the low concentrations of mercury species present in natural waters (ng l^{-1}). Cryogenic trapping [Bloom, 1989] or extraction with an organic solvent (benzene or toluene) have been used. Alternatively, adsorption of the analytes of interest onto ion exchange resins is a convenient way to perform species preconcentration. Moreover, in combination with flow injection (FI), this approach

would seen to offer considerable potential due to the ability to undertake rapid on-line sample manipulation. The time scale for such pretreatments is short and this is attractive in terms of conserving the natural speciation state of the analyte. Different materials, such as sulphydryl cotton fiber (SCF) [Lee and Mowner, 1989; Lee, 1987] and dithiocarbamate resin [Bulska et al., 1991; Emteborg et al., 1993] have been utilized for the preconcentration of mercury in natural waters. Detection limits of 0.05 ng l^{-1} for methylmercury (4 l sample processed) [Lee, 1987] and of 0.05 ng l^{-1} for methyl-, ethyl- and 0.15 ng l^{-1} for inorganic mercury (500 ml sample processed) [Emteborg et al., 1993] were obtained, respectively.

The need for derivatisation can be avoided by using HPLC with ICP-AES [Gast et al., 1979; Krull et al., 1982] or ICP-MS. The use of HPLC with ICP-MS has been reviewed a number of times [Hill et al., 1993; Seubert, 1994; Uden, 1995]. Problems related to the use of organic solvents in the mobile phase to achieve the separation were solved by using a direct injection nebuliser (DIN) [Wiederin et al., 1991; Shum et al., 1992a; Shum et al., 1992b] or ultrasonic nebuliser with a membrane desolvator [Montaser et al., 1991; Botto and Zhu, 1994] as already reported in chapter 1.

Another consideration in the determination and speciation of mercury at trace levels in environmental samples is related to the sampling procedure. Studies on trace mercury speciation have shown that plastic or glass containers can both absorb trace mercury ions from and/or contribute mercury to samples as a result of surface dissolution [Jacgues and Fabrice, 1982], which cause contamination and dramatic changes in sample composition. To date, in the context of mercury speciation very little attention has been given to this point. Thus for environmental waters the use of an in situ

sampling/storage method such as microcolumn field sampling (as already outlined in chapter 1) could solve the inherent analytical problems associated with sampling/storage. The approach could offer the possibility of collection, preservation and preconcentration of mercury species directly at the sampling site avoiding the risk of contamination and changes in the natural speciation state of the sample.

A basic aim of the work described in this chapter is to advance speciation methodology for mercury in natural waters by combining GC-MIP-AES, one of the most powerful techniques for mercury speciation, with microcolumn field sampling. By processing water samples in the field it is hoped to preserve mercury species, avoiding the risk of contamination or analyte loss. Subsequent to method development in the laboratory, survey work was undertaken in the Manchester Ship Canal, a watercourse known to be contaminated and subject to industrial mercury discharges.

2.2. Experimental.

Reagents and Materials.

Standard solutions of inorganic mercury were prepared by appropriate dilution of stock solution (1000 mg l^{-1} as Hg of mercury nitrate in 1% V/V HNO_3). The organomercury stock solutions (RHgCl; R = CH_3 or C_2H_5) were prepared by dissolving the respective compounds in acetone (Merck, ARISTAR) and standard solutions were prepared by appropriate dilution of stock solutions (1000 mg l^{-1} as Hg). Hydrochloric acid (0.01 M and 3 M) was prepared from concentrated reagent (Merck, ARISTAR). Buffer solutions of sodium hydroxide 2.7 M (Merck, ARISTAR)/sodium acetate 3 M (Merck, ARISTAR) were prepared by dissolving the compounds in Millipore water. Sodium tetraphenylborate was obtained from Aldrich and a 1% m/V solution was freshly prepared in Millipore water a few minutes before use.

For the determination of total mercury, tin chloride solution (3% m/V) in hydrochloric acid (15% V/V) was freshly prepared each day from tin chloride 2-hydrate salt (Spectrosol, ARISTAR) and Millipore water. A potassium bromide/potassium bromate solution (0.5% m/V + 0.14% m/V) was made daily by dissolving potassium bromide/ potassium bromate salts (Fisons, ARISTAR) in Millipore water. Hydrochloric acid solution (0.01 M, 3 M and 5 M) was prepared from concentrated reagent. Sample solutions were adjusted to pH 3.5 by adding dropwise dilute nitric acid solution (0.5 M, Merck, ARISTAR).

The sulphydryl cotton adsorbent (SCF) was produced by introducing the sulphydryl

functional group in natural cotton fibres according to the procedure of Lee, 1987. Thioglicollic acid (50 ml, 97% m/m, Merck, ARISTAR), acetic anhydride (30 ml, 36% m/m, Merck, ARISTAR), acetic acid (20 ml, 30% m/m, Merck, ARISTAR) and sulphuric acid (0.15 ml, 96% m/m, Merck, ARISTAR) were measured into a wide-neck flask and then mixed thoroughly (care exothermic reaction). The mixture was cooled to room temperature and absorbent cotton (15 g) was added and left to soak. The stoppered flask was then placed into an oven at 4°C left for 4 days. Then, the cotton fibre was washed with double distilled water until washing were between pH 6-7 and the material dried at a low temperature (35°C). The dried cotton was next transferred to a sealed light-free container for storage. An esterification reaction takes place between the hydroxyl group on the cotton fibre and the carboxylic acid group in thioglycollic acid. The product is known as SCF:

$$\underset{\text{cotton fibre}}{n\ C_6H_9O_4OH} + \underset{\text{thioglycollic acid}}{n\ HOOCCH_2SH} \longrightarrow \underset{\text{sulphydryl cotton}}{n\ C_6H_9O_4O\overset{\overset{O}{\|}}{C}CH_2SH} + n\ H_2O$$

SCF microcolumns were made by putting about 0.015 - 0.018 g of the cotton fibre into a 60 mm length of column. Two other small tubes (20 mm x 0.8 mm) were fitted at both ends of the column to allow connection of the microcolumn to the system.

Instrumentation Equipment and Operating Procedures.

Gas Chromatography-Microwave Induced Plasma- Atomic Emission Spectrometry (GC-MIP-AES).

A commercially available GC-MIP-AES was used for the determination and speciation of mercury species. The basic components of the GC-MIP-AES (HP5890 Series GC),

illustrate in figure 2.2 are: (i) gas chromatograph, (ii) microwave induced plasma, and (iii) spectrometer / detector. A full description of the operating conditions and procedures is given.

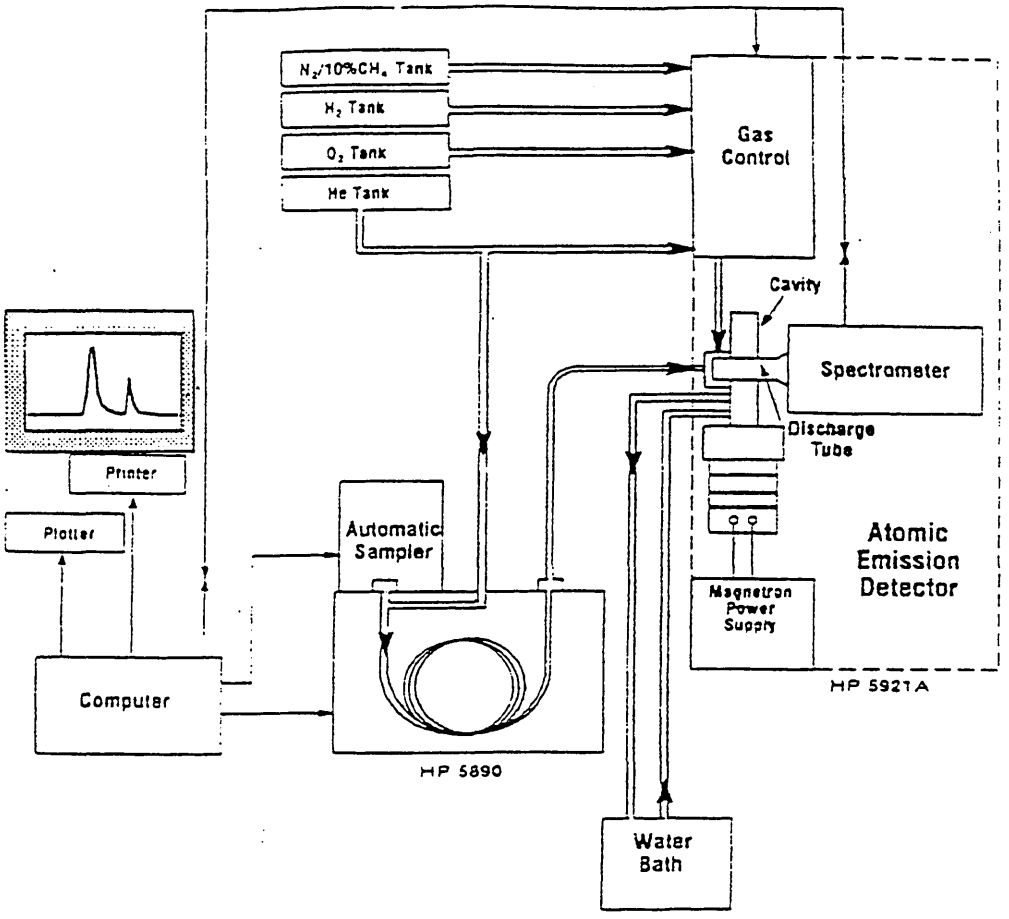


Figure 2.2.
Schematic of GC-MIP-AES.

(i) Gas chromatograph (GC). The gas chromatograph was equipped with an autosampler for sample introduction. The autosampler consisted on a plastic plate, containing holes for introducing sample vials (maximum 100 sample vials; capacity, 2 ml). An automatic arm was used to transport the different sample vials (one at time) to a small plastic plate which contained a n-hexane vial and a waste vial. At the top of this small plastic plate there was a closed box which contained the syringe (1 µl capacity).

Then, the syringe took n-hexane for rinsing steps (n = 3) and the n-hexane was deposited in the waste vial. Finally, the syringe took the sample which was introduced into the GC.

By using automatic injection the liquid sample (1 μl) was introduced, through the injection port, into the GC column. The injection port was equipped with a vaporizing injector, which was suitable for splitless injection (which allowed the analysis of compounds present at trace levels). Here, there was a temperature of 250°C which was needed for the volatilisation of the sample prior to entry into the GC column. Then, the sample was swept into the GC column, by using helium as a carrier gas. Separation of the compounds took place on the GC column due to their different affinities for the stationary phase. The compounds that exhibited stronger affinities for the stationary phase required more time to be eluted. So, compounds with different retention behaviour were separated.

The GC column was a capillary column (fused silica HP-1; non-polar; 25 m length x 0.23 mm id; 0.17 μm film thickness) and was installed between the injection port and the microwave plasma. The GC column was wound on a wire frame which was mounted on a pair of brackets which slip into slots at the to of the oven interior. The column ends come off the bottom of the frame, making smooth curves to the inlet and detector fittings (6.35 mm graphite O-rings for the injector part and 1.0 mm graphite ferrules for the microwave plasma part).

The column was housed in an insulated, thermostatically controlled oven. This was necessary because the temperature of the column during the analysis had to be increased

in order to assist the separation / elution of the components from the GC column. The column temperature programme used for the present studies was: 3 min 40°C, 30°C/min to 270°C hold 2 min 270°C.

Injection volume:	1 µl
Injection port:	splitless
Injection port temperature:	250°C
GC column:	25 m length x 0.23 mm id.; 0.17 µm film thickness; fused silica HP-1; non-polar
GC column flow rate:	2.5 ml min^{-1}
GC column-MIP coupling:	GC column to cavity
Total number of injections:	3
Oven temperature programme:	3 min 40°C, 30°C/min to 270°C hold 2 min 270°C
Scavenger gas:	O_2, air and H_2
Scavenger gas flow:	25 ml min^{-1}
Elements:	C (247.857 nm) Hg (253.652 nm)
Signal output/display:	Emission intensity versus time 2D plot
Window purge helium flow rate:	30 ml min^{-1}

Table 2.1.
Operational conditions for the Hewlett Packard 5890 Series II gas chromatograph.

(ii) Microwave Induced Plasma (MIP). The effluent from the GC was directed to the MIP to effect atomisation and excitation of mercury species. The aim of the microwave cavity is to transfer energy efficiently from the microwave generator to the helium gas, producing the ionisation of helium. Then, there is a transfer of energy by collision from the ionised helium to analyte species thereby effecting atomisation / ionisation / excitation. As a result there is a release of characteristic emission (the wavelength of the principal mercury transitions is 253.65 nm and 184.95 nm) as excited mercury species

decay to the ground state. The emitted light is directed to the spectrometer where it undergoes dispersion before detection by the photodiode array system.

Although plasma gas temperatures are lower in the MIP that in the ICP or DCP, high electron temperatures are available, particularly in the helium plasmas which has kinetic energies between 0.4 and 2.0 eV (these energies correspond to kinetic temperatures between 4600 and 23,000 K) giving high spectral emission intensities for many elements, including many non-metals which respond poorly in the argon ICP or DCP. The partial energy level diagram for mercury showing the prominent transitions (wavelengths) is given in figure 2.3.

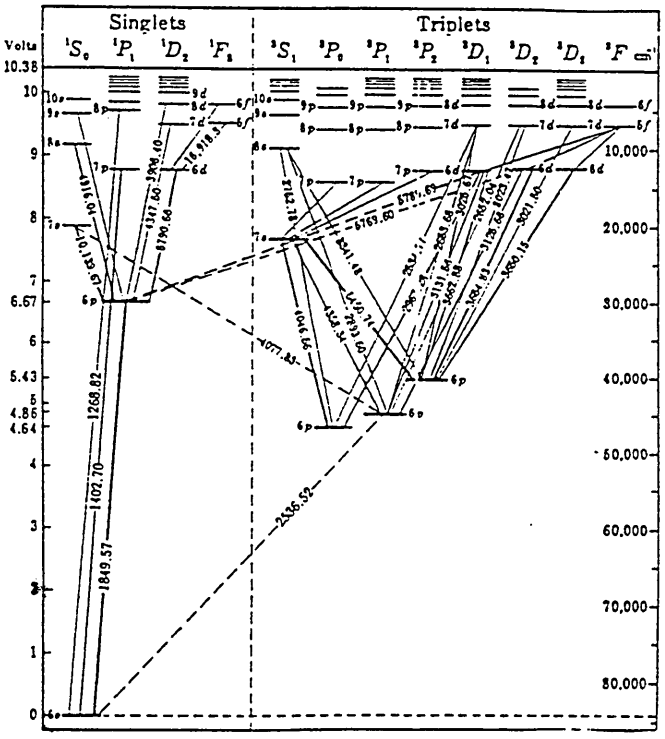


Figure 2.3.
The energy level diagram (Grotrian) for the mercury atom (from reference [Herzberg and Spinks, 1944].

The coupling of the GC to the MIP was based on the work of Quimby and Sullivan, 1990. They proposed a new approach which solved several problems (such as discharge

tube erosion and poor selectivity) reported earlier in the literature [McLead et al., 1973; Quimby et al., 1978; Brenner, 1978; Tanabe et al., 1981; Estes et al., 1981; Ke-Wei et al., 1985; Hagen et al., 1985; Ebdon et al., 1986]. To address these problems Quimby and Sullivan, 1990 incorporated changes in the spectrometer (which are addressed in the next section) and in the MIP. Developments for the MIP included an atmospheric pressured helium plasma and an automated gas handling system.

The plasma is created in a reentrant cavity which resembles the TM-010 cavity first described by Beenakker [Beenakker, 1977; Beenakker and Boumans, 1978] and was designed to eliminate the need for tuning and to mount directly on the magnetron waveguide. Both cavities are shown in figure 2.4.

To produce the plasma, microwave power from a microwave oven magnetron tube (Panasonic 2M211A) is supplied to the cavity via a waveguide, which is a rectangular extrusion with inside dimensions of 95 mm by 45 mm and 3.0 mm thick walls. The magnetron tube is mounted according to the manufacture's instructions at the opposite end of the waveguide. The cavity/waveguide/magnetron assembly is attached to the spectrometer with a hinge at the magnetron end and screws at the cavity end. The screws hold the cavity against a precisely machined locating ring on the spectrometer. This arrangement allowes the cavity to be swung away from the spectrometer for discharge tube replacement and then precisely repositioned. The MIP characteristics for the plasma and the generator are summarised in table 2.2.

(a)

(b)

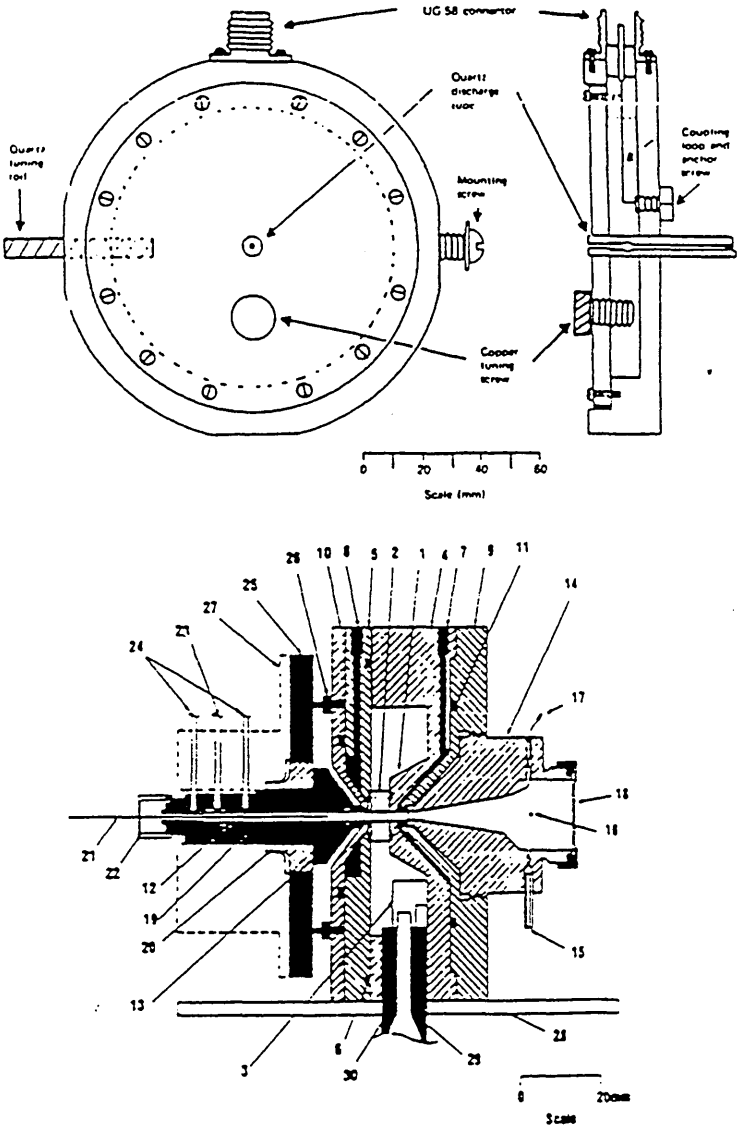


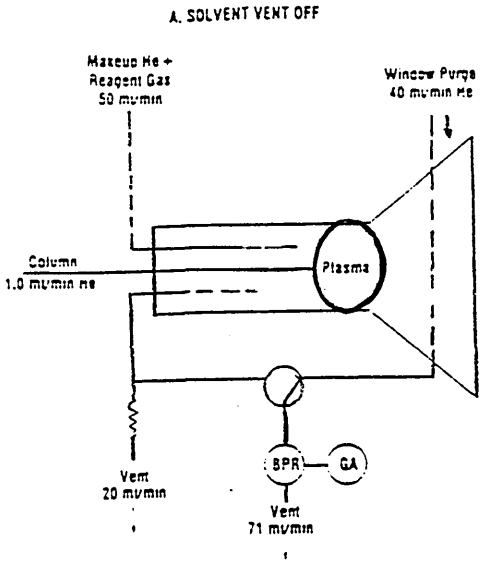
Figure 2.4.
Diagram of (a) Beenakker (from reference [Beenakker, 1977]) and (b) reentrant cavity (from reference [Quimby and Sullivan, 1990]).

Microwave Plasma		
	Cavity:	Reentrant design
	Discharge tube:	Water-cooled walls 0.1 mm thick
	Ignition:	Automatic
	Exhaust:	From discharge tube into a purge chamber to prevent air diffusion
Microwave Generator		
	Frequency:	2450 MHz
	Power level:	50 Watts
	Power tube:	2M211 A magnetron
	Tuning:	none required
	Coupling to cavity:	95 mm x 45 mm x 3.0 mm thick walls

Table 2.2.
MIP characteristics for the plasma and generator.

A computer-controlled gas handling system was also incorporated for obtaining a minimal dead volume solvent venting device and for excluding air from the exit end of the discharge tube. The solvent venting scheme is shown in figure 2.5a,b.

(a)



(b)

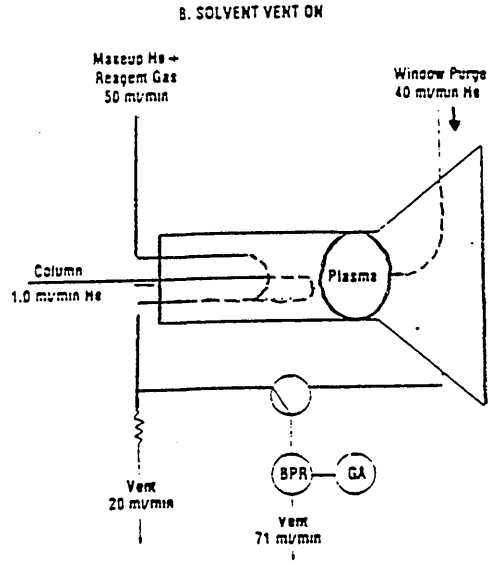


Figure 2.5.
Solvent venting scheme for MIP. (a) Solvent vent off and (b) solvent vent on.

Figure 2.5a shows the gas flow configuration in the solvent vent-off mode (i.e. column effluent going into the plasma). It can be seen that the column effluent and makeup gas (~30 ml min^{-1} helium containing 0.1-0.5% reagent gases, O_2, H_2 and air) enter the plasma. An additional 20 ml min^{-1} of makeup gas (helium) was used to purge the

column connector region and exits along the outside of the column. The rest of the makeup gas and the column effluent, after passing through the plasma, combine with the window purge helium and exit through the solenoid valve and back pressure regulator (BPR).

Figure 2.5b shows the solvent venting mode. For venting, the solenoid valve was switched so that the exit tube on the window side of the chamber was closed. The window purge gas is now forced to flow backward through the plasma. It flows past the end of the column and sweeps the column effluent and the makeup gas through the solenoid valve and the BPR. The solvent venting solenoid valve was controlled by the computer and was time programmable.

The gas flow system also provided makeup helium blended with the appropriate reagent gas. It allowed the flow of makeup gas and reagent gases to be varied independently. Three different reagent gases are used: oxygen, hydrogen and a mixture of 10% methane in nitrogen. The three reagent gases can be turned on and off in any combination by using the appropriate solenoid valves. The flow rates of oxygen, hydrogen and the nitrogen/methane mixture used in this work were 0.25 ml min^{-1}.

The discharge tube, which is a 1.0 mm id. x 1.25 mm o.d. x 42 mm long polyimide-coated fused silica tube (Hewlett-Packard), was placed in the centre and horizontal in the microwave cavity, as shown in figure 2.6. This allowed an axial view of the plasma. The discharge tube is water cooled and under such conditions the internal wall temperature is approximately 350°C. This improves the lifetime of the discharge tube from 8-12 h to 30 days for continuos use.

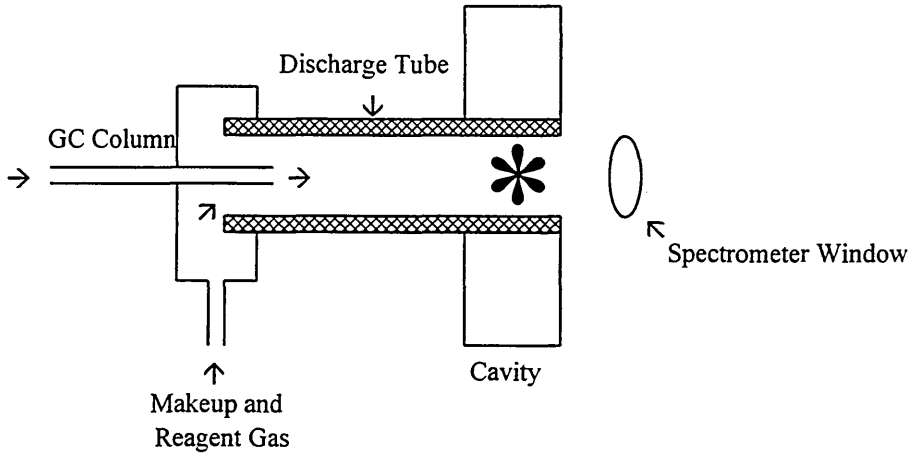


Figure 2.6.
Interface used in the GC-MIP-AES (from reference [Quimby and Sullivan, 1990]).

(iii) *Spectrometer*. The light emitted by the excited atoms and ions in the plasma was directed to the entrance slit (0.05 mm width) and was diffracted and focused by the spectrometer. The spectrometer was made following the design of Sullivan and Quimby, 1990 and is based on a concave holographic grating which has a flat focal plane along which the photodiode array detector can be moved. Figure 2.7 shows a schematic diagram of the spectrometer. It can be seen that the plane of the spectrometer was arranged vertically to minimize the area on the laboratory bench occupied by the instrument. The outside wall of the spectrometer was attached to the cavity so that the focal point of the elliptical mirror was 2 mm into the end of the discharge tube. The spectrometer had some of the aspects of a polychromator because many lines clustered within the width of the photodiode array can be measured

simultaneously. But it also had features of a monochromator because the spectrometer can be set at any wavelength and it can scan a continuos spectrum.

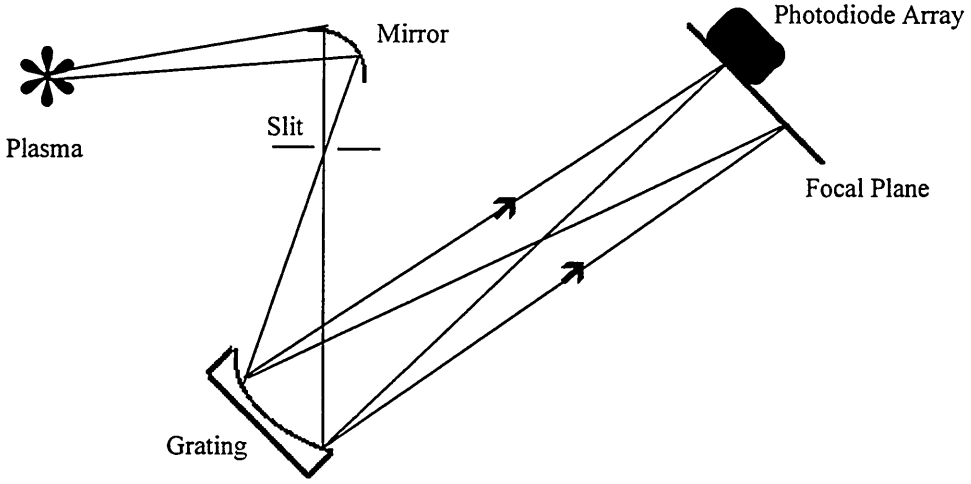


Figure 2.7.
Schematic diagram of the spectrometer (from reference [Sullivan and Quimby, 1990]).

The properties of the concave grating spectrometer are described in table 2.3. As can be seen the light-collecting ability of the spectrometer is indicated by the f / number. In this case the f / number is 3.6, a fast light-collecting spectrometer. As can also be seen the grating is curved, with a curvature of 0.3 m radius, and has grooves with extremely complex spacing and orientation (grating groove density of 550 / mm and grating groove depth of 0.08 μm) which was needed for the dispersion of the spectrum on a flat rather than a concave focal plane (as occurs with classical gratings). The focal curve covered the wavelength ranges of ultraviolet and visible light (160-800 nm, 1st order; 160-400 nm, 2nd order).

Focal length:	0.3 m
f / number:	3.6
Focal plane length:	0.35 m
Grating groove density:	550 / mm
Grating groove depth:	0.08 μm
Wavelength range:	160 - 800 nm
Dispersion:	0.2 (vacuum UV) to 0.06 (near-IR.) nm / pixel
Resolution:	0.1 nm at 400 nm
Entrance slit width:	0.05 mm
Condensing optics:	90° elliptical mirror
Detector:	211 pixel photodiode array
pixel size:	0.6 x 0.006 mm
dark current:	0.5 pA at 25°C
dynamic range:	105

Table 2.3.
Spectrometer components and operating conditions.

A photodiode array (HP5921A) was used for multichannel detection. Each of the 211 detecting diode elements or pixels consist of a photodiode and a capacitor connected in parallel to a silicon-based mechanism. At the beginning of a measuring cycle the capacitor is fully charged. Every 10 milliseconds the light striking a given photodiode is measured. This light creates a photocurrent that in turn discharges the capacitor to a level proportional to the photocurrent created. At the end of the measuring cycle the capacitor is recharged. The photodiode array spectrometer can perform simultaneous multielement detection. There is also provision for undertaking background correction.

Flow Injection (FI) System.

A FI system, used for sample processing both in the laboratory and in the field, is shown in figure 2.8 and consists of a peristaltic pump (Gilson Minipuls or portable minipump ALITEA) and a SCF microcolumn, which was used to preconcentrate mercury species (methyl-, ethyl- and inorganic mercury).

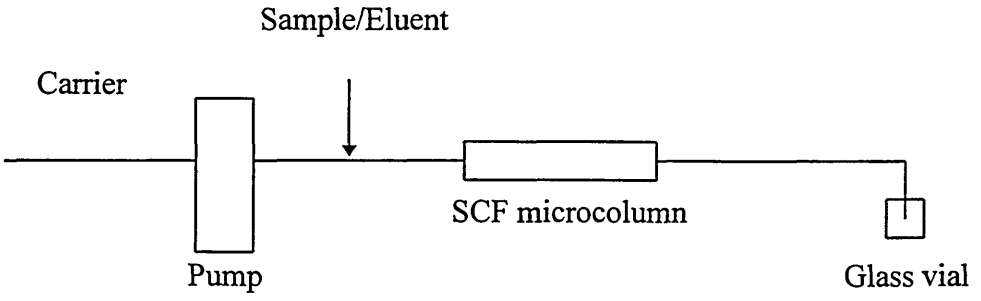


Figure 2.8.
FI system used off-line for sample processing in the laboratory and in the field.

Prior to use the SCF microcolumns are conditioned by twice passing through 2 ml of hydrochloric acid solution (3 M) followed with twice passing through 2 ml of hydrochloric acid (0.01 M) solution (flow rate, 4 ml min^{-1}).

Cold Vapour-Atomic Fluorescence Spectrometry (CV-AFS).

For the determination of total mercury a FI-CV-AFS system was used. The FI system was connected on-line to the CV-AFS detector, as shown in figure 2.9, and consisted of a peristaltic pump (Gilson Minipuls), a rotatory injection valve (Ommnichem) and a gas/liquid separator (PS Analytical). Typical operating parameters for the FI-CV-AFS system are shown in table 2.4.

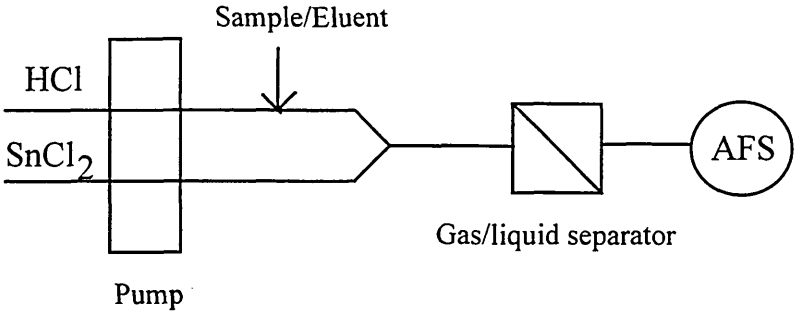


Figure 2.9.
FI-CV-AFS system for the determination of total mercury.

Flow Injection (FI) system	
Flow rate of streams:	1.5 ml min^{-1}
Sampling loop:	0.5 ml
Total number of injections:	3
Merlin Mercury Detector	
source:	low pressure mercury discharge lamp
analytical line:	253.6 nm
sensitivity setting:	2 x 1000
flow rate of sheath argon:	2 l min^{-1}
flow rate of sample aeration:	2 l min^{-1}
Chart Recorder	
sensitivity:	1 V
chart speed:	10 mm min^{-1}

Table 2.4.
Typical operating parameters for the FI-CV-AFS system.

The Merlin mercury fluorescence detector (P.S. Analytical), shown in figure 2.10, was coupled to a vapour generator (P.S.Analytical), which prepared the analyte for measurement by reducing chemically the mercury to a vapour prior to detection. The reductant used to convert the mercury into its vapour state was tin (II) chloride ($SnCl_2$) and the gaseous/liquid mixture then passed to a gas/liquid separator, where gaseous mercury vapour was separated from the liquid stream. The vapour was then transferred by argon carrier gas to the detector via a membrane dryer tube to remove moisture.

A mercury vapour discharge lamp was used as an excitation source. Atomic mercury

vapour entered the atom cell and at room temperature absorbed and fluoresced at the same wavelength to emit fluorescence radiation in all directions. Resonance fluorescence occurred at 253.6 nm which provided the most sensitive detection. The fluorescence was measured at 90° to the incident beam to reduce constant effect of background scatter. Lenses and collimators were used to focus and collect radiation of interest and a photomultiplier tube (PMT) measured the fluorescence. Unlike CV-AAS the Merlin fluorescence detector operated without a flow cell and therefore had a rapid flush out time.

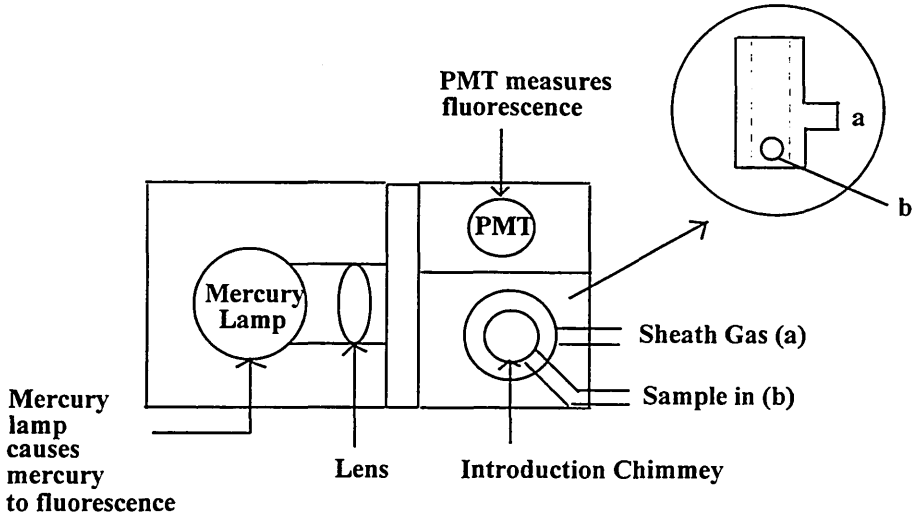


Figure 2.10.
Schematic diagram showing the vapour introduction chimney and layout of the Merlin detector.

Laboratory Procedures.

Derivatisation/extraction of mercury species. A typical GC-MIP-AES analysis was realised as follow: a standard solution containing methyl-, ethyl- and inorganic mercury (e.g.: 1 mg l^{-1} as Hg, 2 ml) was introduced into an empty glass vial (5 ml capacity) which contained buffer solution (sodium acetate / acetic acid; 3 M, 100 µl). Then, sodium tetraphenylborate (5% m/V, 120 µl) and n-hexane (1 ml) were added and the

tube shaken for 30 min. using an automatic shaking machine and centrifuged for 5 min.. Then, the organic phase was withdrawn using a glass pipette and placed in a screw-capped glass vial (2 ml capacity) ready for injection (automatic) to the GC-MIP-AES.

Derivatisation / extraction of mercury species after elution from SCF microcolumns. SCF microcolumns were subjected to injections of hydrochloric acid (3 M, 2 ml) to remove residual contamination, followed by a rinsing step of hydrochloric acid (0.01 M, 2 ml). Then, a standard solution containing methyl-, ethyl- and inorganic mercury (e.g.: 1 mg l^{-1} as Hg, 2 ml) was adjusted to pH 3.5 by dropwise addition of concentrated nitric acid and passed through the SCF microcolumn at a rate of 4 ml min^{-1} (typical sample volume: 20 - 200 ml). The effluent from the microcolumn was then subjected to the procedure described above. Retained mercury species were then eluted with hydrochloric acid (3 M, 0.5 ml; elution flow rate, 1 ml min^{-1}; microcolumn capacity, 5 µg Hg / 0.015 g cotton). The eluate (0.5 ml) was collected in an empty glass vial (5 ml capacity) which contained 1 ml of high purity water (Millipore) and buffer solution (sodium hydroxide/sodium acetate; 2.7 / 3 M, 358 µl) was added to obtain a pH of 2. Sodium tetraphenylborate (5% m/V, 120 µl) and n-hexane (1 ml) were added and the tube shaken for 30 min. using an automatic shaking machine and centrifuge for 5 min. The organic phase was withdrawn using a glass pipette and placed in a screw-capped glass vial (2 ml capacity) ready for injection (automatic) to the GC-MIP-AES.

Field Sampling.

The cruises for the collection and processing of water samples (27.10.93 and 08.06.95) from the Manchester Ship Canal were organised by Dr. Peter Jones of the

Environmental Agency, North West Region-Warrington, who made the arrangements for sampling taking account of the location of industrial discharges, possible mercury dispersion and the movement of tides. The Manchester Ship Canal was selected for the determination and speciation of mercury because it is known that the principal source of inorganic mercury was a discharge chloroalkali plant. The stations at which water samples were collected are shown in figure 2.11: Fisher Wharf, Stanlow Wharf and Stanlow Point.

The collection of the water samples, shown in figure 2.12, was realised as follow: the water sample (1-2 l) was taken using a bucket attached to the end of a rope. A water sample was then adjusted on collection to pH 3-3.5, by adding dropwise, a small amount of concentrated nitric acid (Merck, ARISTAR). Then the SCF microcolumn , which was cleaned previously in the laboratory, was connected to a feed pump. The feed pump was switched on and a non filtered water sample (60 ml) was passed through the SCF microcolumn, at a flow rate of 4 ml min^{-1}, to effect the enrichment of mercury species. Then, the SCF microcolumn (5 per station) was disconnected, placed in a light tight box, returned to the laboratory and placed in fridge. Typical delay before analysis was 24 hours.

For analysis the SCF microcolumns were connected one at time to a feed pump for elution. Retained mercury species were then eluted with hydrochloric acid (3 M, 0.5 ml; elution flow rate, 1 ml min^{-1}). The eluate (0.5 ml) was collected in an empty glass vial (5 ml capacity) which contained 1 ml of high purity water (Millipore) and buffer solution (sodium hydroxide / sodium acetate; 2.7 / 3 M, 358 µl) was added to obtain a pH of 2. Then, sodium tetraphenylborate (5% m/V, 120 µl) and n-hexane (1 ml) were

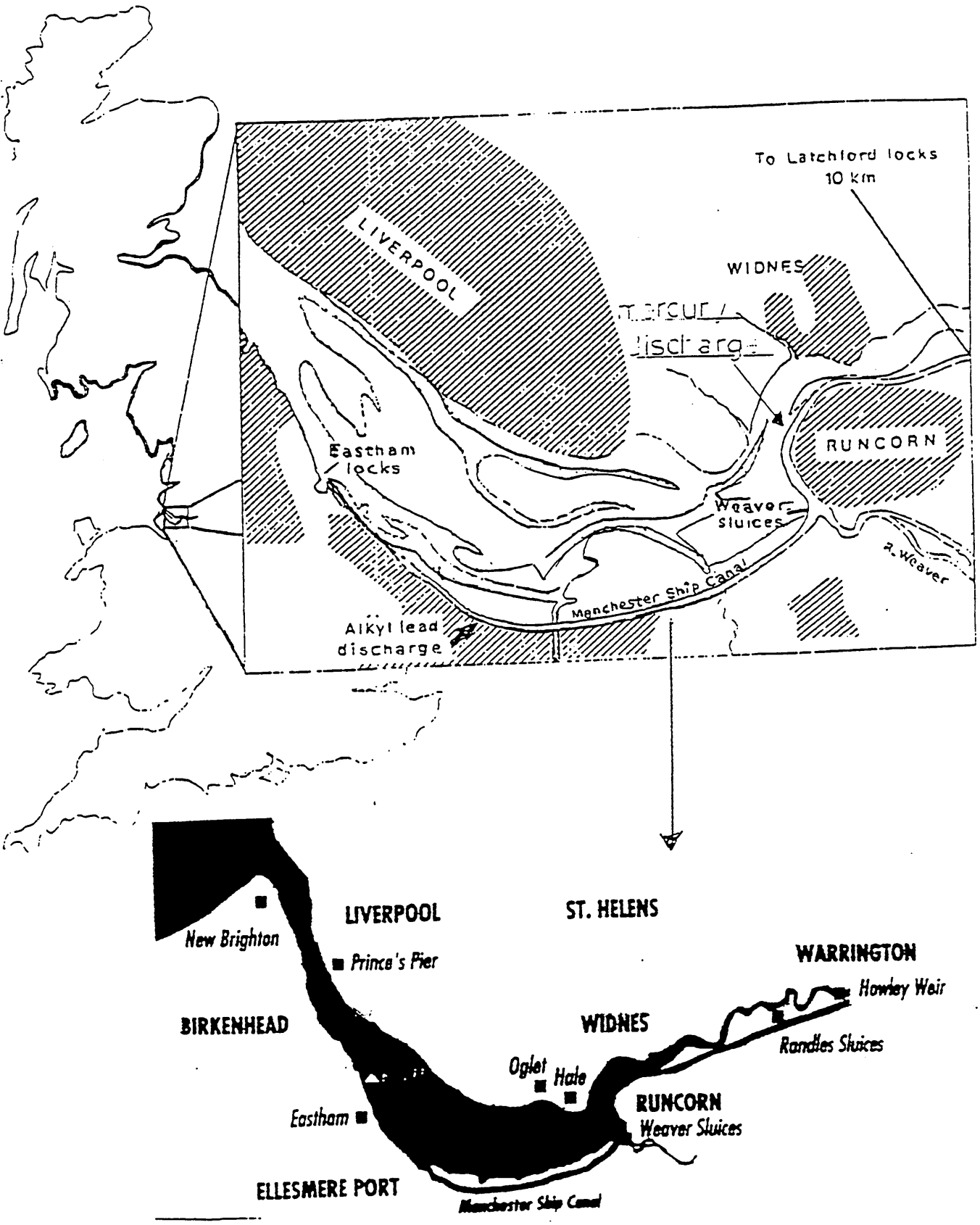


Figure 2.11.
Mersey Estuary and Manchester Ship Canal location map.

added and the tube shaken for 30 min. using an automatic shaking machine and centrifuged for 5 min. The organic phase was withdrawn using a glass pipette and placed in a screw-capped glass vial (2 ml capacity) ready for injection (automatic) to the GC-MIP-AES.

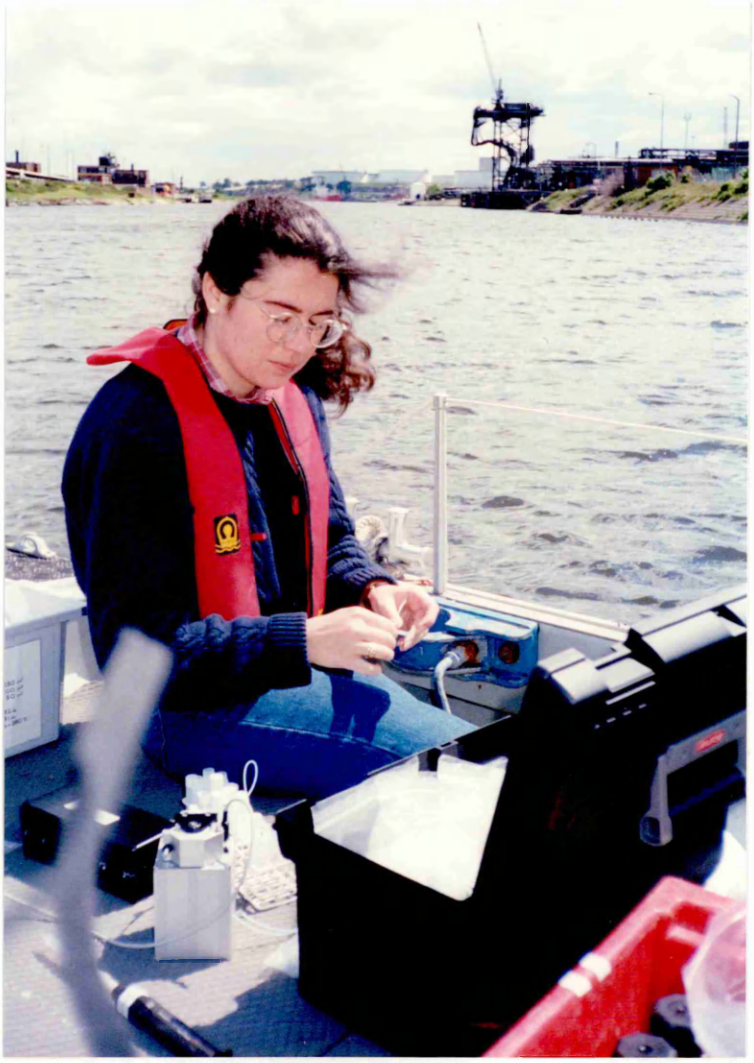


Figure 2.12.
Field sampling in the Manchester Ship Canal.

For the blank SCF microcolumns, the above procedure was repeated using SCF microcolumns made from the same batch of SCF and pretreated with the same batch of hydrochloric acid solution as the microcolumns used for sampling.

On the second cruise samples were also collected for total mercury data. Water samples were collected in a flask (100 ml capacity; 5 per station) which contained concentrated nitric acid (1 ml). On return to the laboratory the determination of total mercury was carried out using a standard pretreatment procedure for the oxidation of organomercury species into inorganic mercury [Wei and McLeod, 1992]. This involved adding hydrochloric acid (5 ml of 3 M) to a precleaned flask (100 ml) followed by the water sample which was added and made up to the mark. Finally, bromide/bromate solution (2.5 ml of 2.5% m/V KBr + 0.7% m/V $KBrO_3$) was added. The flask was left to stand for at least 1 hour to allow oxidation to proceed. Total mercury was determined by introducing the water sample into the FI-CV-AFS. For the blank test, the above procedure was repeated using flasks from the same batch and pretreated with the same batch of nitric acid, hydrochloric acid and bromide/bromate solution as the sampled flasks.

2.3. Results and Discussion.

Work reported in this section is concerned with applying and developing the methodology outlined in figure 2.13 to the speciation of mercury in waters. It can be seen that the scheme involves the use of a GC-MIP-AES for separation/quantitation of mercury species (methyl-, ethyl- and inorganic mercury) following on from various sample pretreatment stages. Pretreatments involve analyte deposition/elution on SCF microcolumns followed by derivatisation/extraction of mercury species. Experiments reported first are concerned with basic instrument performance followed by optimisation of the plasma emission signal. Thereafter method development is directed to derivatisation/extraction. Finally, basic performance characteristics and application to canal water is given.

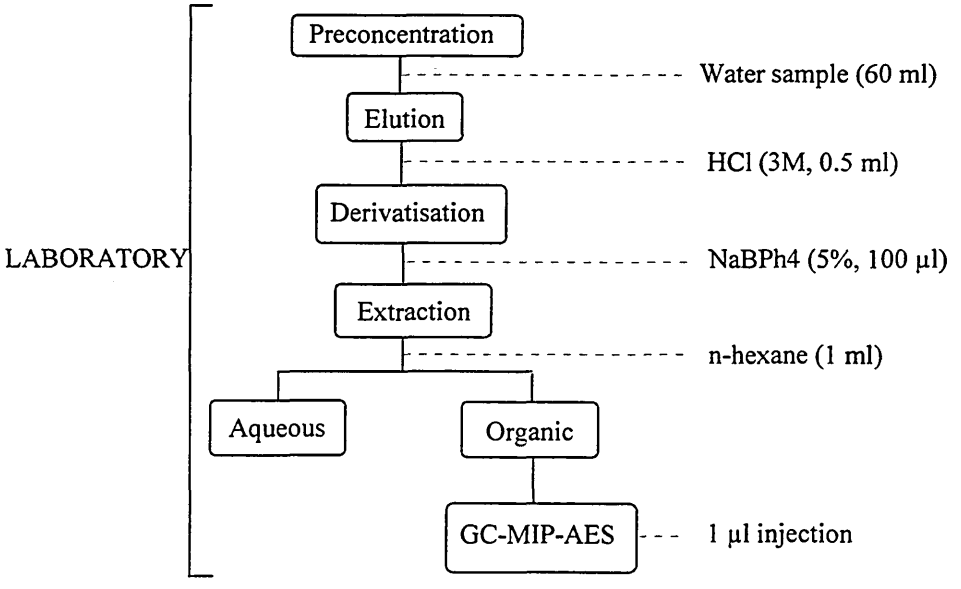


Figure 2.13.
Overview of the speciation scheme for mercury in natural waters.

In order to acquire the basic instrument response, a standard solution of aqueous inorganic mercury (1 mg l^{-1} as Hg, 2 ml) was subjected to sample processing (derivatisation/extraction, see page 60 in Experimental for full details) and then an aliquot of the extract was introduced to the screw-capped glass vial (2 ml capacity) for injection (automatic) to the GC-MIP-AES. Figure 2.14 shows the resultant emission (253.6 nm) versus time (min) response. The peak area or peak height of the transient signal is proportional to the amount of mercury in the sample. Besides quantitation potential, the retention time of 9.44 min is indicative of inorganic mercury species.

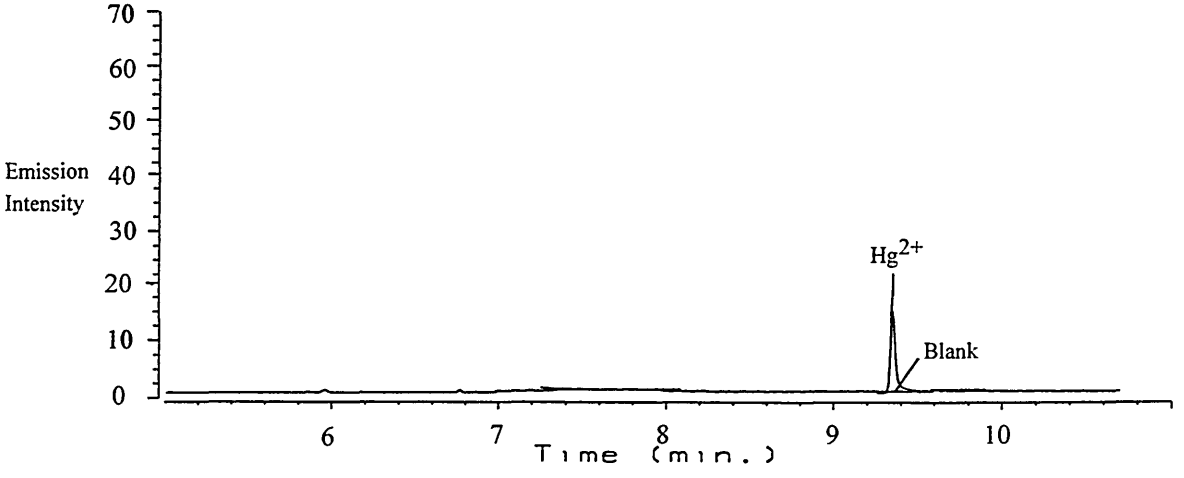


Figure 2.14.
A typical emission-time response (253.6 nm) for a single standard solution of inorganic mercury and distilled water (blank). Standard 1 mg l^{-1} as Hg, 2ml. Extraction, 1 ml n-hexane. Injection, 1 µl.

An important aspect of signal processing is that for each time slice the emission spectrum can be displayed. Thus the emission spectrum corresponding to the transient signal at retention time 9.44 min as shown in figure 2.15 confirms the presence of

mercury. If required instrument software also can provide a 3D plot of emission intensity versus wavelength versus time.

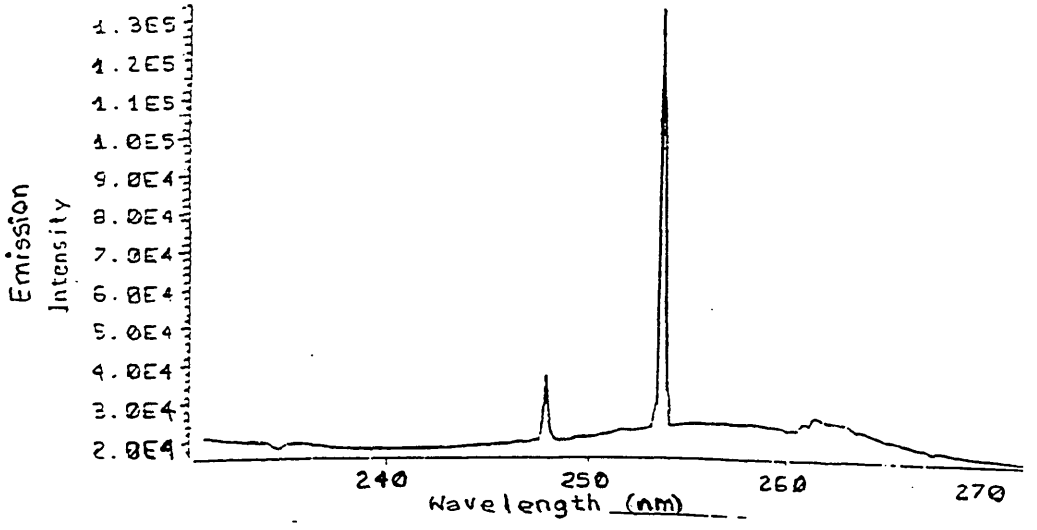


Figure 2.15.
Emission spectrum corresponding to the transient signal (GC-MIP-AES) at retention time 9.44 s.

Characterisation and optimisation of the GC-MIP-AES. McCormack et al., 1965 were the first to describe the use of a microwave induced plasma (MIP) for the spectroscopic detection of organic compounds eluted from a gas chromatographic column. They proposed that the disintegration of the organic compounds in the plasma produced free atoms (such as C, H, O, N, F, Cl, Br, I, S and P) that could be detected by characteristic atomic emission lines. Furthermore, the intensity of such atomic emission lines was linearly proportional to the quantity of the individual elements. Bache and Lisk, 1967 used helium as carrier gas instead of argon and obtained an increase in the emission intensity of elements under investigation (C, H, Cl, Br, I and S). This is due to the fact that the electron energy of the microwave plasma is a function of the ionisation potential of the plasma gas and helium has a higher ionisation potential (24.6 eV) than argon (15.8 eV) [Zhander and Hieftje, 1981]. However, the generation of a stable plasma using helium is only possible at reduced pressure with conventional resonant cavities, producing difficulties in coupling the GC and the MIP. Moreover with conventional

resonant cavities it was necessary to take special precautionary measures for generating the plasma and for maintaining it. For example, McLean et al., 1973 described two different compromise situations: the first one was related to the microwave power (working range 25 - 200 W). The use of high microwave power (>50 W) increased emission intensity for most elements but also increased the erosion of the discharge tube. However, lower microwave power (< 150 W) resulted in an unstable plasma. They also proposed a microwave frequency of 2450 MHz, instead of 8 MHz due to the obtention of higher emission intensities. The second one is related to the introduction of the sample into the MIP which is critical. Sample introduction tend to be restricted to gas samples (because the low power does not afford a high enough plasma energy to vaporize or evaporate solid or liquid samples, or to atomize the analytes species) at low flow rates (working range 20 - 700 ml min^{-1}). The use of high flow rates (> 300 ml min^{-1}) increases emission intensities for most elements but also increases the erosion of the discharge tube. However, for the analysis of trace elements lower flow rates (< 50 ml min^{-1}) would result in a decrease in sensitivity.

Different MIP cavities for interfacing with a GC have been designed in order to overcome these problems [Zhander and Hieftje, 1981]. One of the most useful cavities was proposed by Beenakker [Beenakker, 1977] which is a circular symmetric cylindrical hollow metal container. The discharge tube is mounted along the axis in the center of the resonant cavity where the microwave field is maximum. This design allows an end-on (axial) plasma viewing, gives a small plasma size and operates at atmospheric pressure with helium. Recently, Quimby and Sullivan, 1990 improved the Beenakker cavity shape and produced a new cavity termed the reentrant cavity (see figure 2.4a in Experimental). According to these workers a key experimental parameter in GC-MIP is

the makeup helium flow rate which is needed to maintain chromatographic peak shape. As already mentioned in the Experimental section the makeup helium (which contains reagent gases) is combined with the column effluent and goes to the plasma. It has been noted previously [Van Dalen et al., 1977; Quimby et al., 1978; Estes et al., 1981] that reducing the makeup helium flow rate (e.g. flow rates of 10 ml min^{-1}) can improve detection limits, especially for non-metals. Studies realised by Quimby and Sullivan, 1990 have demonstrated this effect with the present instrumentation for a number of elements within the wavelength range 174.2 nm (N) to 777.2 nm (O), including mercury (253.6 nm) which behaved as a non-metal due to its high volatility. This improvement is possible because the present cavity design prevents back diffusion of air into the plasma, permitting a stable plasma to be sustained at lower flows and because the solvent venting scheme provides minimal dead volume between the end of the column and the plasma.

In the present study optimisation of the makeup helium flow rate was carried out by studying the emission intensities for a fixed concentration of methyl-, ethyl- and inorganic mercury species (100 $\mu g\ l^{-1}$ as Hg, 2 ml). The effect of variation of makeup helium flow rate over the range 30 to 300 ml min^{-1} is shown in figure 2.16. It is seen that mercury emission signals were maximised at a relatively low flow rate of 30 ml min^{-1}. There is an increase in sensitivity of 4 fold for all mercury species (for example the limit of detection, as 3s, for methylmercury varied from 1.2 $\mu g\ l^{-1}$ to 0.3 $\mu g\ l^{-1}$ as Hg by using 30 ml min^{-1}). This is in agreement with the work reported for mercury by Quimby and Sullivan, 1990. At very high makeup flow rate the residence time of the mercury species in the plasma is short therefore there is insufficient time for efficient excitation, giving rise to a small signal. As the flow rate decreases the residence time

increases, giving rise to enhanced signals. However, the use of flow rates much below 30 ml min^{-1} is not recommended by the manufacturer because it would shorten the discharge tube life and might result in interaction between analyte and tube walls and give rise to memory effects.

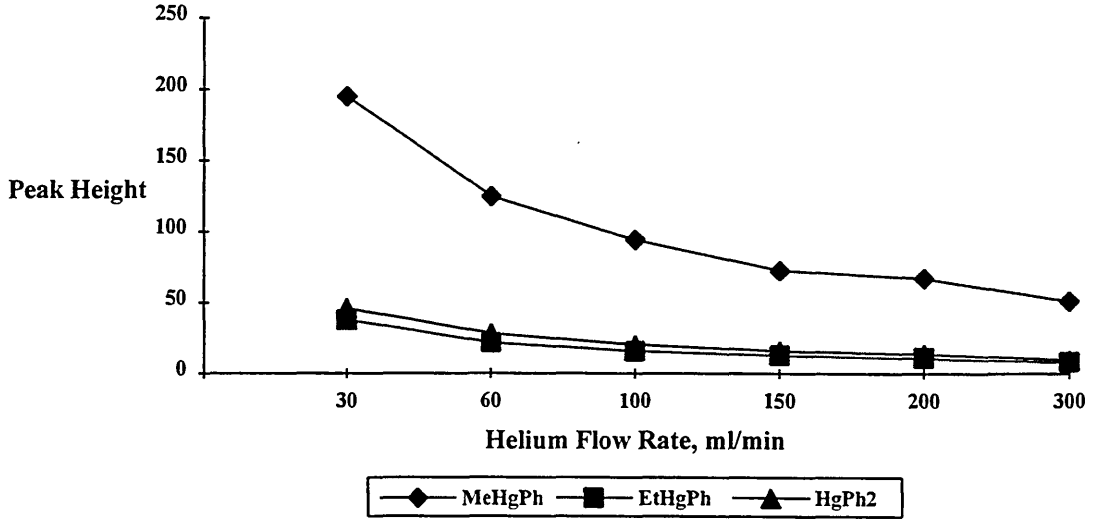


Figure 2.16.
Effect of makeup helium flow rate on signal intensities: mixed standard solutions were subjected to sample processing (derivatisation / extraction, see page 62 in Experimental for full detail) and then an aliquot of the extract was introduced to the screw-capped glass vial (2 ml capacity) for injection (automatic) to the GC-MIP-AES. Standard, 100 µl l^{-1} as Hg, 2 ml. Extraction, 1 ml n-hexane. Injection, 1 µl.

Other considerations in developing and optimising GC-MIP methodology are related to the chromatographic characteristics of the compounds. Earlier investigations [Luckow and Rüssel, 1977; Luckow and Rüssel, 1978; Bulska et al., 1991] indicated that the chromatographic characteristics of organomercury halides (such as methyl- and ethylmercury chloride) is difficult using packed or capillary columns because of their poor chromatographic characteristics (severe tailing, decomposition, low column efficiencies). This is due to the fact that in these compounds the mercury-halide bond is

of very high polar character and may interact strongly with the column, leading to severe tailing and apparent decomposition. These problems can be temporarily alleviated by column passivation [Bulska et al., 1991] prior to analytical measurements using a concentrated solution of mercury (II) chloride in benzene (later replaced with toluene due to its high toxicity). Unfortunately the passivation procedure must be repeated frequently because the column efficiency deteriorates with time. Actually, the use of capillary columns (which have shown a better performance on account of their more inert character and high efficiencies) in combination with derivatisation (which is treated in detail in the next section and can be explained as the formation of non-polar dialkyl derivatives which exhibit superior chromatographic characteristics) have been found to be the most effective way of performing chromatographic separation of organomercury halides.

In the present work the effect of varying the helium flow rate through the GC column was considered an interesting parameter for study because mercury adsorption/memory onto the GC column could influence chromatographic peak shape. The helium flow rate through the GC column was therefore varied from 0.5 to 2.5 ml min^{-1} and resultant responses are shown in figure 2.17. It is seen that mercury emission signals were maximised at a relatively high flow rate of 2.5 ml min^{-1} and, furthermore, a decrease of the tailing of mercury peaks was found (5%).

In figure 2.18 it can also be seen that for increasing flow rate, there is a decrease in retention times (from 9.90 to 6.98 min for methyl-, from 10.44 to 7.58 min for ethyl- and from 12.37 to 9.44 min for inorganic mercury) due to a more rapid transit of analyte species through the column.

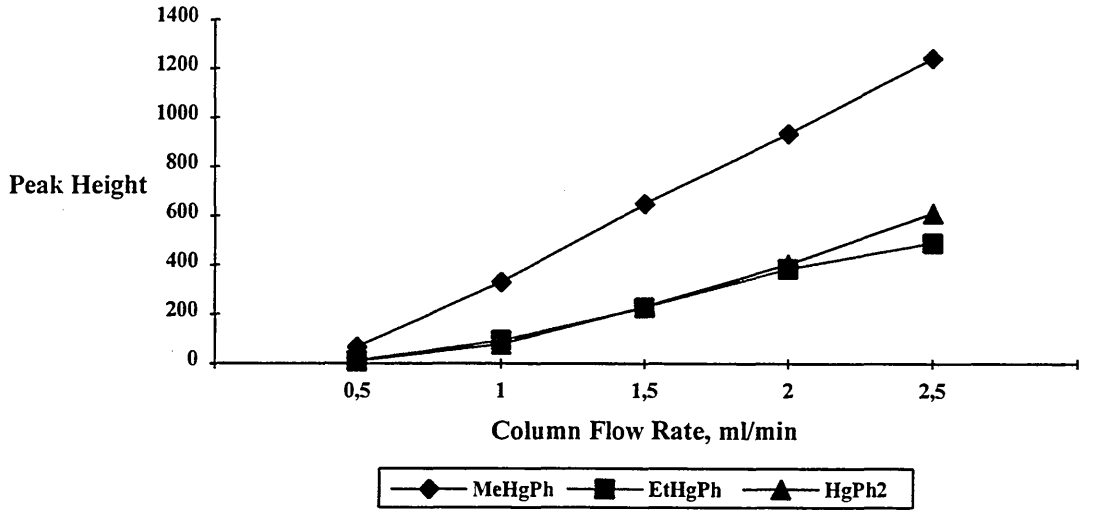


Figure 2.17.
Effect of column flow rate on signal intensities: mixed standard solutions were subjected to sample processing (derivatisation / extraction, see page 61 in Experimental for full detail) and then an aliquot of the extract was introduced to the screw-capped glass vial (2 ml capacity) for injection (automatic) to the GC-MIP-AES. Standard, 100 µl l^{-1} as Hg, 2 ml). Extraction, 1 ml n-hexane. Injection, 1 µl.

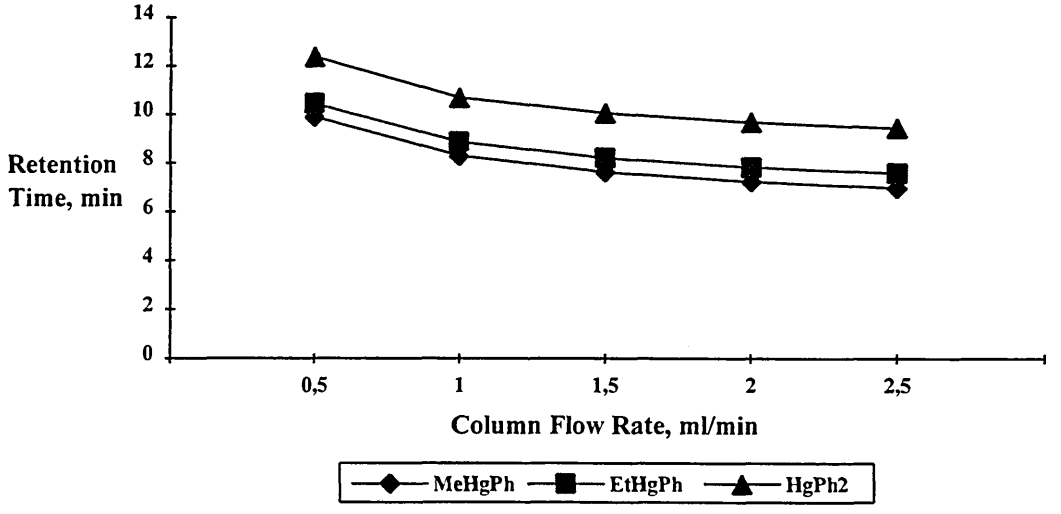


Figure 2.18.
Effect of column flow rate on retention time: mixed standard solutions were subjected to sample processing (derivatisation / extraction, see page 61 in Experimental for full detail) and then an aliquot of the extract was introduced to the screw-capped glass vial (2 ml capacity) for injection (automatic) to the GC-MIP-AES. Standard, 100 µl l^{-1} as Hg, 2 ml). Extraction, 1 ml n-hexane. Injection, 1 µl.

Sample Pretreatment: Derivatisation, Extraction and Preconcentration.

Derivatisation. Prior to analysis by GC-MIP mercury species are converted to a form amenable to GC by derivatisation and separated from the sample matrix normally by liquid-liquid extraction. In the present study derivatisation and extraction of mercury species are performed simultaneously by adding the derivatising agent and the n-hexane simultaneously in the aqueous solution containing the mercury species.

With most separation methods for organic and inorganic mercury speciation based on gas chromatography, the compounds are preferentially converted to alkylated mercury to facilitate chromatography and separation from the matrix. This chemical reaction, usually called derivatisation must preserve the chemical identity of the mercury species initially present in the sample. Two main derivatisation tendencies are currently used to produce volatile organomercury species. The first one is based on Grignard reaction by derivatisation with propylmagnesium chloride in tetrahydrofuran or n-butylmagnesium chloride in tetrahydrofuran [Bulska et al., 1991; Emteborg et al.,1993] as substitute and is performed in solvent media. The second one is based on ethylation and is mostly performed in aqueous media [Fishers et al., 1991; Filipelli et al., 1992].

For derivatisation reactions with a Grignard reagent the organo and inorganic mercury species are extracted from the matrix into an organic solvent which is usually done with complexing agents like tropolone or dithiocarbamate. The alkylmercury ions ($R_xHg^{(2-x)+}$) are then converted in the solvent to volatile mixed alkylmercury ($R_xHgR'_{(2-x)}$) after reaction with a Grignard reagent (R'MgX) according to the following:

$$R_xHg^{(2-x)+} + R'MgX \xrightarrow[H^+]{\text{solvent}} R_xHgR'_{(2-x)}$$

inorganic mercury x = 0

organomercury x = 1 R = Me, Et R′= Pro-, n-Bu

After the derivatisation, the organic phase is usually separated from the aqueous phase, dried with a drying agent and reduced to a smaller volume by evaporation of the solvent to preconcentrate the derivatised analytes. Extraction of the analytes in a solvent followed by Grignard derivatisation minimises interference reactions associated in the liquid phase. However, it is a complicated process, which involves many sample pretreatment steps requiring many manipulations of small amounts of analytes leading to potential analyte loss or contamination.

The introduction of sodium tetraethylborate ($NaB(CH_3CH_2)_4$) as a derivatising reagent by reaction with inorganic and organic analytes in water is simpler than the approach above. It was first introduced for the determination of methyl- and inorganic lead [Rapsomanikis et al., 1986] and methyl- and inorganic mercury [Rapsomanikis and Craig, 1991] species. The ethylated organo and inorganic mercury species are generated according to the following reaction:

$$R_xHg^{(2-x)+} + (2-x)\,NaBEt_4 \xrightarrow{H^+} R_xHgEt_{(2-x)} + (2-x)\,Na^+ + (2-x)\,BEt_3$$

inorganic mercury x = 0

organomercury x = 1 R = Me, Et

It has to be noted, however, that with direct ethylation it is not possible to differentiate the inorganic from the ethylated forms. This problem can be, partially overcome using

sodium tetraphenylborate ($NaB(C_6H_5)_4$) instead of sodium tetraethylborate for derivatisation. In the present study, derivatisation of mercury species is based on the work of Lückord and Russell, 1978 who utilised sodium tetraphenylborate for phenylation of inorganic mercury. As a result phenylation may be applicable to other mercury species, such as methyl- or ethylmercury according to the following reaction:

$$R_xHg^{(2-x)+} + (2\text{-}x)\,NaBPh_4 \xrightarrow{H^+} R_xHgPh_{(2-x)} + (2\text{-}x)\,Na^+ + (2\text{-}x)\,BEt_3$$

inorganic mercury	x = 0	
organomercury	x = 1	R = Me, Et

In order to perform the derivatisation reaction, a mixed standard solution of aqueous methyl-, ethyl- and inorganic mercury (10 μg l^{-1} as Hg, 2 ml) was subjected to sample processing (derivatisation and extraction see page 61 in Experimental for full details) and then an aliquot of the extract was introduced to the screw-capped glass vial (2 ml capacity) ready for injection (automatic) to the GC-MIP-AES. Figure 2.19 shows the resultant GC-MIP-AES response confirming the presence of the three derivatised mercury species. Attempts to optimise the phenylation yield were based on studies of solution parameters i.e. pH and hydrochloric acid concentration.

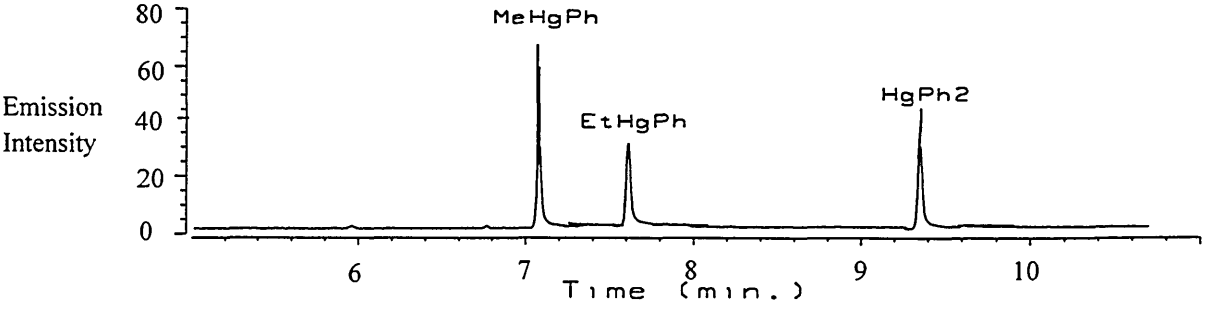


Figure 2.19.
GC-MIP-AES response for a mixed standard solution containing methyl-, ethyl- and inorganic mercury. Standard, 10 μl l^{-1} as Hg, 2 ml. Extraction, 1 ml n-hexane. Injection, 1 μl.

Solution pH. Optimal pH values according to the literature for phenylation [Luckow and Rüssel, 1978] range from pH 2 to 3. For this experiment the pH values of a mixed standard solution of methyl-, ethyl- and inorganic mercury (100 μg l^{-1}, as Hg) were adjusted to 1.5, 2, 2.5, 3, 4, 5, 6, 7 and 10 with hydrochloric acid / sodium acetate / sodium hydroxide. After sample processing (derivatisation and extraction see page 61 in Experimental for full details) an aliquot of the extract was introduced to a screw-capped glass vial (2 ml) ready for injection (automatic) into the GC-MIP-AES. The results are shown in figure 2.20. As can be seen, there is similar results for all mercury species which is a decrease in analyte yield with increase of pH and at very low values. The yield of the phenylation reaction may be diminished at low pH because of a rapid destruction of the phenylating reagent by H^+ [Luckow and Rüssel, 1978]. The low pH values could accelerate the degradation of $NaBPh_4$ to BH_3 according to the following mechanism:

$$NaBPh_4 + H^+ \longrightarrow BPh_3 + PhH + Na^+$$

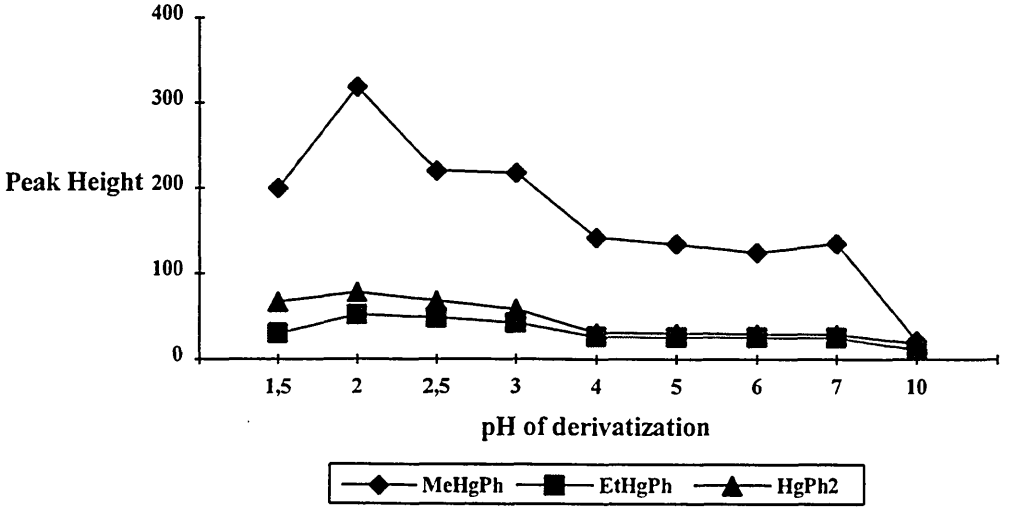


Figure 2.20.
Influence of the pH of derivatisation on signal response. Mixed standard solution, 100 μl l^{-1} as Hg, 2 ml). Extraction, 1 ml n-hexane. Injection, 1 μl.

The pH value of 2 may ensure good reagent stability and facilitate the transfer of phenyl groups from $NaBPh_4$. In the case of neutral or basic pH the loss in yield could be attributed to the inhibition of the phenylation reaction by formation of unreactive multihydroxyl organometallic anions [Bloom, 1989].

Effect of hydrochloric acid concentration. It is necessary to test whether high concentrations of hydrochloric acid affect signal response. This is due to the fact that samples after processing using SCF microcolumns, as shown in figure 2.11, are contained in hydrochloric acid solution. For this experiment, mixed standard solutions of methyl-, ethyl- and inorganic mercury (150 $\mu g\ l^{-1}$ as Hg, 1 ml) were prepared in differing concentrations of hydrochloric acid (0, 0.1, 0.5, 1, 1.5 and 3 M).

After sample processing (derivatisation and extraction see page 62 in Experimental for full details) an aliquot of the extract was introduced to a screw-capped glass vials (2 ml) ready for injection (automatic) to the GC-MIP-AES. The results are shown in figure 2.21.

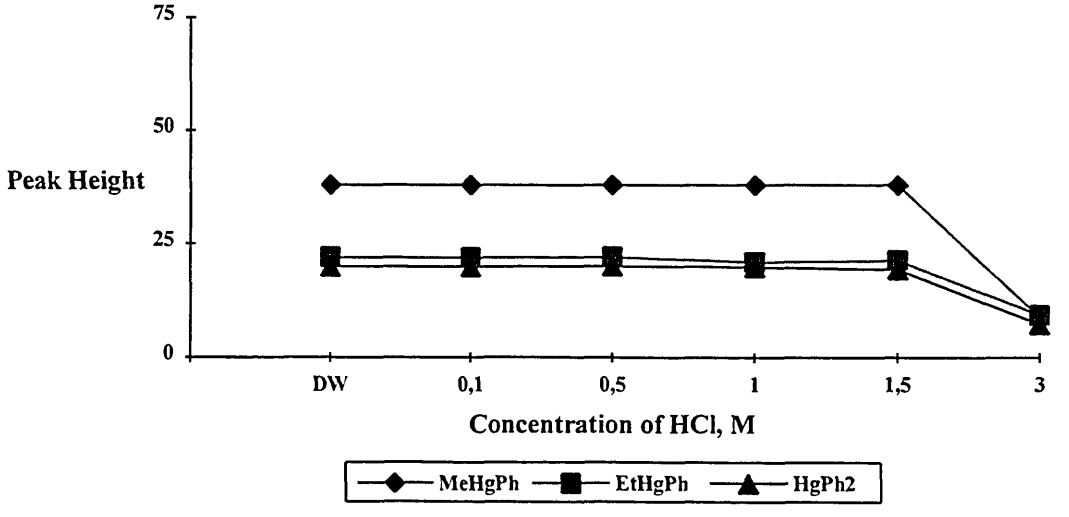


Figure 2.21.
Effect of hydrochloric concentration on signal intensities. Mixed standard solution (150 $\mu g\ l^{-1}$ as Hg, 2 ml). Extraction, 1 ml n-hexane. Injection, 1 μl.

As can be seen, there is a relatively constant response for all mercury species at different concentrations except at 3 M. At this concentration of hydrochloric acid there is a substantial decrease in response for all mercury species (methyl-, ethyl- and inorganic mercury) due to the instability of the reagent (sodium tetraphenylborate) at very acid pH, as explained above. As a result, eluate from the SCF microcolumn (0.5 ml, 3 M HCl) was therefore diluted with 1 ml of high purity water before derivatisation / extraction in order to obtain a final acid strength of about 1 M (for which we obtain the same signal as for distilled water).

Extraction. In order to eliminate matrix interferences prior to GC analysis two different approaches have been used to separate mercury species from the sample matrix. The first one is the purge and trap technique as proposed by Rapsomanikis et al., 1986. The purge and trap technique is based on the trapping of the volatile derivatives in an U-trap chromatographic column which is immersed in liquid nitrogen. Then, the trap is electrically warmed and the species are separated on the basis of their boiling points and their chromatographic properties. However, the most usual method for elimination of matrix interferences is liquid-liquid extraction in which the phases (aqueous and organic) are immiscible and are brought into contact by manual or automatic shaking. A time of 0.5 - 5 min is usually sufficient to ensure equilibrium is reached. For the extraction step the use of toluene or n-hexane is recommended rather than the toxic benzene. In this work n-hexane was selected due to its lower boiling point which allows the use of a lower initial oven temperature (40°C). However, the use of toluene is recommended when using a Grignard reaction because the Grignard reagent solvent (tetrahydrofuran) is more soluble in toluene than in n-hexane [Bulska et al., 1991].

In this work in order to assess the number of extractions needed to transfer all mercury species from the aqueous to the organic phase (extraction efficiency) a mixed standard solution (10 μg l^{-1} as Hg, 2 ml) was subjected to three consecutive extractions with n-hexane (1 ml). For the three consecutive extractions, the organic phase was withdrawn using a glass pipette and placed in three different screw-capped glass vials (2 ml) ready for injection (automatic) to the GC-MIP. It was found that on injection of the extracts (consecutively) most of the mercury species were contained in the first extraction, as shown in figure 2.22.

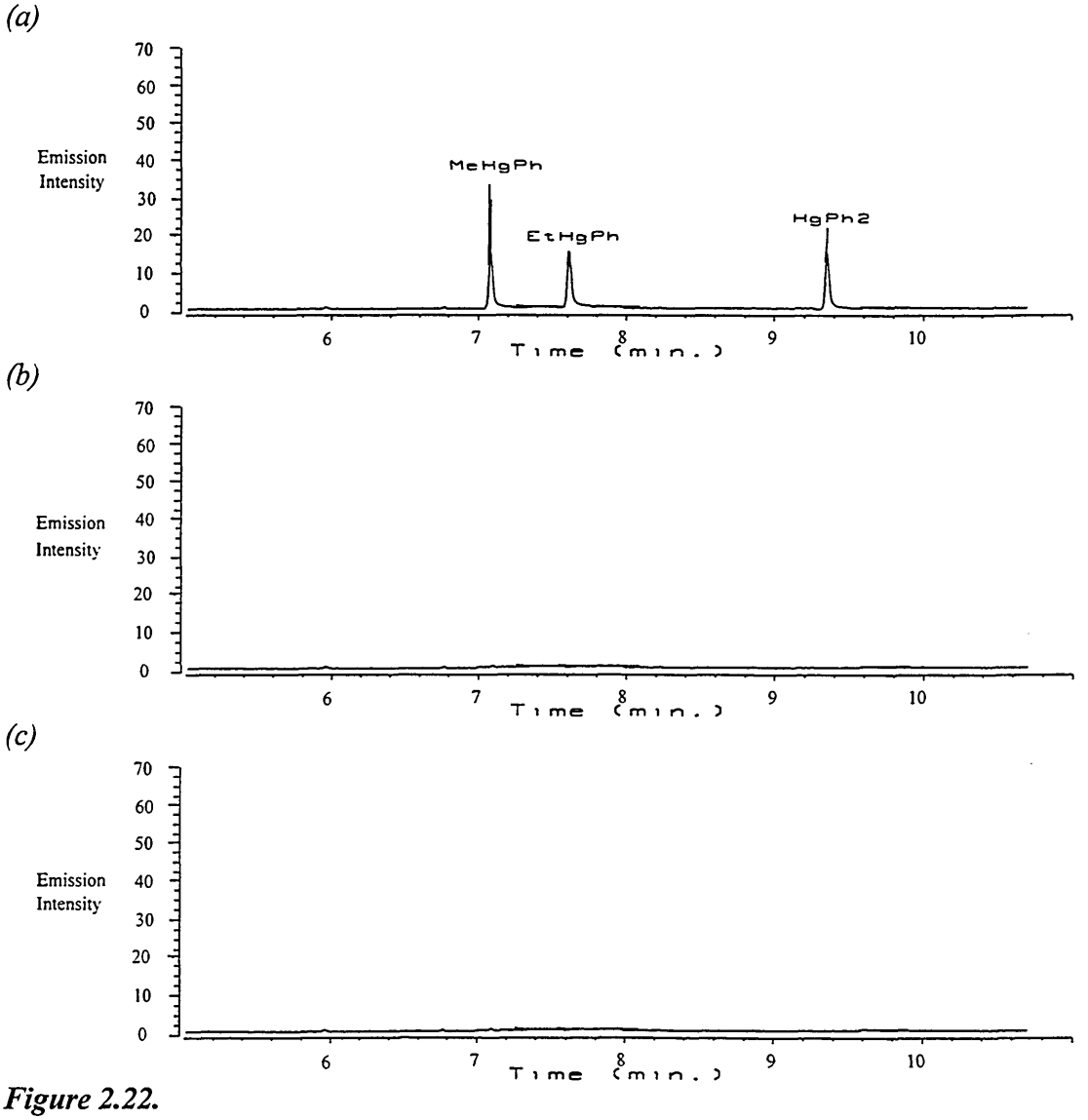


Figure 2.22.
Emission-time response corresponding to: (a) first, (b) second and (c) third extraction. Standard, 10 μl l^{-1} as Hg, 2 ml). Elution volume, 0.5 ml. Extraction, 1 ml n-hexane. Injection, 1 μl.

Recovery values for a single extraction were calculated by subtracting the amount of mercury presented in the second extraction (which correspond to 3 - 5% of the total signal presented in the first extraction) and were 97% for methyl-, ethyl- and 95% for inorganic mercury. Hence only a single extraction step was utilised in further work.

Preconcentration. GC-MIP-AES has been proposed as a very sensitive detection technique for mercury enabling levels of 0.1 pg as Hg to be detected [Quimby and Sullivan,1990]. This value corresponds to absolute detection limits when highly efficient capillary columns are used. However, they have a limited sample capacity. Only 1 µl of the analyte solution may be injected without signal distortion reducing the possibility of a straightforward ultratrace analysis of water samples and creating a need for more efficient sample preparation procedures. Two different preconcentration procedures has been used for mercury. The first one used cryogenic trapping for preconcentration of methyl- and inorganic mercury prior analysis by GC-AAS [Rapsomanikis et al., 1986]. Cryofocussing allows preconcentration factors of 50 to 100 fold and is based on the trapping of the volatile derivatives in an U-trap chromatographic column which is immersed in liquid nitrogen as mentioned already. However, this method is not applicable when complex matrices (e.g. biological tissues or sediments) have to be analysed. Alternatively, adsorption of the mercury species onto ion exchange resins is a convenient way to perform species preconcentration. Moreover, in combination with flow injection (FI), this approach would seen to offer considerable potential due to the ability to undertaken on line sample manipulation [Olsen et al., 1983]. The time scale for such pretreatments is short and this is attractive in terms of conserving the natural speciation state of the analyte. Different materials such as dithiocarbamate resin [Bulska et al., 1991; Emteborg et al., 1993] and sulphydryl cotton

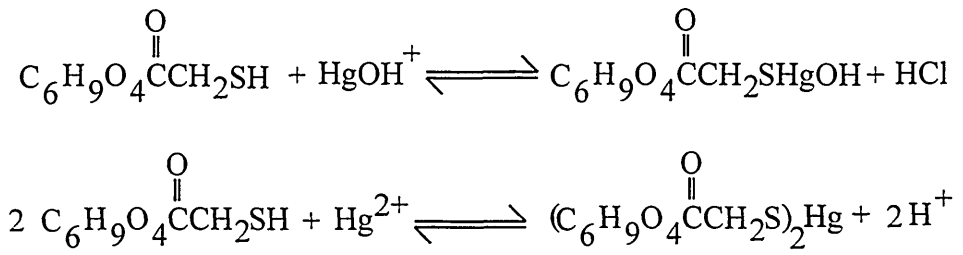
fibre (SCF) [Lee, 1987; Lee and Mowner, 1989] have been utilised for the preconcentration of mercury in natural waters. Detection limits of 0.05 ng l^{-1} for methyl-, ethyl- and 0.15 ng l^{-1} for inorganic mercury (500 ml sample processed) [Emteborg et al., 1993] and of 0.05 ng l^{-1} for methylmercury (4 l sample processed) [Lee and Mowner, 1989] have been achieved, respectively.

In this work preconcentration of mercury species has been carried out using a microcolumn of SCF in a FI system as follows: water sample containing methyl-, ethyl- and inorganic mercury was introduced into the SCF microcolumn, which is incorporated in a FI system (described earlier in Experimental). Mercury species were deposited on the SCF microcolumn with passage of the matrix to waste. Next, there is a complete release of the analytes from the microcolumn using an eluent (HCl 3 M). Deposition / elution of methyl- or ethylmercury at pH 3.5 is carried out according to the following reaction (where R = methyl- or ethyl-):

$$C_6H_9O_4\overset{O}{\overset{\|}{C}}CH_2SH + RHgCl \rightleftharpoons C_6H_9O_4\overset{O}{\overset{\|}{C}}CH_2SHgR + HCl$$

At acidic pH methyl- and ethylmercury tend to ionise into RHg^+ and Cl^- due to the considerable ionic character of the Hg-Cl bond and deposit on the SCF microcolumn. At higher pH the ionisation to RHg^+ and Cl^- is not as pronounced as in acidic medium and the uptake of methyl- and ethylmercury on the SCF microcolumn decreases.

Inorganic mercury at pH 3.5 appears as $HgOH^+$ and Hg^{2+} and is deposited / eluted on the SCF microcolumn according to the following reactions:



At basic pH, $Hg(OH)_2$ is produced and inorganic mercury is not deposited on the SCF microcolumn.

In order to study the on-line enrichment of mercury species on SCF microcolumns, a mixed standard solution containing methyl-, ethyl- and inorganic mercury (120 $\mu g\ l^{-1}$ as Hg, 0.5 ml) was passed through a SCF microcolumn. The effluent collected during the deposition stage was subjected to sample processing (derivatisation / extraction, see page 62 in Experimental for full details) and then an aliquot of the extract was introduced to the screw-capped glass vial (2 ml capacity) ready for injection (automatic) to the GC-MIP-AES. The response for the analysis of the effluent collected during the SCF microcolumn deposition stage is given in figure 2.23a. As can be seen there is no mercury response indicating an effective deposition for the three different mercury species. The accompanying figures (figure 2.23b,c) refer to processing of eluate. Retained mercury species were then eluted with HCl (3 M, 0.5 ml, elution flow rate, 1 ml min^{-1}). In the first elution, the eluate (0.5 ml) was passed through the SCF microcolumn, collected in an empty glass vial (5 ml capacity) and subjected to sample processing (derivatisation / extraction see page 62 in Experimental for full details) before injected into the GC-MIP-AES. The same procedure was repeated for the second elution. The responses for processing of eluates is given in figure 2.23b,c. As can be seen mercury species corresponding to the first elution are detected (figure 2.23b) whereas there is essentially no response for the second elution (figure 2.21c). This

finding indicated that a single injection of hydrochloric acid was effective for elution and recovery of the three mercury species.

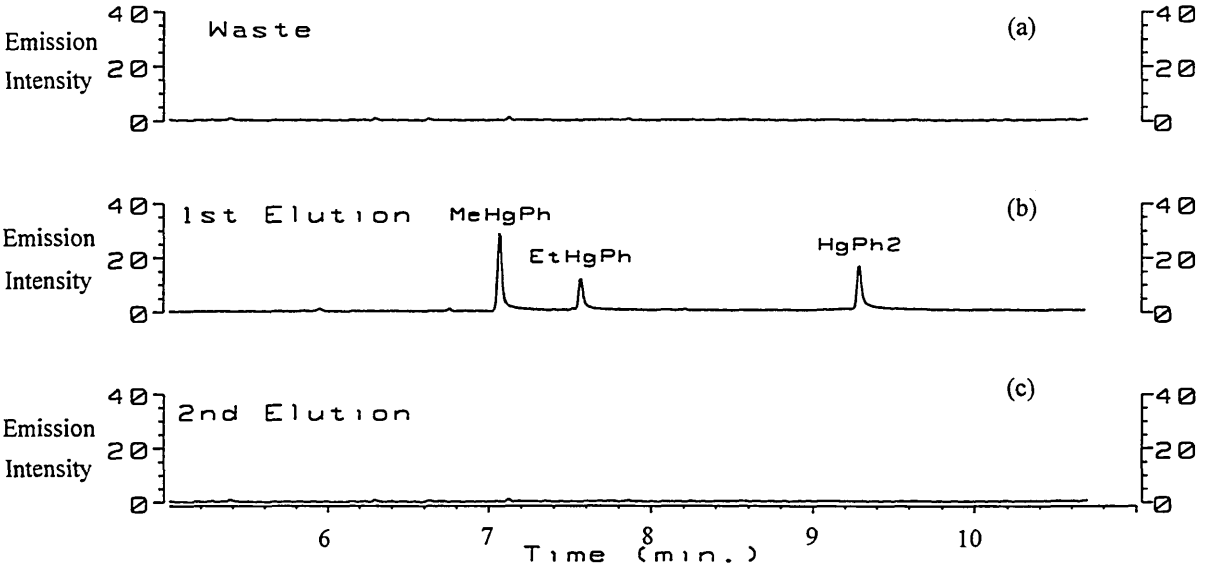


Figure 2.23.
Typical emission-time responses for: (a) effluent collected during SCF microcolumn deposition stage, (b) eluate corresponding to the first elution from the SCF microcolumn and (c) eluate corresponding to the second elution from the SCF microcolumn. Mixed standard solution, 120 μg l^{-1}as Hg , 0.5 ml. Extraction, 1 ml n-hexane. Injection, 1 μl.

Recovery values for the first elution were calculated by comparing the amount of mercury detected for the first elution with the response for direct injection of a mixed standard solution containing 120 μg l^{-1} as Hg. The recovery values were 98% for methyl-, 97% for ethyl- and 96% for inorganic mercury. Hence only a single elution step was utilised in further work.

Analytical Performance. The previous sections have been concerned with optimising the sample pretreatment stage and the basic instrument response. In order to demonstrate the value of the new methodology in natural water analysis basic

performance data, such as preconcentration factor, linearity of response, limits of detection, precision and accuracy are next discussed.

Preconcentration factor. Based on considerations of detection capability of the GC-MIP-AES (limit of detection for mercury is 300 ng l^{-1} as Hg) and concentrations of mercury species in natural waters (10 - 100 ng l^{-1} as Hg) then substantial preconcentration factors of about 400 are required in sample pretreatment. Therefore, studies based on processing sample volumes of 20 - 200 ml were performed. A mixed standard solution (1 μg l^{-1} as Hg of methyl-, ethyl- and inorganic mercury) was processed in the FI system using SCF microcolumns. Then, the retained mercury species were eluted by injection of hydrochloric acid (3 M, 0.5 ml) and subjected to sample pretreatment before injection (automatic) into the GC-MIP-AES. Figure 2.24 shows the improvement in method sensitivity for processing increasing volumes (20 - 200 ml, n = 3) of a mixed standard solution containing methyl-, ethyl- and inorganic (1 μg l^{-1} as Hg).

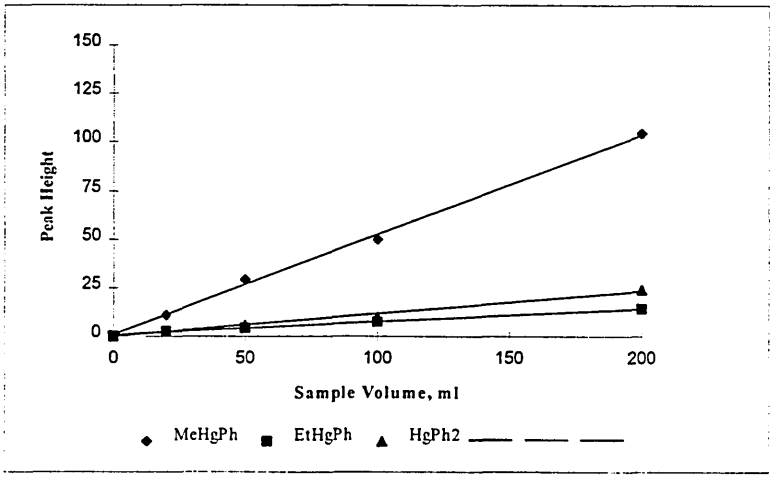


Figure 2.24.
Effect of sample volume on signal intensities for a mixed standard solution (1 μg l^{-1} as Hg of methyl-, ethyl- and inorganic mercury). Extraction, 1 ml n-hexane. Injection, 1 μl

In the case of a 20 ml sample, processing took about 5 min and resulted in a nominal enrichment factor of ca. 40 (eluent, 0.5 ml) for mercury species. For a 200 ml sample processing took about 50 min and a nominal enrichment factor of 400 (eluent, 0.5 ml) resulted. Typical emission-time responses for 20 and 200 ml are also presented in figure 2.25 a,b respectively.

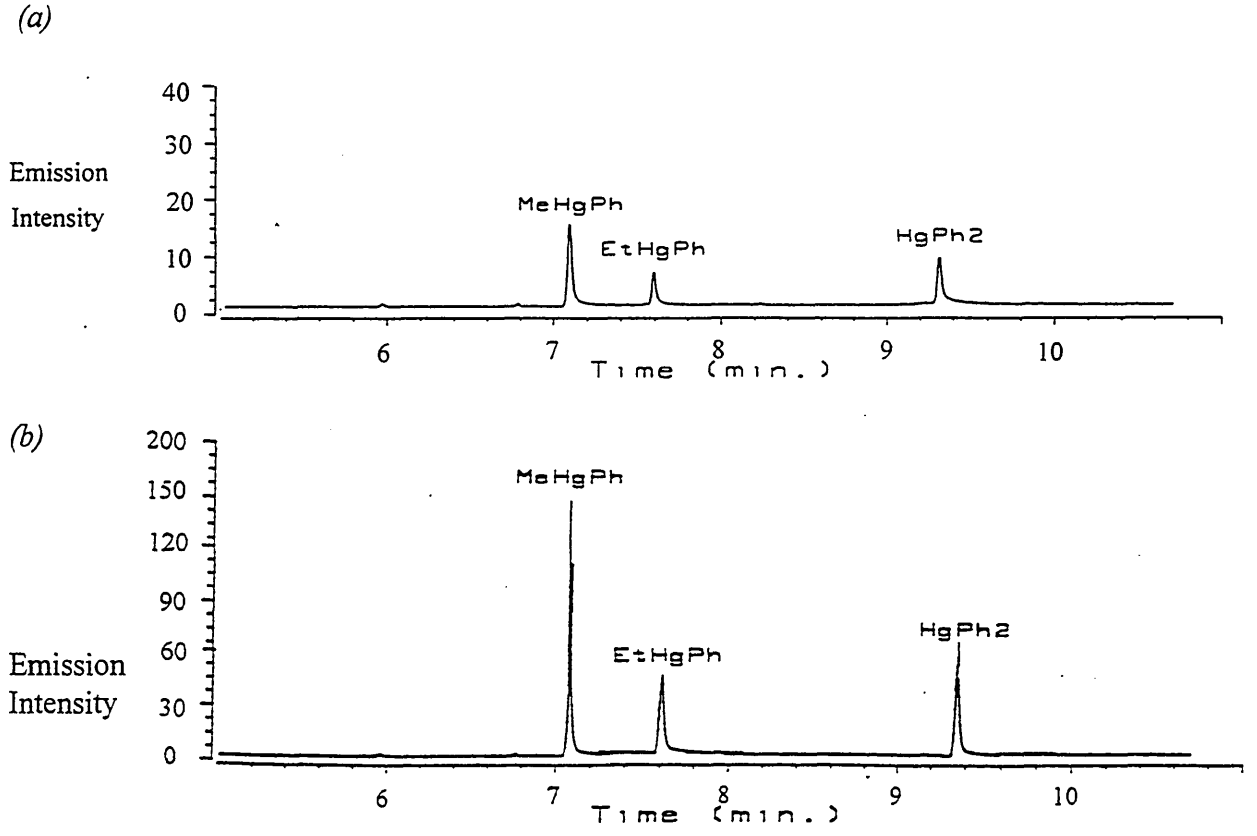


Figure 2.25.
Typical emission-time responses for processing (a) 20 ml and (b) 200 ml of a mixed standard solution (1 μg l^{-1} as Hg of methyl-, ethyl- and inorganic mercury). Extraction, 1 ml n-hexane. Injection, 1 μl.

The previous studies refer to processing of simple aqueous standard solutions. The same experiment was carried out by for a water from the Manchester Ship Canal. This experiment is necessary since the presence of co-existing cations (such as Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}) and anions (Cl^{-}, SO_4^{2-}) might interfere with the uptake / deposition process for the mercury species. The cations could effect the deposition process by competing with

mercury species for the SCF site. Also the presence anions could effect the deposition process by complexing with mercury species such that they are not retained on SCF sites. One way to study the possibilities of such interferences is to calculate gradients of the response curves for standard solutions and canal water. If the gradients are the same the conclusion is that there is the same sensitivity, but if there is a lower gradient for canal water thus would suggest that the uptake / deposition process was subject to interference.

Increasing values of standard solution (20 - 200 ml, n = 3) and spiked canal water (1 μg l^{-1} as Hg of methyl-, ethyl- and inorganic mercury) were therefore introduced into different SCF microcolumns (n=3). Then, the retained mercury species were eluted by injection of hydrochloric acid (3 M, 0.5 ml) and subjected to sample pretreatment before injection (automatic) into the GC-MIP-AES. Figure 2.26 shows the improvement in method sensitivity for processing increasing volumes (20 - 200 ml) of the spiked canal water.

The gradients for the response curves for the standard solution (obtained from figure 2.24) and for the spiked water from the Manchester Ship Canal (obtained from figure 2.26) for all mercury species (methyl-, ethyl- and inorganic mercury) are shown in table 2.5. By comparing gradients it is seen that similar values were obtained for methyl- and inorganic mercury species (0.524 and 0.561 for methyl-, and 0.117 and 0.103 for inorganic mercury) suggesting that preconcentration of such analytes on SCF microcolumns does not suffer interferences when applied to a real water sample. In the case of ethylmercury a reduced gradient was realised for the spiked water samples (0.068 and 0.046) suggesting an interference effect/reduced recovery. To date it has not

been possible to clarify why there is a reduced recovery in the case of ethylmercury (but not with methylmercury) but given that ethylmercury species are not detected in the canal water the effect is more academic than practical interest.

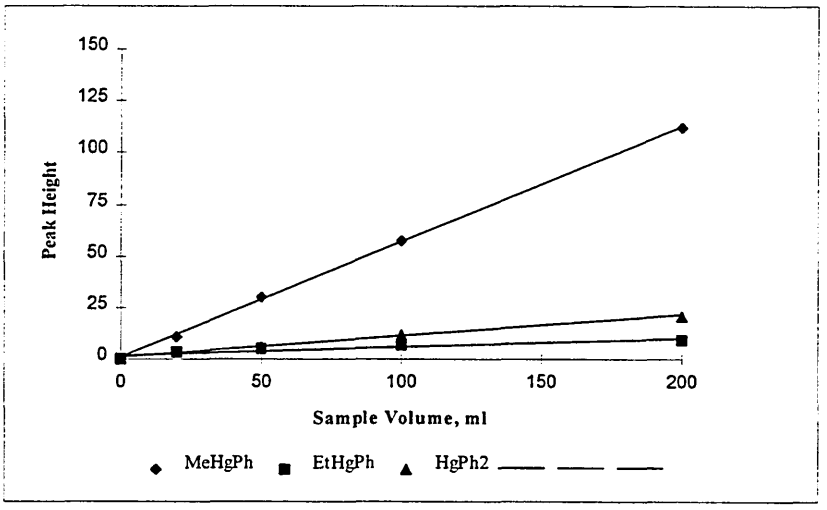


Figure 2.26.
Effect of sample volume on signal intensities for spiked water from the Manchester Ship canal (1 μg l^{-1} as Hg of methyl-, ethyl- and inorganic mercury, 0.5 ml). Extraction, 1 ml n-hexane. Injection, 1 μl.

Mercury species	Standard solution	Spiked canal water
Methyl-	y = 0.524x + 0.546; r = 0.9989	y = 0.561x + 0.567; r = 0.9998
Ethyl-	y = 0.068x + 0.663; r = 0.9796	y = 0.046x + 1.728; r = 0.9388
Inorganic	y = 0.117x - 0.271; r = 0.9969	y = 0.103x + 0.642; r = 0.9986

Table 2.5.
Calibration data for the standard solution and for spiked water from the Manchester Ship Canal for methyl-, ethyl- and inorganic mercury.

Calibration and limit of detection. To study signal as a function of concentration, different mixed standard solutions containing methyl-, ethyl- and inorganic mercury were prepared over the concentration range of 0, 0.1, 0.2, 0.5, 1, 5 and 10 μg l^{-1} as Hg. The different standard solutions were then processed (60 ml sample volume) in the FI system using different SCF microcolumns (n = 3). Then, the retained mercury species

were eluted by injection of hydrochloric acid (3 M, 0.5 ml) and subjected to sample pretreatment before injection (automatic) into the GC-MIP-AES. The calibration graphs are shown in figure 2.27 and were linear (r = 0.998 for methyl-, r = 0.997 for ethyl- and 0.997 for inorganic mercury) over the concentration range examined. The limits of detection for mercury species were calculated as 3σ, where σ is the standard deviation of the blank. Limits of detection for mercury species (methyl-, ethyl- and inorganic mercury) were based on passing 60 ml of dilute hydrochloric acid (0.01 M) through 10 different SCF microcolumns (n = 10). Then, the SCF microcolumns were eluted by injection of hydrochloric acid (3 M, 0.5 ml) and subjected to sample pretreatment before injection (automatic) into the GC-MIP-AES. The emission intensities of the blank solutions were measured (n = 10) and concentrations calculated with reference to the appropriate calibration curve. The analytical data and response curves are shown in table 2.6 and figure 2.27 respectively.

Methylmercury		***Ethylmercury***		***Inorganic mercury***	
Emission intensity	*Concentration equivalent, ng/l*	*Emission intensity*	*Concentration equivalent, ng/l*	*Emission intensity*	*Concentration equivalent, ng/l*
0.28	*8.1*	*0.58*	*18.3*	*0.38*	*17.9*
0.34	*9.8*	*0.61*	*19.3*	*0.65*	*30.7*
0.61	*17.6*	*0.88*	*27.8*	*0.69*	*32.6*
0.46	*13.3*	*0.69*	*21.8*	*0.45*	*21.2*
0.57	*16.5*	*0.79*	*24.9*	*0.85*	*40.2*
0.37	*10.7*	*0.78*	*24.6*	*0.79*	*37.3*
0.75	*21.7*	*0.30*	*9.5*	*0.95*	*44.9*
0.69	*19.9*	*0.79*	*24.9*	*0.33*	*15.6*
0.85	*24.6*	*0.63*	*19.9*	*0.44*	*20.8*
0.27	*7.8*	*0.19*	*6.1*	*0.75*	*35.4*
$\sigma = 0.205$	$\sigma = 5.9$	$\sigma = 0.287$	$\sigma = 6.9$	$\sigma = 0.215$	$\sigma = 10$

Table 2.6.
Emission intensities (peak height responses) and concentration equivalent for different SCF microcolumns used for calculating the limit of detection. Blank, hydrochloric acid 3 M. Blank volume, 60 ml. Elution volume, 0.5 ml. Extraction, 1 ml. Injection, 1 µl.

By analysing the method blank against the calibration blank permits a check of the sample preparation reagents and procedures for contamination control. Moreover statistical evaluation of the blank permits an estimation of the limit of detection. The limits of detection for mercury species were 18 ng l^{-1} as Hg for methyl-, 21 ng l^{-1} as Hg for ethylmercury and 30 ng l^{-1} as Hg for inorganic mercury.

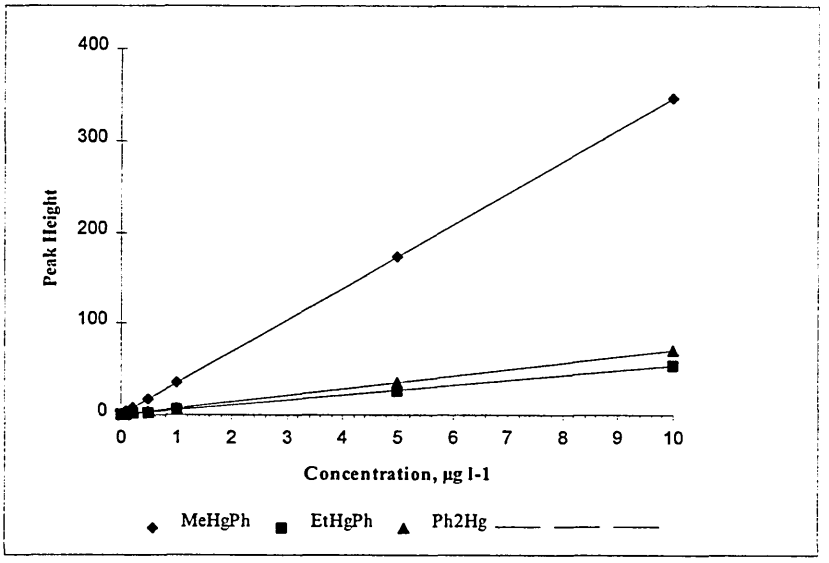


Figure 2.27.
Calibrations graphs for mixed standard solutions (methyl-, ethyl- and inorganic mercury) for the concentration range 0, 0.1, 0.2, 0.5, 1, 5 and 10 µl l^{-1} as Hg, 0.5 ml. Extraction, 1 ml n-hexane. Injection, 1 µl.

Accuracy and Precision. Precision is a measure of the reproducibility of results and is expressed as the RSD (relative standard deviation, s / X x 100). A measurement process should be sufficiently precise to minimise the number of replicate measurements required for the intended use. Method precision was calculated by passing 60 ml of a mixed standard solution of 1 µg l^{-1} as Hg through 10 different SCF microcolumns (n = 10). Then, the retained mercury species were eluted by injection of hydrochloric acid (3 M, 0.5 ml) and subjected to sample pretreatment before injection (automatic) into the GC-MIP-AES. The data are shown in table 2.7 and were 5.3% for methyl-, 10.5% for

ethyl- and 12% for inorganic mercury. Table 2.7 also shows data for injection without SCF microcolumn preconcentration and it can be seen that similar values were obtained: 4.9% for methyl-, 10.9% for ethyl- and 13.7% for inorganic mercury. Both values are in consistent with earlier values reported in the literature [Emteborg et al., 1993] using GC-MIP-AES involving sample pretreatment such as preconcentration, derivatisation and extraction.

Methylmercury		**Ethylmercury**		**Inorganic mercury**	
Without microcolumn	With microcolumn	Without microcolumn	With microcolumn	Without microcolumn	With microcolumn
55.1	57.4	11.5	10.7	17.6	18.2
59.9	62.7	12.9	12.3	14.5	19.5
60.4	64.8	13.5	13.9	18.3	20.0
62.3	60.7	13.4	14.3	20.2	24.4
60.2	63.4	16.1	13.9	19.6	22.2
57.0	60.4	14.6	14.4	16.6	18.6
63.8	69.2	15.8	15.6	16.5	17.4
58.4	63.2	12.4	12.4	12.9	18.9
61.4	62.2	12.4	12.3	18.8	20.5
55.0	58.7	13.7	13.6	15.0	16.3
x = 59.4	x = 62.3	x = 13.6	x = 13.4	x = 17.0	x = 19.6
s = 2.9	s = 3.3	s = 1.5	s = 1.1	s = 2.3	s = 2.3
RSD(%) = 4.9	RSD(%) = 5.3	RSD(%) = 10.9	RSD(%) = 10.5	RSD(%) = 13.7	RSD(%) = 12.0

Table 2.7.
Precision data for mercury species (methyl-, ethyl- and inorganic mercury) based on processing sample (a) without and (b) with microcolumn preconcentration.Mixed standard solution of (a) 120 μg l^{-1} as Hg and (b) 1 μg l^{-1} as Hg respectively. Sample volume, 60 ml. Elution volume, 0.5 ml. Extraction, 1 ml n-hexane. Injection, 1 μl.

Accuracy is the degree of agreement of a measured value with the true or expected value of the quantity of concern. Usually the accuracy of a method is calculated by analysing reference materials (RMs) certified for elemental / species composition. In the case of environmental waters few RMs are available for mercury speciation due to the problems associated with species stability, especially methylmercury. In this work as a test of

accuracy drinking water and canal water were spiked with 1μg l^{-1} as Hg of a mixed standard solution (containing methyl-, ethyl- and inorganic mercury) in order to check the recovery. A 60 ml sample of water (1 μg l^{-1} as Hg of methyl-, ethyl- and inorganic mercury) was processed using different SCF microcolumns (n = 3). Then, the retained mercury species were eluted by injection of hydrochloric acid (3 M, 0.5 ml) and subjected to sample pretreatment before injection (automatic) into the GC-MIP-AES. A calibration graph using the same concentration of mercury species was also obtained and the spiked values were introduced in their equations and the % was calculated. The data are shown in table 2.8 and as can be seen these values indicate that the proposed method may be valid for the determination and speciation of mercury in such waters.

Species	Tap water (%recovery)	Manchester ship canal (%recovery)
Methylmercury	92.4±4.2	93.6±4.5
Ethylmercury	90.0±3.9	92.1±5.1
Inorganic mercury	93.0±5.0	91.6±3.3

Table 2.8.
Recovery data for spiked additions to waters. Tap water and canal water spiked with mixed standard solutions (methyl-, ethyl- and inorganic mercury, 1 μg l^{-1}, as Hg). Sample volume, 60 ml. Elution volume, 0.5 ml. Extraction volume, 1 ml. Injection, 1 μl.

In this section the application of the proposed methodology to the analysis of natural waters is described. That is, instead of implementing microcolumn preconcentration in the laboratory, as described in the previous section, water samples are processed directly in the field by passage through the microcolumns in order to immobilise the analytes. The microcolumns with retained analytes are returned to the laboratory for elution/quantitation. In the next section the use of SCF microcolumns for sampling / preconcentration of mercury species (methyl-, ethyl- and inorganic mercury) in combination with GC-MIP-AES is described. Moreover, if the proposed in situ preconcentration process is to be valid, the mercury species in the sampled waters need to be immobilised on the SCF microcolumns and rendered stable during the interval between collection in the field and analysis in the laboratory. An outline of the integrated field / laboratory procedure is given in figure 2.28.

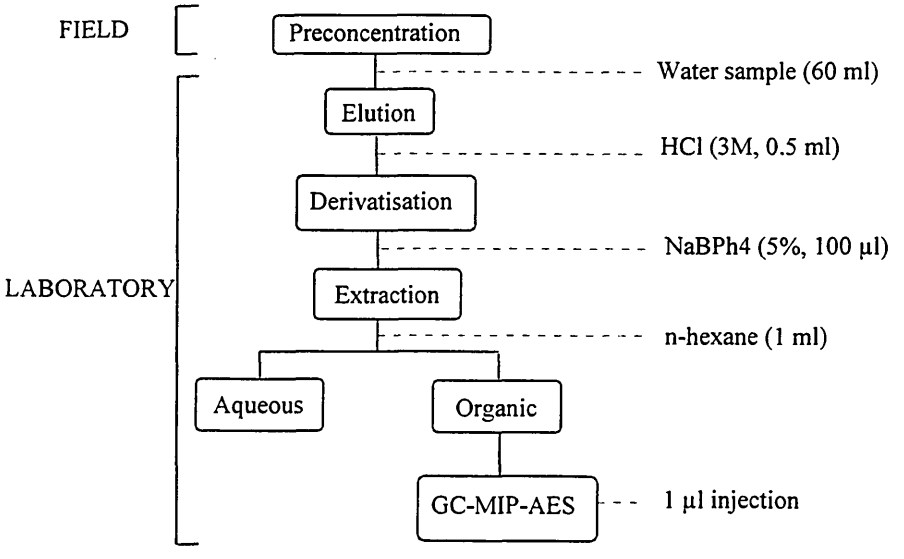


Figure 2.28.
An overview of the integrated field/laboratory procedure.

Stability of Immobilised Mercury Species. It was therefore first necessary to establish a simple and a rapid way to check stability of the immobilised mercury species. Various time periods between sampling and analysis of water samples were therefore examined. In this experiment ten separate SCF microcolumns were loaded with a mixed mercury standard solution (10 μg l^{-1} as Hg of methyl-, ethyl- and inorganic mercury) and ten with spiked Manchester Ship Canal water. Ten SCF microcolumns (five containing standard solution and five containing spiked canal water) were then immediately eluted by injection of hydrochloric acid (3 M, 0.5 ml) and subjected to sample pretreatment before injection (automatic) into the GC-MIP-AES. The other ten SCF microcolumns (five containing standard solution and five containing spiked canal water) were removed from the FI system and stored in polyethylene bags for 1 week. After this time, the SCF microcolumns were inserted into the FI system, one at a time, for elution by injection of hydrochloric acid (3 M, 0.5 ml) and subjected to sample pretreatment before injection (automatic) into the GC-MIP-AES. The data are presented in table 2.9 and recovery values were calculated by comparing the amount of mercury detected for a freshly prepared SCF microcolumn containing the same amount of mercury species. That is the study essentially compared columns that had been left standing for over one week with there that had been processed directly.

	1 hour, % recovery		**1 week, % recovery**	
Species	**Standard**	**MSC**	**Standard**	**MSC**
Methyl-	100±8	99±15	103±10	98±13
Ethyl-	100±12	98±16	100±8	96±12
Inorganic	99±12	97±16	99±6	97±9

Table 2.9.
Effect of storage time on the recovery of mercury species (methyl-, ethyl- and inorganic mercury) retained on SCF microcolumns. Mixed standard solution, 10 μg l^{-1}, as Hg. Storage conditions, 4°C.

The data show that once the mercury species (methyl-, ethyl- and inorganic mercury) have been effectively immobilised on the SCF microcolumns they were held stable for at least 1 week, resulting in reliable and meaningful speciation data for methyl-, ethyl- and inorganic mercury. Typical GC-MIP-AES responses for fresh and aged microcolumns for spiked Manchester Ship Canal are given in figure 2.29.

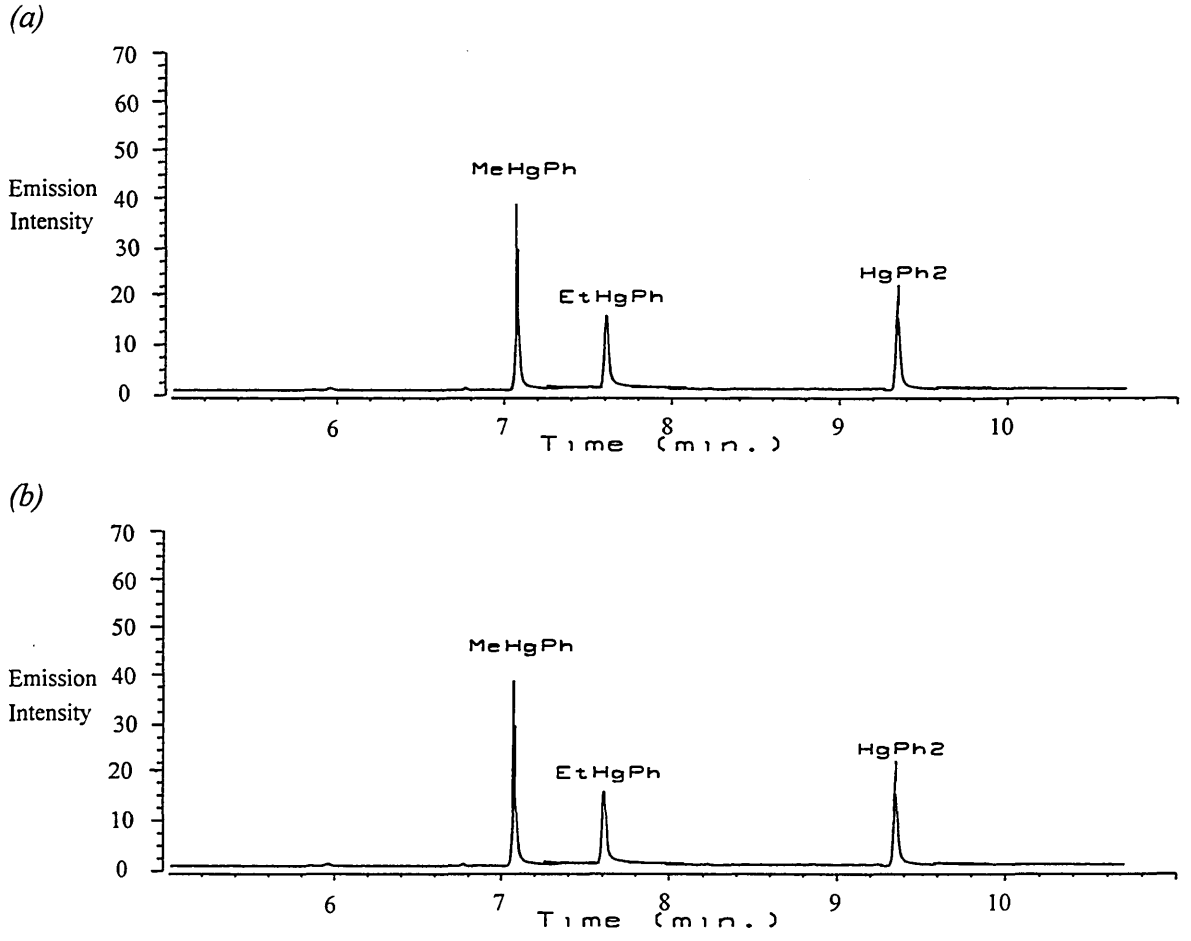


Figure 2.29.
Typical GC-MIP-AES response for fresh and aged microcolumns for spiked Manchester Ship Canal water (10 μg l^{-1} as Hg of methyl-, ethyl- and inorganic mercury; 3 ml). Elution volume, 0.5 ml. Extraction, 1 ml n-hexane. Injection, 1 μl.

Speciation and Distribution of Mercury in the Surface Water of the Manchester Ship canal. To test the new methodology the Manchester Ship Canal was selected. The canal, represented in figure 2.11, is a highly polluted watercourse and is known to be

contaminated with mercury due to the presence of a chloroalkali plant. Pollution problems of the Manchester Ship Canal are linked to the Industrial Revolution of the 18th century. Runcorn and Ellesmere port began to develop as major centres for the chemical industry with the establishment of the Castner-Kellner Alkali company in 1897. This company produced caustic soda and chlorine by electrolysis of brine which used a rocking mercury cathode. Waste brine from the process becomes contaminated with mercury. Discharges of mercury in the Runcorn and Ellesmere Port sites have been monitored since early 1970s by the Environment Agency (formerly National Rivers Authority) [National Rivers Authority, 1995]. In the mid-1970s the load of mercury discharged was estimated to be nearly 60 tonnes/year. This has been progressively reduced by an improved effluent treatment process and the Company now discharges less than 1 tonne/year. In 1993, the Environmental Agency (formerly National Rivers Authority) reported an average total mercury concentration of 0.07 $\mu g\ l^{-1}$ along the Manchester Ship Canal [National Rivers Authority, 1995].

Although there has been a substantial reduction in the amount of mercury discharged to the canal in recent times, there remains an accumulated reservoir of mercury in sediments (about 1 $mg\ kg^{-1}$) [National Rivers Authority, 1995]. These data reported by the Environmental Agency are for total mercury only.

In order to obtain data on mercury speciation microcolumn field processing was performed on two different cruises (27.10.93 and 08.06.95). For both cruises water samples (sub-surface) were collected at three sampling sites (Fisher Wharf, S2; Stanlow Wharf, S3 and Stanlow Point, S4) (see figure 2.11) where relatively high values of organomercury species (22 - 58 $ng\ l^{-1}$ as Hg) were earlier reported [Wei et al., 1994].

The study focused on a small area rather than on a wide area as the aim was mainly to investigate the possible occurrence of methylation processes and not to perform a survey of metal concentrations. Water samples (1 l), on collection, were adjusted to pH 3.0 - 3.5 by dropwise addition of concentrated nitric acid. By using a portable pump, 60 ml of water sample was introduced into the SCF microcolumn. On completion of sampling, SCF microcolumns were stored in a light tight box and returned to the laboratory for analysis. For each sampling site five replicate analysis (corresponding to processing of 5 different SCF microcolumns) were performed. The determination of methyl-, ethyl- and inorganic mercury species was based on the elution of mercury species from the SCF microcolumn by injection of hydrochloric acid (3 M, 0.5 ml) and subjecting the eluate to sample pretreatment before injection (automatic) into the GC-MIP-AES. The data on the first cruise (27.10.93) are presented in table 2.10a. It can be seen that the canal is contaminated with inorganic mercury (0.101 - 0.340 $\mu g\ l^{-1}$ as Hg). The data do correlate well with values obtained for the second cruise (08.06.95) as shown in table 2.10b. By comparing data for inorganic mercury for both cruises it can be seen that the higher concentration is detected at station S2 (Fisher Wharf) in the first cruise while for the second cruise station S4 yields the maximum value. This could be explained by the fact that the waters have been moved during tidal movements. It is known that inorganic mercury in the Manchester Ship Canal is derived from the operation of chloroalkali plant either through direct discharge of effluent containing mercury or from mobilization of mercury from the bottom sediments [Falchuk et al., 1977]. In addition for the three sampling stations monitored, elevated concentrations of methylmercury were detected (0.040 - 0.160 $\mu g\ l^{-1}$ as Hg), in contrast to that of ethylmercury (see table 2.10a,b). These values obtained for methylmercury concentrations for the first and the second cruise (0.052 - 0.182 $\mu g\ l^{-1}$ as Hg) are consistent as can be seen from table

2.10a,b. A typical emission-time response for GC-MIP-AES analysis of the canal water is shown in figure 2.30.

(a)

Water Sample	Methylmercury, $\mu g\ l^{-1}$ as Hg	Ethylmercury $\mu g\ l^{-1}$ as Hg	Inorganic mercury $\mu g\ l^{-1}$ as Hg
S2	0.160±0.005	-	0.340±0.003
S3	0.100±0.003	-	0.320±0.004
S4	0.040±0.002	-	0.101±0.002

(b)

Water Sample	Methylmercury, $\mu g\ l^{-1}$ as Hg	Ethylmercury $\mu g\ l^{-1}$ as Hg	Inorganic mercury $\mu g\ l^{-1}$ as Hg
S2	0.052±0.003	-	0.179±0.005
S3	0.106±0.005	-	0.333±0.003
S4	0.182±0.002	-	0.366±0.001

Table 2.10.
Speciation data for mercury for: (a) the first cruise (27.10.93) and (b) the second cruise (08.06.95) in the Manchester Ship Canal. Data, mean±s (n = 5). Sample volume, 60 ml. Elution volume, 0.5 ml. Extraction, 1 ml n-hexane. Injection, 1 µl.

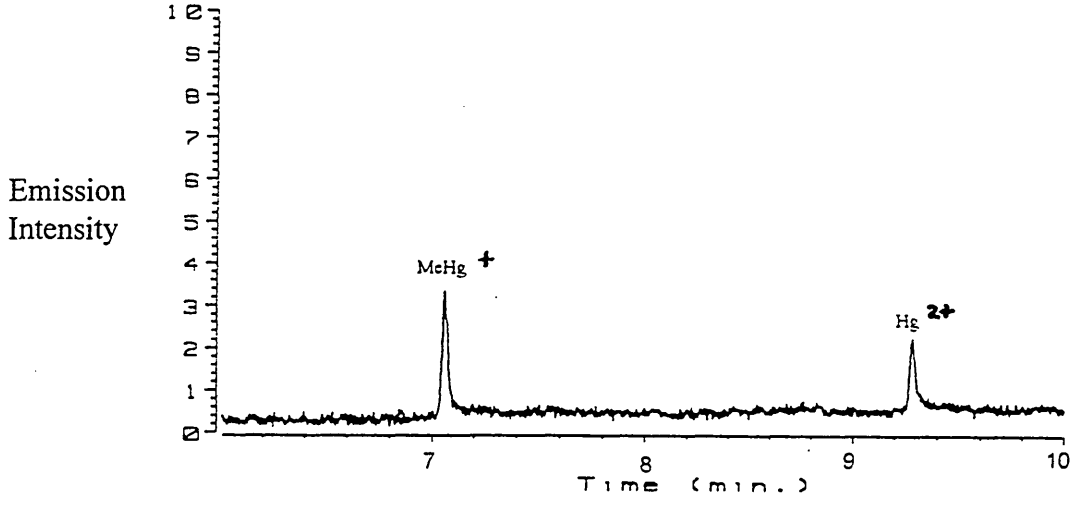


Figure 2.30.
Typical emission-time response for Manchester Ship canal water from Fishers Wharf. Sample volume: 60 ml. Elution volume, 0.5 ml. Extraction, 1 ml n-hexane. Injection, 1 µl.

For this cruise (08.06.95) the sampling procedure was also designed in order to allow determination of total mercury. Water samples (1 l), on collection, were introduced into 200 ml flask (n = 5 for each station), which contained concentrated nitric acid (1 ml). The flasks were stored in a light tight box and returned to the laboratory for analysis.

For each sampling site collected three replicate analyses were performed. The FI-AFS measurements were performed within 48 h of sample collection. Total mercury concentrations determined were 0.352±0.006 μg l^{-1} as Hg for Fisher Wharf (S2), 0.427±0.001 μg l^{-1} as Hg for Stanlow Wharf (S3) and 0.611±0.008 μg l^{-1} as Hg for Stanlow Point (S4). Although only a limited number of samples were analysed the data indicate a degree of consistency in that total mercury concentrations and the summations of the methyl- and inorganic mercury data (0.231±0.008 μg l^{-1} as Hg for Fisher Wharf, 0.439±0.008 μg l^{-1} as Hg for Stanlow Wharf and 0.548±0.003 μg l^{-1} as Hg for Stanlow Point) are of similar magnitude. Thus microcolumn sampling/enrichment in combination with GC-MIP-AES may provide a valid route for determination of individual mercury species. However, the good correlation of results could also suggest that concentrations of particulate mercury (many mercury compounds -mercury complexes with humic substances- are associated with particulate matter derived from soils and sediments) are very low/negligible because SCF microcolumns may act as a filter, retaining also particulate mercury which is eluted at the same time as the immobilised mercury with hydrochloric acid. The concentrations of total mercury found confirm that the Manchester Ship Canal is still highly contaminated (fresh waters generally contains <5 ng l^{-1} total mercury [Bloom, 1989]).

The presence of methylmercury in the canal is further a significant finding from an environmental standpoint due to the high toxicity of methylmercury species, indicating a new possible pathway for the transformation of mercury in the environment. Different pathways have already been discussed to explain the formation of methylmercury: biological methylation by bacteria and higher organisms [Gilmour and Henry, 1991] and chemical methylation in the presence of tin and lead alkyls [Jewett and Brinkman,

1974]. It has also been suggested that a mixed chemical / biological mechanism might operate [Falchuk et al., 1977]. However, in this watercourse transmethylation from other organometals (tetraalkyllead species) may be the responsible for producing methylmercury [Jewett and Brinkman, 1974]. It is known that the surface waters in question (sampling sites 2, 3 and 4) contain relatively high concentrations of alkyllead compounds (1.4 - 2.7 $\mu g\ l^{-1}$ as Pb) due to the discharge of lead from the manufacture of tetraalkyllead compounds by Associated Octel at Ellesmere Port. This discharge is unusual in containing stable, water soluble, organic lead species and their distribution profiles were noted to be similar to that obtained for methylmercury in this study [National Rivers Authority, 1995]. This similarity implies that an in situ alkylation reaction of the type:

$$Hg^{2+} + Me_3Pb^{+} \longrightarrow MeHg^{+} + Me_2Pb^{2+}$$

might be operating whereby there is a transfer of alkyl groups to inorganic mercury. Such as possibility was demonstrated in the laboratory [Lee and Hultberg, 1990] and confirmed by ourselves [Wei et al., 1994]. In order to consider this possibility, it is necessary to study in detail the distribution of organolead compounds in the Manchester Ship Canal and a method for speciation of tetraalkyl- and inorganic lead is proposed in chapter 3.

2.4. Concluding Remarks.

The combination of the SCF microcolumn field sampling technique and GC-MIP-AES has been used for the determination and speciation of mercury in environmental waters. The approach offered both preservation and high sensitivity combined with high separation ability for organomercury (methyl- and ethyl-) and inorganic mercury species.

Application to monitoring of the Manchester Ship Canal (a watercourse known to be contaminated with mercury) was demonstrated. It was found that both methyl- and inorganic mercury were present in the canal in concentrations ranging from 0.052 - 0.182 $\mu g\ l^{-1}$ as Hg for methylmercury and 0.179 - 0.366 $\mu g\ l^{-1}$ as Hg for inorganic mercury. The presence of methylmercury is considered to be due to a transmethylation reaction, a route which till now has not been widely reported in the literature [Mena, 1996].

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Chapter 3: Lead Speciation

3.1. Introduction.

Lead has always been present in the environment as a result of natural inputs (such as the erosion of minerals such as galena - lead sulphide- or volcanic eruptions) and human activities. Human activities include the wide use of lead for centuries for a variety of purposes. The ancient Romans used lead for water pipes and cooking utensils. In present times lead is used for the manufacture of acid accumulators, household paints, roofing materials and piping materials, including pipes used for potable water. Recently alkyllead compounds have been used in gasoline (due to their antiknock properties of tetramethyllead, tetraethyllead and some mixed methylethyllead compounds), solders, pigments, ammunition and cable sheathing. Accordingly by there is significant environmental contamination of air, water, soil, dust and foods by lead.

Lead pollution in air (typically 0.1 $\mu g\ m^{-3}$ in rural areas and 0.5 - 2 $\mu g\ m^{-3}$ in cities) is due mainly to the exhaust gases from motor vehicles. Most of the lead in air is in the form of fine particles and some 20 - 60 % of the particles are deposited in the respiratory system [World Health Organisation, 1977; Harrison and Laxen, 1978]. The present lead content of lake and river water, world-wide, has been estimated to be 1 - 10 $\mu g\ l^{-1}$ [World Health Organisation, 1973], although higher values have been recorded where specific contamination has occurred, particularly from industrial sources. The concentration of lead in finished water (i.e. water after treatment) prior to its distribution is generally lower than in source waters because lead is partially removed by most conventional water treatment processes [National Research Council, 1977]. However, the levels of lead in drinking water (tap water) can be much higher (10 - 20 $\mu g\ l^{-1}$) due to the use of lead pipes running from the street, household plumbing and / or lead lined

storage tanks, especially when the water has a low pH or is soft (i.e. In certain regions of Scotland tap water has been shown to contain more than 300 $\mu g\ l^{-1}$ Pb). Soil and roadside dust contain elevated levels of lead (2 - 10 $g\ kg^{-1}$) as a result of the deposition of air particulates.

The presence of lead in food is dependent on the type of food. Many fresh vegetables, cereals and fruits contain small quantities of lead as a result of its absorption from the soil in which they are grown and because of the deposition on surfaces from lead in the air. Canned foods tend to contain the highest levels if lead solders have been used in the manufacture of the can. Additional lead in food can arise from contamination by cooking vessels, such as pots that have soldered joints, some glazed earthenware utensils and from tap water used for preparing the food. Typical daily intakes for adults are on average about 200 $\mu g\ day^{-1}$ and in children about 90 $\mu g\ day^{-1}$ [Underwood, 1977].

The different fractions that make up total lead are shown in figure 3.1. Dissolved lead in the environment exists mainly in the inorganic form, but significant amounts of organo lead compounds result from the use of leaded gasoline. Once in the environment organolead compounds undergo a variety of degradation processes which continue in the troposphere under the influence of sunlight and some atmospheric constituents (such as hydroxyl radicals and ozone) to give the most stable trialkyllead species (R_3Pb^+), as shown in figure 3.2. Further conversion finally yields inorganic lead via dialkyllead (R_2Pb^{2+}) intermediates [Harrison and Laxen, 1978; Jarvie et al, 1981; Nielsen et al., 1981; De Jonghe et al, 1983; Harrison et al., 1986; Van Cleuvenbergen and Adams,

1989]. From the atmosphere they pass to the aquatic environment by wet and dry deposition [Jensen and Luoma, 1977]. It has also been reported that many lead compounds (lead complexes with humic substances or lead complexes such as $PbCl^+$) are associated with particulate matter (which is retained in a 0.45 μm filter, as shown in figure 3.1) in water samples. They are derived from soils and sediments, where they form through the chemical and biologically mediated breakdown of biological tissue [Boggs et al., 1985].

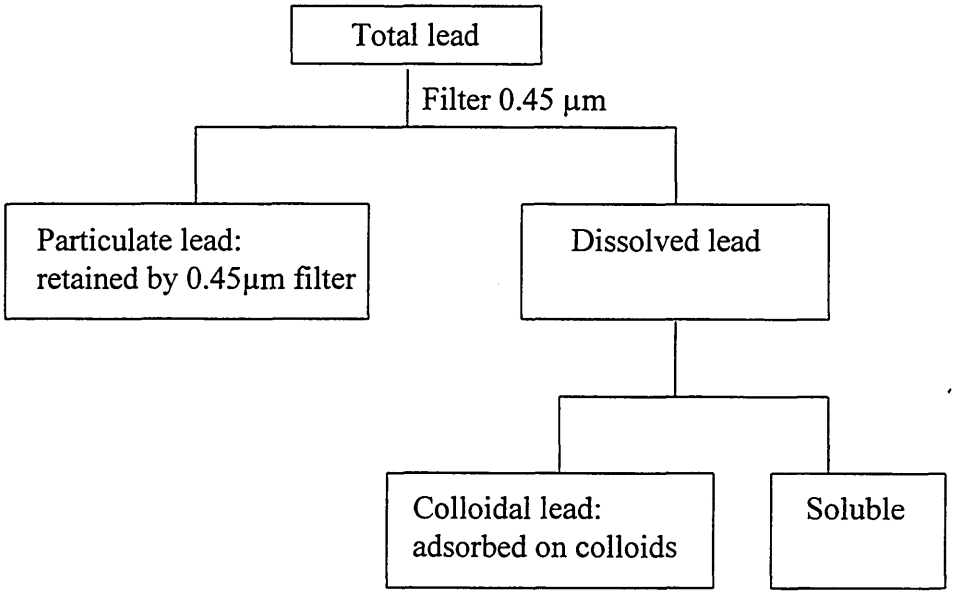
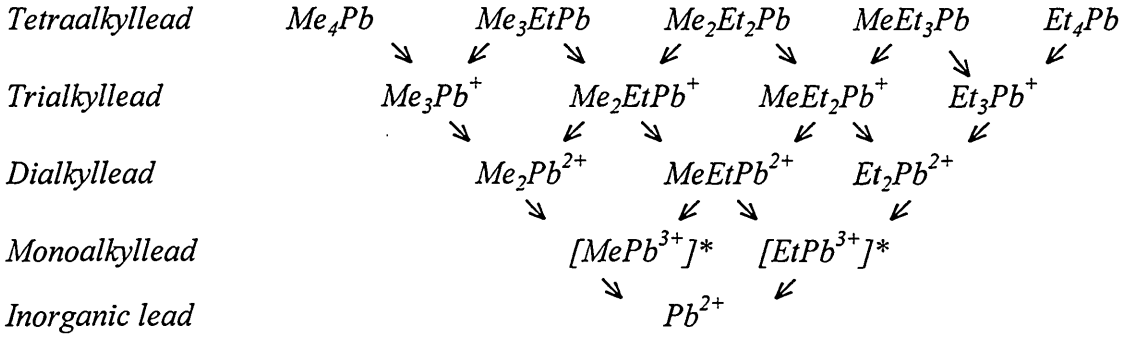


Figure 3.1.
Different fractions that make up total lead concentration in water samples.



** very unstable, evidence for the presence only circumstantial.*

Figure 3.2.
Organolead species occurring in environmental samples (e.g. water samples) from ref [Lobinski et al., 1994].

Lead is not known to be essential for the functioning of biological systems and its toxicity depends on its chemical form. Organo lead is more toxic than its inorganic form. Moreover, the toxicity of organolead compounds diminishes in the sequence $R_4Pb > R_3Pb^+ > R_2Pb^{2+}$ ($R = C_2H_5 > CH_3$) but the ionic species are more persistent in the environment. Similarly, methylated lead compounds are less toxic than the corresponding ethylated species, but are more stable, volatile and have longer half-lives [Berman, 1980; Radojevic and Harrison, 1987; Van Cleuvenbergen and Adams, 1989]. Lead is absorbed when ingested by the respiratory and gastrointestinal tracts and enters the blood from where is distributed to soft tissues and bone. Moreover, lead in high doses acts as an accumulative general metabolic poison because, even at low concentration levels, reduces the activity of enzymes (involved in normal haeme synthesis) and aminoacids containing sulphur (leading to interference in the regulation of oxygen transport) [Berman, 1980; Radojevic and Harrison, 1987]. However, an additional and significant exposure to lead has been found in children including infants (up to one year old): the ingestion and chewing of household paint (containing high levels of lead) due to the "hand to mouth" activity of such children. There is a special risk in their exposure to lead because its absorption is higher than for adults and they have a higher susceptibility, due in part to their rapid growth rate [World Health Organisation, 1972]. Pregnant women and developing foetuses also appear to be more sensitive to lead because of increased maternal food intake and changes in hormonal status. In this case lead has been associated with subtle behavioural effects and chromosomal aberrations. Significantly higher blood lead levels (> 400 $\mu g\ l^{-1}$ Pb) have been found in mentally retarded children [Moore, 1977].

With respect to the environmental impact of lead large scale mortality of estuarine birds

(such as wildfowl and gulls) occurred from 1979 to 1982 (2400 birds died in 1979, 864 in 1980, 85 in 1981 and 140 in 1982) in the Manchester Ship Canal [Wilson et al., 1986]. Mortality on this scale was unprecedented in the area and was due to the discharge to the canal of alkyllead species (predominantly trialkyllead) from a factory [Wilson et al., 1986]. Waste from the process became contaminated with stable, water soluble, organo lead species. Discharges of lead in the Runcorn and Ellesmere Port sites have been monitored since the early 1970s by the Environment Agency (formerly National Rivers Authority). In 1986, the EA reported an average total lead concentration of 6 mg l^{-1} along the canal [EA, 1995]. Although there has been a substantial reduction in the amount of lead discharged to the canal in recent times, there still remains an accumulated reservoir of lead in eel and flounders (> 2 mg kg^{-1}) [EA, 1995].

In order to control industrial discharges of lead into the aquatic environment the European Commission which sets the legislative program for the European Union (EU) has recognised the potential global impact of lead pollution. The Dangerous Substances Directives (76/464/EEC) has also set standards for List II Substances which have been identified as having a deleterious effects on the aquatic environment. Lead is included in List II and the environmental quality standard (EQS) for lead, as dissolved metal, is 25 μg l^{-1}. It is necessary to remark that these Directives are for total lead only. But as already mentioned for natural waters lead may be present both in organic and inorganic forms and hence data on lead speciation is necessary.

Modern methods for the determination of total lead at the μg l^{-1} level are based on atomic absorption spectrometry (AAS), inductively coupled plasma-atomic emission spectroscopy (ICP-AES) or inductively coupled plasma-mass spectrometry (ICP-MS) in

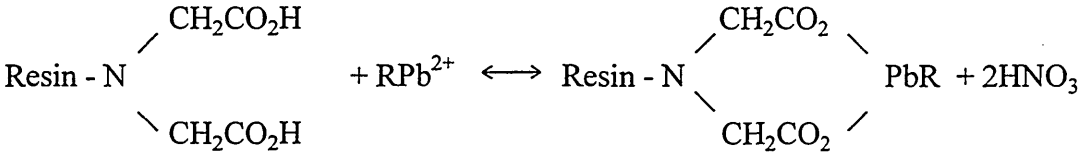
combination with sample preconcentration schemes including evaporation [Analytical Quality Control, 1985], solvent extraction [Standing Committee of Analyst, 1976; Fukkamachi and Ishibashi, 1980; Nord and Karlberg, 1981] and atom trapping techniques [Marshall and Mottola, 1985; Jiang et al., 1990]. Numerous hyphenated techniques have been developed for the determination and speciation of lead in environmental samples. This is due to the fact that lead speciation clearly must involve separation followed directly by specific determination and quantification of organic and inorganic lead species. The hyphenated techniques used for lead speciation can be classified according to the separation step which involves chromatographic or non-chromatographic separations. Some examples of separation using chromatography of lead species include the use of GC [Kolb et al, 1966; Reamer et al., 1978; Estes et al., 1981; Ebdon et al., 1982; Estes et al., 1982; Andersson et al., 1984; Chakraborti et al., 1984; Ibrahim et al., 1985; Rapsomanikis et al., 1986; Allen et al.,1988; Lobinski and Adams, 1992] or HPLC [Vickrey et al., 1980] with sensitive detection / quantitation systems (such as AAS, MIP-AES, ICP-AES or GFAAS). Much of this subject area has been reviewed by Radojevic, 1989. It can be concluded that the procedures developed until now for the determination and speciation of lead species at the sub ng l^{-1} level have required from 0.1 (GC-MIP-AES) to 10 cm^3 (GC-AAS) for sample injection, always preceded by a tedious and time consuming preconcentration scheme involving large sample volumes (0.5 - 10 l) to offer the necessary method sensitivity.

From the speciation standpoint then there is a need to devise and develop rapid on-line sample pre-treatments/ separations which can be fully integrated with the final measurement step. In recent years, improved sensitivity and reduction of analysis times have been obtained with the use of on-line ion exchange separation / preconcentration

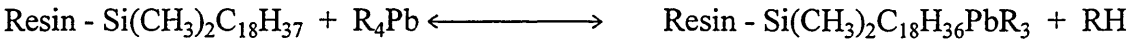
procedures which are easily implemented in continuous flow systems. The ion exchange material is usually contained within a microcolumn and the analytes are retained via an electrostatic or complexation process [Valcárcel and Luque de Castro, 1991]. The first report of the development of a FI manifold for on-line ion exchange with direct coupling to an atomic absorption spectrometer (AAS) was by Olsen et al., 1983. They used Chelex-100 for preconcentration of lead and cadmium in sea water. Since then, numerous papers have appeared in the literature describing the use of ion exchange separation / preconcentration in conjunction with AAS, ICP-AES and ICP-MS. The most frequently used packing materials for on-line preconcentration of lead are: activated alumina (basic form) [Zhang et al., 1988], fibrous alumina [Dadfarnia et al., 1994], and chelating ion exchangers such as Chelex-100 [Olsen et al., 1983; Schulze and Elsholz, 1986; Ruzicka and Arndal, 1989], 8-quinolinol-5-sulphonic acid [Fang et al., 1984; Hartensein et al., 1985a; Hartensein et al., 1985b], 8-hydroxyquinoleine covalently immobilized on to controlled pore glass [Nelms, Greenway and Hutton, 1995], C18 bonded silica gel [Malamas et al., 1984], immobilised cysteine [Elmahadi and greenway, 1993], chloroxine [Elmahadi and Greenway, 1996] and iminodiacetate resin based on controlled pore glass [Greenway, Nelms, Skhosana and Dolman, 1996; Nelms, Greenway and Koller, 1996]. These microcolumns systems have been used in combination with flame AAS, GFAAS, ICP-AES and ICP-MS. However, the major limitation is that to date a speciation potential for lead has not been demonstrated using such FI systems.

In this work it is proposed to develop rapid speciation methodology for lead using a FI system. Success of the scheme depends on identifying an appropriate microcolumn chemistry, that displays differing affinities for inorganic and organolead species. In an

ideal situation column chemistry would be such that one class of species would be completed retained on sample injection whereas the other class would be unretained and hence proceed to the detector for quantitation. Thereafter an injection of eluent would complete the two stage measurement cycle. Several packing materials were selected for investigation: Muromac A-1, C18 (octadecyl bonded silica gel), sulphydryl cotton fibre (SCF) and activated alumina. Muromac A-1 is a cation exchanger which contains an imminodiacetic acid functional group and should selectively retain cationic species (such as Me_2Pb^{2+}, Et_2Pb^{2+} or Pb^{2+}) according to the following scheme:



C18 is apolar containing bonded silica with octadecyl functional group [Fang and Welz, 1989] and may retain neutral lead species (Me_4Pb or Et_4Pb) according to:



where R = methyl- or ethyl-

SCF has a complexing capability for organolead compounds due to the high affinity of such compounds for the sulphydryl functional group and hence may retain neutral lead species (Me_4Pb or Et_4Pb):

Activated alumina can be function as an anion or a cation exchanger due to its amphoteric character [Laurent et al., 1983; Schmitt and Pietrzyk, 1985]. This is due to the fact that alumina surface, as shown in figure 3.3, has hydroxyl ions chemisorbed on acidic sites (aluminium ions) and protons chemisorbed on oxygen atoms. When neutral alumina is washed with an acidic solution (e.g. HNO_3) the hydroxyl groups are then replaced by more loosely NO_3^- ions that give rise to the anion exchange properties of acidic alumina. As a result acidic alumina can reject cationic species. Conversely, when the neutral alumina is washed with a basic solution (e.g. NH_4OH), the protons are then replaced by NH_4^+ ions that give rise to the cation exchange properties of basic alumina. The retention of inorganic lead using basic alumina proceeds according to:

$$2\ Al\text{-}O^-H^+ + Pb(NO_3)_2 \longleftrightarrow 2\ Al\text{-}O^-Pb^{2+} + 2\ HNO_3$$

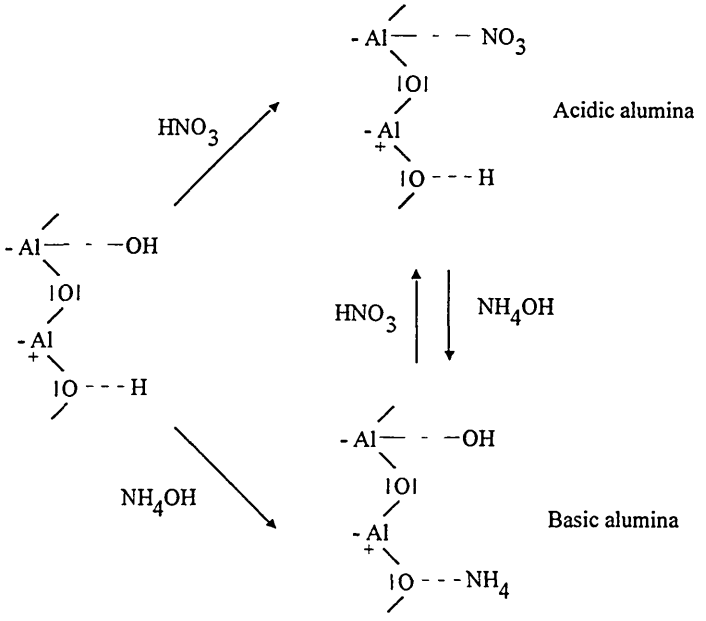


Figure 3.3.
Surface behaviour of alumina in basic and acidic media, respectively (from ref [Schmitt and Pietrzyk, 1985].

Moreover, as a result of the very complex alumina surface physical adsorption between neutral analytes and alumina surface, via van deer Waals forces may also operate

[Schmitt and Pietrzyk, 1985]. A subsequent injection of methanol can then be used to effect elution of the neutral lead species.

Another consideration in the determination and speciation of lead at trace levels in environmental samples is related to the sampling procedure. Studies on trace lead speciation have been shown that plastic (Teflon or polyethylene) or glass containers can both absorb trace lead ions from and/or contribute lead to samples as a result of surface dissolution [Florence and Baley, 1977]. Very little attention has been given to this point in most of the published papers. Most studies are simply concerned with processing inorganic lead or total lead concentrations. In other words there is not any substantial information related to species preservation. Thus a further and potentially highly significant development for speciation methodology concerns the use of microcolumns to collect, in the field, analyte species of interest, i.e. instead of undertaking sample processing in the laboratory samples are processed immediately on sample collection in an attempt to stabilise/immobilise species of interest. Microcolumns with retained analytes are then returned to the laboratory and inserted into the FI system for elution / final measurement. In this way it is hoped to avoid the problems associated with maintaining species integrity during the time delay between sampling and laboratory analysis. Viability for this approach has been successfully demonstrated in the case of mercury speciation in the Manchester Ship Canal (see chapter 2).

A basic aim of the work described in this chapter then is to advance speciation methodology for inorganic and organolead species, in natural waters by combining and developing microcolumn separations/fields sampling with FI-ICP-MS.

3.2. Experimental.

Reagents and Materials.

Standard solutions of inorganic lead were prepared by appropriate dilution of stock solution (1000 mg l^{-1} as Pb of lead nitrate in 1% V/V HNO_3). The tetraalkyllead stock solutions (R_4Pb; R = CH_3 and C_2H_5) were 1498 mg l^{-1} as Pb for $(CH_3)_4Pb$ and 998 mg l^{-1} as Pb for $(C_2H_5)_4Pb$ (in n-hexane). Standard solutions of 10 mg l^{-1} as Pb were prepared in methanol (Merck, HPLC grade, ARISTAR) and 10 μg l^{-1} as Pb in distilled water (Millipore). The dialkyllead stock solutions (R_2PbCl_2; R = CH_3 and C_2H_5) were prepared by dissolving the compounds in distilled water (Millipore) and standard solutions of 10 μg l^{-1} as Pb were prepared by appropriate dilution of stock solutions (1000 mg l^{-1} as Pb). Hydrochloric acid (0.01 and 3 M), nitric acid (0.02 M and 2 M) and ammonium hydroxide (0.02 M) were prepared from concentrated reagents (Merck, ARISTAR).

Separate microcolumns containing the different packing materials were prepared as follow:

Muromac A-1 microcolumns. Microcolumns were made by putting about 0.005 - 0.008 g of Muromac A-1 (100 - 200 μm) into a 20 mm length of column, the adsorbent being packed evenly along a 10 mm length of column. Two other small tubes (20 mm x 0.8 mm) were fitted at both ends of the column to allow connection of the microcolumn to the FI system. Two small pieces of sponge were added at both ends of the column in order to retain the Muromac A-1.

C18 (octadecyl bonded silica gel) microcolumns. Microcolumns were made by putting about 0.005 - 0.008 g of C18 (Bio-Rad Laboratories, Richmond, California, USA; particle size range 50 - 150 μm) into a 20 mm length of column, the adsorbent being packed evenly along a 10 mm length of column. Two other small tubes (20 mm x 0.8 mm) were fitted at both ends of the column to allow connection of the microcolumn to the FI system. Two small pieces of sponge were added at both ends of the column in order to retain the C18.

Sulphydryl cotton (SCF) microcolumns. Microcolumns were made by putting about 0.005 - 0.008 g of SCF (prepared as in chapter 2, Experimental section) into a 20 mm length of column, the adsorbent being packed evenly along a 10 mm length of column. Two other small tubes (20 mm x 0.8 mm) were fitted at both ends of the column to allow connection of the microcolumn to the FI system. A sponge support to retain the material was not necessary at both ends of the column.

Alumina microcolumns. Microcolumns were made by putting about 0.005 - 0.008 g of activated alumina (Merck, Brockman grade 1; particle size range 180 - 212 μm) into a 20 mm length of column, the adsorbent being packed evenly along a 10 mm length of column. Two other small tubes (20 mm x 0.8 mm) were fitted at both ends of the column to allow connection of the microcolumn to the FI system. Two small pieces of sponge were added at both ends of the column in order to retain the alumina.

Equipment and Operating Procedures.

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

An ICP-MS was used for the determination of lead species. The basic components of the ICP-MS (VG Elemental PlasmaQuad 2 Plus), illustrated in figure 3.4, are: (i)

inductively coupled plasma and (ii) mass spectrometer / detector. Typical operating parameter for the system are shown in table 3.1.

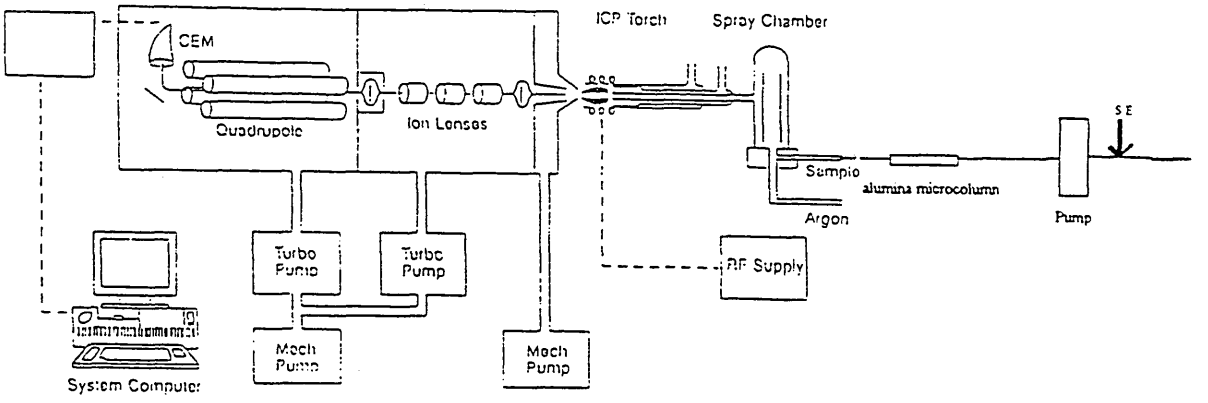


Figure 3.4.
ICP-MS diagram.

Element:	Pb			
Mass:	208			
Ion Lenses Settings:	Extraction Setting	1.00	L1 Setting	7.70
	Collector Setting	7.70	L2 Setting	5.40
	Front Plate Setting	8.00	L3 Setting	5.00
	Pole Bias Setting	6.00	L4 Setting	3.80
Nebuliser Gas Flow:	0.907 l min^{-1}			
Nebuliser Gas Pressure:	42 psi.			
Auxiliary Gas Flow:	0.6 l min^{-1}			
Coolant Gas Flow:	13 l min-1			
Power:	1300 W			
Injection Volume:	25 - 150 µl			
Elution Volume:	25 µl			
Injection/elution Flow Rate:	100 µl min-1			
Total number of injections:	5			
Signal Output / Display:	Ion Current or Intensity versus time 2 D plot.			

Table 3.1.
*Operational conditions for the ICP-MS (*VG Elemental PlasmaQuad 2 Plus*).*

(i) *Inductively Coupled Plasma (ICP).* The inductively coupled plasma was equipped with a flow injection (FI) system in order to effect sample injection. The FI system (figure 3.5) was a single line system and consisted of peristaltic pump (Gilson Minipuls) which was used to control the carrier stream flow rate (usually 100 μl min^{-1}), rotary injection valve (Omnichem; 100 μl sample loop) which was used for sample injection and a microcolumn containing the different packing materials (Muromac A-1, C18, SCF or alumina) which was used to separate / preconcentrate lead species (neutral - Me_4Pb, Et_4Pb- and cationic (organic -Me_2Pb^{2+}, Et_2Pb^{2+}- or inorganic -Pb^{2+}-) lead). To minimise sample dispersion in the FI system, these tube lengths were kept to a practical minimum.

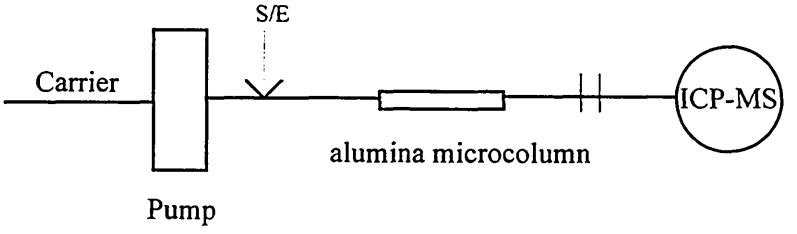


Figure 3.5.
FI system for speciation of lead.

The liquid sample was delivered into a microconcentric nebuliser (MCN-100, CETAC technologies) where the sample was dispersed by a stream of argon gas to produce a spray of droplets in the spray chamber. The spray chamber was a double pass type made from borosilicate glass and its purpose was to ensure that only the smallest solution droplets (less than 10 μm diameter) reached the plasma. The sprayed solution was transported to the plasma and the unused solution condensed and was pumped away from the spray chamber by the peristaltic pump.

The argon gas stream containing the sample was then directed through the centre tube (18 mm inner diameter and 100 mm length) of the torch into the core of the very high temperature plasma. The plasma torch, made from quartz, was of the normal Fassel configuration. Tubes of 13 and 1.5 mm inner diameter which terminated short of the injector tip were used for introduction of the coolant gas flow (which protected the tube walls) and the auxiliary gas flow (which was mainly used to ensure that the hot plasma was kept clear of the tip of the central capillary injector tube, to prevent melting).

The radiofrequency (RF) generator provided power for the generation and sustainment of the plasma discharge. The RF generator was connected to the load coil, which surrounded the top end of the torch. When RF power (typically 1300 W) was applied to the load coil, an alternating current moved back and forth within the coil at a rate corresponding to the frequency of the generator (27 MHz). This RF oscillation of the current in the coil caused RF electric and magnetic fields to be set up in the area at the top of the torch. With argon gas being swirled through the torch, a spark was applied to the gas causing some electrons to be stripped from the argon atoms. These electrons were then accelerated in the magnetic field. These high energy electrons in turn collided with other argon atoms, stripping off still more electrons and the process continued in a chain reaction, breaking down the gas and yielding a plasma consisting of argon atoms, electrons and argon ions. Energy was then transferred from the plasma to the sample aerosol effecting desolvation, vaporisation, atomisation, excitation and ionisation in turn.

(ii)Mass Spectrometer. The plasma core containing the sample ions was extracted through a sample cone of the mass spectrometer into a reduced pressure region (2 mbar)

which was evacuated by a single rotatory pump. With this pressure differential across the aperture, the plasma expanded into the low pressure region as a directed supersonic jet, cooling rapidly and thus reducing the opportunities for the ion concentrations to move away from their bulk plasma values. Then the plasma was extracted through the skimmer cone with 0.75 mm aperture and there was a further drop in pressure. Both the sample and the skimmer cones were made from pure nickel, a metal which has a good thermal conductivity and mechanical robustness, and a reasonable resistance to the commonly used acids.

In the region behind the tip of the skimmer cone a negative voltage was applied to ion lenses to ensure that only positively charged species (negatively charged species were repelled and neutral species were diffused away from the system axis towards the intermediate stage diffusion pump) were extracted and focused into the entrance aperture of the quadrupole mass filter.

The quadrupole mass filter consisted of four straight molybdenum rods (12 mm diameter, 230 mm long) which were suspended parallel to and equidistant from the ion beam axis and the opposite pairs were connected together. DC and RF voltages of amplitude U and V, respectively, were applied to each pair. Then, the DC voltage was positive for one pair and negative for the other pair. By applying the proper RF and DC voltages only the positively charged species of a given mass to charge (m/z) ratio will have stable paths through the rods and will emerge from the other end. Other ions will be deflected too much and will strike the rods and be neutralised and lost there. In this way it is possible to obtain a mass spectrum.

The ions transmitted by the quadrupole were detected by a channeltron electron multiplier, as illustrated in figure 3.6.

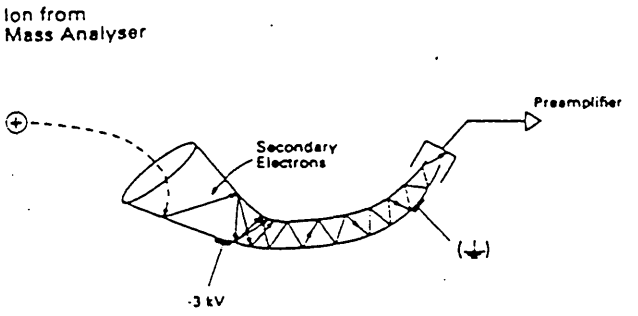


Figure 3.6.
Channeltron electron multiplier.

The operating principle is similar to that of a photomultiplier, except that there were no discrete dynodes. Instead, an open glass tube with a cone at the end was used. The interior of the tube and cone were coated with a lead oxide semiconducting material (exact composition was proprietary). When ionising ions strike the detector, one or more secondary electrons are ejected. Inside the tube, the potential varied continuously with position, so the secondary electron(s) move further into the tube to regions closer to ground. These secondary electrons hit another section of the coating and more secondary electrons are emitted. This process is repeated many times as the secondary electrons pass down the tube. The result is a discrete pulse containing as many as 10^8 electrons at the collector after an ion strikes the detector. Then, the pulse of 10^8 electrons is sensed and shaped by a fast amplifier. The output pulse from the pre-amplifier then goes to a digital discriminator and counting circuit, which counts only pulses with amplitudes above a certain threshold. The threshold level was chosen high

enough to discriminate against low-amplitude pulses caused by spurious emissions of electrons from inside the tube. The dark current count rate can be very low with a channeltron, typically 1 counts s^{-1} or less. Very weak ion signals can be sensed in this way. Electrical connections were made to the semiconducting coating through metal strips. For detection of positive ions, the cone was biased at a high negative potential (~ -3 kV) and the back of the tube near the collector was held near ground.

Laboratory Procedures.

Muromac A-1 microcolumns were inserted in the FI system and were subjected to injections of nitric acid (1 M) to remove residual contamination, followed by a rinsing step (HNO_3 0.02 M). Then, single standard solutions containing tetramethyl-, tetraethyl- dimethyl-, diethyl- or inorganic lead (typical sample volume: 20 - 1000 µl) were adjusted to pH 5 by dropwise addition of concentrated nitric acid and then passed through the Muromac A-1 microcolumn at a rate of 100 µl min^{-1}. Retained lead species were eluted from the microcolumn using nitric acid (1 M) and (100 µl; elution flow rate, 100 µl min^{-1}) and measured by ICP-MS.

C18 microcolumns were treated in the same way as Muromac A-1 microcolumns (described above).

SCF microcolumns were treated in the same way as Muromac A-1 microcolumns.

Acidic alumina microcolumns were inserted in the FI system and were subjected to injections of methanol to remove residual contamination, followed by a rinsing step (HNO_3, 0.02 M). Then, a standard solution containing tetramethyl-, tetraethyl-, dimethyl-, diethyl- or inorganic lead (typical sample volume: 20 - 1000 µl) was adjusted to pH 5 by dropwise addition of concentrated nitric acid and then passed through the acidic alumina microcolumn at a rate of 100 µl min^{-1}. Cationic (organic and inorganic) lead was not retained in the microcolumn and was measured by ICP-MS. Then, retained neutral (tetramethyl- and tetraethyl-) lead species were eluted with methanol (100 µl; elution flow rate, 100 µl min^{-1}) and measured by ICP-MS.

Field Procedures.

The cruises for the collection and processing of water samples (08.06.95) from the Manchester Ship Canal were organised by Dr. Peter Jones of the Environmental Agency (North West Region; Warrington) who made the arrangements for sampling taking account of the location of industrial discharges, possible lead dispersion and the movement of tides. The Manchester Ship Canal was selected for the determination and speciation of lead because it is known that the principal sources of tetraalkyllead in the canal originated from a plant manufacturing additives for petrol. The stations at which water samples were collected were Fishers Wharf, Stanlow Wharf and Stanlow Point as shown in figure 2.11 (chapter 2).

The collection of the water samples, for lead speciation, was shown in figure 2.12 and

was realised as follows: the water sample (1 - 2 l) was collected using a plastic bucket attached to the end of a tow rope. A water sample (1 ml) was then adjusted on collection to pH 5, by adding dropwise, a small amount of concentrated nitric acid (Merck, ARISTAR). Then, the acidic alumina microcolumn, which was cleaned previously in the laboratory, was connected to the FI system. The feed pump was switched on and an unfiltered water sample (1 ml) was passed through the microcolumn, at a flow rate of 0.5 ml min^{-1} to effect the immobilisation of neutral lead species. The effluent from the acidic alumina microcolumn, containing cationic (organic and inorganic) lead, was collected in Nalgene vials (5 ml capacity) which contained 10 µl of nitric acid). Then the acidic alumina microcolumns (5 per station) were disconnected and with the Nalgene vials (5 per station) were placed in a light tight box, returned to the laboratory and placed in the fridge. The ICP-MS measurements were performed within 48 hours of sample collection. For microcolumn analysis, the acidic alumina microcolumns, one at time, were connected to the FI system. For each sampling site five replicate analysis (corresponding to processing of five different microcolumns) were performed. The determination of neutral lead species was based on subjecting acidic microcolumns to injections of methanol (50 µl; elution flow rate, 100 µl min^{-1}) and subsequent measurements by ICP-MS. Measurements of the effluent from the microcolumn containing cationic (organo and inorganic) lead species were performed by nebulising the water sample from the corresponding Nalgene vials using the FI-ICP-MS system (nitric acid solution, 0.02 M, served as the carrier stream).

Calibration graphs were made by using mixed standard solution in the range 0 - 100 µg l^{-1}, as Pb. For blank and mixed standard solutions measurements, the above procedure was repeated using acidic alumina microcolumns made from the same batch of alumina

and Nalgene vials and pretreated with the same batch of nitric acid solutions as the microcolumns used for sampling. For each concentration value five replicate analysis (corresponding to processing of five different microcolumns) were also performed.

Measurements of total lead were performed by nebulising the water sample from the corresponding Nalgene vials using the FI-ICP-MS system. Nitric acid solution (0.02 M) served as the carrier stream and duplicate measurements were performed for each sample. Calibrations graphs were made by using mixed standard solutions in the range 0 - 100 $\mu g\ l^{-1}$. For the blank and mixed standard solutions test, the above procedure was repeated using Nalgene vials containing distilled water and treated in the same way as for real samples.

3.3. Results and Discussion.

In this section it is proposed to develop rapid speciation methodology for lead in environmental waters using the FI system illustrated in figure 3.7. Success of the scheme depends on identifying microcolumn chemistry, that display differing affinities for neutral and cationic (organic and inorganic) lead species. In an ideal situation column chemistry would be such that one class of species would be completed retained on sample injection whereas the other class would be unretained and hence proceed to the detector for quantitation. Thereafter an injection of eluent would complete the two stage measurement cycle (see figure 3.7). Studies are first directed at evaluation of various candidate column packings. Thereafter laboratory and field based studies using the preferred material, activated alumina, are reported.

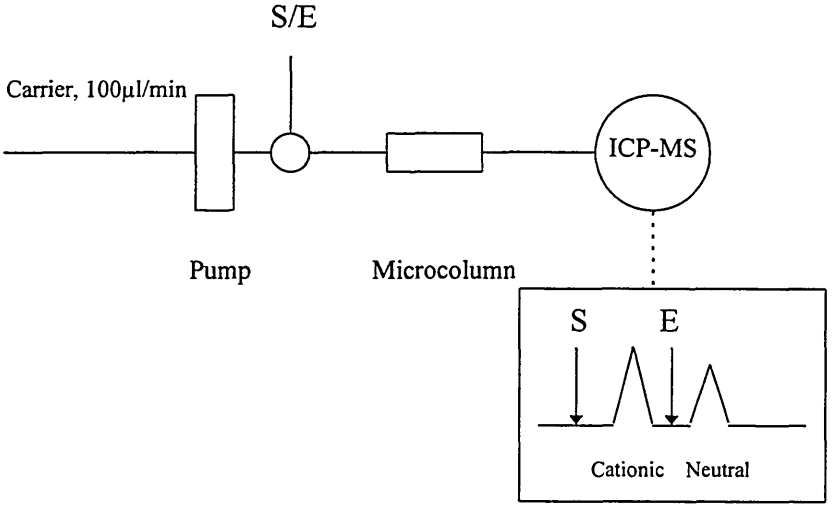


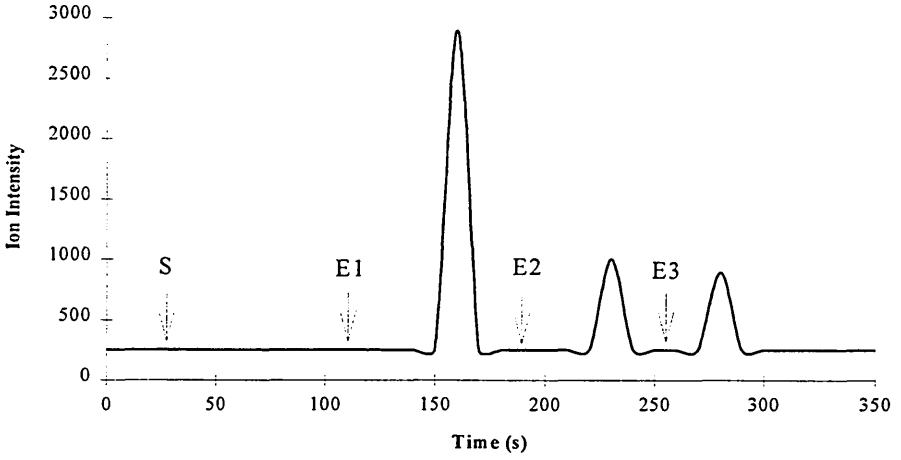
Figure 3.7.
Overview of the speciation approach for lead in natural waters.

3.3.1. Initial Studies

The evaluation of various candidate column packings (Muromac A-1, C18, SCF and activated alumina) is reported for different neutral (Me_4Pb, Et_4Pb) and cationic (organic - Me_2Pb^{2+}, Et_2Pb^{2+} - and inorganic - Pb^{2+} -) lead species. Single standard solutions of the proposed species (10 μg l^{-1}, as Pb) were processed in the FI system (full details in Experimental) independently for each material. Results for Muromac A-1, C18, SCF and activated alumina for the different lead species are shown in figures 3.8, 3.9, 3.10 and 3.11 respectively. It can be seen from figure 3.8 that there is the same behaviour for the different lead species. In all cases the absence of lead signal immediately after the injection of the sample (S) indicates the retention of lead species. The on-line injection of eluent, nitric acid 1 M (E1), removes both the neutral and cationic (organic or inorganic nature) lead species that appear in the detector after about 150 seconds. Moreover lead species are eluted completely in the first elution. The responses for the second and third elutions (E2 and E3) correspond to residual contamination. It can also be seen from figures 3.9 and 3.10 that there is the same behaviour for the different lead species using C18 and SCF respectively. As result of these data it be concluded that separation of lead species (Me_4Pb, Et_4Pb, Me_2Pb^{2+}, Et_2Pb^{2+}, Pb^{2+}) using Muromac A-1, C18 or SCF is not possible because all of them present the same behaviour independently of the organic group attached to them.

Experiments were then performed using alumina microcolumns. Single standard solutions of the proposed species (10 μg l^{-1}, as Pb) were processed in the FI system (full details in Experimental). Results for the different lead species are shown in figure 3.11.

(a)



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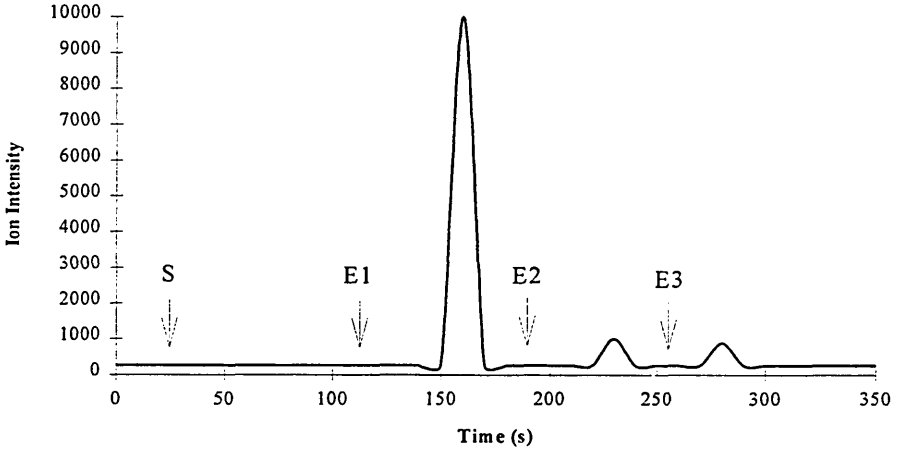


(c)

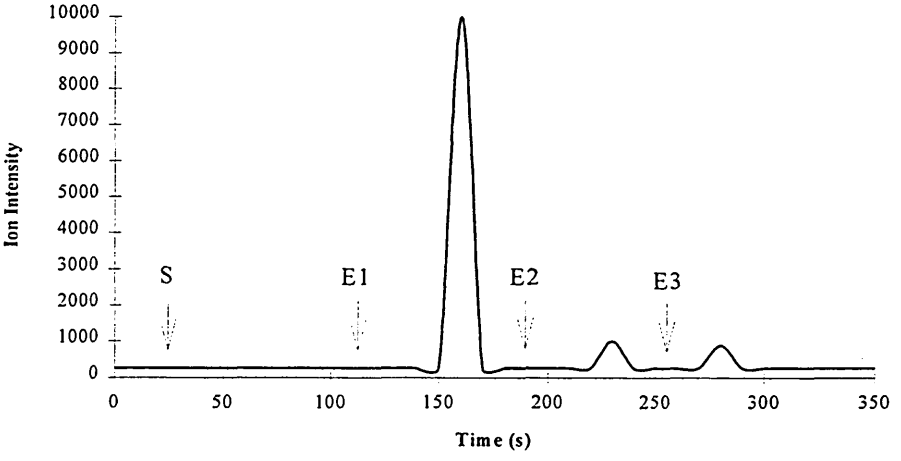
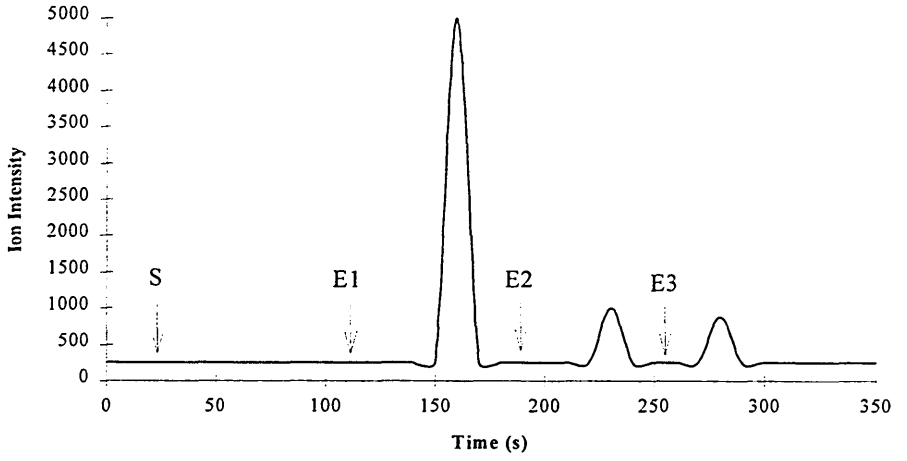


Figure 3.8.

Ion response (^{208}Pb) versus time (seconds) for processing single standard solution (10 $\mu g\ l^{-1}$, as Pb) in Muromac A-1 microcolumn for (a) neutral (Me_4Pb, Et_4Pb), (b) organic cationic (Me_2Pb^{2+}, Et_2Pb^{2+}) and (c) inorganic cationic(Pb^{2+}) lead species. (S, sample injection; E1 - E3, injections of eluent).

(a)



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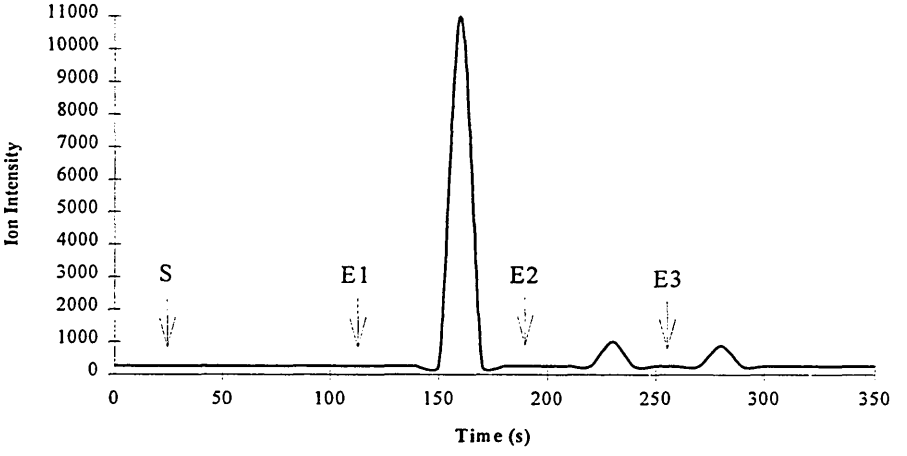


(c)

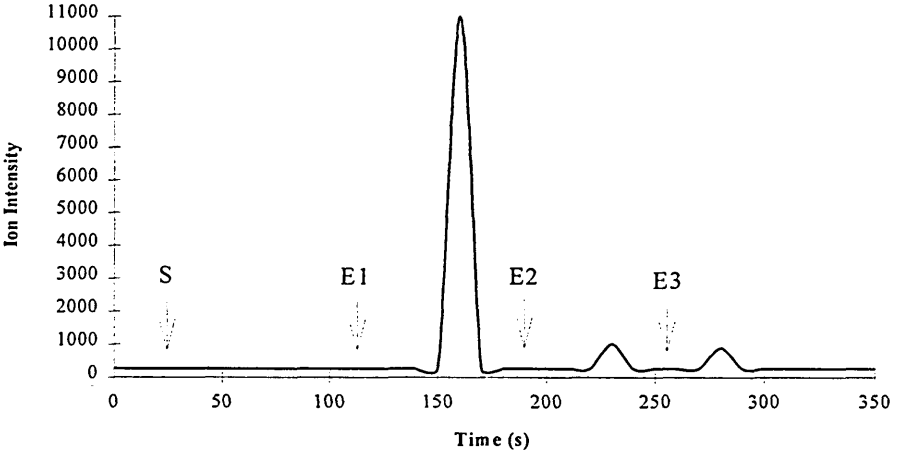
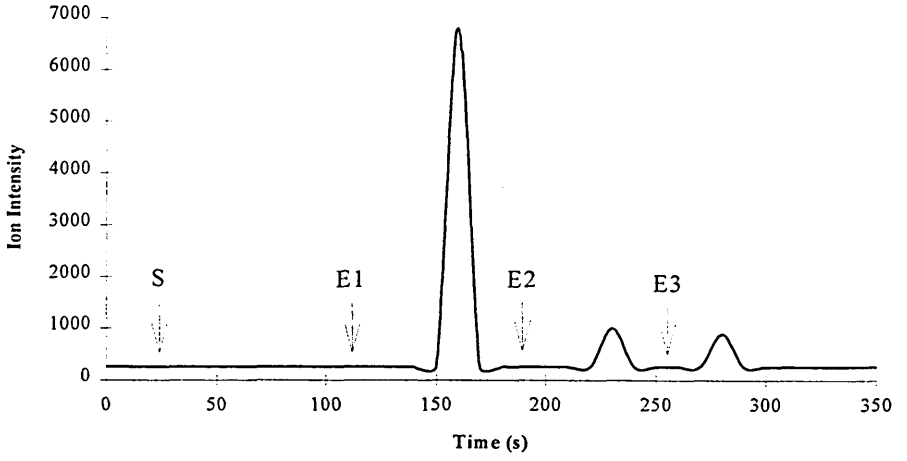


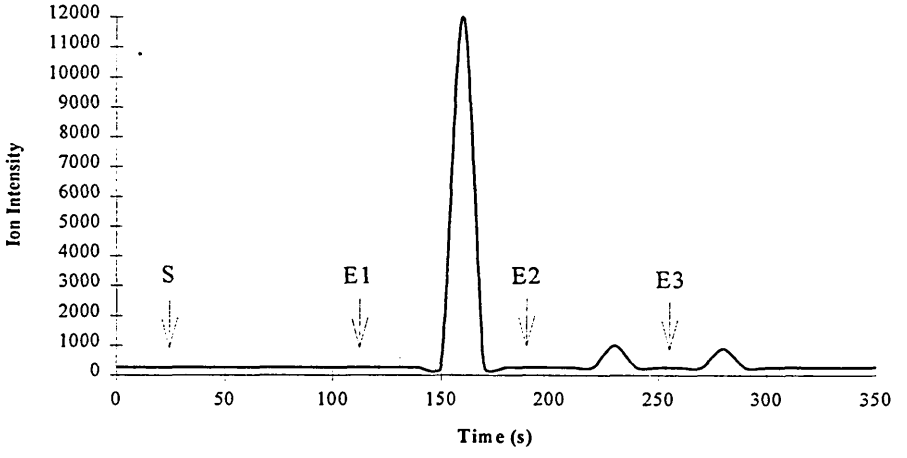
Figure 3.9.

Ion response (^{208}Pb) versus time (seconds) for processing single standard solution (10 µg l^{-1}, as Pb) in C18 microcolumn for (a) neutral (Me_4Pb, Et_4Pb), (b) organic cationic (Me_2Pb^{2+}, Et_2Pb^{2+}) and (c) inorganic cationic(Pb^{2+}) lead species. (S, sample injection; E1 - E3, injections of eluent).

(a)



(b)



(c)

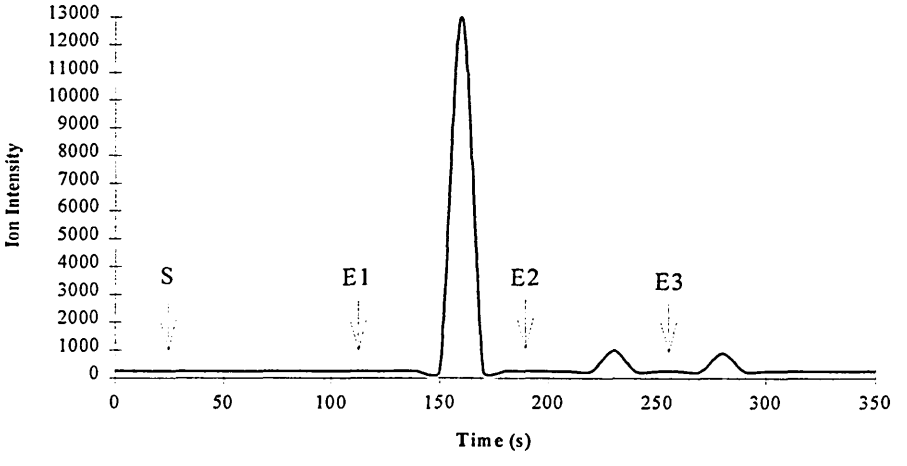
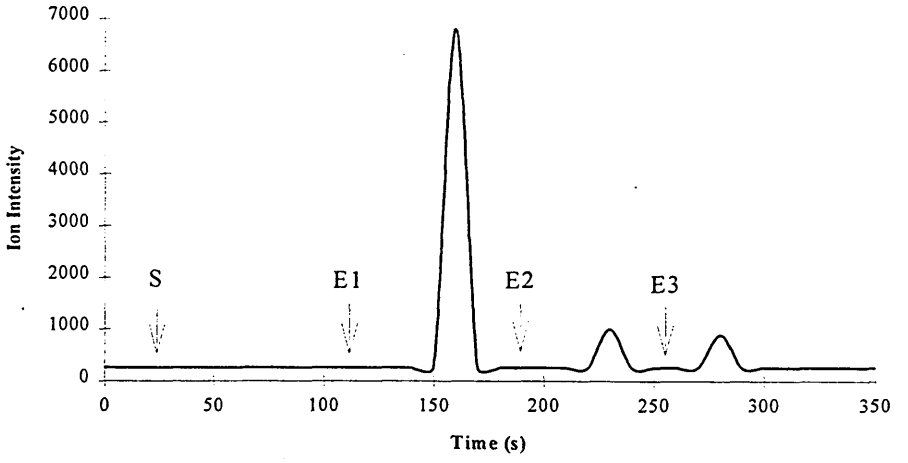
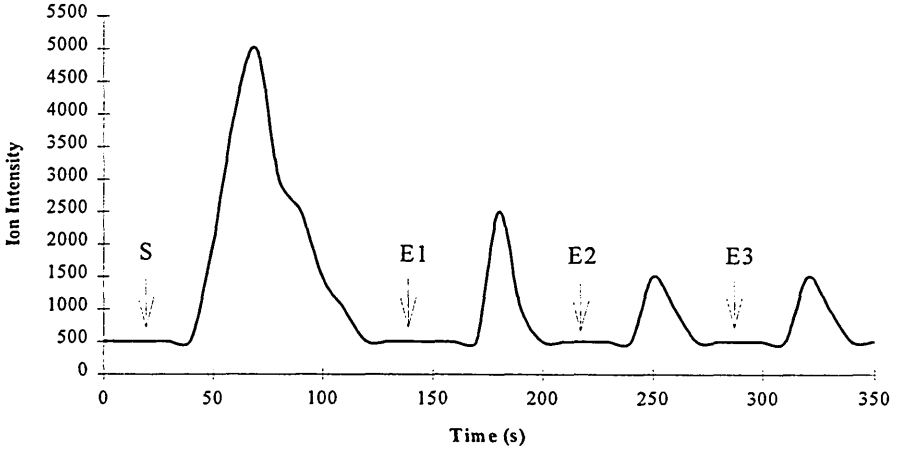


Figure 3.10.
Ion response (^{208}Pb) versus time (seconds) for processing single standard solution (10 μg l^{-1}, as Pb) in SCF microcolumn for (a) neutral (Me_4Pb, Et_4Pb), (b) organic cationic (Me_2Pb^{2+}, Et_2Pb^{2+}) and (c) inorganic cationic(Pb^{2+}) lead species. (S, sample injection; E1 - E3, injections of eluent).

(a)



(b)



(c)

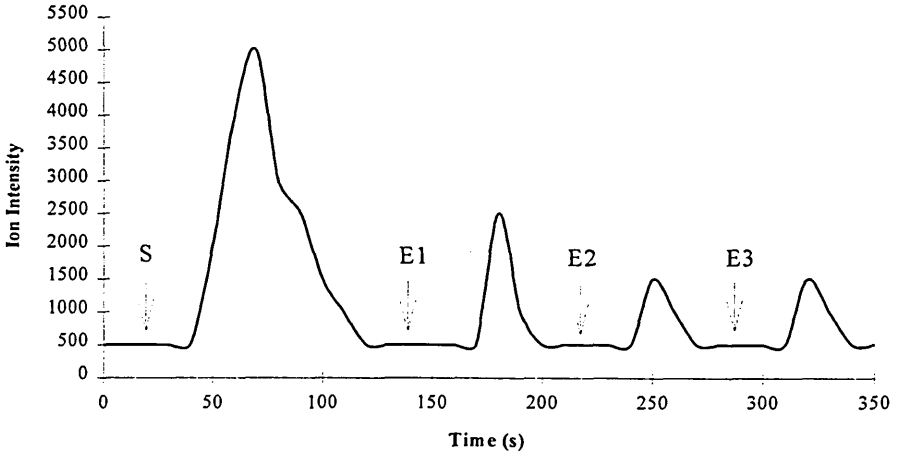


Figure 3.11.
Ion response (^{208}Pb) versus time (seconds) for processing single standard solution (10 μg l^{-1}, as Pb) in acidic alumina microcolumn for (a) neutral (Me_4Pb, Et_4Pb), (b) organic cationic (Me_2Pb^{2+}, Et_2Pb^{2+}) and (c) inorganic cationic(Pb^{2+}) lead species. (S, sample injection; E1 - E3, injections of eluent).

It can be seen that there is different behaviour for neutral (figure 3.11 a) and cationic (figure 3.11 b, c) lead species. For neutral species the absence of a lead signal immediately after the injection of the sample (S) indicates the retention of such species presumably due to physisorption via Van der Waals forces. The on-line injection of eluent, methanol (E1), removes neutral lead species which then are detected after about 150 seconds. Moreover the lead species are essentially eluted completely in the first elution. The response for the second and third elution correspond to residual contamination. It can also be seen that individual separation of neutral lead species (tetramethyl- or tetraethyllead) is not possible because both of them present the same behaviour independently of the organic group attached to them. This inability to resolve the signals of the neutral lead species is clearly a limitation of the proposed FI method. Inspection of figures 3.11 b, c indicate that cationic lead species are not retained on the acidic alumina microcolumn since there is lead response immediately after sample injection. Analyte recovery, calculated by comparing the peak area response for processing via the microcolumn relative to direct injection (without microcolumn) was 58% for Me_2Pb^{2+}, 55% for Et_2Pb^{2+} and 57% for Pb^{2+}. It can also be seen from figures 3.11 b, c that cationic lead corresponding to the first elution is also detected whereas the response for the second and third elution correspond to incomplete analyte recovery acting as a residual contamination.. This finding indicated that there is a small retention of cationic lead on acidic alumina. However, it can also be deduced from figure 3.11 that on-line separation of cationic (organic - Me_2Pb^{2+}, Et_2Pb^{2+} - or inorganic - Pb^{2+}-) lead species is not possible since they exhibit the same behaviour independently of the organic group attached to them. As result of these experiments tetramethyl- and inorganic lead were selected as a representantative of neutral and cationic lead species

respectively. Further experiments were therefore performed in an attempt to identify conditions that minimised retention for inorganic lead.

According to previous studies [Zhang et al., 1988] deposition of inorganic lead on basic alumina is dependent on sample pH (they recommended a sample pH 6). It was therefore decided to study systematically the effect of sample pH on inorganic lead deposition. In this study the pH values of standard solutions of inorganic lead (10 $\mu g\ l^{-1}$, as Pb) were adjusted to 2, 3, 4, 5, 6, 7 and 10 with nitric acid / ammonium hydroxide before processing in the FI-ICP-MS system as before. The lead response immediately after sampling was measured and analyte (Pb^{2+}) recovery was calculated by comparing the peak area response for processing via the microcolumn relative to direct injection (without microcolumn). The results are shown in figure 3.12. As can be seen there is a maximum of non-retained analyte yield at pH 5, indicating that the 90% is not retained on the acidic alumina microcolumn.

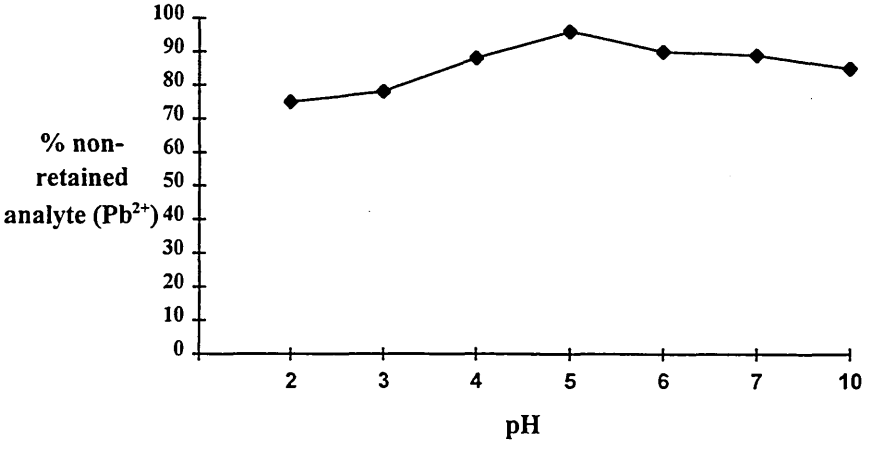


Figure 3.12.
Effect of sample pH during the deposition stage for a single standard solution of inorganic lead (10 $\mu g\ l^{-1}$, as Pb); sample volume, 50 μl.

It can also be seen in figure 3.12 that there is a decrease in non-retained analyte yield with increase of pH which is an indication of analyte retention during the deposition stage. A pH 5 was selected for further experimental work.

It is now necessary to study the deposition characteristics of tetramethyllead at pH 5. A single standard solution containing tetramethyllead (10 μg l^{-1}, as Pb; pH 5) was processed in the FI system (full details in Experimental). As shown in figure 3.13 tetramethyllead underwent deposition on the acidic alumina microcolumn because there was essentially no response corresponding to the deposition stage, as shown in figure 3.13. It can also be seen from figure 3.13 that in the first elution there is significant release of analyte whereas the response for the second and third elution correspond to incomplete analyte recovery acting as a residual contamination. Analyte (tetramethyllead) recovery is calculated by comparing the peak area response for processing via the microcolumn relative to direct injection (without microcolumn) and was 75%.

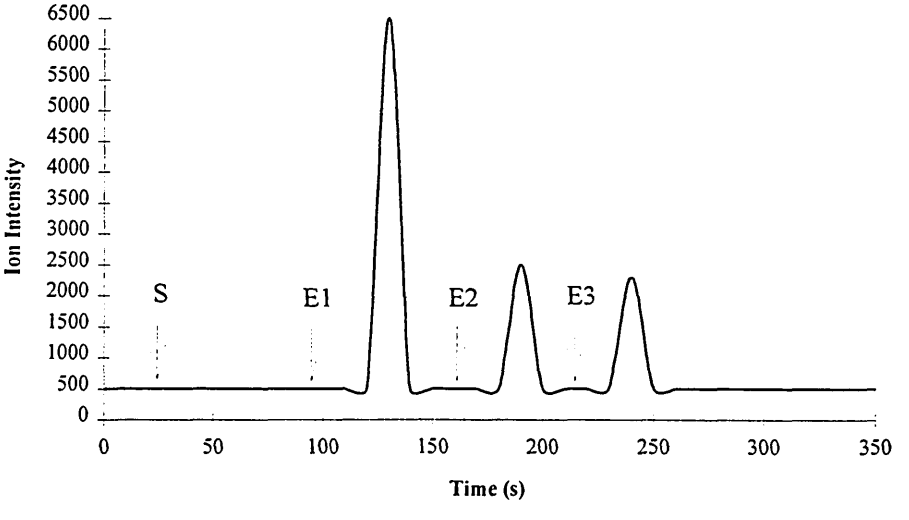


Figure 3.13.
Ion response (^{208}Pb) versus time (seconds) for processing single standard solution (10 μg l^{-1}, as Pb). S, sample injection (50 μl); E1 - E3, injections of eluent (50 μl).

As result of the above studies it is suggested that acidic alumina microcolumns may allow a rapid on-line separation of neutral (Me_4Pb) and cationic (Pb^{2+}) lead species. A mixed standard solution containing tetramethyl-, and inorganic lead (10 μg l^{-1},as Pb; pH 5) was processed in the FI system (full details in Experimental). Figure 3.14 shows a typical separation. As can be seen there is lead response immediately after sampling injection, which based on previous experiments, can be attributed to inorganic lead. Retained tetramethyllead was then eluted by injection of methanol (50 μl). As shown in figure 3.14 subsequent injections of methanol (second and third elutions) correspond to residual contamination. Analyte (inorganic and tetramethyllead) recovery, calculated by comparing the peak area response for processing via the microcolumn relative to direct injection (without microcolumn) was 79% and 82% respectively, i.e. higher than that observed in the previous experiment (figure 3.13). In the case of tetramethyllead the recovery value indicated that a single injection of methanol was effective for elution and recovery of tetramethyllead and hence only a single elution step was utilised in further work.

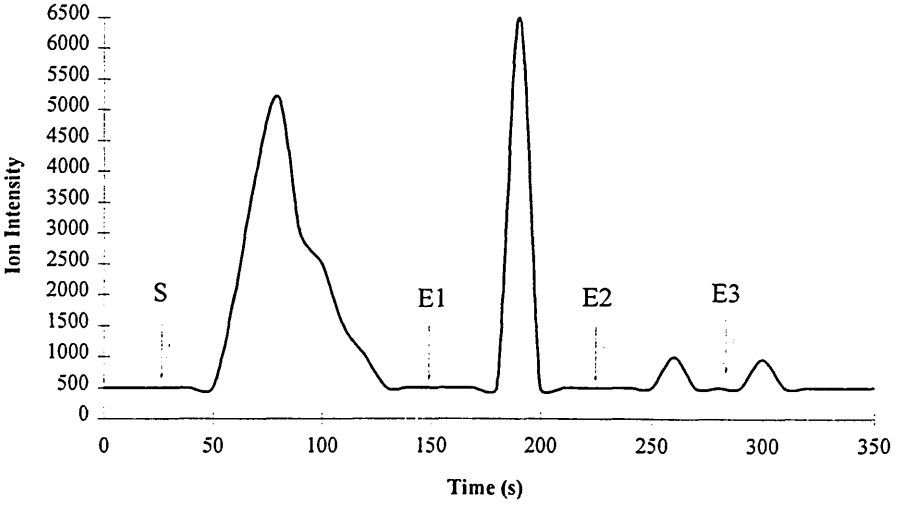


Figure 3.14.
Ion response (^{208}Pb) versus time (seconds) for processing mixed standard solution of tetramethyl- and inorganic lead (10 μg l^{-1},as Pb; pH 5). S, sample injection (50 μl); E1 - E3, injections of eluent, methanol (50 μl).

3.3.2. Basic Analytical Performance

The previous sections have been concerned with identifying analytically useful the on-line separation conditions. In order to demonstrate the potential value of the new methodology in natural water analysis basic performance data, such as linearity of response, limits of detection, precision and accuracy are next addressed.

To study signal as a function of concentration, different mixed standard solutions containing tetramethyl- and inorganic lead were prepared in the concentration range of 0, 1, 5, 10, 20 and 100 μg l^{-1} as Pb. The different standard solutions were then processed (25 μl sample volume) in the FI-ICP-MS system using different acidic alumina microcolumns (n = 3). Inorganic lead was measured and then the retained tetramethyllead was eluted by injection of methanol (25 μl) and measured by ICP-MS. The calibration graphs are shown in figure 3.15 and were linear (r = 0.999 for tetramethyl- and 0.998 for inorganic lead) over the concentration range examined.

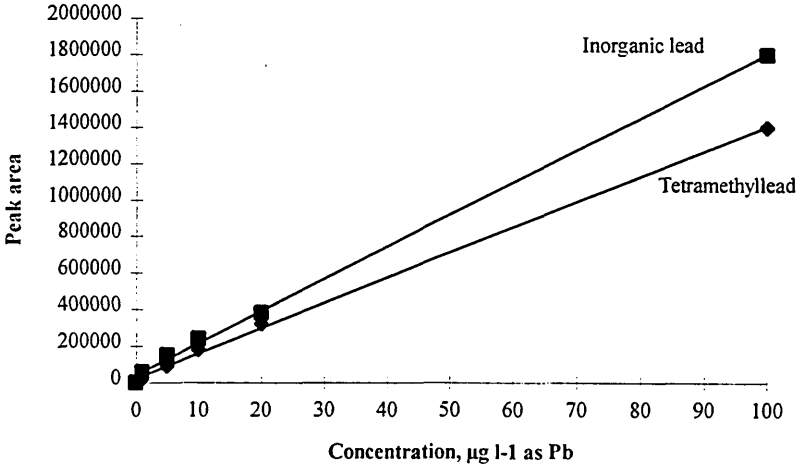


Figure 3.15.
Calibrations graphs for a mixed standard solution (tetramethyl- and inorganic lead) for the concentration range 0, 1, 5, 10, 20 and 100 μl l^{-1} as Pb.

The limits of detection for lead species were calculated as 3σ, where σ is the standard deviation of the blank and were 42 ng l^{-1} as Pb for tetramethyl- and 65 ng l^{-1} as Pb for inorganic lead. The blank measurements were based on passing 25 µl of dilute nitric acid (0.02 M) through 10 different acidic alumina microcolumns (n = 10) and referring the ion intensities for deposition (tetramethyllead) and elution (inorganic lead) (methanol) stages to the appropriate calibration curve. The analytical data are shown in table 3.2. Comparison of the method blank against the calibration blank permits a check of the sample preparation reagents and procedures for contamination control.

Tetramethyllead		***Inorganic lead***	
Ion intensity	*Concentration equivalent, µg/l*	*Ion intensity*	*Concentration equivalent, µg/l*
22131	*0.049*	*39550*	*0.042*
22110	*0.047*	*40102*	*0.073*
22230	*0.056*	*39853*	*0.059*
22310	*0.062*	*39630*	*0.046*
21753	*0.022*	*39125*	*0.018*
21842	*0.028*	*39011*	*0.011*
21803	*0.026*	*40037*	*0.069*
21945	*0.036*	*39631*	*0.046*
22155	*0.051*	*39765*	*0.054*
22161	*0.052*	*40107*	*0.073*
s = 193	*s = 0.014*	*s = 380*	*s = 0.021*

Table 3.2.
Ion intensity response and concentration equivalent for different acidic alumina microcolumns used for calculating the limit of detection of the blank (nitric acid 0.02 M; sample volume, 25 µl)

Based on considerations of detection capability of the ICP-MS (limit of detection for lead is 40 ng l^{-1}, as Pb) and concentrations of lead species in contaminated water (high µg l^{-1} levels) then substantial preconcentration factors are not required in sample pretreatment.

A measurement process should be sufficiently precise to minimise the number of replicate measurements required for the intended use. Method precision was calculated by passing 25 µl of a mixed standard solution (10 µg l^{-1} as Pb of tetramethyl and inorganic lead) through 10 different acidic alumina microcolumns (n = 10) and compared with 10 injections through a single microcolumn.

Then, the signal corresponding to the unretained lead fraction was measured by ICP-MS and the retained lead was eluted by injection of methanol (25 µl) and measured by ICP-MS. The data shown in table 3.3 yielded % RSD values of 0.33% (tetramethyl-) and 0.55% (inorganic lead). Table 3.3 also shows data for injection without acidic alumina microcolumn and it can be seen that similar RSD values were obtained: 0.33% for tetramethyl- and 0.54% for inorganic lead.

Tetramethyllead		**Inorganic lead**	
Without microcolumn	With microcolumn	Without microcolumn	With microcolumn
186872	183135	247433	240010
184246	180543	260964	248021
184227	180735	255692	249031
189219	182954	249417	261329
184423	170033	256733	270336
191829	174043	292538	252431
186688	189633	269411	243121
193503	187993	250640	283762
173503	185435	278697	241935
177595	180561	260238	253135
X = 1852105	X = 1815065	X = 262176	X = 2543111
σ = 6066	σ = 5945	σ = 14288	σ = 13859
RSD(%) = 0.33	RSD(%) = 0.33	RSD(%) = 0.54	RSD(%) = 0.55

Table 3.3.
Precision data for lead species (tetramethyl- and inorganic lead) based on processing sample without and with microcolumn. A mixed standard solution of 10 µg l^{-1} as Pb sample, 25 µl.

Accuracy is the degree of agreement of a measured value with the true or expected value. Usually the accuracy of a method is calculated by analysing reference materials (RMs) certified for elemental / species composition. In the case of environmental waters, few RMs are available for lead speciation measurement due to the problems associated with species stability, especially tetramethyllead. In this work as a test of accuracy a water sample (real water sample), which did not contain lead species, was spiked with a mixed standard solution (containing 10 μg l^{-1} as Pb of tetramethyl- and inorganic lead) in order to check analyte recoveries i.e. 50 μl samples of water were processed using different acidic alumina microcolumn (n = 5). Inorganic lead was not retained, passed through the acidic alumina microcolumn and was quantified by ICP-MS. Then, the retained tetramethyllead was eluted by injection of methanol (25 μl) and quantified by ICP-MS. A calibration graph using the same concentration of lead species was also obtained and recovery values for samples were calculated as 95±0.35 for tetramethyl- and 93±0.52 for inorganic lead. As can be seen these values indicate that the proposed method may be valid for the determination and speciation of gross organo and inorganic lead fractions in such waters. Interestingly, these results also suggest the presence of co-existing compounds present in natural waters do not affect deposition and recovery of the added lead species.

3.3.3. Field Investigations.

In this section the application of the proposed methodology to the analysis of natural waters is described. Water samples (sub-surface) were collected at three sampling sites (Fisher Wharf, S2; Stanlow Wharf, S3 and Stanlow Point, S4) in the Manchester Ship

Canal, a watercourse known to be subjected to lead discharges (the EA reported an average total lead concentration of 6 mg l^{-1} Pb along the canal), as already was pointed out in the introduction. First, data for total lead and lead speciation are reported in order to clarify whether microcolumn sampling provides a route for determination of total or dissolved lead species. Thereafter the impact of organolead in the environment is discussed.

For total lead concentration water samples (sub-surface) were collected as described earlier in the Experimental section (page 127). The data reveal a total lead concentration data of 42.0±5.9 μg l^{-1}, as Pb (Fisher Wharf), 35.5±0.23 μg l^{-1}, as Pb (Stanlow Wharf) and 70.7±2.16 μg l^{-1}, as Pb (Stanlow Point). The data are consistent with earlier data reported by the EA, 1995 and indicated that the Manchester Ship Canal is significantly contaminated with lead. As already pointed out a significant lead contribution originates from the factory manufacturing tetraalkyllead compounds (methylated, ethylated and some mixed methylethylated) for use as antiknock agents in petrol.

For lead speciation, water samples (sub-surface) were collected as described earlier in the Experimental section (page 126) and were separated into two different fractions at the sampling site: (1) the effluent from the alumina microcolumn was collected in Nalgene vials and contains cationic lead species (organic - Me_2Pb^{2+}, Et_2Pb^{2+} - and inorganic - Pb^{2+} - lead) and (2) the microcolumn which contain the immobilised neutral lead species (Me_4Pb, Et_4Pb). For lead speciation a typical ion intensity - time response for the canal water (Fisher Wharf, S2) is shown in figure 3.16 and it can be seen that the on-line injection of methanol (E1) removes the immobilised neutral lead species that appear in the detector after about 150 seconds.

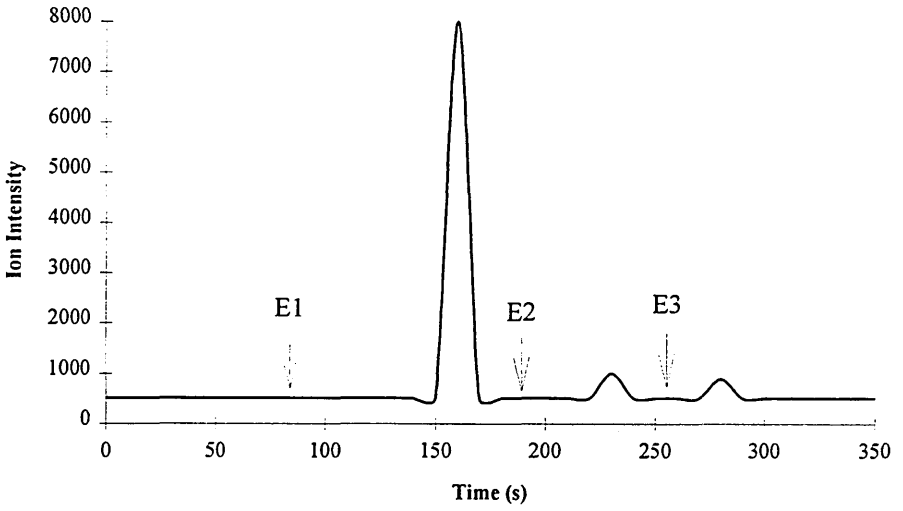


Figure 3.16.
Typical emission-time response for Manchester Ship canal water (off-line) for neutral lead species from sampling station (Fisher Wharf, S2). Sample volume: 1 ml; elution volume: 25 µl).

Two further injections of methanol (E2 and E3) serve to remove residual lead. It should also be noted that there is an absence of signals on sample injection because the separation of the different lead fractions was realised off-line at the sampling site.

The respective data for neutral, cationic (organo and inorganic) and total lead species are presented in table 3.4. It can be seen that the canal is contaminated with neutral lead species (1.33 - 34.4 µg l^{-1} as Pb) indicating that the original discharge to the Manchester Ship Canal is in this form. More information can be obtained from table 3.4.

Water Sample	*Cationic lead†, µg l^{-1} as Pb*	*Neutral lead† µg l^{-1} as Pb*	*Total lead*, µg l^{-1} as Pb*
S2	*29.0±3.97*	*1.33±0.044*	*42.0±5.91*
S3	*28.6±3.51*	*3.10±0.21*	*35.5±0.23*
S4	*39.2±6.70*	*34.4±4.12*	*70.7±2.16*

Table 3.4.
*Speciation and total lead data for the cruise (08.06.95) in the Manchester Ship Canal. Data, mean±s (n = 5). For neutral lead (sample volume, 1 ml; elution volume, 25 µl) and for cationic lead (sample volume, 1 ml). †by FI-ICP-MS, *by conventional ICP-MS.*

The data showed a total lead concentration of 42.0±5.91 μg l^{-1} as Pb (Fisher Wharf), 35.5±0.23 μg l^{-1} as Pb (Stanlow Wharf) and 70.7±2.16 μg l^{-1} as Pb (Stanlow Point). Although the data are based on only a limited number of analyses there is a degree of consistency in results for total lead and the summations of the cationic and neutral lead data (30.3±4.01 μg l^{-1} as Pb for Fisher Wharf, 31.7±3.72 μg l^{-1} as Pb for Stanlow Wharf and 73.6±10.8 μg l^{-1} as Pb for Stanlow Point). Clearly more extensive sampling and analysis is needed before any definitive conclusions can be discuss concerning possible relationship between total lead and the individual lead fractions. Nevertheless results suggest that concentrations of particulate lead (many lead compounds -lead complexes with humic substances- are associated with particulate matter derived from soils and sediments) are very low/negligible because acidic alumina microcolumns may acts as a filter, retaining also particulate lead which is not eluted by methanol at the same time as dissolved neutral lead species.

Moreover, for the three sampling stations in which water samples were analysed, elevated concentrations of cationic lead species (28.6 - 39.2 μg l^{-1} as Pb) were observed. This may reflect the fact that neutral lead species undergo transformations to cationic forms as a direct result of irradiation by UV/visible light. This is due to the fact that the concentration for cationic (organic and inorganic) lead is higher than for neutral lead species indicating their instability, as already was pointed out in the introduction (page 109). The cationic lead fraction should contains trialkyl-, dialkyl- (methylated, ethylated and some mixed methylethylated forms) and inorganic lead species, however the identification of these individual lead species is not possible using the present methodology. Future work should include the use of a hyphenated technique for individual determination of lead species.

As a result of the above data it can be said that the three sampling stations analysed in the Manchester Ship Canal water are contaminated with neutral organic lead species and as result of that there are different cationic (organic or inorganic nature) lead species in such water. This is important in terms of environmental impact of lead not only because of the high toxicity of the organolead species. The presence of high concentrations of organolead species could affect the environment in other ways and for instance may give rise or promote synergistic reactions involving other environmental contaminants e.g. the methylation of inorganic mercury to methylmercury in the Manchester Ship Canal (reported earlier in chapter 2). As already mentioned in chapter 1, the formation of methylmercury could be explained by biological methylation by bacteria and higher organisms in the presence of methyl donor molecules such as methylcobalamin [Chau, 1986; Craig, 1986]. The methyl-Co bond, shown in figure 1.1 (chapter 1), is stable but allows the transfer of CH_3^- by direct substitution according to the following reaction:

$$CH_3CoB_{12} + Hg^{2+} + H_2O \longrightarrow CH_3Hg^+ + H_2OCoB_{12}$$

It can be assumed that if the biological process was surely responsible for the formation of methylmercury in the Manchester Ship canal, the presence of methylmercury would be different, with the maximum values the highest concentration of inorganic mercury (eastern part) [Wei and McLeod, 1992]. However, by comparing the presence of neutral and cationic lead species and methylmercury in the Manchester Ship canal were noted to be similar. This similarity implies that an in situ methylation reaction of the type:

$$Hg^{2+} + (CH_3)_3Pb^+ \longrightarrow CH_3Hg^+ + (CH_3)_2Pb^{2+}$$

might be operating whereby there is a transfer of one methyl group (CH_3^-) to inorganic mercury.

In an attempt to confirm this possibility as a potential pathway of formation of methylmercury in the Manchester Ship canal a short experiment involving a simulation study in the laboratory was undertaken. Water from the Manchester Ship canal (Stanlow Point, S4) was spiked with high concentrations of inorganic mercury (mg l^{-1}, as Hg) and samples were analysed for methylmercury at regular intervals (12 and 36 hours) using GC-MIP-AES. The results are shown in figure 3.14 and it can be seen that after 12 hours the presence of methylmercury (at μg l^{-1} level) is observed and the signal increases after 36 hours which confirm the transfer of one methyl group from trimethyllead to inorganic mercury.

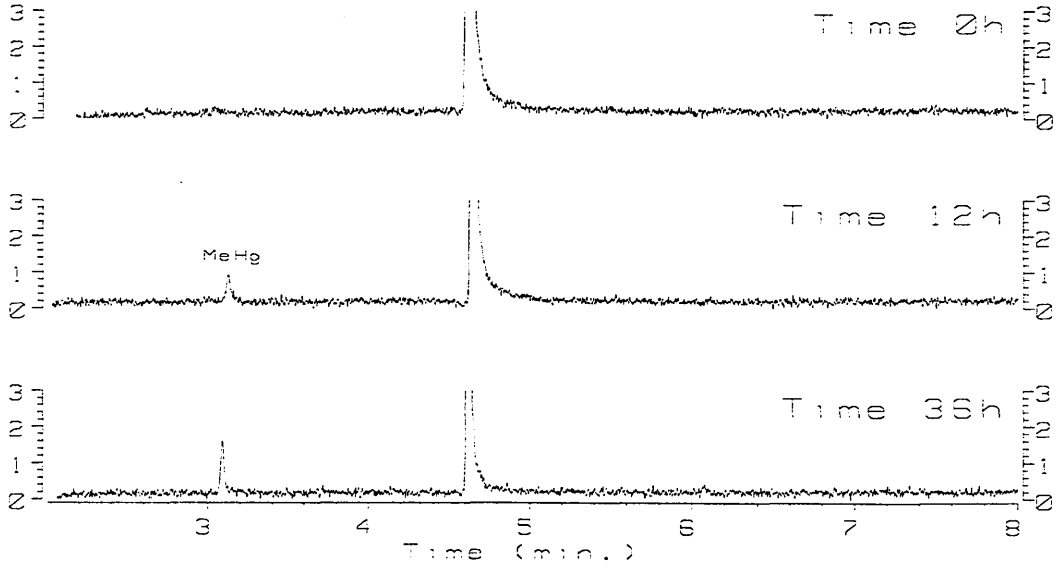


Figure 3.14.
Simulation study: canal water spiked with inorganic mercury (mg l^{-1}, as Hg).

3.4. Concluding Remarks.

The combination of acidic alumina microcolumn field sampling and FI-ICP-MS has been found successfully used for the determination and speciation of lead in environmental waters. The approach offered preservation combined with high separation ability for neutral and cationic (organic and inorganic nature) lead species. This rapid separation allows a rapid knowledge of the toxicity of the water samples indicating moreover that possible environmental pathways may be operating. However, the identification of all individual lead species is not possible using the present methodology.

Application to monitoring of the Manchester Ship Canal was demonstrated, a watercourse known to be contaminated with lead. It was found that both neutral and cationic (organic and inorganic) lead species were present in the canal in concentration ranged from 1.33 - 34.4 $\mu g\ l^{-1}$, as Pb for neutral and 28.6 - 39.2 $\mu g\ l^{-1}$, as Pb for cationic (organic and inorganic) lead species.

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Chapter 4: Reference Materials for Trace Element Speciation

4.1. Introduction.

In the last decade the determination and speciation of trace elements in environmental samples has received considerable attention. The main purpose of speciation is to provide additional information and knowledge concerning the occurrence and distribution of trace elemental species since it is this data and not total element concentration that correlates with toxicity and bioavailability. Such work in general requires the use of sensitive hyphenated analytical systems as already described in earlier chapters. However, despite the remarkable progress experienced in instrumental analytical techniques reviewed of much of the speciation data in the literature indicated that are few mechanisms available to check accuracy.

In order to correctly evaluate the state of the environment, accurate analytical data on the properties under measurement are first of all required. Accuracy can be defined as the closeness of the agreement between the result of a measurement and a true value of the measurable quantity of an analyte [ISO, 1991]. The establishment of compatible accuracy-based chemical measurement systems is become increasingly essential for environmental analysis [Quevauviller et al., 1992]. When results differs so much, they are not trustworthy and poor performance by analytical laboratories creates economic losses: extra analyses, destruction of food and goods, court actions, etc. Typical examples have illustrated the lack of accuracy that occurs in the determination of inorganic [Griepink, 1990] and organic traces [Maier, 1991] in environmental matrices. These examples are by no means exceptional and the problem is common to many field of analysis.

As a check on accuracy several approaches have been described [Rasberry, 1986]. The first one involves the use of different analytical techniques. Agreement of data obtained indicates strong evidence of accurate results. However, only a few laboratories are so well equipped to do that and an alternative is to organise interlaboratory exercises which involve a number of analytical centres with expertise in a given methodology. These two approaches can be combined in a third one: the incorporation in the analytical scheme of suitable reference materials (RM) and certified reference materials (CRM). In the most general terminology, a reference material is a substance for which one or more properties are established sufficiently well for use to calibrate a chemical analyser or to validate a measurement process. A certified reference material is a RM issued and certified by an organisation generally accepted to be technically competent to do so [Rasberry, 1986]. The CRMs are certified using the most accurate and reliable measurements techniques available consistent with the end use requirements. The RMs were used for the first time early in this century. The analytical needs were progressively approaching the domain of trace analysis and there was an increasing awareness for the obtainment of comparable results for similar materials over extended periods of time and among different laboratories. As a result of that the American Foundrymen's Association in 1905 notified the US National Bureau of Standards, formed only four years earlier, indicating that a better agreement in the analyses of cast iron was needed. This gave birth to the first four standards, consisting of cast iron, certified for chemical content. This opened up a new era in modern analytical chemistry.

In recent years, environmental pollution by heavy metals is having adverse effects on animals, plants, fish and also on human health and there is a need a need for sediments, plants, tissue and human fluid CRMs. Table 4.1 gives an overview of producers of RMs

and CRMs to date. The main organisations that have issued and certified environmental CRMs are National Research Council of Canada (NRCC), National Institute of Standards and Technology (NIST), Standards, Measurements and Testing Programme (SM&T) and National Institute for Environmental Studies (NIES) and they have proposed a CRM programme, the objective being the preparation and certification of environmental standards, to serve the need of environmental scientists and laboratories, as shown in table 4.2.

Manufacturer	Coverage	Country
Behring Institute (BI)	Biology	Germany
Bundesanstalt für Materialprüfung und -forschung (BAM)	Industry	Germany
Bureau of Analysed Samples (BAS)	Industry	UK
Central Bureau for Nuclear Measurements (CBNM)	Biology, environment, nuclear and isotopic applications	Belgium
Ferroetalon Vaskut (FV)	Industry	Hungary
Geological Survey of Japan (GSJ)	Geology	Japan
Institute of Geological Sciences (IGS)	Geology	UK
Institute of Radioecology and Applied Nuclear Techniques (IRANT)	Environment	Czechoslovakia
Instytut Metali Niezelaznych (IMN)	Industry	Poland
International Atomic Energy Agency (IAEA)	Biology, environment, nuclear and isotopic applications	International
Kaulson Laboratory (KL)	Biology, environment	USA
Laboratory of the Government Chemist (LGC)	Biology, environment, food and agriculture	UK
National Institute for Environmental Studies (NIES)	Biology, environment	Japan
National Institute of Standards and Technology (NIST)	Biology, environment, food and agriculture, industry, physical chemistry	USA
National Research Council of Canada (NRCC)	Biology, environment	Canada
National Research centre for Certified Reference Materials (NRCCRM)	Biology, environment, food and agriculture, physical chemistry	China
New Brunswick Laboratory (NBL)	Nuclear and isotopic applications	USA
Nyegaard (NYE)	Biology	Norway
South African Bureau of Standards (SABS)	Environment	South Africa
Standards, Measurements and Testing Programme (SM&T)	Biomedicine, food and agriculture, industry, environment, physical chemistry	European Community

Table 4.1.
A selection of organisations producing RMs.

CRMs	Certified components	Organisation
River Sediment (1645)	Al, Ca, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Si, Ti and Zn	NIST
Estuarine Sediment (1646)	-	NIST
Pond Sediment (No. 2)	Al, Ca, Co, Cr, Cu, F, Fe, Hg, Mn, Ni, Pb, Si, Ti and Zn	NIES
Lake Sediment (SL-1)	-	IAEA
River Sediment (S-1)	-	SM&T
Lake Sediment (S-2)	-	SM&T
Marine Sediment (MESS-1)	-	NRCC
Marine Sediment (BCSS-1)	-	NRCC
Pepperbush	-	NIES
Chlorella	-	NIES
Human Serum (No. 4)	-	NIES
Hair (No. 5)	-	NIES
Mussel	-	NIES
Nearshore water (CASS-2)	As, Cd, Cr, Co, Cu, Fe, Mo, Mn, Ni, Pb, and Zn	NRCC
Seawater (CRM 403)	Cd, Cu, Mo, Ni, Pb and Zn	SM&T
Open ocean water (NASS-4)	As, Cd, Cr, Co, Cu, Fe, Mo, Mn, Ni, Pb and Zn	NRCC
Estuarine water (SLEW-1)	As, Cd, Cr, Co, Cu, Fe, Mo, Mn, Ni, Pb and Zn	NRCC
Estuarine water (CRM 505)	Cd, Cu, Ni and Zn	SM&T
Riverine water (SLRS-2)	Al, As, Ba, Ca, Cd, Cr, Co, Cu, Fe, K, Na, Mg, Mo, Mn, Ni, Pb, Sb, Sr, U, V and Zn	NRCC
Freshwater (CRMs 399-400)	Al, Ca, Cl, Fe, K, Mg, Mn, Na and P	SM&T
Freshwater (CRMs 479-480)	NO_3^-	SM&T
Simulated rainwater (SRM 2694[a])	Ca, F, NO_3^-, SO_4^{2-}, K, Mg and Na	NIST
Simulated rainwater (CRMs 408-409)	NH_4^+, Ca, Cl, H_3O^+, Mg, NO_3^-, K, Na and SO_4^{2-}	SM&T

Table 4.2.
Different CRM for environmental analysis of trace elements [Okamoto, 1982; Quevauviller, 1994].

It can be seen from table 4.2 that the NIST (USA) has issued River Sediment Reference Material in which 13 trace elements are certified [NIST, 1978]. This material was collected from the heavily polluted area in Indiana of the United States and contains approximately 3% of chromium, which is anomalous for a typical soil or sediment. The

IAEA has issued Lake Sediment Reference Material, in which recommended values are given for 28 trace elements. The NIES has produced Pepperbush Reference Material, a botanical sample. The most typical characteristics of Pepperbush relative to the botanical Standard RMs issued by NIST are the high concentration of manganese, zinc, cobalt, nickel and cadmium. Chlorella is a typical green algae which are widely distributed in lakes, rivers and ponds. Freeze-dried Serum and Hair have also been prepared as a RM for trace element analysis.

The main limitations in the use of RM or CRM is that they are not always available due to the instability of the material. Stability is an indispensable condition to ensure that the figures attached to the certified properties are not prone to alteration in the course of time. Chemical phenomena consequent on contact with air, moisture, adsorption, reactions among different components, growth of fungi and bacteria and decomposition by exposure to light may all seriously affect the original status of the CRM. Even in the best case the full validity of the CRM expires after a certain period of time (often several years).

As can also be seen from table 4.2 all these RMs are certified for total element concentration only but it is necessary to produce RMs certified for trace element species. The nature of this task differs from procedures adopted for total element analyses. Two important considerations in the preparation of biological and environmental RM for use in the study of the chemical speciation have been proposed. The first one is that the chemical speciation of the analyte has to be similar in both the RMs and the sample. This is necessary in order to ensure the accuracy of the quantitative analyses of the various chemical species and avoids errors. The second one

is that is necessary to completely identify and characterise the chemical species of the analytes to be certified in the material. In the case of environmental samples few RMs are available for trace element speciation measurements, as shown in table 4.3, due to the problems associated with production of natural materials and maintenance of species stability.

CRMs	Organisation	Species	Concentration
TORT-1 (lobster hepatopancreas)	NRCC	Methylmercury	0.128±0.014 μg g^{-1} as Hg
DOLT-1 (dogfish liver)	NRCC	Methylmercury	0.080±0.011 μg g^{-1} as Hg
DORM-1 (dogfish muscle)	NRCC	Methylmercury Arsenobetaine	0.731±0.060 μg g^{-1} as Hg 15.7±0.8 μg g^{-1} as As
CRM 463 (tuna fish)	SM&T	Total mercury Methylmercury	2.85 μg g^{-1} total Hg 3.04 μg g^{-1} as MeHg
CRM 464 (tuna fish)	SM&T	Total mercury Methylmercury	5.24 μg g^{-1} total Hg 5.50 μg g^{-1} as MeHg
PACS-1 (marine harbour sediment)	NRCC	Tributyltin Dibutyltin Monobutyltin	1.21±0.24 μg g^{-1} as Sn 1.14±0.20 μg g^{-1} as Sn 0.28±0.17 μg g^{-1} as Sn
CRM 462 (estuarine sediment)	SM&T	Tributyltin Dibutyltin	70.5±13.2 ng g^{-1} as Sn 128±16 ng g^{-1} as Sn
CRM 11 (fish tissue)	NIES	Tributyltin Triphenyltin	1.3±0.1 μg g^{-1} as Sn 6.3 μg g^{-1} as Sn
SLRS-1 (river water)	NRCC	As(III) As(V) MMA[a] DMA[b] TMA[c] Total	0.16±0.01 ng ml^{-1} 0.18±0.02 ng ml^{-1} < 0.02 0.05±0.01 ng ml^{-1} < 0.01 0.52±0.03 ng ml^{-1}

[a] *MMA, monomethylarsenic;* [b] *DMA, dimethylarsenic;* [c] *TMA, tetramethylarsenic*

Table 4.3.
CRMs available for chemical speciation

It can be seen from table 4.3 that environmental samples such as biological tissues and sediments with certified concentrations of particular species (e.g. methylmercury, As(III), trimethyltin,...) at low concentrations have been recently produced. DORM-1, derived from spiny dogfish (Squalus acanthias) from the south-west coast of nova

Scotia (Canada), was filleted, minced, homogenised, spray-dried, acetone extracted three times, screened, tumbled, bottled and radiation sterilised. The principal arsenic species in DORM-1 was identifiable as arsenobetaine (AB). This arsenic compound has been identified in many marine animals including crabs, lobsters, fishes, sharks and shrimps. After arsenic species were extracted and concentrated, AB was determined by HPLC-ICP-MS and GFAAS. The concentration of AB was 15.7±0.8 μg g^{-1} as As and this constitutes 84% of the total arsenic in the sample. The methylmercury contents of three marine standard biological tissues (DORM-1, DOLT-1 and TORT-1) have also been reported in table 4.3. TORT-1 derived from edible grade lobster tomalley, was homogenised, spray-dried, acetone extracted three times, screened, tumbled, bottled and radiation sterilised. Methylmercury contents of the three CRMs were extracted and concentrated in either toluene or aqueous solution. Toluene solutions were required for gas chromatography analyses. The determination were made by GC-ECD, CV-AAS and ICP-MS. Agreements amongst the data from the various techniques and collaborating laboratories were excellent. The results showed that methylmercury was the only organomercury compound present in the standards in significant amounts. It constitutes about 39%, 92% and 36% of total mercury in TORT-1, DORM-1 and DOLT-1, respectively. CRMs containing tributyltin (TBT) in fish tissue and sediment have also recently become available, as shown in table 4.3. The NIES has certified the content of tributyltin (TBT) in fish tissue. Sea bass (Leteolabrax japonicus) was selected because this fish species is known to accumulate both inorganic and organic contaminants from the environment. The analysis of the tissue included extraction of TBT with methanolic HCl/ethylacetate, cleanup by means of anion and cation exchanger columns and alkylation of the TBT with propylmagnesium bromide. The propyl TBT was determined by GC-FPD, GC-ECD and GC-MS. The concentrations of TBT and dibutyltin (DBT) in

marine standard reference material PACS-1 (a sediment sample collected from the Esquimalt Harbour in British Columbia, Canada) have been determined by HPLC-ICP-MS and GC-FPD.

It can also be seen from table 4.3 that CRMs for chemical speciation are no so far available for water samples due to the problems associated with maintenance of species stability. Typical examples are methylmercury [Olson, 1977; Lansens et al., 1990] and chromium(VI) [Hem, 1977; Nriagu and Nieboer, 1988]. Lansens et al., 1990 undertook stability tests on mercury standard solutions and recommended that samples be prepared in PTFE containers and stored in a refrigerator at 4°C. If glass volumetric flasks were used for storage the glass had to be pre-treated with nitric acid. Stability studies on seawater conflict with the previous in that conversion of methylmercury chloride to inorganic mercury and adsorption to container walls were reported [Olson, 1977]. Adsorption losses were rapid for polyethylene vessels and hence glass was recommended as the preferred container medium. It was also suggested that samples, on collection, be stored in brown bottles because light may promote the conversion of methylmercury to inorganic mercury.

In the case of chromium, some progress was obtained by Dyg et al., 1990 with the development of aqueous and lyophilised chromium(III) and chromium(VI) reference materials. The study followed the changes in concentrations of the two species as function of time, solution composition, adsorption losses, temperature and choice of the material of the container. Concentrations of 25 $\mu g\ l^{-1}$ of each species were chosen as being representative. The samples contained 50 nmol l^{-1} of a HCO_3^- / H_2CO_3 buffer at a pH of 6.4 under a blanket of carbon dioxide. The choice of carbonate as an agent that

would prevent the hydrolysis of chromium(III) meant that the matrix resembled that of real water. The chromium concentrations were measured over a period of 140 days. The composition of the proposed standard was stable when it was stored at 5°C under a carbon dioxide blanket in a PTFE container. The stability studies [Dyg et al., 1990] demonstrated the reliability of the materials, which were subjected to an European interlaboratory comparison that confirmed the validity of the methodology for preparing such candidate reference materials for chromium(III)/chromium(VI) speciation.

Given the problems associated with maintaining species stability in aqueous media the possibility of achieving potential stabilisation through immobilisation on a solid support was considered an interesting research topic. Microcolumns with retained analytes could offer the ability to recover species upon elution (via elution in a FI system) with a verified, certified recovery after specified time period. The aim of this section then is to clarify whether species immobilised on solid supports can be quantitatively recovered after extended periods of storage. Work is directed at the chemistries examined in previous chapters i.e. immobilisation of mercury species on sulphydryl cotton and immobilisation of chromium species on activated alumina.

4.2. Experimental.

Reagents and Materials.

Standard solutions of inorganic mercury were prepared by appropriate dilution of stock solution (1000 mg l^{-1}, as Hg of mercury nitrate in 1% v/v HNO_3). The organomercury stock solutions (1000 mg l^{-1}, as Hg of RHgCl; R=CH_3 and C_2H_5) were prepared by dissolving the respective compounds in acetone (1 ml, ARISTAR, Merck) and standard solutions were prepared by appropriate dilution of stock solutions (1000 mg l^{-1}, as Hg). Hydrochloric acid (0.01M and 3M) was prepared from concentrated reagents (ARISTAR, Merck). Buffer solutions of sodium hydroxide 2.7 M (Merck, ARISTAR) / sodium acetate 3M (Merck, ARISTAR) were prepared by dissolving the compounds in Millipore water. Sodium tetraphenylborate was obtained from Aldrich and a 1% solution was freshly prepared in Millipore water a few minutes before use. The SCF adsorbent was produced as earlier described in chapter 2 (Experimental) [Lee and Mowrer, 1989]. SCF microcolumns (n = 30) were made by putting about 0.015 - 0.018 g of the cotton fibre into a 60 mm length of PTFE tubing (id. 1.5 mm), the absorbent being packed evenly along a 50 mm length of the column. Two other small tubes (20 mm x 0.8 mm) were fitted at the both ends of the column to allow connection of the microcolumn to the FI system.

For the determination of methylmercury by FI-CV-AFS, tin chloride solution (3% m/V) in hydrochloric acid (15% V/V) was freshly prepared each day from tin chloride 2-hydrate salt (Spectrosol, ARISTAR) and Millipore water. A potassium bromide/potassium bromate solution (0.5% m/V + 0.14% m/V) was made daily by

dissolving potassium bromide/potassium bromate salts (Fisons, ARISTAR) in Millipore water. Hydrochloric acid solution were adjusted to pH 3.5 by adding dropwise dilute nitric acid solution (0.5 M, Merck, ARISTAR).

Standard solutions of chromium(III) and chromium(VI) (200 μg l^{-1}) were prepared by appropriate dilutions of stock solutions (1000 mg l^{-1} of potassium dichromate and chromic nitrate; Merck, ARISTAR). Nitric acid (8, 2 and 0.02 M), ammonium hydroxide (2 and 0.02 M) and potassium hydroxide (2 M) were prepared from concentrated reagents (Merck, ARISTAR). Activated alumina was chromatographic grade (Merck, Brockman grade 1, particle size range 180 - 212 μm). Alumina microcolumns (n = 300) were prepared by putting about 0.095 - 0.097 g of the activated alumina into a 60 mm length of column. Two other small tubes (20 mm x 0.8 mm were fitted at the both ends of the column to allow connection of the microcolumn to the FI system.

Equipment and Operating Procedures.

Cold Vapour-Atomic Fluorescence (CV-AFS).

A CV-AFS was used to determine mercury. The basic components of the CV-AFS (Merlin, P.S.Analytical) are described in detail in chapter 2 (see Experimental). Typical operating parameters for the system are shown in table 4.3.

The CV-AFS was equipped with a flow injection (FI) system in order to effect sample injection. The FI system (figure 4.1) was a three line system and consisted of peristaltic

pump (Gilson Minipuls), rotary injection valve (Omnichem; 500 µl sample loop), a SCF microcolumn and a mixing coil (4 m x 0.8 mm id in length) which was used to ensure the total conversion of methylmercury to inorganic mercury. To minimise sample dispersion in the FI system, tube lengths were kept to a practical minimum.

Flow Injection (FI) system	
microcolumn length	50 mm (1.5 mm id.)
sulphydryl cotton packed	0.015 - 0.018 g
sampling loop	500 µl
flow-rate of streams	1.5 ml min^{-1}
Merlin Mercury Detector	
source	low pressure mercury discharge lamp
analytical line	253.6 nm
sensitivity setting	2 x 1000
flow-rate of sheath Argon	2 l min^{-1}
flow-rate of sample aeration	2 l min^{-1}
integration time	136 s
Chart Recorder	
sensitivity	1 V
chart speed	10 mm min^{-1}

Table 4.3.
Operating conditions for microcolumn-FI-CV-AFS.

The FI-CV-AFS system was set up without insertion of the SCF microcolumn, and cleaned by firstly pumping hydrochloric acid solution (5 M) through the three feed tubes for 20 min and then pumping Millipore water for 10 min. The carrier (HCl 0.01 M), the oxidant (KBr 0.5% m/V + $Curb_3$ 0.14% m/V) and the reductant ($since_2$ 3% m/V in HCl 15% w/v) streams were continuously pumped, each at a flow rate of 1.5 ml min^{-1}. They are introduced into the carrier stream which is mixed to the oxidant stream in a mixing coil of PTFE tubing (4 m x 0.8 mm) in order to ensure the conversion of

methylmercury to inorganic mercury. After passing the mixing coil the stream is mixed to the reductant stream to convert inorganic mercury to elemental mercury and enters to the liquid/gas separator for extraction of elemental mercury. Mercury signals were recorded with a Hitachi 0.56 strip chart recorder and processed by standard software routines. Typical transient signals: peak height versus time. When a smooth baseline was observed the system was ready for calibration and sample analysis.

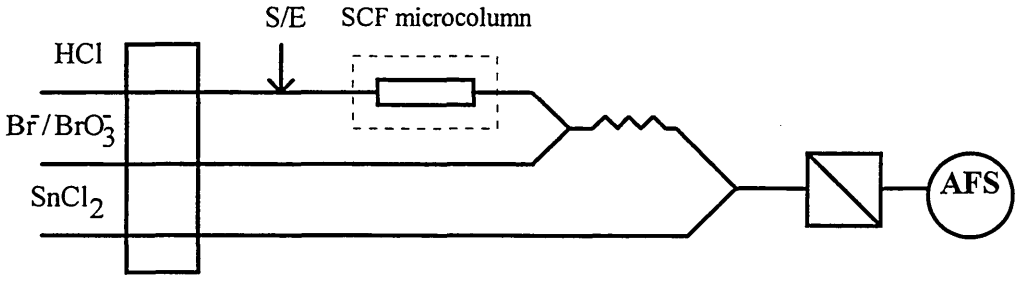


Figure 4.1.
FI system used on-line with CV-AFS for determination of methylmercury.

Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES).

An ICP-AES was used for the determination of chromium species (chromium(III) and chromium(VI)). The basic components of the ICP-AES (Spectro Analytical P), illustrated in figure 4.2, are: (i) inductively coupled plasma and (ii) spectrometer / detector. Typical operating parameters for the system are shown in table 4.4.

(i) <u>*Inductively coupled plasma*</u>. The inductively coupled plasma was equipped with a flow injection (FI) system in order to effect sample injection. The FI system (figure 4.3), a single line system, consisted of peristaltic pump (Gilson Minipuls) which was used to control the carrier stream flow rate (usually 1 ml min^{-1}), rotary injection valve

(Omnichem; 250 and 1000 µl sample loop) which was used for sample injection and alumina microcolumn which was used to separate / preconcentrate chromium species (chromium(III) and chromium(VI)). To minimise sample dispersion in the FI system, tube lengths were kept to a practical minimum.

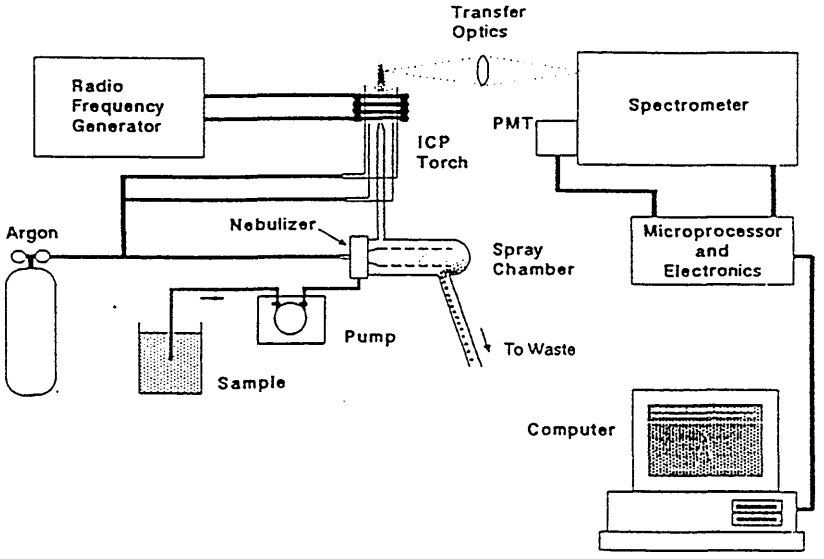


Figure 4.2.

ICP-AES diagram.

Element:	Cr
Analytical Wavelength:	267.72 nm
Nebuliser Gas Flow:	1 l min^{-1}
Auxiliary Gas Flow:	0.9 l min^{-1}
Coolant Gas Flow:	18 l min^{-1}
Power:	1100 W
Injection Volume:	250 µl
Elution Volume:	250 µl
Injection/elution Flow Rate:	1 ml min^{-1}
Total Number of Injections:	5
Signal Output / Display:	Intensity versus time 2 D plot.
Preflush Time:	13 sec
Integration Time:	30 sec

Table 4.4.
Operational conditions for the ICP-AES (Spectro Analytical P).

The liquid sample was delivered into a pneumatic nebuliser (cross flow) where the sample was dispersed by a stream of argon gas to produce a spray of droplets in the spray chamber. The spray chamber was a double pass type made from borosilicate glass and its purpose was to ensure that only the smallest solution droplets (less than 10 μm diameter) reached the plasma. Around 1% of the sprayed solution was transported to the plasma and the unused solution condensed and was pumped away from the spray chamber by the peristaltic pump.

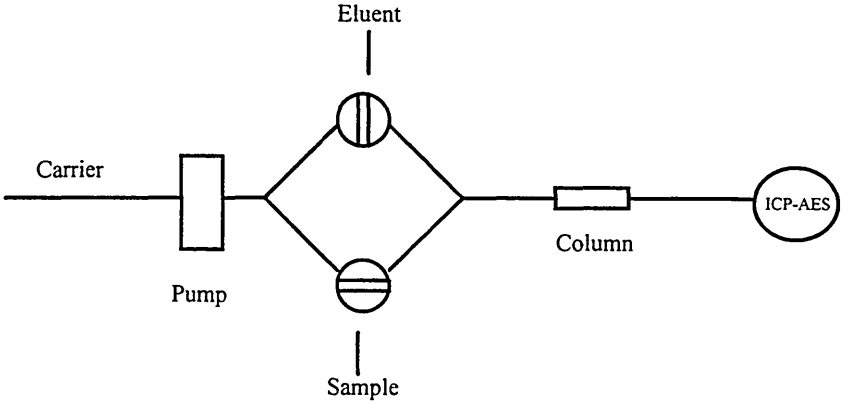


Figure 4.3.
Flow injection system for chromium speciation.

The argon gas stream containing the sample was then directed through the centre tube (18 mm inner diameter and 100 mm length) of a torch into the core of a very high temperature plasma. The plasma torch was made in one piece from quartz glass in the normal Fassel configuration. Tubes of 13 and 1.5 mm inner diameter which terminate short of the torch mouth were used for the introduction of the coolant gas flow (which protected the tube walls) and the auxiliary gas flow (which was mainly used to ensure that the hot plasma was kept clear of the tip of the central capillary injector tube, to prevent its being melted).

The radiofrequency (RF) generator provided power for the generation and sustainment of the plasma discharge. The RF generator was connected to a copper coil, called the load coil, which surrounds the top end of the torch. When RF power (typically 1000 W) was applied to the load coil, an alternating current moved back and forth within the coil at a rate corresponding to the frequency of the generator (27 MHz). This RF oscillation of the current in the coil caused RF electric and magnetic field to be set up in the area at the top of the torch. With argon gas being swirled through the torch, a spark was applied to the gas causing some electrons to be stripped from their argon atoms. These electrons were then caught up in the magnetic field and accelerate by them. These high energy electrons in turn collided with other argon atoms, stripping off still more electrons and continued in a chain reaction, breaking down the gas into a plasma consisting of argon atoms, electrons and argon ions. Energy is transferred from the plasma to the sample aerosol desolvating, vaporising, atomising, excitating and ionizating in turn.

(ii) *Spectrometer*. The radiation emitted by the excited atoms and ions in the plasma was focused onto an entrance slit and then directed to a diffraction grating where dispersion into constituents frequencies occurs. The Spectro instrument uses a monochromator based on the multiple spectrometer system and contains a single grating, multiple entrance slits and a slew-driven mount with six photomultipliers. The mount is attached through a connecting rod to a slide rest, which is driven by a stepping motor through an accurately cut spindle. Fibers optics illuminate five entrance slits (10 μm), while the six exit slits(20 μm) are mounted on a section of the arc that can rotate through 8° of the Rowland circle. The entrance slits are equipped with shutters so that the slit of interest can be opened on command. The monochromator has the same focal length (0.75 m) as the multispectrometer unit. With a 2400 groove / mm holographic

grating and an effective ruled area of 40 mm x 40 mm, the monochromator has a reciprocal dispersion of 5.5 mm/mm in the first order and a theoretical resolving power of 96,000. After the separation of the light into its components wavelengths detection is a photomultiplier tube. Typical transient signals: peak height versus time.

Microwave Oven Microdigest A-301.

An open focused monomode microwave oven Microdigest A-301 (2450 MHz, maximum power 200 Watts) (Prolabo, France) was used for the on-line digestion of chromium(III) and chromium(VI) enriched alumina microcolumns. With this system the sample is placed in the wave-guide were microwave energy is focused with maximum intensity. This focused microwave system is displayed in figure 4.4. The microwave power delivered by the magnetron is very reproducible and is controlled by a TX32 programmer. This control allows power to be incremented from 20 to 200 Watts by steps of 10 Watts. The time of exposure of the sample can be set by steps of 1 minute. The samples are placed in 50 ml open vessels system made of borosilicate glass. A refluxing unit on top of the sample holder prevents possible losses of the analytes by volatilisation.

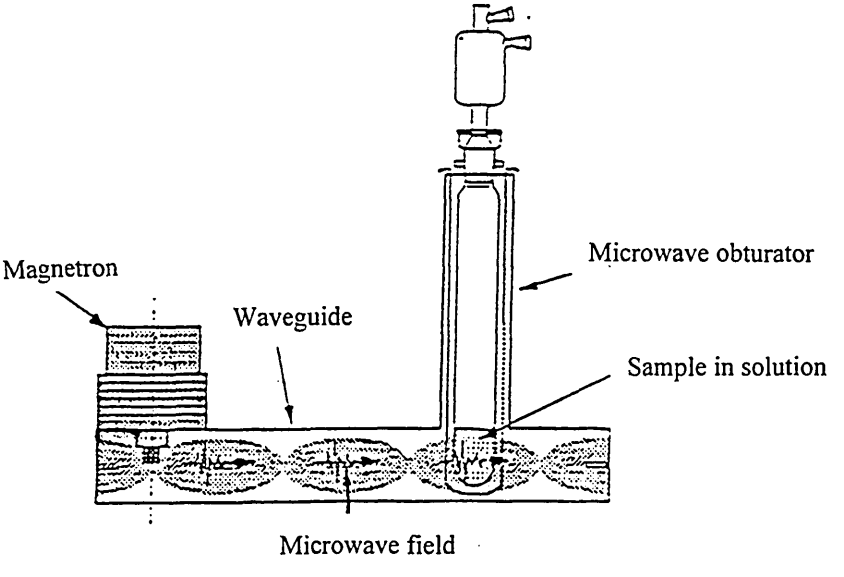


Figure 4.4.
Microwave diagram (Prolabo, France).

Analytical Procedures.

Mercury

A typical CV-AFS analysis was realised as follow: SCF microcolumns (n = 10) were inserted in the FI system and subjected to injections of hydrochloric acid (3 M) to remove residual contamination, followed by a rinsing step (HCl 0.01 M). Then, a methylmercury standard solution (10 μg l^{-1}, as mercury) was adjusted to pH 3.5 by dropwise addition of concentrated nitric acid and then passed through the SCF microcolumn at a rate of 1.5 ml min^{-1} (typical sample volume: 0.5 ml). Retained methylmercury was then eluted with hydrochloric acid (3 M; typical elution volume: 0.5 ml; elution flow rate, 1.5 ml min^{-1}). The methylmercury was oxidised to inorganic mercury by bromide / bromate solution and then reduced to elemental mercury by tin chloride reagent. Transient signals were registered on a chart recorder and quantitative measurements were based on evaluation of peak area using standard instrument software (Merlin P.S. Analytical). For comparison purposes, a single SCF microcolumn was used for processing (n=10) of the methylmercury standard solution in an identical manner to the above.

In a separate experiment, to check stability of immobilised mercury species (methyl-, ethyl- and inorganic mercury), SCF microcolumns were analysed using GC-MIP-AES. A typical GC-MIP-AES analysis was realised as follow: SCF microcolumns (n = 30) were subjected to injections of hydrochloric acid (3 M) to remove residual contamination, followed by a rinsing step (HCl 0.01 M). Then, a standard solution (containing methyl-, ethyl- and inorganic mercury, 10 μg l^{-1}, as Hg) was adjusted to pH 3.5 by dropwise addition of concentrated nitric acid and then passed through the SCF microcolumn at a rate of 1.5 ml min^{-1} (typical sample volume: 3 ml). A new fresh SCF

microcolumn was then inserted and the process repeated until loading of the 30 SCF microcolumns was completed. SCF microcolumns were placed in polyethylene bags in a light tight box and stored at 4°C in a refrigerator until analysis (over a 4 month period). Then, retained mercury species were eluted with hydrochloric acid (0.5 ml, 3M; elution flow-rate, 1.5 ml min^{-1}). The eluate (0.5 ml) was collected in an empty glass vial (5 ml capacity) which contained 1 ml of high purity water (Millipore) and buffer solution (sodium hydroxide/sodium acetate, 380 µl, 2.7 M/3 M) was added to obtain a pH of 2. Then sodium tetraphenylborate (120 µl, 5% m/V) and n-hexane (1 ml) were added and the tube shaken for 30 min using an automatic shaking machine. Excess borate reagent was added in accordance with work of Lückord and Russel, 1978 in an attempt to maximise derivatisation yield. After centrifugation (2000 rev min^{-1}) for approximately 5 min (MSE, Mistral 200, Fisons Scientific Equipment, UK) the organic phase was withdrawn using a glass pipette and placed in a screw-capped glass vial (2 ml) ready for injection (automatic) to the GC-MIP-AES.

Chromium.

A typical ICP-AES analysis was realised as follow:

Chromium (III) determination.

Alumina microcolumns (n = 10) were subjected to injections of nitric acid (2 M) to remove residual contamination, followed by a rinsing step (ammonium hydroxide 0.02 M). Then, a chromium(III) standard solution (200 µg l^{-1}) was passed through the alumina microcolumn at a rate of 1 ml min^{-1} (typical sample volume, 250 µl). Retained chromium(III) was then eluted with nitric acid (2 M; typical elution volume, 250 µl) and quantified using ICP-AES. For comparison purposes, a single alumina microcolumn

was used for processing (n = 10) of the chromium(III) standard solution in an identical manner to the above.

Chromium (VI) determination.

Alumina microcolumns (n = 10) were subjected to injections of ammonium hydroxide (2 M) to remove residual contamination, followed by a rinsing step (nitric acid 0.02 M). Then, a chromium(VI) standard solution (200 μg l^{-1}) was passed through the alumina microcolumn at a rate of 1 ml min^{-1} (typical sample volume, 250 μl). Retained chromium(VI) was then eluted with ammonium hydroxide (2 M; typical elution volume, 250 μl) and quantified using ICP-AES. For comparison purposes, a single alumina microcolumn was used for processing (n = 10) of the chromium(VI) standard solution in an identical manner to the above.

In a separate study to check long term stability of immobilised chromium species alumina microcolumns (three of each chromium species) were packed in polyethylene bags and stored under the following regimes until analysis: (1) storage at 4°C in refrigerator, (2) storage at -70°C in refrigerator and (3) oven drying at 65°C (overnight) and subsequent storage in desiccator at room temperature.

Thereafter quantitation of chromium(III) or chromium(VI) species was carried out by using three different methods:

(i) *FI-ICP-AES*.

Aged alumina microcolumns were inserted into the FI system for elution/quantitation (for chromium(III), 250 μl of HNO_3 2 M; for chromium(VI), 250 μl NH_4OH 2 M) after 1 day, 2 days, 4 days, 8 days, 11 days, 16 days, 3 months and 6 months. In order to calculate analyte recoveries, transient signals for the aged microcolumns were evaluated

with reference to signals derived from freshly charged microcolumns. Results for each analysis were based on elution for 3 separate microcolumns.

(ii) *FI-ICP-AES after acid digestion.*

Chromium(III) microcolumns.

Alumina from fresh (n=3) and aged microcolumns (n=3, oven drying at 65°C (overnight) and subsequent storage in desiccator at room temperature over an 8 days period) were removed from PTFE tube and treated in a beaker with 1 ml HNO_3 and 1 ml distilled water and heated gently for 5 minutes. The solution was transferred to a volumetric flask (5 ml of capacity). In order to calculate analyte recoveries, emission intensity signals were evaluated with reference to signals derived from freshly prepared chromium(III) standard (200 $\mu g\ l^{-1}$).

Chromium(VI) microcolumns.

Alumina from fresh (n=3) and aged microcolumns (n=3, oven drying at 65°C (overnight) for 12 hours and subsequent storage in desiccator at room temperature over an 8 days period) were removed from PTFE tube and treated in a beaker with 1 ml HNO_3 and 1 ml distilled water and heated gently for 5 minutes. The solution was transferred to a volumetric flask (5 ml of capacity). In order to calculate analyte recoveries, emission intensity signals were evaluated with reference to signals derived from freshly prepared chromium(VI) standard (200 $\mu g\ l^{-1}$).

(iii) *FI-ICP-AES with on-line Microwave Digestion.*

Chromium(III) microcolumns.

Fresh and aged microcolumns (n = 45, oven drying at 65°C (overnight) and subsequent storage in desiccator at room temperature over an 1 and 6 month period) were inserted into the FI system, incorporating a microwave oven (Microdigest, Prolabo, figure 4.5), and subjected to elution at different microwave powers and for different length of the

heating coil. In order to calculate analyte recoveries, transient signals were evaluated with reference to signals derived from freshly prepared chromium(III) standard (200 μg l^{-1}, 250 μl).

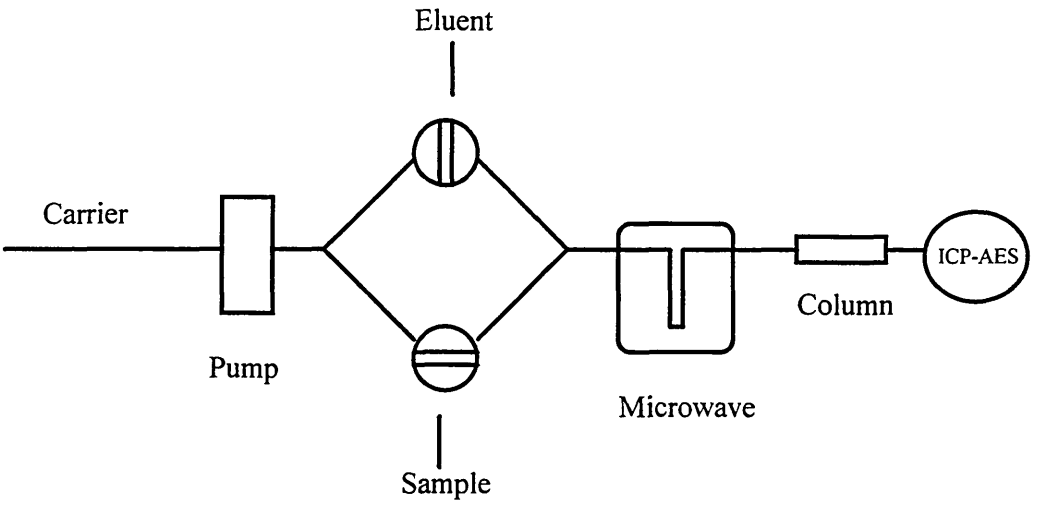


Figure 4.5.
Flow injection system using microwave oven.

Chromium(VI) microcolumns.

Fresh and aged microcolumns (n=45, oven drying at 65°C (overnight) and subsequent storage in desiccator at room temperature over an 1 and 6 month period) were inserted into the FI system, incorporating a microwave oven (Microdigest, Prolabo, figure 4.5), and subjected to elution at different microwave powers and for different length of the heating coil. In order to calculate analyte recoveries, transient signals were evaluated with reference to signals derived from freshly prepared chromium(VI) standard (200 μg l^{-1}, 250 μl).

4.3. Results and Discussion.

The work reported in this section is concerned to ascertain whether immobilisation of mercury and chromium species on a solid support would provide an effective means for stabilisation of such species at the trace level and thus offer a possibility of developing a new class of RM. Experiments are reported separately for mercury and chromium species and are first concerned to check column to column variability (homogeneity studies) in terms of recovered species. Then stability studies and examination of the effect of storage regimes on analyte recoveries are reported.

Laboratory Studies: Mercury.

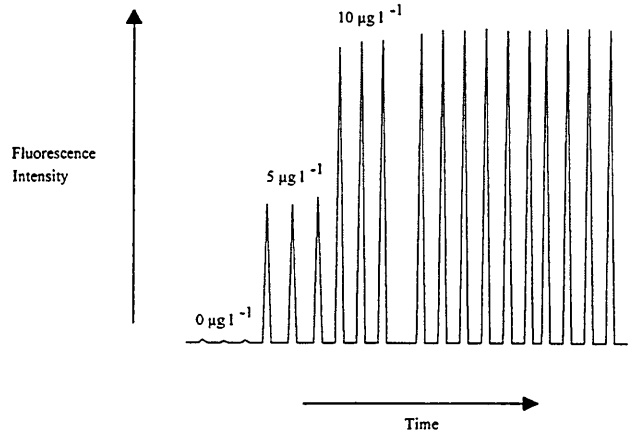
In the production of RMs one of the essentials is that the proposed material is homogeneous within the accepted range of end use requirements [Rasberry, 1986]. Homogeneity can be assessed by establishing the variation of elemental content for samples selected randomly. For assessment of homogeneity of methylmercury immobilised on SCF microcolumns, FI-CV-AFS analysis was adopted because, it offers good precision [Wei and McLeod, 1992] and hence any significant intercolumn variability should be readily detected. Ten different SCF microcolumns SCF were selected randomly and were charged with the same methylmercury standard solution (10 $\mu g\ l^{-1}$; sample volume, 0.5 ml) and analysis was repeated one time to allow calculation of relative standard deviation (RSD). For comparison purposes a single SCF microcolumn was charged repeatedly with the same standard solution and analysis was repeated 10 times to allow calculation and comparison of respective RSDs. The two data sets are shown in table 4.5 and it can be seen that the variation within SCF

microcolumns (experimental error) in terms of the RSD was 0.3% while the variation between SCF microcolumns (sample homogeneity) was 0.4%.

	Repeat analysis (single column)	Replicate analysis (10 columns)
intensity (mean)	1025	1024
s	2.88	4.31
RSD	0.3%	0.4%

Table 4.5.
Precision data for methylmercury species (n = 10; 10 μg l^{-1}, as Hg).

(a)



(b)

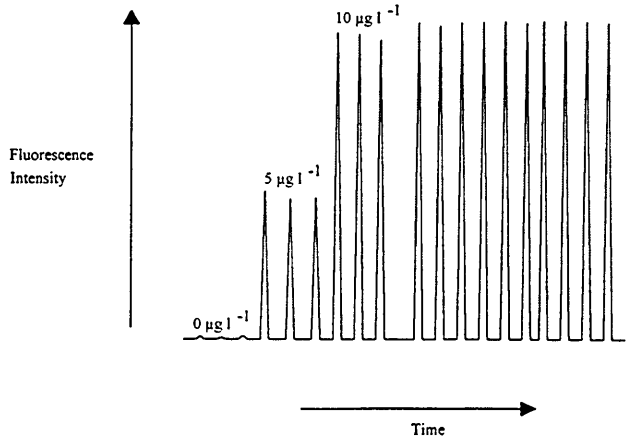


Figure 4.6.
Typical FI-CV-AFS transient signal (fluorescence versus time) for the (a) repeat analysis (single column) and (b) replicate analysis (10 columns) (10 μg l^{-1}, as Hg; sample volume, 0.5 ml).

These results indicate that the variation associated with the sample variability was similar to the analytical error. These results also indicate the high precision of the FI-CV-AFS method and to the fact that prepared SCF microcolumns were homogeneous and contained essentially identical quantities of analyte. A typical FI-CV-AFS for the repeat and replicate analysis is shown in figure 4.6. On the basis of this finding it was considered appropriate to proceed with stability studies.

Stability of immobilised mercury species (methyl-, ethyl- and inorganic mercury).

For stability studies a batch (n = 30) of SCF microcolumns were prepared and charged with a mixed mercury standard solution (10 $\mu g\ l^{-1}$, as Hg containing methyl-, ethyl- and inorganic mercury; sample volume, 3 ml) and stored at 4°C in a light tight box. At regular time intervals (1 hour, 1 week, 2 months and 4 months) microcolumns were removed and mercury species were quantified by GC-MIP-AES (after elution/derivatisation/ extraction). Unlike the FI-AFS procedure the GC method does provide quantitation of individual organic compounds, however, method precision is typically around 5% RSD for methyl-, 10% RSD for ethyl- and 12% RSD for inorganic mercury.

At each time interval methyl-, ethyl- and inorganic mercury were determined by subjecting microcolumns (n = 3) to the GC-MIP-AES procedure. For each microcolumn/final extract, automatic injection (1 µl) was performed in triplicate thus a total of 9 measurements for each species were acquired. Analyte signal intensities (peak height) were referred to emission intensities derived from freshly prepared mixed mercury standard solution in order to calculate recoveries. The standard solution was processed three times and, for each extract, automatic injection was performed in

triplicate again to give a total of 9 measurements (for each species). Recovery data are summarised in table 4.6 while a typical GC response for fresh and aged (2 months) microcolumns is presented in figure 4.7.

Mercury Species	1 hour	1 week	2 months	4 months
Methyl-	100±8	103±10	100±6	96±6
Ethyl-	100±12	100±8	97±6	7±1
Inorganic	99±12	99±6	97±8	102±10

Table 4.6.
Analyte recoveries as function of storage time for mercury species (10 μg Hg l^{-1} methyl-, ethyl- and inorganic mercury; 3 ml) immobilised on sulphydryl cotton over a 4 months period.

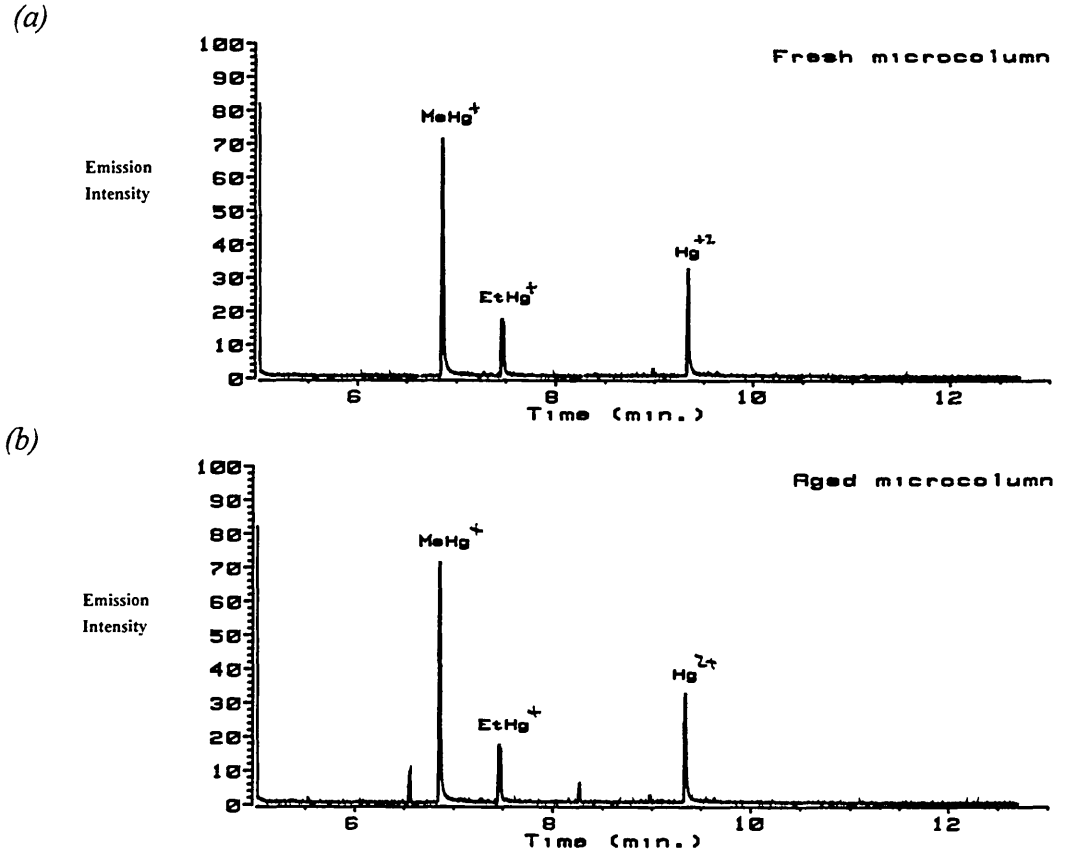


Figure 4.7.
Typical GC-MIP-AES response for (a) fresh and (b) aged microcolumns over a 2 months period (10 μg Hg l^{-1} methyl-, ethyl- and inorganic mercury; 3 ml) immobilised on sulphydryl cotton over a 4 months period.

It is clear that analyte recoveries are essentially quantitative throughout the 4 months study, the only exception being ethylmercury chloride. While ethylmercury species appear to be stable for up to 2 months, poor recovery data are obtained for 4 months (recovery, 7%). This result may be interpreted as loss of analyte or poor recovery in the method itself. Further work would be needed to clarify this point.

Given the relatively long time needed for analysing samples using GC-MIP-AES (30 minutes per run) the possibility of instability of mercury species in the organic extract was examined. For this study a batch (n = 30) of glass vials containing the organic extracts were prepared with a mixed mercury standard solution (10 μg l^{-1}, as Hg containing methyl-, ethyl- and inorganic mercury; sample volume, 3 ml) containing buffer solution (sodium hydroxide/sodium acetate, 370μl, 2.7 M/3 M) was added to obtain a pH of 2. Then sodium tetraphenylborate (120 μl, 5% m/V) and n-hexane (1 ml) were added and the tube shaken for 30 min using an automatic shaking machine. After centrifugation (2000 rev min^{-1}) for approximately 5 min the organic phase was withdrawn using a glass pipette and placed in a screw-capped glass vial (2 ml), ready for injection (automatic) to the GC, and stored at -70°C and at room temperature over a 4 moth period. At regular time intervals (1 hour, 1 week, 2 months and 4 months) glass vials were removed and mercury species were quantified by GC-MIP-AES. At each time interval methyl-, ethyl- and inorganic mercury were determined by subjecting glass vials (n = 3) to the GC-MIP-AES analysis.

For each vial, automatic injection (1 μl) was performed in triplicate. Thus a total of 9 measurements for each species were acquired. Analyte signal intensities (peak height) were referred to emission intensities derived from freshly prepared mixed mercury

standard solution in order to calculate analyte recoveries. The standard solution was processed three times and, for each extract, automatic injection was performed in triplicate again to give a total of 9 measurements (for each species). Recovery data for vials stored at (a) -70°C and (b) room temperature are summarised in table 4.7. It is clear that analyte recoveries are essentially quantitative throughout the 4 months study, although the storage at room temperature would not be recommended due to a partial loss of the solvent (n-hexane).

Storage regimes	Species	0 hours	1 week	2 months	4 months
-70°C	Methyl-	100±1	103±3	99±5	99±5
	Ethyl-	100±3	102±1	105±5	105±5
	Inorganic	100±2	105±2	103±3	103±3
Room Temp	Methyl-	100±3	101±4	104±4	104±4
	Ethyl-	100±2	103±2	101±3	101±3
	Inorganic	100±1	105±3	104±3	104±3

Table 4.7.
Analyte recoveries as function of storage time for mercury species extracted into n-hexane (10 μg Hg l^{-1} methyl-, ethyl- and inorganic mercury; 3 ml) and stored at -70°C and room temperature on glass vials over a 4 months period.

Based on this short study it is clear that immobilisation of mercury species (methylmercury chloride, inorganic mercury) on SCF provides an effective means for analyte stabilisation/preservation. The possibility, therefore, exists to develop new RMs and calibrants based on immobilisation technology. Microcolumn sample processing is seen as an increasingly important trend in ultratrace analysis methodologies and hence new demands for RMs prepared in column format can be anticipated. As shown in the present study column format is versatile and practically useful in that it is compatible with both on-line/FI methodology and also with classical/batch operations. For follow up work it is planned to circulate enriched microcolumns (low level, high level) to

specialist laboratories and subject samples to rigorous interlaboratory study. At the same time alternative solid support media will be tested.

Laboratory Studies: Chromium.

Speciation of chromium species (chromium(III) and chromium(VI)) is based on FI-ICP-AES methodology using alumina microcolumns used earlier [Cox et al., 1985; Cox and McLeod, 1986; Cox and McLeod, 1992].

For assessment of homogeneity of chromium species immobilised on alumina microcolumns, ICP-AES analysis was adopted because it offers good precision [earlier [Cox et al., 1985; Cox and McLeod, 1986; Cox and McLeod, 1992] and hence any significant intercolumn variability should be readily detected. Ten different basic alumina microcolumns were selected randomly and were charged with the same chromium (III) standard solution (200 $\mu g\ l^{-1}$; sample volume, 250 μl) and analysis was repeated one time to allow calculation of respective relative standard deviation (RSD). For comparison purposes a single basic alumina microcolumn was charged repeatedly with the same chromium(III) standard solution and analysis was repeated ten times to allow calculation and comparison of respective RSD. The two data sets are shown in table 4.8 and it can be seen that the variation within basic alumina microcolumns (experimental error) in terms of the RSD was 3% while the variation between basic alumina microcolumns (sample homogeneity) was 3%. These results indicate that the variation associated with the sample variability is the same to the analytical error. These results also indicate that prepared basic alumina microcolumns were homogeneous and

contained essentially identical quantities of analyte. Typical ICP emission-time responses corresponding to elution of chromium(III) is given in figure 4.8.

	Repeat analysis (single column)	Replicate analysis (10 columns)
intensity (mean)	2310	2290
s	61	72
RSD	3%	3%

Table 4.8.
Precision data for chromium(III) species (n = 10; 200 μg ml^{-1}; sample volume, 250 μl).

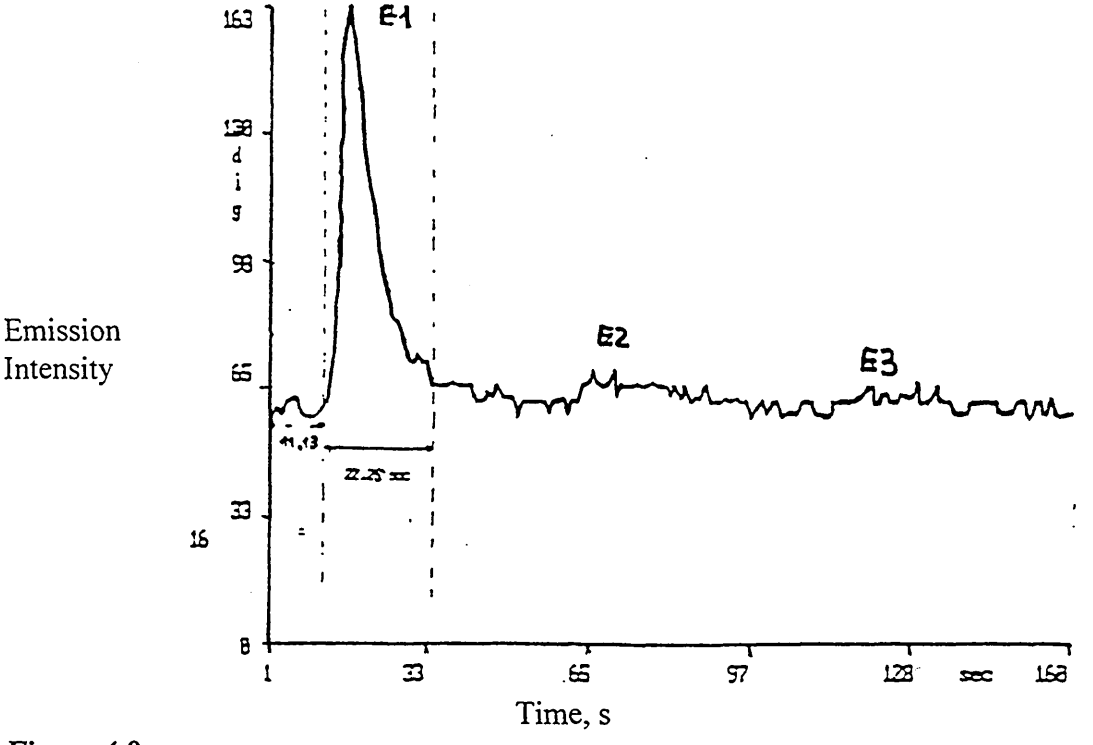


Figure 4.8.
Typical ICP emission-time responses corresponding to three elutions (E1-E3) of Cr(III). Standard solution, 200 μg l^{-1}, 250 μl.

In a separate experiment, ten different acidic alumina microcolumns were selected randomly and were charged with the same chromium (VI) standard solution (200 μg l^{-1}; sample volume, 250 μl) and analysis was repeated one time to allow calculation of respective relative standard deviation (RSD). For comparison purposes a single acidic alumina microcolumn was charged with the same chromium(VI) standard solution and analysis was repeated ten times to allow calculation and comparison of respective RSD.

The two data sets are shown in table 4.9 and it can be seen that the variation within acidic alumina microcolumns (experimental error) in terms of the RSD was 2% while the variation between acidic alumina microcolumns (sample homogeneity) was 3%. These results indicate that the variation associated with the sample variability is similar to the analytical error. These results also indicate that prepared acidic alumina microcolumns were homogeneous and contained essentially identical quantities of analyte. Typical ICP emission-time responses corresponding to elution of chromium(VI) is given in figure 4.9. On the basis of this finding it was considered appropriate to proceed with stability studies.

	Repeat analysis (single column)	Replicate analysis (10 columns)
intensity (mean)	2336	2346
s	57	78
RSD	2%	3%

Table 4.9.
Precision data for chromium(VI) species (n = 10; 200 μg ml^{-1}; sample volume, 250 μl).

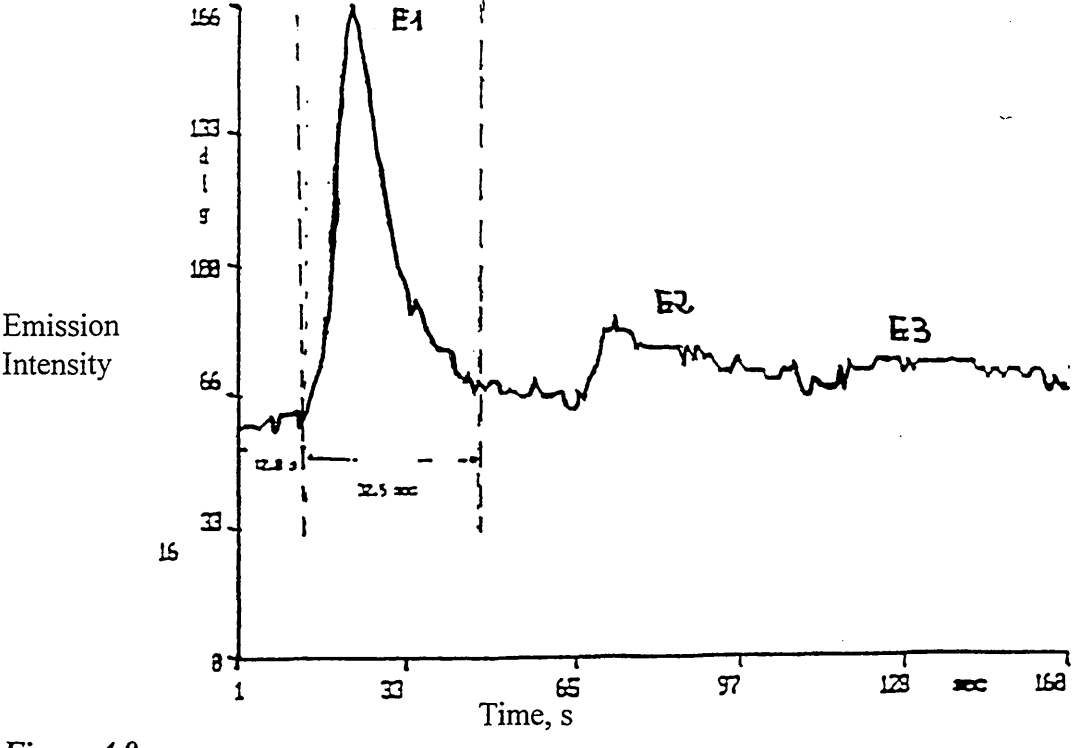
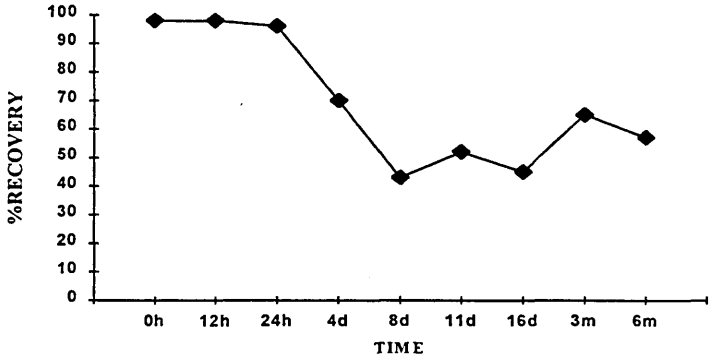


Figure 4.9.
Typical ICP emission-time responses corresponding to three elutions (E1-E3) of Cr(VI). Standard solution, 200 μg l^{-1}, 250 μl.

Stability of Immobilised chromium Species.

Alumina microcolumns (n=108) were charged with chromium (acidic alumina: chromium(VI) 200 μg l^{-1}, 250 μl; basic alumina: chromium(III) 200 μg l^{-1}, 250 μl) and then stored at 4°C until analysis. The charged microcolumns were then inserted into the FI system at appropriate time intervals and subjected to elution/quantitation. From the analytical data presented in figure 4.10 (a, b) there would seem to be a progressive decline in analyte recovery with time over a 16 day period. For the first 24 hours analyte loss in the case of chromium(VI) (figure 4.10a) was marginal but within 8 days recoveries for both chromium(VI) (figure 4.10a) and chromium(III) (figure 4.10b) were of the order 47±3 and 47±4 respectively.

(a)



(b)

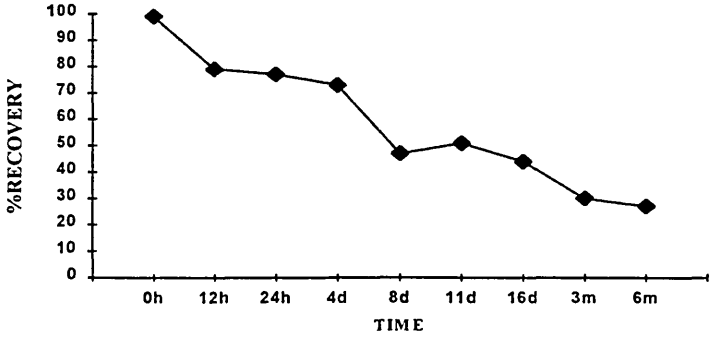


Figure 4.10.
Effect of storage time on recoveries of (a) Cr(VI) and (b) Cr(III) species. Standard solution, 200 μg l^{-1}, 250 μl.

Generally between 8 days and six months recovery values were relatively constant for chromium(VI) (40 - 60 %) whereas there was a progressive decline for chromium(III). A possible explanation for reduced recovery is a gradual and increasing loss of analyte to residual fluid in the microcolumns (0.02 M NH_4OH or 0.02 M HNO_3). Another possibility is the incomplete removal of analyte during the elution step. Further experiments concerning variation in storage conditions were next attempted in order to gain insight into the loss mechanisms.

Alumina microcolumns (n = 180) were charged as above and then subjected to 3 different storage regimes before analysis: (1) storage at 4°C as before, (2) storage at -70°C and (3) oven drying at 65°C (overnight) and subsequent storage in desiccator at room temperature. Aged microcolumns were then inserted into the FI system at appropriate time intervals and subjected to elution (for chromium(III), 250 µl, HNO_3 2M; for chromium(VI), 250 µl, NH_4OH 2M). The results for the analysis performed over an 8 days period, are summarised in table 4.10.

		24 hours	48 hours	4 days	8 days
Cr(III)	**I**	79±4	77±1	73±3	54±3
	II	61±6	62±5	62±3	49±5
	III	26±1	27±1	25±1	21±1
Cr(VI)	**I**	98±5	97±2	73±5	57±3
	II	47±3	50±4	54±1	50±2
	III	44±3	49±3	51±1	21±2

I. Storage at 4°C, II. Storage at -70°C, III. Oven drying (70°) and storage in desiccator. See experimental for full details.

Table 4.10.
Analyte recovery (%) for chromium species under various storage regimes (n=3).

It can be seen that for both chromium forms and for all storage conditions there still remains considerable signal loss. The magnitude of analyte recoveries for the experiments performed at 4°C (for both chromium(VI) and chromium(III), of the order 57±3% and 54±3% respectively) correlate well with the data of figure 4.10a,b. Storage of microcolumns at -70°C tended to yield relatively constant recoveries within the 8 days period for both chromium(VI) (47±3% - 54±1%) and chromium(III) (49±5% - 62±3%). Oven drying and subsequent storage in a dessicator also resulted in relatively constant but low recoveries: chromium(III) (21±1% - 27±1%) and chromium(VI) (44±3% - 51±1%).

On the basis of the above results it would seen that for aged microcolumns the strength of bonding between chromium species and the alumina structure altered with storage time and thus in order to achieve effective elution more vigorous conditions for the elution step are needed. This was confirmed by subjecting aged columns to conventional hot plate digestion with nitric acid. Recoveries for chromium(III) were 101±1% and 96±5% for fresh and aged microcolumns, respectively and for chromium(VI) 99±5% and 98±5%.

Attempts were made to see whether improved recoveries under continuous flow conditions could be obtained through variation in key FI parameters: (i) eluent strength, elution flow-rate and elution volume (these variables affect the electrostatic attraction phenomena), and (ii) temperature (which affects the kinetics of the reaction because first, it affects the dispersion by changing the diffusion coefficient and introducing temperatures gradients. Secondly, it affects the rate of chemical reactions). For the study

of the temperature, a microwave reactor was incorporated into the FI system as shown in figure 4.5.

Eluent strength (figure 4.11a,b), elution flow-rate (figure 4.12a,b) and elution volume (figure 4.13a,b) were optimised separately for chromium(VI) and chromium(III). Optimisation was studied by the unvariate method in all cases. Aged microcolumns (oven drying at 65°C overnight and storage in desiccator at room temperature over an 1 month period) were eluted with different:

Eluent strength. For chromium(VI), aged microcolumns were eluted with 0.5, 1, 2 and 4 M NH_4OH and with 0.5, 1 and 2 M KOH. The results are represented in figure 4.11a. The black line represents KOH and the line with marks represents NH_4OH. The maximum in analytes recoveries are 40% and 80% for NH_4OH (4M) and KOH (2M) respectively. For chromium(III), aged microcolumns were eluted with 0.5, 1, 2, 4 and concentrate HNO_3. The results are represented in figure 4.11b. The maximum in analyte recoveries are lower 20% for HNO_3 (4 M).

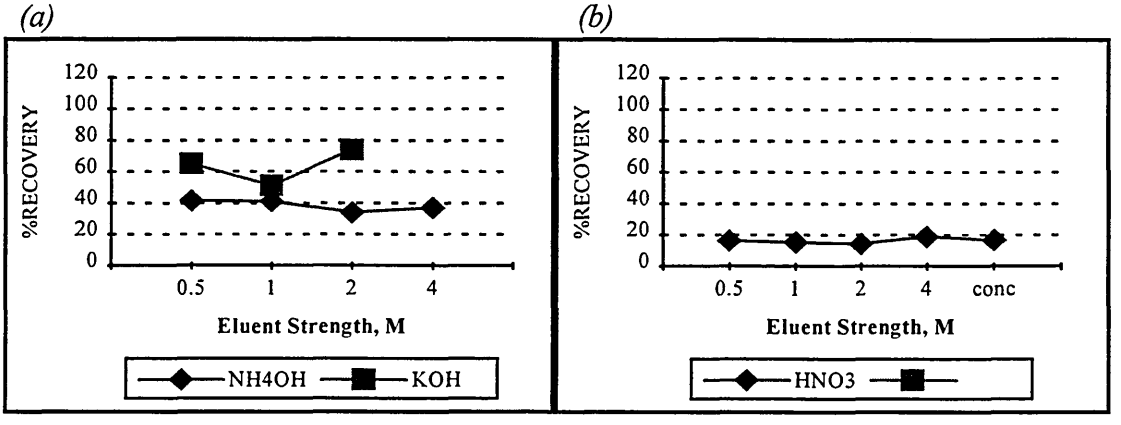


Figure 4.11.
Effect of eluent strength (M) on analyte recoveries (a) chromium(VI) and (b) chromium(III). Standard solution, 200 μg l^{-1}, 250 μl.

Elution flow-rate. For chromium(VI), aged microcolumns were eluted with 0.3, 0.5, 1

and 2 ml min^{-1} of NH_4OH (4M) and KOH (2M). The results are represented in figure 4.12a. The black line represents KOH and the line with marks represents NH_4OH. The maximum in analyte recoveries are 40% and 80% for 0.3 and 0.5 ml min^{-1} for NH_4OH and KOH respectively. For chromium(III), aged microcolumns were eluted with 0.3, 0.5, 1 and 2 ml min^{-1} of HNO_3 (4 M). The results are represented in figure 4.12b and the maximum in analyte recoveries are 30% for 0.5 ml min^{-1}.

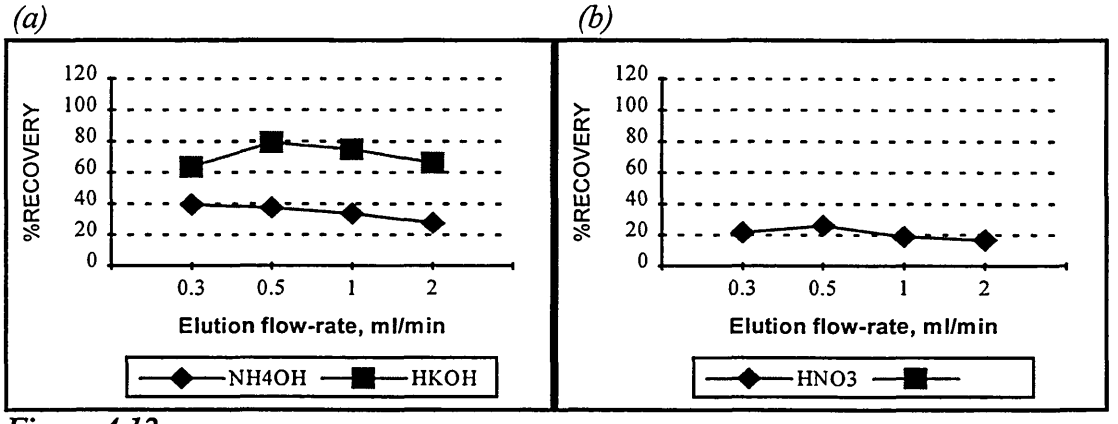


Figure 4.12.
Effect of elution flow-rate (ml min^{-1}) on analyte recoveries (a) chromium(VI) and (b) chromium(III). Standard solution, 200 µg l^{-1}, 250 µl.

(iii) *Elution Volume*. For chromium(VI), aged microcolumns were eluted with 0.25, 1 and 2.5 ml of NH_4OH (4M, 0.3 ml min^{-1}) and KOH (4M, 0.5 ml min^{-1}) respectively. The results are represented in figure 4.13a.

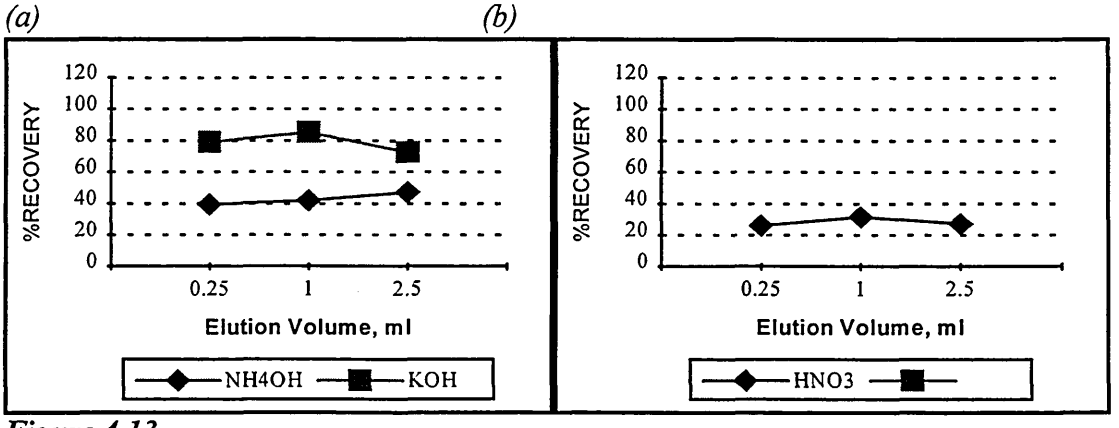


Figure 4.13.
Effect of elution volume (ml) on analyte recoveries (a) chromium(VI) and (b) chromium(III). Standard solution, 200 µg l^{-1}, 250 µl.

The black line represents KOH and the line with marks represents NH_4OH. The maximum in analyte recoveries are 50% and 85% for 2.5 and 1 ml of NH_4OH and KOH respectively. For chromium(III) aged microcolumns were eluted with 0.25, 1 and 2.5 ml of HNO_3 (4M, 0.5 ml min^{-1}). The results represented in figure 4.13b show a maximum in analytes recoveries (30%) for 1ml.

As a result of the above experiments it can be said that for both chromium species there is a higher analyte recovery using an strong eluent (KOH and HNO_3 for chromium(VI) and chromium(III) respectively), a low elution flow rate (0.5 ml min^{-1}) and a high elution volume (1 ml). This is due to the fact that there is more vigorous conditions in combination with a high contact time between the eluent and the immobilised analyte which results in a more effect in the nature of the binding strength. However, analyte recoveries are higher for chromium(VI) than for chromium(III) due to the different nature of the binding strength which is stronger for cations that for anions.

In a further attempt to improve recovery under continuos flow conditions a microwave reactor was incorporated into the FI system as shown in figure 4.5. (KOH 2M was used as eluent for chromium(VI) while HNO_3 4M, for chromium(III)). Alumina microcolumns (n=90) were charged as before (acidic alumina: chromium(VI) 200 μg l^{-1}, 250 μl; basic alumina: chromium(III) 200 μg l^{-1}, 250 μl) and after 1 month storage were inserted into the FI system for elution/quantitation. Elution response was studied as function of microwave power (0, 10, 20 and 30%) and length of the heating coil (0.4, 1 and 3 m). The results are summarised in table 4.11. It can be seen that for both chromium forms 100% analyte recovery can be obtained but there is dependency on microwave power and length of the heating coil. Analyte recoveries for the experiments

performed in the absence of microwave power (for both chromium(VI) and chromium(III) were of the order 55±12% and 23±3%, respectively) and again were similar to the data of figure 4.11a,b. An increase of the microwave power to 10% for chromium(VI) and to 30% for chromium(III), (the length of the heating coil for both species was independently 1 or 3 m) resulted in an analyte recoveries of 106±7% - 108±8% for chromium(VI) and 107±15% and 110±3% for chromium(III). Aged microcolumns (6 months) were also studied at 30% power and with 3 m of heating coil. Relatively poor recoveries of 90±8% for chromium(VI) and 80±10% for chromium(III) were obtained.

		0% Power	10% Power	20% Power	30% Power
Cr(III)	I	23±3	43±5	35±2	47±5
	II	23±3	82±7	88±9	107±15
	III	23±3	82±14	88±9	110±3
Cr(VI)	I	55±12	49±6	67±4	86±2
	II	55±12	108±8	98±12	102±14
	III	55±12	106±7	-	-

I. Heating coil: 0.4 m, II. Heating coil:1 m and III. Heating coil: 3 m. See experimental for full details.

Table 4.11.
Analyte Recoveries (%) for chromium species using microwave heating.

Chromium studies have demonstrated that, for alumina microcolumns charged with chromium(III) and chromium(VI), there is a difference in binding strength between freshly prepared and aged microcolumns. As a results, unless vigorous elution conditions are established (e.g. microwave heating) under FI conditions, analyte

recoveries will be incomplete. Prospects for developing alumina microcolumns, in their present format, as a new candidate reference material are therefore compromised unless elution is performed in a batch/off-line manner so that 100% elution efficiency is achieved. Further work aimed at modifying/deactivating the alumina structure could prove useful for FI measurements as would be investigation of alternative column packings.

4.4. Concluding Remarks.

The studies have shown that microcolumns with retained analytes may serve as external calibrants and offer potential for developing new formats for delivery of RMs. In the case of mercury, SCF microcolumns charged with methyl- and inorganic mercury were found to be stable over a 4 month period and hence provide a convenient new route for instrument calibration and method validation. In the case of ethylmercury stability was observed for over a two month period.

In contrast to mercury, however, work with chromium revealed that binding strength associated with chromium-alumina interaction increased progressively with time such that there was a gradual reduction in analyte recoveries with time. That is in order to achieve a high analyte yield on elution. Vigorous elution conditions were necessary using either wet or microwave assisted digestion. Clearly the uselfuness of using chromium enriched alumina microcolumns for method validation/calibration is compromised. Further work to identify a more appropriate support medium is needed.

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Chapter 5: Conclusions and Future Work

5.1. Conclusions.

Separate but related investigations for mercury, lead and chromium speciation involving flow injection systems containing microcolumns in combination with GC-MIP-AES, ICP-MS or ICP-AES have been reported in this thesis, advancing speciation measurement capabilities for these elements in natural waters.

The first investigation was related to the preconcentration and individual determination of mercury species (methyl-, ethyl- and inorganic mercury) in synthetic solutions and in natural waters. The mercury species were preconcentrated on a conditioned SCF microcolumn. Retained analytes were eluted from the microcolumn using hydrochloric acid and then were subjected to derivatisation with sodium tetraphenylborate ($NaBPh_4$) and extracted into n-hexane. The determination of the three species was performed by hyphenated GC-MIP-AES. Limits of detection for mercury species, defined as three times the standard deviation of the blank, were 10 ng l^{-1} for methyl- and ethyl- mercury and 16 ng l^{-1} for inorganic mercury. The precision at the 10 μg l^{-1} level based on the use of ten different microcolumns was 5.33% (methyl-), 10.5% (ethyl-) and 12% (inorganic mercury). The approach offered high sensitivity combined with high separation ability and simplicity in the derivatisation/extraction step.

The second investigation was related to the development and evaluation of a novel manifold for a rapid on-line speciation of neutral and cationic (organic and inorganic nature) lead species in synthetic solutions and in natural waters. The manifold was based on the direct coupling of an acidic alumina microcolumn separator with the ICP-MS detector, using a FI system. The neutral lead species were completed retained on sample

injection on the microcolumn whereas cationic (organic and inorganic nature) lead species were unretained and hence proceed to the detector for quantitation. Thereafter an injection of eluent (methanol) completed the two stage measurement cycle. In this way both neutral and cationic (organic and inorganic nature) lead species were determined in a single run. Limits of detection for lead species, defined as three times the standard deviation of the blank, were 49.4 ng Pb l^{-1} for tetraalkyl- and 68.2 ng Pb l^{-1} for inorganic lead. The precision at the 10 μg l^{-1} level based on the use of ten different microcolumns was 0.33% (tetramethyllead) and 0.55% (inorganic lead). The approach allowed a rapid knowledge of the toxicity of water samples in a simple way.

For both investigations microcolumn technology was transferred to the field, and speciation of mercury and lead in surface waters of the Manchester Ship Canal was undertaken. The microcolumn field sampling technique offered the preservation of the natural speciation state of the water sample directly at the sampling site and during the interval between collection and analysis. For analytical chemistry the field sampling technique developed for this work was an important topic of research because of the simplicity of manipulation respect to the classic sampling methodology, taking into account the low concentrations of species relative to the total amount of the element, the contamination and analyte loss problems at such low concentrations, the rapid interchange of species under natural conditions, etc. With the field sampling technique developed, toxicity and environmental behaviour of trace mercury and lead can be monitored and assessed.

It is necessary to remark that it has been the first time in which the mercury and lead speciation in this seriously polluted waterway in Britain has been measured

simultaneously. A high degree of correlation between distributions of methylmercury and organolead compounds in this waterway suggest a transfer of methyl- groups from organolead species to the inorganic mercury.

A further investigation was related to the use of microcolumns as external calibrants and certified reference materials (CRMs). Given the interest and increasing acceptance of methodology based on on-line FI procedures there will be an increasing demand for CRMs in microcolumn format. Microcolumns with retained analytes offered the ability to recover species upon elution with a verified, certified recovery after a long term period. Target elements for this investigation were mercury and chromium. In the case of mercury, for establishing the ability of SCF microcolumns as external calibrants and candidate CRMs of mercury species, stability studies for immobilised methyl-, ethyl- and inorganic mercury analytes were performed maintaining 30 ng of each species stored in dark at 4°C for a 4 month period. Similar investigations were performed to establish if activated alumina could be proposed as external calibrant and candidate CRMs for chromium(III) and chromium(VI) (basic and acidic alumina microcolumns respectively). Stability studies were performed maintaining 50 ng of each species stored under different storage regimes for a 6 month period. After usual developed elution it was found that analyte recoveries for chromium(III) and chromium(VI) were of the order 27% and 57%, respectively. To achieve quantitative recoveries (100%) it was necessary to incorporate microwave heating in the FI system.

The possibility, therefore, exits to develop new RMs and calibrants based on immobilisation technology. Microcolumn sample processing is seen as an increasingly

important trend in ultratrace analysis methodologies and hence new demands for RMs prepared in column format can be anticipated.

5.2. Future Work.

Extension of the work described in this thesis is possible in a number of ways:

Because the data are based on only limited surveys, the concentrations of different mercury and lead species in surface water of the Manchester Ship Canal must be interpreted with caution. A long-term monitoring programme at selected site in this waterway might be beneficial to obtain more information about the natural and anthropogenic flux of mercury and lead in this system. Future research must also include studies on mercury and lead speciation and distribution on sediments which may also be responsible for the origin of methylmercury in the Manchester Ship Canal.

The identification of all lead (tetraalkyl-, trialkyl- dialkyl- and inorganic lead) species in the Manchester Ship Canal in their ethylated and methylated forms could give an improved understanding of the chemical/biological reactions responsible for the methylation of mercury in this polluted waterway. The approach could be developed by using preconcentration of all lead species onto Muromac A-1, C18 or sulphydryl cotton in combination with GC-MIP-AES. A derivatisation procedure is needed, such as Grignard butylation, which allows the differentiation of methylated and ethylated forms of the lead species.

Further investigation concerning the development of CRMs for mercury and chromium

species is needed. In the case of mercury, while ethylmercury species appear to be stable for up to 2 months, poor recovery data are obtained for 4 months (recovery, 7%). This result may be interpreted as loss of analyte or poor recovery in the method itself. Further work is needed to clarify this point.

As shown in the present study column format is versatile and practically useful because it is compatible with both on-line/FI methodology and also with classical/batch operations. For follow up work it is planned to circulate enriched microcolumns (low level, high level) to specialist laboratories and subject samples to rigorous interlaboratory study. At the same time alternative solid support media will be tested. In the case of chromium studies have demonstrated that unless vigorous elution conditions are established (e.g. microwave heating) under FI conditions, analyte recoveries will be incomplete. Prospects for developing alumina microcolumns, in their present format, as a new candidate reference material, are therefore compromised unless elution is performed in a batch/off-line manner so that 100% elution efficiency is achieved. Further work aimed at modifying/deactivating the alumina structure could prove useful for FI measurements as would be the investigation of alternative column packings.

Finally, future research must also include studies concerning the possibility to use the acidic alumina microcolumn as external calibrant and candidate CRMs for neutral lead species (tetraalkyllead). It must be also verified experimentally, that the original concentration of tetraalkyllead species (tetramethyl- and tetraethyllead) retained on acidic alumina, are stable for a long time period in controlled storage conditions.

Study Programme

As part of the research programme the author has :

a) Attended selected lecture courses and departmental research meetings.

b) Presented work at research group meetings.

c) Attended workshop on "Method Development and Improvement of Enviromental Analysis" within the BCR Programme, Brussels, Belgium, February 17-19, 1993.

d) Attended "XXVIII Colloquium Spectroscopicum Internationale" held in York, UK, June 29-July 4, 1993.

e) Attended "Seminar on New Developments in Elemental Analysis" held in Sheffield, UK, September 16, 1993.

f) Attended workshop on "Trends in Speciation Analysis" within the BCR Programme, Rome, Italy, February 19-22, 1994.

g) Attended workshop on "Sample Presentation in ICP Mass Spectrometry" within Sheffield Hallam University and Fisons Instruments, Novotel, Sheffield, UK, June 20-22, 1994.

h) Attended the "4th International Conference on Plasma Source Mass Spectrometry" held in Durham, UK, September, 1994.

i) Presented work at:

- The "12th International Symposium on Microchemical Techniques (ISM'92)", held in Córdoba, Spain, 5-9 September, 1992.
- The "1994 Winter Conference on Plasma Spectrochemistry" held in San Diego, California, US, January 10-15, 1994.
- The "6th International Conference on Flow Analysis" held in Toledo, Spain, June 8-11, 1994.
- The "Analytical Quality Control and Reference Materials: Life Sciences" held in Rome, Italy, December 5-7, 1994.

j) Completed work at the Istituto di Analisis e Technologie Farmaceutiche ed Alimentari, Università di Genova, Via Brigata Salerno (ponte), 16147 Genova, Italy, October, 1993.

k) Participated in Intercalibrations organised by the Community Bureau of Reference (EC).

l) Publications:

- "Microcolumn field sampling and flow injection-atomic fluorescence spectrometry for speciation of mercury in surface waters"; Wei Jian, M.L.Mena, C.W.McLeod and J.Rollins; *Intern. J. Environ. Anal. Chem.*, (1994), 57, 99.

- "Microcolumn preconcentration and gas chromatography-microwave induced plasma-atomic emission spectrometry (GC-MIP-AES) for mercury speciation in waters"; M.L.Mena, C.W.McLeod, P.Jones, A.Widers, V.Minganti, R.Capelli and Ph.Quevauviller; *Fresenius J. Anal. Chem.*, (1995), 351, 456.

- "Rapid determination of mercury in environmental materials using on-line microwave digestion and atomic fluorescence spectrometry"; A.Morales-Rubio, M.L.Mena and C.W.McLeod; *Anal. Quim. Acta*, (1995), 308, 364.

- "Stability of chromium species immobilised on microcolumns of activated alumina"; M.L.Mena, A.Morales-Rubio, A.G.Cox, C.W.McLeod and Ph.Quevauviller; *Química Analítica*, (1995), 14, 164.

- "Mercury species immobilised on sulphydryl cotton: a new candidate reference material for mercury speciation"; M.L.Mena and C.W.McLeod; *Mikrochim. Acta*, (1996), 123, 103.

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MICROCOLUMN FIELD SAMPLING AND FLOW INJECTION-ATOMIC FLUORESCENCE SPECTROMETRY FOR SPECIATION OF MERCURY IN SURFACE WATERS

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A novel approach to mercury speciation in waters based on microcolumn field sampling and flow injection-atomic fluorescence spectrometry is described. Microcolumns of sulphydryl cotton are used as a new sampling tool in order to collect, enrich and immobilise organic mercury species. On return to the laboratory the microcolumns are inserted into the flow injection system for elution/quantitative analysis. The technique, applied to survey analyses of the Manchester Ship Canal, revealed relatively high concentrations of organomercury (0.006–0.058 μg Hg l^{-1}) and inorganic mercury (0.0038–0.530 μg Hg l^{-1}). The distribution profiles for the inorganic and organic fractions did not correlate well.

KEY WORDS: Mercury speciation, microcolumn field sampling, flow injection, atomic fluorescence, waters.

INTRODUCTION

There is a continuing need to improve our understanding of mercury transport and cycling in the biosphere on account of the toxicity of mercury. As is well known, organomercury compounds and particularly methylmercury are highly toxic relative to inorganic forms and hence analytical methodology capable of differentiating between the various species is essential. Many approaches have been reported for the determination and speciation of mercury and include: cold vapour atomic absorption/atomic fluorescence with selective chemical reductants/pretreatments[1–3], chromatographic methods based on gas chromatography[4–8] and high performance liquid chromatography[9], solvent extraction and inductively coupled plasma mass spectrometry[10] and electrochemical separation and atomic absorption

spectrometry[11]. Recently, flow injection atomic fluorescence (FI-AFS) with microcolumn separation has been proposed as a novel speciation technique[12]. In this latter approach, a microcolumn of sulphydryl cotton fibre (SCF) is incorporated in the FI system to effect on-line separation of organomercury and inorganic mercury species. The SCF microcolumn is essentially a scaled-down version of that utilised by Lee[6] for gas chromatographic separation/enrichment of organomercury compounds.

With reference to mercury concentrations in natural waters, reliable speciation data are scarce. This is partly because of the extremely low concentrations of individual species (ng l^{-1}) but also is a reflection of the difficulty after sampling of maintaining the natural speciation state until analysis is performed. As is often practised in official monitoring programmes for mercury, chemical species are actually destroyed at the time of sampling (addition of powerful oxidants to the sample) and only total mercury data are reported. A notable exception to the above are the monitoring studies of Lee and Hultberg[6,13] where methylmercury concentration in Swedish surface waters were reported to be in the sub ppt range (0.16–0.41 ng l^{-1}). More recently Frech *et al.*[8] utilised microcolumns of dithiocarbamate resin for trace enrichment of methyl-, ethyl-, and inorganic mercury in freshwater and seawater prior to determination by GC-MIP-AES and observed methylmercury concentrations of 0.08–1.15 ng l^{-1} and inorganic mercury concentrations of 0.31–1.98 ng l^{-1}.

In the present work a new speciation approach based on the combination of microcolumn field sampling and FI-AFS has been developed. Instead of implementing microcolumn enrichment/separation in the laboratory, water samples are processed at the sampling site by passage through microcolumns of sulphydryl cotton in order to immobilise and stabilise organomercury species. The microcolumns with retained analyte are then returned to the laboratory and incorporated into a FI-AFS system for elution/quantitative analysis. In this way it is hoped to preserve and maintain the original organomercury species until analysis is performed as already demonstrated in the case of chromium speciation studies[16]. In addition to determination of organomercury concentrations the measurement scheme also allows for determinations of inorganic mercury. As a trial study the new methodology is applied to the Manchester Ship Canal, a water course known to be contaminated by mercury.

EXPERIMENTAL

Reagents

Tin chloride solution (3% m/V) in hydrochloric acid (15% v/V) was freshly prepared on a daily basis from tin chloride 2-hydrate salt (Spectrosol, Merck, Poole, UK), hydrochloric acid (36% m/m, Aristar, Merck) and high purity water. A potassium bromide/potassium bromate solution (0.5% +0.14% m/V) was made by dissolving respective reagents (Analytical grade, Fisons, Loughborough, UK) in water. Hydrochloric acid solution (0.01 M) was prepared from hydrochloric acid (36% m/m, Aristar, Merck). Hydrochloric acid solution (3 M) was freshly prepared by diluting 50 ml of hydrochloric acid (36% m/m) in a 200 ml pre-cleaned flask prior to analysis.

Preparation of sulphydryl cotton fibre and microcolumn field sampling kit

Sulphydryl cotton fibre was prepared according to the procedure of Lee[6] as follows: thioglycollic acid (50 ml, General Purpose Reagent, UK, 97% m/m, Merck), acetic anhydryde (30 ml, 36% m/m, General Purpose Reagent, Merck), acetic acid (20 ml, 30% m/m, Merck) and sulphuric acid (0.15 ml, 96% m/m, General Purpose Reagent, Merck) were measured into a wide-neck flask and then mixed thoroughly (care exothermic reaction). The mixture was cooled to room temperature and absorbent cotton (15 g) was added and left to soak. The stoppered flask was then placed into an oven at 40°C and left for 4 days. Thereafter the cotton fibre was washed with Millipore water until washings were between pH 6–7 and the material dried at a low temperature (40°C). The dried cotton was next transferred to a sealed light-free container for storage.

Sulphydryl cotton microcolumns were made by inserting the cotton fibre (0.015–0018 g) into PTFE tubing (60 mm × 1.5 mm ID), the absorbent being packed evenly along a 50 mm length of column. Two further PTFE tubes (20 mm × 0.8 mm) were fitted at both ends of the column to allow connection of the microcolumn to the field sampling kit and the flow injection system. The field sampling kit, consisting of an on-line filter (0.45 µm, Anachem, Luton, UK), a syringe (60 ml of capacity) and a sulphydryl cotton microcolumn (60 mm × 1.5 mm), was conditioned by passing through, twice, 2 ml of hydrochloric acid solution (3 M, Aristar, Merck) followed by 2 ml of Millipore water.

Equipment

The determination of organomercury was performed with a 3-line FI system illustrated in Figure 1. Main components were a peristaltic pump (Ismatec, London, UK), rotary injection valve (OmniFit, Cambridge, UK) and an atomic fluorescence detector incorporating gas liquid separator (Merlin, P.S. Analytical, Sevenoaks, UK). Transient signals derived from elution of organomercury from the sulphydryl cotton microcolumn were recorded on a strip

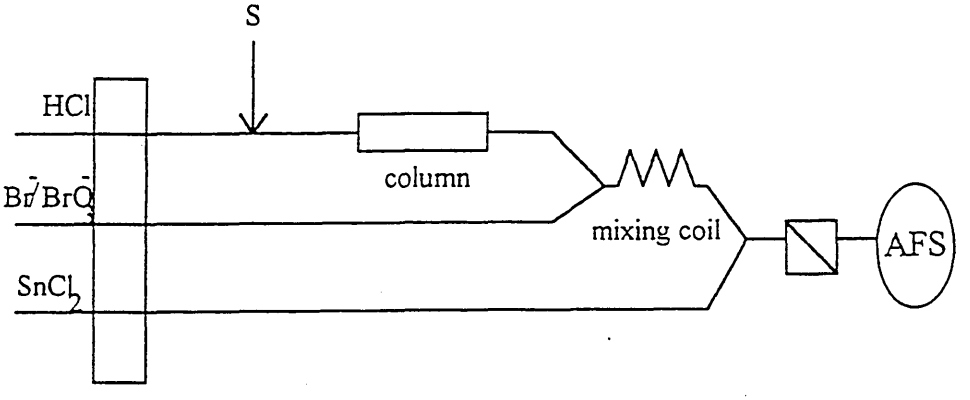

Figure 1. Flow injection system incorporating microcolumn of sulphydryl cotton for determination of organomercury species (see text for operating parameters).

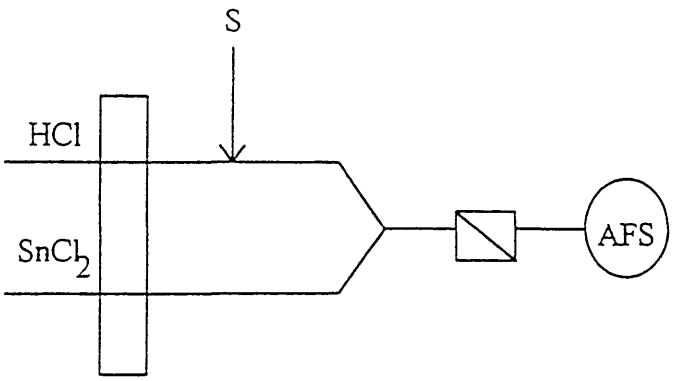

Figure 2. Flow injection system for determination of inorganic mercury (see text for operating parameters).

chart recorder (Hitachi 056) and quantitative measurements were based on evaluation of peak area using standard software routines (Merlin, P.S. Analytical). Key FI parameters were: eluent, hydrochloric acid (0.5 ml, 3 M); mixing coil length 4 m; carrier/reagent stream flow rates, 1.5 ml min^{-1}. Full details concerning the operation and performance of the FI system are detailed elsewhere[11].

The determination of dissolved inorganic mercury was performed using a 2-line FI system (on-line oxidation omitted) as shown in Figure 2. Flow injection operating parameters were as above.

Sampling and pretreatment procedures

The sampling procedure was designed in order to allow determination of dissolved organomercury (corresponding to fraction retained on the microcolumn) and dissolved inorganic mercury (corresponding to fraction in effluent after microcolumn processing).

Sample solutions (1 l), on collection, were adjusted to pH 3.0–3.5 by dropwise addition of concentrated nitric acid. A plastic syringe (60 ml capacity) was filled and rinsed with acidified sample before assembly of the field sampling kit. An aliquot of sample solution (30 ml) was then processed by passage through the filter and microcolumn. At the same time column effluent was collected in a pre-cleaned 50 ml flask, which contained nitric acid (0.5 ml) (for determination of dissolved inorganic mercury). On completion of sampling, microcolumns, stored in a light tight box, and flasks were returned to the laboratory for analysis. For each sample collected 3 replicate analyses (corresponding to processing of microcolumns in triplicate) were performed. The FI-AFS measurements were performed within 48 h of sample collection.

Water samples (sub-surface) were collected at 10 sampling stations throughout a 20 km stretch of the Manchester Ship Canal.

Measurement procedure

The determination of organomercury was based on the elution of organomercury from the sulphydryl cotton microcolumn. Prior to use the FI system (Figure 1) was cleaned and rinsed by pumping hydrochloric acid solution (5 M) for 25 min and Millipore water for 10 min. Then reductant ($SnCl_2$), oxidant ($Br^-/BrO3^-$ and carrier (0.01 M HCl) streams were continuously pumped, each at a low flow rate of 1.5 ml min^{-1}. When a smooth baseline was observed the FI system was ready for calibration and sample analysis.

Standard solutions of methylmercury chloride (0.00, 0.010 and 0.020 μg Hg l^{-1}) were processed as for water samples and fluorescence signal responses for respective columns were used to generate a calibration graph. The peristaltic pump was then switched off to allow insertion of microcolumns (see Figure 1). The pump was then reactivated and when a smooth baseline occurred hydrochloric acid (0.5 ml, 3M) was injected to effect elution of retained mercury species.

Determination of dissolved inorganic mercury was based on direct injections of samples (0.5 ml) into the FI system (Figure 2) and calibration utilised inorganic mercury standard solutions (0.00, 0.10, 0.25 μg Hg l^{-1}).

RESULTS AND DISCUSSION

Prior to survey analysis, reliability of the measurement procedure was checked by analysing synthetic standard solutions of methylmercury chloride and spiked waters of the Manchester Ship Canal. The transient signals for processing the afore mentioned waters are shown in

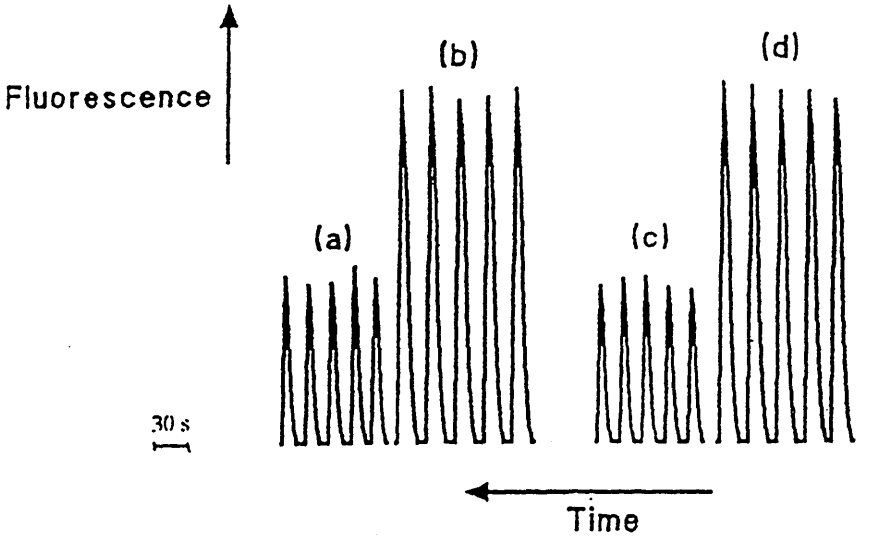

Figure 3. Typical transient signals for standard solutions and spiked waters: (a) 50 ng l–1 MeHgCl, (b) 100 ng l–1 MeHgCl, (c) 50 ng l–1 spike canal water and (d) 100 ng l–1 spike to canal water. Each transient signal correspond to elution of methylmercury from a separate microcolumn.

Table 1. Analytical data for standard solutions of methylmercury chloride using field sampling technique and FI-AFS.

Standard solutions, μg Hg l^{-1}	*Analysed Value, μg Hg l^{-1}*	*% RSD**
0.010	0.007	8.6
0.030	0.026	6.9
0.060	0.058	2.8

* No. of replicates, 10 (10 different microcolumns). Sample volume processed, 30 ml

Table 2. Recovery data for analysis of water of Manchester Ship Canal.

Sample	*Analysed Value, ng Hg l^{-1}*	*Recovery (%)*
Sample (unspiked), ng Hg l^{-1}	<0.002	—
Spiked sample (50 ng Hg l^{-1}	47.43±6.2	94.8±6.2
Spiked sample (100ng Hg l^{-1}	102.6±5.1	102.6±5.1

* No. of replicates, 5 (5 different microcolumns). Sample volume processed, 10 ml. Methylmercury chloride as spike.

Figure 3 and the corresponding analytical data are given in Tables 1 and 2. It is clear that the proposed sampling/FI-AFS measurement scheme is rapid, precise and sensitive and may offer a new and effective approach for determination of methylmercury in waters.

In the case of the Manchester Ship Canal the data of Table 3 reveal significant concentrations of mercury at all sampling stations, confirming the extensive contamination of the water course throughout the 20 km stretch examined. As one might expect, inorganic mercury constituted the major mercury fraction. Concentrations for organomercury were at the low ppt level except for stations 2, 3 and 4, where relatively high values (22–58 ng l^{-1}) were observed (a second survey confirmed elevated concentrations of organomercury at stations 2, 3 and 4). The fact that organomercury data did not correlate well with inorganic mercury data may have significance with respect to biogeochemical cycling/production pathways.

Table 3. Speciation data for mercury in Manchester Ship Canal.

Station	*Organomercury*, μg Hg l^{-1}*	*Inorganic mercury#, μg Hg l^{-1}*
1.	0.009±0.001	0.325±0.006
2.	0.022±0.003	0.320±0.019
3.	0.058±0.003	0.530±0.008
4.	0.035±0.003	0.250±0.017
5.	0.005±0.001	0.140±0.011
6.	0.004±0.001	0.495±0.001
7.	0.005±0.001	0.270±0.025
8.	0.006±0.001	0.265±0.160
9.	<0.002	0.245±0.020
10.	<0.002	0.045±0.004

*Data are mean values for 3 replicate analyses. # Data are mean values for 4 measurements derived from 2 separate column effluents. Uncertainty limits ± σ

It is generally accepted that a principle source of inorganic mercury contamination in the canal is derived from the operation of chloroalkali plants either through direct discharge of effluent containing mercury or from mobilisation of mercury from the bottom sediments. The latter is a known route for formation of methylmercury and the fact that organomercury data do not correlate well with inorganic mercury data does suggest an alternative production pathway may be operative. Apart from direct input from a point source, one possibility is an in-situ alkylation reaction. The surface waters in question (stations 2, 3, 4) are known to contain relatively high concentrations of alkyllead compounds and their distribution profiles [15] (stations 1, 2, 3, 4, 5) were noted to be similar to that obtained for organomercury in this study. This similarity implies that an in-situ alkylation reaction of the type:

$$Hg^{2+} + Me_3Pb^{+} \text{———} MeHg^{+} + Me_2Pb^{2+}$$

might be operating whereby there is a transfer of alkyl groups from alkyl lead to inorganic mercury. Such a possibility although demonstrated in the laboratory [13], and confirmed by ourselves, has to our knowledge, not been reported for environmental waters.

CONCLUSIONS

It is clear that the combination of microcolumn field sampling and flow injection-atomic fluorescence spectrometry provides an elegant yet powerful solution to the determination and speciation of mercury in environmental waters. The approach is extremely efficient both at the sampling and the measurement stages by virtue of the continuous flow/flow injection procedures. The microcolumn field sampling technique is being developed for use with other FI-based detection systems (eg. FI-IC-MS) and hyphenated techniques such as GC-atomic emission spectrometry[17], the latter allowing determination of individual organomercury species unlike the present methodology. Work is in progress to assess long term stability of the immobilised mercury species.

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Microcolumn preconcentration and gas chromatography-microwave induced plasma-atomic emission spectrometry (GC-MIP-AES) for mercury speciation in waters

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Abstract. A novel method for the direct determination of mercury species at the ng l^{-1} level in natural waters is described. Methyl-, ethyl- and inorganic mercury are preconcentrated on a sulphhydryl cotton microcolumn incorporated in a flow injection system. Retained mercury species are then eluted with hydrochloric acid solution (3 mol/l) and subjected to phenylation before determination by gas chromatography-microwave induced plasma-atomic emission spectrometry. Limits of detection for mercury species are 10 ng l^{-1} for methyl- and ethyl-mercury and 16 ng l^{-1} for inorganic mercury based on processing 200 ml of sample. Application of the methodology to waters of the Manchester Ship Canal revealed elevated levels of methylmercury and inorganic mercury.

1 Introduction

It is essential for environmental monitoring purposes to speciate and hence distinguish between organic and inorganic forms of mercury because, generally, methylmercury is more toxic than inorganic mercury [1]. Speciation also assists in understanding the biogeochemical cycling of mercury and the identification of the "environmental compartments" where transformations of the mercury species can take place. The chemical pathways for the methylation of mercury in the environment have been reviewed [2–4].

The most widely used methods for the determination of individual mercury species are based on gas chromatography (GC) with detection by electron capture (EC) [5,6], microwave induced plasma-emission spectrometry (MIP-AES) [7] or atomic absorption spectrometry (AAS) [8]. However, derivatization of the analytes is recommended for GC work due to thermal instabilities of mercury species in the inlet system and/or the GC column.

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The most common derivatisation procedures used are hydride generation using sodium borohydride ($NaBH_4$), aqueous-phase ethylation using sodium tetraethylborate ($NaBEt_4$) and Grignard alkylation. The latter must be preceded by extraction of the organomercury species into an organic solvent. This is usually done with complexing agents like tropolone or dithiocarbamates [9,10]. Sample preparation procedures using Grignard derivatisation involve a great number of steps, making the procedure tedious and time consuming. Hydride generation and aqueous-phase ethylation are much faster and can be performed directly in the aqueous phase. Organomercury hydrides [11] or ethylates are extracted into an organic solvent or are caught in a cryogenic trap after purging with nitrogen [12, 13]. However, in hydride generation problems with quantitative recovery are likely to occur due to the instability and volatility of organomercury hydrides and for direct aqueous-phase ethylation, differentiation between ethyl- and inorganic mercury speciation is precluded.

The use of high-performance liquid chromatography (HPLC) for mercury speciation is a recent development which avoids the need for derivatisation. Determination of 10 different organomercury compounds [14] (inorganic, methyl-, methoxyethyl-, benzoic, mersalylic acid, ethyl-, ethoxyethyl-, phenyl-, nitromersol- and tolyl-mercury) in soils was carried out using HPLC with photometric detection (UV at 230 nm) after extraction into toluene. The combination of HPLC with inductively coupled plasma (ICP-AES) [15], atomic absorption (AA) [16] or atomic fluorescence (AF) [17] is not particularly sensitive and hence it has proved necessary to implement post-column vapour generation to improve the detection capability. Krull et al. [15], for example, have reported three to four orders of magnitude improvements in detection limits for mercury species using the cold vapour generation technique.

Invariable for all the afore-mentioned procedure some form of preconcentration step is necessary. Extraction with an organic solvent (benzene or toluene) is a popular preconcentration approach. However, because the parti-

tion coefficient for methylmercury between benzene and water is low, (ca. 5–10) [18], the approach is not adequate for concentrations ≤ 0.5 ng l^{-1}. Cryogenic trapping [11] and adsorption onto ion exchange/chelating resins such as sulphhydryl cotton fibre (SCF) [19] and dithiocarbamate resin [10, 20] have also been utilised. Lee and Mowner [19] preconcentrated methylmercury from water onto an SCF adsorbent using either a column technique or a two stage, batch-column procedure. The latter procedure involved mounting pieces of SCF gauze onto a glass frame, which was immersed in the sample contained in a 4 l reservoir. The methylmercury was eluted with hydrochloric acid and water (batch procedure). After adjusting to pH 4, the eluate was passed through a small column containing SCF wool. Methylmercury was then extracted into benzene and determined, as the chloride, by GC-ECD. By using a 4 l sample and 400 µl of benzene in the final extraction step, a detection limit of 0.05 ng l^{-1} of methylmercury was achieved. The batch-column procedure provided a variable, non-quantitative recovery of methylmercury, but was preferred for the analysis of humic-rich waters. The use of a non-specific detector, i.e. the ECD, did result in the chromatograms exhibiting numerous interfering peaks. Methylmercury concentrations in Swedish surface waters were reported to be in the sub-ppt range (0.16–0.41 ng l^{-1}).

More recently microcolumn enrichment techniques based on the flow injection (FI) methodology have been developed for mercury speciation purposes. Frech et al. [10] utilised a microcolumn of dithiocarbamate resin in a FI system to preconcentrate mercury species in waters prior to GC-MIP-AES. Sample pretreatment included an elution step with acidic thiourea, extraction into toluene and butylation with a Grignard reagent. Limits of detection for methyl- and ethylmercury were 0.05 ng l^{-1} and 0.15 ng l^{-1} in the case of inorganic mercury for a 500 ml sample. Results for the determination of mercury species in freshwater and seawater were reported and observed methylmercury concentrations were 0.08–1.15 ng l^{-1} with inorganic mercury concentrations of 0.31–1.98 ng l^{-1}.

Earlier Wei et al. [21] developed a FI-AFS method for the rapid sequential determination of inorganic mercury and organomercury in natural waters. Trace enrichment and separation of mercury species was achieved using a microcolumn of SCF, essentially a scaled down of that utilised by Lee [19] for GC separation of organomercury compounds. The limit of detection for methylmercury based on processing a 0.5 ml sample volume was 6 ng l^{-1}. The same group also proposed microcolumn field sampling [22] as a means to collect and preserve organomercury species until the laboratory measurement was performed. The technique, applied to survey analyses of the Manchester Ship Canal [23], revealed relatively high concentrations of organomercury (0.006–0.058 µg Hg l^{-1}) and inorganic mercury (0.0038–0.530 µg Hg l^{-1}). Unfortunately the separative capability afforded by the FI system does not permit discrimination of individual organomercury species and hence, in order to further develop and exploit microcolumn preconcentration/field sampling, coupling with chromatographic techniques is essential.

In the present study a FI system incorporating microcolumns of SCF is used to preconcentrate mercury species prior to GC-MIP-AES. The analytes after elution from the microcolumn were subjected to derivatisation with sodium tetraphenylborate ($NaBPh_4$) and then extracted into n-hexane. Method development studies and application to waters of the Manchester Ship Canal are presented.

2 Experimental

2.1 Reagents and materials

Standard solutions of inorganic mercury were prepared by appropriate dilution of a stock solution (1000 mg l^{-1} of mercury nitrate in 1% v/v HNO_3) with high-purity water (Millipore). The organomercury stock solutions (1000 mg l^{-1} of RHgCl; R = CH_3 and C_2H_5) were prepared by dissolving the compounds in acetone (Merck, ARISTAR) and standard solutions were prepared by appropriate dilution with high-purity water (Millipore). Hydrochloric acid (0.01 mol/l and 3 mol/l) was prepared from concentrated reagents (Merck, ARISTAR). A buffer solution of sodium hydroxide (2.7 mol/l)/sodium acetate (3 mol/l) was prepared by dissolving the compounds in high-purity water (Millipore). Sodium tetraphenylborate (5% solution in Millipore water, Aldrich) was freshly prepared a few minutes before use. The sulphhydryl cotton fibre (SCF) adsorbent was produced by introducing the sulphhydryl functional group into natural cotton fibres according to the procedure of Lee et al. [19].

2.2 Flow injection system

A FI system consisting of peristaltic pump (Gilson Minipuls), rotary injection valve, and SCF microcolumn was used for preconcentration of mercury species. Microcolumns were prepared by packing a PTFE tube (5 cm × 1.5 mm i.d.) with SCF (~0.015 g). Columns were inserted into the FI manifold and subjected to injections of hydrochloric acid (3 mol/l) to remove residual contamination. Hydrochloric acid (0.01 mol/l) was used as carrier stream.

2.3 Sampling and preconcentration

Water samples (non-filtered, 1 l) were collected in glass containers at 3 sampling stations of the Manchester Ship Canal and returned to the laboratory for analysis (within 24 h). Water samples (1 l) and standard solutions were adjusted to pH 3.5 by dropwise addition of conc. nitric acid. The solutions (20–200 ml) were then processed in the FI system with a deposition flow rate of 4 ml min^{-1}. Retained mercury species were then eluted with hydrochloric acid (0.5 ml, 3 mol/l; elution flow rate, 1 ml min^{-1}). The eluate (0.5 ml) was collected in a glass vial (5 ml capacity) which contained 1 ml of high-purity water (Millipore).

2.4 Derivatization and extraction

To the glass vial containing the eluate and 1 ml of water, buffer solution (sodium hydroxide/sodium acetate,

380 µl, 2.7 mol/l/3 mol/l) was added to obtain a pH of 2. Then sodium tetraphenylborate (120 µl, 5% m/V) and n-hexane (1 ml) were added and the tube shaken for 30 min using an automatic shaking machine (Gallenkamp, UK). Excess borate reagent was added in accordance with the work of Lückow and Russel [24] in an attempt to maximise the derivatisation yield. After centrifugation (2000 rev min^{-1}) for approximately 5 min (MSE, Mistral 200, Fisons Scientific Equipment, UK) the organic phase was withdrawn using a glass pipette and placed in a screw-capped glass vial (2 ml) ready for injection (automatic) into the GC.

2.5 Instrumentation and measurement

Extracts were analysed by GC-MIP-AES (HP5890 Series GC). The analytical column was a non-polar column (25 m length × 0.23 mm i.d. × 0.17 µm film thickness HP-1), with helium as carrier gas at 2.5 ml min^{-1} (linear velocity 43 cm s^{-1}). Splitless injection was employed (purge time = 60 s) and during elution of the solvent (5 min), the column outlet was vented (to prevent the plasma from being extinguished) and an auxiliary helium flow (30 ml min^{-1}) was directed to the plasma. After the solvent peak had passed, the valve was switched, thereby re-routing the column effluent to the plasma through a fused-silica capillary transfer line (HP).

Atomic emission for mercury was monitored at 253.6 nm using the diode array spectrometer (HP5921A). Standard software routes were used for evaluation of chromatographic peaks. Unless stated otherwise, the peak height was used for quantitation. Operating conditions for the CG-MIP-AES are specified in Table 1.

Table 1. Operating conditions for the GC-MIP-AES (Hewlett Packard 5890)

Instrumental configuration	
Gas chromatograph:	HP 5890A
Automatic sampler:	HP 7673A
Detector:	HP 5921A
GC parameters	
Injection port:	Split/Splitless
Injection port temperature:	250 °C
Column:	25 m × 0.32 mm × 0.17 µm film thickness HP-1
Column-detector coupling:	Column to cavity
Oven temperature program:	3 min 40 °C, 30 °C/min to 270 °C hold 2 min 270 °C
Helium flow rate:	2.5 ml min^{-1}
Split ratio:	100:1
Injection volume:	1 µl
AE parameters	
Elements analyzed	

Element	Wavelength (nm)	Scavenger gas	Makeup flow (ml/min)
Hg	253.652	O_2/H_2	25

Total number of injections:	2
Spectrometer purge flow:	nitrogen 2 l/min
Helium flow rate:	30 ml/min
Transfer line temperatue:	250 °C
Cavity temperature:	250 °C
Water temperature:	65 °C

3 Results and discussion

3.1 Sample pretreatment

In order to exploit the speciation measurement capability afforded by GC-MIP-AES, analytes were preconcentrated on SCF and, following elution with hydrochloric acid, were subjected to derivatisation with sodium tetraphenylborate. A typical GC-MIP-AES response for processing a mixed standard solution of methyl-, ethyl- and inorganic mercury (60 µg l^{-1}) is given in Fig. 1. The accompaning figures refer to the processing of effluent collected during the deposition stage (Fig. 1 a) and to eluate collected after injection of a second aliquot (0.5 ml) of hydrochloric acid (Fig. 1 c). It is clear that the respective mercury species undergo effective deposition/elution on the sulphhydryl cotton microcolumn. In preliminary studies different concentrations of hydrochloric acid (0.5, 1, 2, 3 and 5 mol/l) were used to effect the elution of mercury species

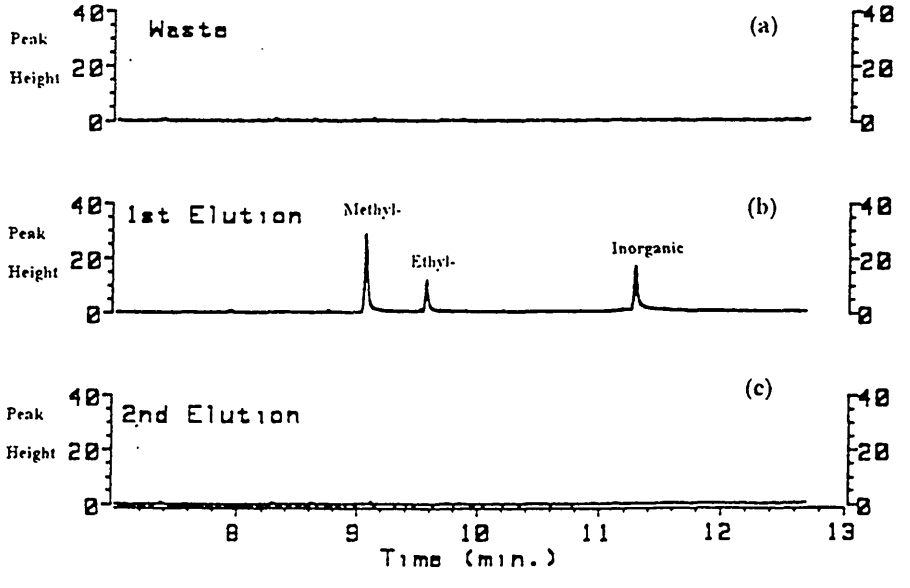

Fig. 1a–c. Typical GC-MIP-AES response for mercury species: a effluent collected during sample processing, b response corresponding to first elution from microcolumn and c response corresponding to second elution from microcolumn. Mixed standard solution (1 µg l^{-1}); sample volume, 60 ml

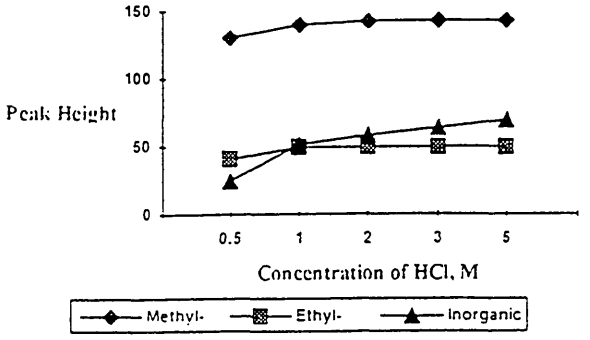

Fig. 2. Effect of hydrochloric acid concentration on the elution step. Mixed standard solution (10 μg l^{-1}); sample volume, 3 ml

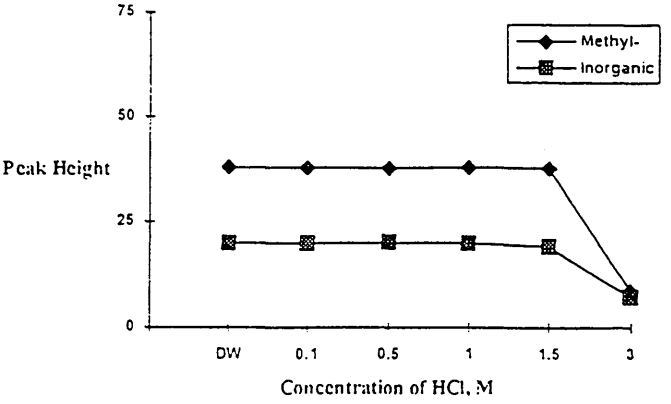

Fig. 3. Effect of hydrochloric acid concentration on signal intensities. Mixed standard solution (150 μg l^{-1}), sample volume, 2 ml

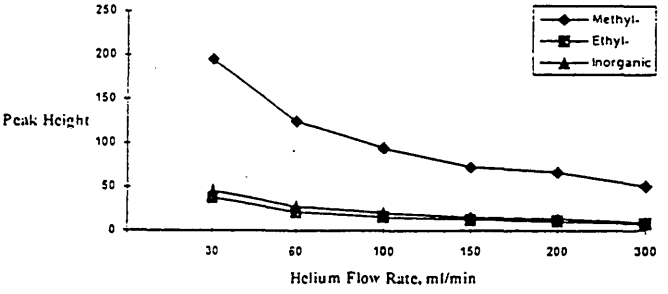

Fig. 4. Effect of helium flow rate on signal intensities. Mixed standard solution (100 μg l^{-1}); sample volume, 2 ml

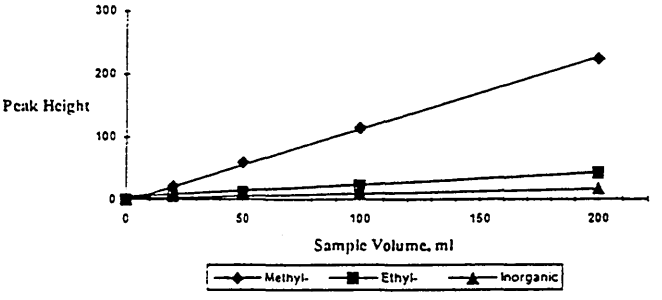

Fig. 5. Effect of sample volume on signal intensities for canal water spiked at the 1 μg Hg l^{-1} with MeHgCl, EtHgCl and Hg^{2+}

from the microcolumn. The effect of the concentration of hydrochloric acid on the elution step is shown in Fig. 2. As can be seen, there is a relatively constant response for methyl- and ethyl-mercury for 2, 3 and 5 mol/l hydrochloric acid while for inorganic mercury there is an improved signal at 3 and 5 mol/l. It was therefore decided to use 3 mol/l hydrochloric acid in further work.

Derivatisation was based on the work of Lückow and Russel [24] who utilised an excess of sodium tetraphenylborate at acidic pH for phenylation of inorganic mercury prior to GC analysis. As shown in the present work, phenylation is equally applicable to methyl- and ethylmercury, although for real samples it would not be possible to differentiate between phenylmercury and inorganic species. Derivatisation based on sodium tetraethylborate has previously been applied to mercury speciation studies [9] and in that case differentiation between ethyl and inorganic mercury was precluded.

To check the extraction efficiency, the eluate (derived from mixed mercury standard solution, 10 μg l^{-1} for 3 species) was subjected to three consecutive extractions with n-hexane (1 ml). It was found that on injections of the extracts (consecutively) into the GC-MIP-AES most of the mercury species were present in the first extraction (recovery values for single extraction: 97.1% for methyl-, 93.8% for ethyl- and 95.1% for inorganic mercury) and hence only a single extraction step was utilised in further work.

The extraction efficiency was affected by the co-existence of hydrochloric acid, the acid being required to effect elution. In order to test for the influence of hydrochloric acid on signal response, mixed standard solutions of methyl- and inorganic mercury (150 μg l^{-1}) were prepared in differing concentrations of hydrochloric acid. The results are shown in Fig. 3. As can be seen, there is a relatively constant response for both mercury species at different concentrations except at 3 mol/l. Eluate from the microcolumn (0.5 ml, 3 mol/l HCl) was therefore diluted with 1 ml of high-purity water before derivatisation/extraction in order to obtain a final acid strength of about 1 mol/l.

3.2 Analytical performance

Operational parameters for the GC-MIP-AES measurement are given in Table 1 and were as recomended by the manufacturer except for the helium gas flow rate (in the MIP and in the GC column). As shown in Fig. 4, mercury emission signals were maximised at a relatively low flow rate of 30 ml min^{-1}. Use of flow rates much below 30 ml min^{-1} would shorten the discharge tube life and might result in interactions between the analyte and the tube walls and give memory effects. A relatively high column flow rate of 2.5 ml min^{-1} was found to yield the best sensitivity for mercury emission.

The FI system with SCF afforded a simple, convenient and rapid means for analyte preconcentration. Figure 5 shows the improvement in method sensitivity for processing increasing volumes (20–200 ml) of a spiked (1 μg l^{-1} methyl-, ethyl- and inorganic mercury) canal water. In the case of a 200 ml sample, processing took about 50 min and resulted in a nominal enrichment factor of ca. 400

Table 2. Recovery data for spike additions to waters. Tap water and canal water spiked at the 1 μg l^{-1} level with mixed standard solutions. Data, mean ± s (n = 5). Sample volume, 60 ml

	Tap water	Manchester Ship Canal
Methylmercury	92.4 ± 4.2	93.6 ± 4.5
Ethylmercury	90.0 ± 3.9	92.1 ± 5.1
Inorganic mercury	93.0 ± 5.0	91.6 ± 3.3

Table 3. Speciation data for mercury in the Manchester Ship Canal. Data, mean ± s (n = 3). Sample volume, 60 ml

Water samples	Methylmercury μg l^{-1}	Ethylmercury μg l^{-1}	Inorganic mercury, μg l^{-1}
S1	0.160 ± 0.005	–	0.340 ± 0.003
S2	0.100 ± 0.003	–	0.320 ± 0.004
S3	0.040 ± 0.002	–	0.101 ± 0.002

(eluent, 0.5 ml) for the mercury species. That good deposition efficiency was maintained for mercury species under real sample conditions and high sample loading (i.e. 200 ml) testifies to the effectiveness of SCF for on-line preconcentration. Calibration graphs based on processing a mixed standard solution (60 ml) of methyl-, ethyl- and inorganic mercury were linear (r = 0.998 for methyl-, 0.997 for ethyl- and 0.997 for inorganic) over the concentration range examined (0, 0.1, 0.2, 0.5, 1, 5 and 10 μg Hg l^{-1}). Limits of detection for mercury species (based on processing 200 ml of carrier (HCl, 0.01 mol/l as described in sections 2.3 and 2.4 of Experimental) were 10 ng l^{-1} for methyl- and ethyl-mercury and 16 ng l^{-1} for inorganic mercury. The precision at the 10 μg l^{-1} level based on the use of 10 different microcolumns (i.e. n = 10) was 5.33% (methyl-), 10.5% (ethyl-) and 12% (inorganic mercury).

As a test of accuracy experiments were performed to check the recovery of spikes (1 μg Hg l^{-1}) added to drinking water and canal water. The data of Table 2 indicate that the proposed method may be valid for the determination and speciation of mercury in such waters.

3.3 Analytical application

In a preliminary trial the methodology was applied to waters of the Manchester Ship Canal, a watercourse known to be contaminated with inorganic mercury [23, 25]. The results of Table 3 confirm this. In addition, for the 3 sampling stations analysed elevated concentrations of methylmercury were detected (0.040–0.160 μg l^{-1}) in contrast to that of ethylmercury. This is a significant finding from an environmental standpoint, given the high toxicity of methylmercury species. These concentrations are considerably higher than reported in other studies [10, 19], confirming the extensive pollution of the watercourse not only by inorganic mercury but also by methylmercury.

The data are consistent with earlier studies of Wei Jian et al. [22, 23] in that relatively high concentrations (high ppt-level) of mercury species were detected in the canal. However the behaviour of SCF in terms of affinity to inorganic mercury was different for the two studies. In the present work, all mercury species underwent effective deposition on the SCF whereas for earlier work [21–23] inorganic mercury was not retained on SCF thus permitting rapid separation of respective mercury species. Further work on column preparation and conditioning is needed in order to clarify differences in uptake behaviour.

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Rapid determination of mercury in environmental materials using on-line microwave digestion and atomic fluorescence spectrometry

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Abstract

A procedure for on-line microwave digestion and determination of mercury in environmental materials by atomic fluorescence spectrometry has been developed. The method is based on the direct injection of slurried samples (acidified) into a carrier stream of hydrochloric acid. Microwave digestion in a Teflon coil (4 m) permits, in only 50 s, the quantitative extraction of Hg from the solid samples. In combination with atomic fluorescence detection the on-line digestion/procedure permits the determination of mercury with a throughput of 15 samples per hour. Based on a sample injection volume of 400 μl, precision was 0.5–1.5% R.S.D. and the limit of detection was 0.09 ng g^{-1}. Application to a range of certified environmental reference materials was demonstrated.

Keywords: Fluorimetry; Flow injection; Mercury; Environmental analysis

1. Introduction

The rapid growth in agriculture and industrial activity has resulted in chemical pollution. In this context mercury is one of the most toxic elements and can produce serious and irreversible neurological damage depending on its chemical form [1]. High concentrations of mercury in the aquatic environment are often associated with chemical plants manufacturing chlorine, sodium hydroxide and hydrogen, but the total concentration of the element in natural waters is low, typically between 0.02–0.5 ng l^{-1} in fresh water and < 10 ng l^{-1} in sea water [2], while for sediments concentrations are 0.23–3.42 μg g^{-1} [3]. Because the toxicity of mercury is well known and large amounts are discharged into the environment it is necessary to develop rapid and automated procedures for the determination and speciation of this element.

In the past 5 years or so, microwave dissolution procedures have been developed to reduce sample preparation/pretreatment times from hours to several minutes [4–8]. Pressure dissolution procedures have been employed for the determination of mercury in fish using nitric acid [9,10] and mixtures of nitric acid–hydrogen peroxide [11,12]. Microwave digestion at atmospheric pressure has been employed with nitric–sulphuric acid mixtures for determination of mercury in sediments [13], marine sediments [14]

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and oily wastes [15]. A further advance concerns the on-line digestion of liquid samples as reported by Burguera et al. [16] whereby a blood sample was injected into the flow injection (FI) system and mineralised on passage through the microwave reactor for determinations of Cu, Fe and Zn. On-line microwave digestion with flow injection-cold vapour-atomic absorption spectrometry (FI-CV-AAS) has also been applied for determinations of mercury in water and urine samples [17].

More recently there has been interest in the on-line digestion of solid samples as slurries. Burguera et al. [18] employed a domestic microwave oven (Panasonic) for digestion of biological tissues to determine Cd and Zn. Carbonell et al. [19,20] used a closed flow system whereby slurried sludge samples were passed through a domestic microwave oven (Balay) continuously until complete digestion was achieved in order to determine Cu, Mn and Pb. Karanassios et al. [21] utilised a stopped-flow approach with a domestic microwave oven (Toshiba) in the digestion of botanical and biological samples for determinations of Al, Ba, Ca, Cu, Fe, Mg, Mn and Zn. A similar application by Haswell and Barclay [22] utilised FI system with relatively long digestion coil (20 m) and in-line filter for determinations of Ca, Fe, Mg and Zn. The method of de la Guardia et al. [23], in contrast to the latter, utilised a relatively short digestion coil (1 m) for the determination of Cu, Mn, Pb and Zn in plant, dietary and sewage sludge samples.

The aim of the present work was to build on the findings of the aforementioned study [23] and devise similar methodology for the determination of mercury. Rapid and efficient extraction of mercury was realised for a range of environmental materials, in slurried form, using focused microwave heating at relatively low power. The paper describes the effects of key FI parameters on extraction efficiency and reports analytical data for certified reference materials.

2. Experimental

2.1. Apparatus

Flow injection-cold vapour atomic fluorescence (FI-CV-AFS) measurements were performed with a Merlin mercury detector with instrumental conditions given in Table 1. A Microdigest microwave oven was employed for the digestion of samples. A peristaltic pump, equipped with two vinyl chloride flexible tubes (1.42 mm i.d.), was employed for carrier and reductant solution transport.

Table 1
Instrumental parameters for the determination of mercury

Parameter	
Peristaltic pump	Gilson Minipuls 3
Carrier flow	2.5 ml min^{-1} (HCl 0.01 M)
Reductant flow	2.5 ml min^{-1} ($SnCl_2$, 3% in 15% HCl solution)
Ar flow	600 ml min^{-1}
Rotary injection valve	Omnifit
Sample injection volume (L1)	400 μl
Microwave oven	Prolabo (Microdigest 301)
Power supply	10% (20 W)
Digestion coil (L2)	4 m (PTFE coil 0.8 mm i.d.)
Cooling system coil (L3)	2 m (PTFE coil 0.8 mm i.d.)
Degassing trap volume	500 μl
Reduction coil (L4)	10 cm (PTFE coil 0.8 mm i.d.).
Gas liquid separator	PS Analytical
Atomic fluorescence detector	Merlin PS Analytical (253.7 nm)

The manifold employed for on-line digestion /on-line detection was a 2-line system as shown in Fig. 1. Samples and standards, injected into the carrier stream using a rotary injection valve (L1), passed through the PTFE digestion coil located inside the microwave oven (L2) and after that through the PTFE coil (L3), which was located in an ice/water bath, to avoid possible mercury loss in the degassing trap. The reductant stream merged with the carrier and the mixture passed through the reduction coil (L4) before entry to the gas liquid separator

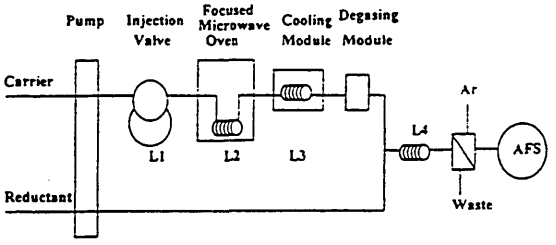

Fig. 1. Flow injection manifold for on-line digestion of solid samples with the cold vapour atomic fluorescence detector system.

and fluorescence detection. To avoid mercury vapour dilution from the bubbles produced in digestion, a degassing trap was located between the ice bath and the T-connector for the reductant stream (see Fig. 1). All manifold lines were of Teflon tube (0.8 mm i.d). As a safety measure, 5 ml of water was added to the reactor, and this small quantity of water was sufficient for 5 h work at 10% microwave power.

2.2. Reagents

Tin(II) chloride solution (3%, w/v) in hydrochloric acid (15%, v/v) was prepared daily from $SnCl_2 \cdot 2H_2O$ (Spectrosol, BDH) and high purity water (Millipore). Hydrochloric acid (0.01 M) prepared from concentrated hydrochloric acid (Aristar, BDH) and Millipore water served as the carrier stream. Standard solutions of mercury (from 5 to 10 $\mu g\ l^{-1}$) were prepared by diluting commercial stock solution (Spectrosol, BDH, 1000 mg l^{-1}) with Millipore water and with the same quantity of concentrated nitric acid (Aristar, BDH) as samples.

2.3. Samples

Samples investigated were (1) BCR sewage sludge of domestic origin (CRM 144), (2) BCR sewage sludge amended soil (CRM 143R), (3) State Bureau of Metrology (China) polluted farmland soil (GBW 08303), and (4) Canada Centre for Mineral and Energy Technology lake sediment (LKSD 4).

2.4. Procedures

In order to study the effect of key experimental parameters on the efficiency of on-line digestion, a sewage sludge slurry (BCR CRM 144, 1.2 g/250 ml), in a nitric acid–water (4 + 1) mixture, was fed continuously through the microwave reactor. Parameters investigated were: carrier flow, digestion coil length and microwave power level. For each set of conditions samples (typical volume, 20 ml) were collected in beakers for determination of mercury by FI-CV-AFS. Studies were also performed with on-line detection, i.e., discrete sample volumes were injected into the carrier stream and AF signals were registered. In these experiments, the effect of varying the sample injection volume and concentration of nitric acid used in slurry preparation were investigated.

A typical analysis run was as follows: a homogenised sample was weighed (70–300 mg) into a volumetric flask (25 ml), concentrated nitric acid was added (15 ml) and the flask was shaken to obtain a homogeneous dispersion, and then diluted to the mark with Millipore water. The slurry was injected (400 μl) into the carrier stream (0.01 M HCl) and irradiated at 10% power level (20 W) for 50 s (the time that a sample takes to pass through the microwave oven reactor at a carrier flow of 2.5 ml min^{-1}).

Standard solutions (2–10 $\mu g\ l^{-1}$) were prepared with the same concentration of nitric acid as samples and processed similarly. Calibration data were based on evaluation of peak height signals. Extraction efficiency for mercury was obtained by calculating the ratio between the concentration value calculated from interpolation of the peak height signal in the calibration graph divided by the actual mercury concentration.

3. Results and discussion

3.1. On-line digestion of slurry samples

Initial work was concerned with the continuous feeding of the slurry sample through the microwave reactor, and for variation in key system parameters for determining mercury (off-line) in the processed solutions. Fig. 2 shows the influence of digestion time and power level on mercury yield for the digestion of the CRM 144 sewage sludge sample. As can be seen, time is a critical parameter and an increase in digestion time results in an increase in mercury yield. However, use of relatively high power levels resulted in lower yield (i.e., 80% recovery), possibly as a result of diffusional losses of mercury.

The results for the digestion of the same sewage sludge sample using different digestion coil lengths (from 2 to 6 m), different carrier flow rates (from 2 to 4 ml min^{-1}) and different power levels (from 0 to 35%) are shown in Fig. 3. The data show that a higher carrier flow rate results in an increase in yield for relatively long digestion coil lengths (Fig. 3c). Conversely, lower carrier flow rates (2.2 ml min^{-1})

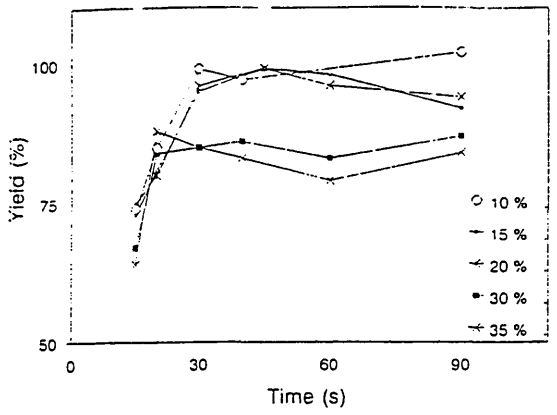

Fig. 2. Effect of digestion time at different microwave power levels on the Hg yield obtained from a sewage sludge slurry sample.

do not influence the yield when altering the coil length (Fig. 3a). Furthermore it was noted that in all cases a low power level (10–15%) gave the best yield.

Based on the above findings a sample residence time within the microwave oven cavity of 50 s (4 m coil digestion length; 2.5 ml min^{-1} carrier flow rate) together with low microwave power (10%) was selected for further studies involving injection of discrete sample volumes into the FI-CV-AFS system.

3.2. Effect of nitric acid concentration and sample injection volume

In initial studies of mercury determination in domestic sewage sludge (CRM 144) using different acid strengths (6.4–16 M) and different sample injection volumes (150–1150 μl) it was found that a minimum nitric acid concentration of 9.6 M was

Table 2
Effect of the sample injection volume ($n = 3$) on Hg atomic fluorescence (mean peak height intensity)

Injection volume (μl)	Hg conc. ($\mu g\ l^{-1}$)			
	5	7.5	10	Sample
1150	495±3	753±6	1014±18	654±20
650	326±9	519±12	692±4	507±20
400	174±1	264±4	336±2	306±1
150	102±3	154±2	197±1	148±2

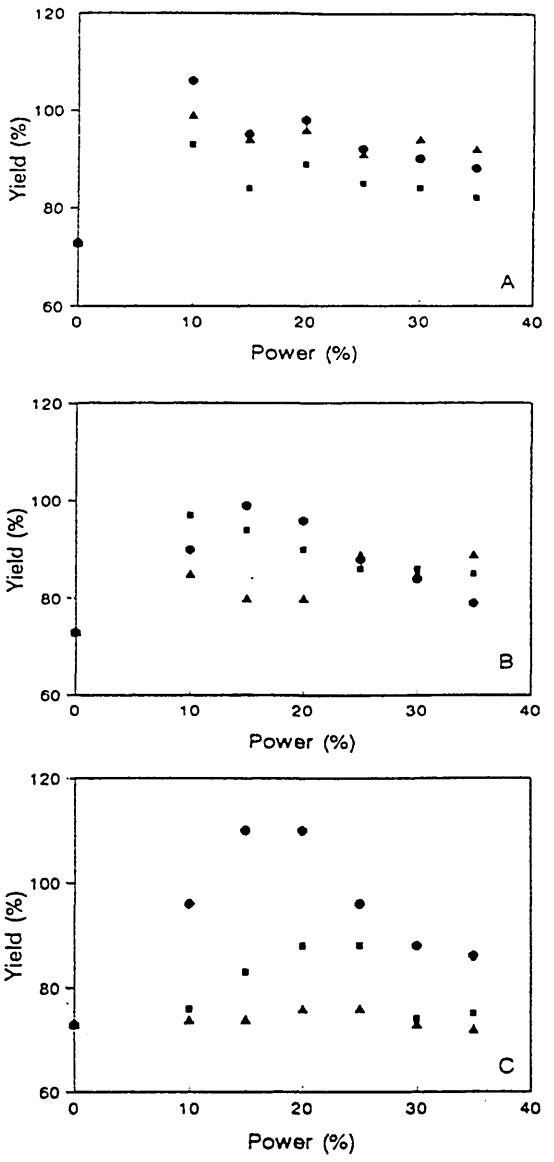

Fig. 3. Effect of mixing coil length (digestion time) and microwave power level at different carrier flows on the recovery of Hg in a sewage sludge sample. (A) 2.2 ml min^{-1}, (B) 3.8 ml min^{-1}, (C) 4.1 ml min^{-1} (●, 6 m; □, 4 m; ▲, 2 m coil length).

necessary for the quantitative extraction of mercury. Furthermore, using lower concentrations of acid did result in some clogging problems within the manifold. On the other hand, no improvement in yield was found at higher nitric acid concentrations. Thus a mixture of nitric acid–water (15 ml + 10 ml) was recommended for preparation of slurries.

Table 3
Performance parameters of the developed procedure for various sample injection volumes ($n = 3$)

Injection volume (μl)	Calibration line	r	LOD ($\mu g\ g^{-1}$)	R.S.D. (%)
1150	$F = 7.8 + 109.1\ C$	0.9995	0.094	1.4
650	$F = 27.0 + 79.6\ C$	0.9996	0.23	1.0
400	$F = 10.2 + 30.8\ C$	0.9997	0.23	0.5
150	$F = 10.5 + 17.3\ C$	0.9989	0.23	1.5

Table 4
Results ($\mu g\ g^{-1}$) obtained for the determination of mercury in certified reference materials (number of sample injections in parentheses)

Reference Material	Certified Value, ($\mu g\ g^{-1}$)	This study, ($\mu g\ g^{-1}$)	Average of obtained values, ($\mu g\ g^{-1}$)	R.S.D. (%)	Error (%)
CRM-144	1.49	1.52 ± 0.06 (4) 1.42 ± 0.02 (4) 1.54 ± 0.02 (4)	1.49 ± 0.06	4.0	–
CRM-143 R	1.10 ± 0.07	0.91 ± 0.03 (4) 0.92 ± 0.01 (4) 0.97 ± 0.02 (4)	0.93 ± 0.03	3.2	−6.4
GBW-08303	2.15 ± 0.12	2.14 ± 0.06 (4) 2.18 ± 0.07 (4) 2.03 ± 0.03 (4)	2.12 ± 0.08	3.8	−1.4
LKSD-4	0.19	0.212 ± 0.009 (4) 0.202 ± 0.009 (4) 0.201 ± 0.005 (4)	0.205 ± 0.006	2.9	7.9

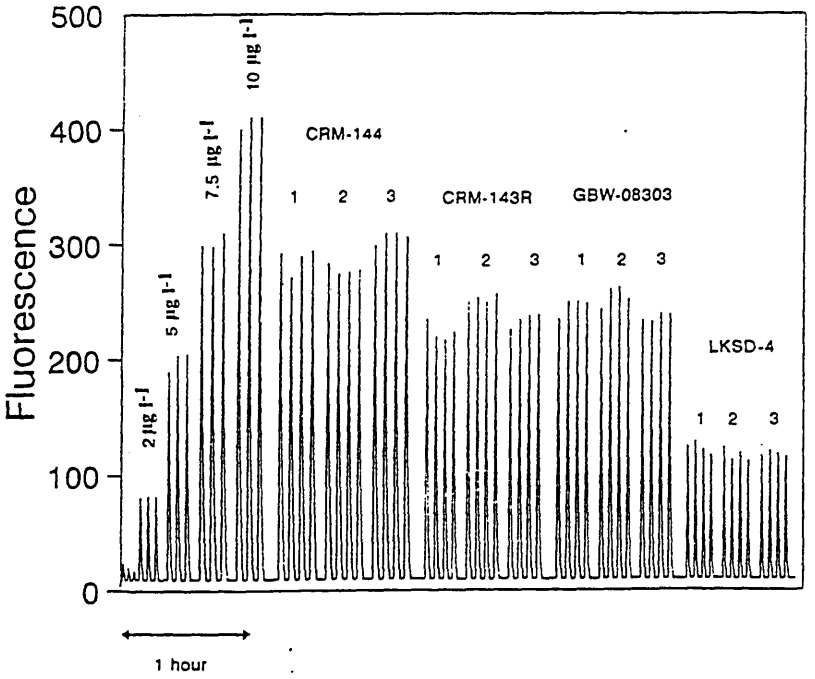

Fig. 4. FI responses for the determination of Hg in certified reference materials.

Table 2 shows the effect of variation of sample injection volume on peak height response. For these experiments BCR domestic sewage sludge (CRM 144) was dispersed in 25 ml of nitric acid while calibration standards (5, 7.5 and 10 $\mu g\ l^{-1}$) were prepared at the same acid concentration as the sample. The sensitivity (expressed as the slope of the regression line), detection limit (three times the standard deviation of the blank ($n = 10$) divided by the slope of calibration line), and %R.S.D. ($n = 3$) were determined for four different injection volumes. Generally, as shown in Table 3, sensitivity improved with increase in sample injection volume. Furthermore, linearity of response was good for the concentration range examined and the R.S.D. ranged between 0.5 and 1.5%. For analytical application, an injection volume of 400 μl was selected because this permitted the analysis of more than 15 samples per hour with sufficient sensitivity to quantify Hg in a range of environmental materials.

3.3. Analysis of certified reference materials

Four environmental certified reference materials were analysed using the recommended procedure and typical FI-CV-AFS responses are given in Fig. 4. As can be seen from Table 4 the accuracy and precision data are extremely good for the different sample types examined, considering the low levels of mercury present. These results indicate that the on line microwave digestion procedure is highly effective for extraction of mercury from the different sample matrices examined, i.e., soil, sewage sludge and lake sediment, and suggest that the developed procedure may have wide applicability.

4. Conclusions

On-line microwave digestion in combination with FI-CV-AFS provides a new and efficient approach to rapid determinations of mercury in environmental materials such as soils, sediments and sewage sludge. Furthermore the developed procedure reduces sample preparation and handing time to a minimum with the consequence that complete automation of the methodology with a throughput of 15 samples per hour is possible. It is proposed to extend investigations to the speciation of mercury in environmental solid samples.

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Stability of Chromium Species Immobilised on Microcolumns of Activated Alumina

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Abstract: A batch of alumina microcolumns was charged with chromium(III) and chromium(VI) (basic and acidic alumina microcolumns respectively, 200 $\mu g \cdot L^{-1}$, 250 µl) and stored under different storage regimes. At regular time intervals over a 6 month period, microcolumns were removed and chromium species were quantified by flow injection-inductively coupled plasma-atomic emission spectroscopy (FI-ICP-AES). It was found that analyte recoveries for chromium (III) and chromium(VI) were of the order 57% and 27%, respectively. In order to achieve effective elution under continuos flow conditions it was necessary to incorporate microwave heating in the FI system. A conventional acid digestion of the chromium enriched alumina powder was effective for recovery chromium species.

Key Words: Chromium speciation, stability, microcolumns, flow injection, inductively coupled plasma atomic emission spectrometry, microwave heating.

Introduction

The development of rapid, robust and highly sensitive techniques for quantification of toxic substances in the environment is a priority. In recognition of this the European Commission (EC) through the Standards, Measurements and Testing (SMT) programme co-ordinate projects aimed at improving analysis capability and producing new types of certified reference materials (CRMs). A concise summary of recent projects sponsored by the EC is available [1].

With respect to new methodology for the determination and speciation of trace elements in environmental systems, work at Sheffield has focused on the use of flow injection (FI) systems for implementing rapid on-line analyte enrichment/species separation. For instance, microcolumns of activated alumina are particularly relevant to chromium speciation and have been used to determine concentrations of chromium(III) and (VI) in waters via flow injection-inductively coupled plasma-emission spectrometry (FI-ICP-AES) [2, 3]. Chromium speciation studies have also been performed with FI-ICP-MS using microcolumns packed with conventional ion exchangers (ANX1606, CETAC Technologies) and reliability was assessed in a recent interlaboratory exercise [4].

Mercury speciation is another topic of interest. Wei et al [5] utilised FI-cold vapour-atomic fluorescence spectroscopy (CV-AFS) with a microcolumn of sulphydryl cotton for the rapid sequential determination of inorganic mercury and organomercury in natural waters at the $ng \cdot L^{-1}$ level. Unfortunately the separative capability afforded by the FI system did not permit discrimination of individual organomercury species and hence the FI procedure was combined with gas chromatography-microwave induced plasma-atomic emission spectroscopy (GC-MIP-AES) [6].

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Another interesting development concerns the incorporation of an on-line microwave reactor into FI systems to effect rapid extraction/detection of inorganic mercury for environmental materials [7]. The newly developed procedure was based on the direct injection of slurries samples (acidified) into a carrier stream of hydrochloric acid with on-line microwave extraction in a Teflon coil (4 m). Quantitative extraction of mercury from solid samples was obtained in about 50 seconds. In combination with atomic fluorescence detection the on-line extraction/procedure permitted the determination of inorganic mercury with a throughput of 15 samples per hour.

A further and potentially highly significant development for speciation research concerns the use of microcolumns to collect, in the field, analyte species of interest, i.e. instead of undertaking sample processing in the laboratory samples are processed immediately on sample collection in an attempt to stabilise/immobilise species of interest. Microcolumns with retained analytes are then returned to the laboratory and inserted into the FI system for elution/final measurement. In this way it is hoped to avoid the problems associated with maintaining species integrity during the time delay between sampling and laboratory analysis. Viability has been successfully demonstrated in the case of chromium speciation in rivers [8], mercury speciation in rivers [9], gold determination in lakes [10] and determination of reactive aluminium in natural waters [11].

A further potential application of microcolumns is their use as external calibrants and certified reference materials (CRMs). Given the interest and increasing acceptance of methodology based on on-line FI procedures there will be an increasing demand for CRMs in microcolumn format. Microcolumns with retained analytes could offer the ability to recover species upon elution (by inserted the microcolumn into a FI system) with a verified, certified recovery after a long term period. In this way it is hoped to avoid the problems associated with maintaining species integrity in aqueous solution. Recently a batch of sulphydryl cotton microcolumns was prepared and charged with a mixed mercury calibrant solution (methyl-, ethyl- and inorganic mercury, 10 $\mu g \cdot L^{-1}$ as Hg, 3 ml) and stored at 4°C in a light tight box. At regular time intervals over a 4 month period microcolumns were removed and mercury species were quantified by gas chromatography-microwave induced plasma-atomic emission spectrometry (GC-MIP-AES) (after elution/ extraction/ derivatisation). It was found that analyte recoveries for methyl- and inorganic mercury were essentially quantitative over the 4 month period while ethylmercury species were stable for up to 2 months [12].

The aim of the present work is to immobilise chromium(III) and chromium(VI) species on microcolumns of activated alumina and, based on long term stability data, demonstrate the possibility of developing a new class of reference material.

Experimental

Reagents and Materials.

Calibrant solutions of chromium(III) and chromium(VI) (200 $\mu g \cdot L^{-1}$) were prepared by appropriate dilutions of stock solutions (1000 $mg \cdot L^{-1}$ of potassium dichromate and chromic nitrate; Merck, ANALAR). Nitric acid (4 $mol \cdot L^{-1}$, 2 $mol \cdot L^{-1}$ and 0.02 $mol \cdot L^{-1}$), ammonium hydroxide (2 $mol \cdot L^{-1}$ and 0.02 $mol \cdot L^{-1}$) and potassium hydroxide (2 $mol \cdot L^{-1}$) were prepared from concentrated reagents (Merck, ARISTAR). Activated alumina was chromatographic grade (Merck, Brockman grade 1, particle size range 180-212 μm).

Alumina Microcolumns

Microcolumns were prepared by packing PTFE tube (6 cm x 1.5 mm id.) with activated alumina (ca. 95 mg/column). Columns were each inserted into the FI manifold (described later) and subjected to injections of nitric acid (2 ml, 2 $mol \cdot L^{-1}$) and ammonium hydroxide (2 ml, 2 $mol \cdot L^{-1}$) to remove residual contamination. Approximately 300 microcolumns were prepared and treated in this way.

Instrumentation

The FI manifold (Figure 1), a single line system, and consisting of peristaltic pump (Gilson Minipuls), rotary injection valve (Omnifit: 250 µl sample loop) and alumina microcolumn was connected directly to nebuliser tubing of an ICP emission spectrometer (SpectroAnalytical, Spectro P).

The spectrometer utilised the chromium 267.72 nm line and was operated using standard plasma operating conditions. Data acquisition and monitoring of transient signals were accomplished using resident software. The preflush time was 13 s and the integration time was 30 s.

Immobilisation

Chromium(III) microcolumns. A microcolumn was inserted into the FI manifold and conditioned by passing eluent (HNO_3 2 $mol \cdot L^{-1}$, 3 injections of 250 µl) and then carrier solution (NH_4OH 0.02 $mol \cdot L^{-1}$, 2 ml). Chromium(III) calibrant solution (250 µl, 200 $\mu g \cdot L^{-1}$) was

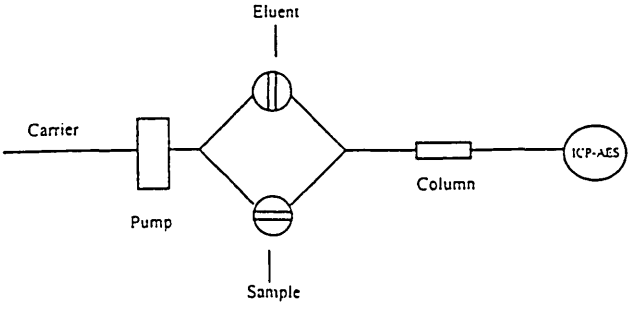


Figure 1. Flow injection manifold for chromium speciation.

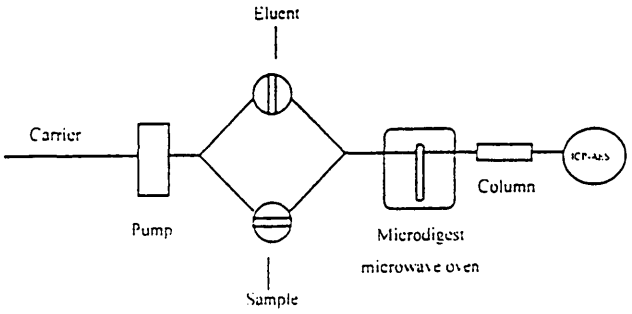


Figure 2. Flow injection manifold with microwave reactor.

then injected into the FI system. The alumina microcolumn was then removed from the system for storage. The process was repeated for a further 150 microcolumns.

Chromium(VI) microcolumns. A microcolumn was inserted into the FI manifold and conditioned by passing eluent (NH_4OH 2 mol· L^{-1}, 3 injections of 250 µl) and then carrier solution (HNO_3 0.02 mol· L^{-1}, 2 ml). Chromium(VI) calibrant solution (250 µl, 200 µg· L^{-1}) was then injected into the FI system. The alumina microcolumn was then removed from the system for storage. The process was repeated for a further 150 microcolumns.

Storage conditions.

Microcolumns were placed in polyethylene bags and stored under various regimes until analysis: (1) storage at 4°C in refrigerator. (2) storage at -70°C in refrigerator and (3) oven drying at 65°C (overnight) and subsequent storage in a desiccator at room temperature.

Elution and quantitation.

Aged microcolumns were inserted into the FI system for elution/quantitation (for chromium(III), 250 µl of HNO_3 (2 mol· L^{-1}); for chromium(VI), 250 µl of NH_4OH (2 mol· L^{-1}) after 1 day, 2 days, 4 days, 8 days, 11 days, 16 days, 3 months and 6 months. In order to calculate analyte recoveries, transient signals for the aged microcolumns were evaluated with reference to signals derived from freshly charged microcolumns. Results for each analysis were based on elutions for 3 separate microcolumns.

On-line microwave digestion.

Chromium(III) microcolumns. Fresh and aged alumina microcolumns (n=45, oven drying at 65°C (overnight) and subsequent storage in desiccator at room temperature over an 1 and 6 month period) were inserted into the FI system incorporating a microwave oven (Microdigest, Prolabo,) (Figure 2), and subjected to elution at different microwave powers and for different length of heating coil. In order to calculate analyte recoveries, transient signals were evaluated with reference to signals derived from freshly prepared chromium(III) calibrant (200 µg· L^{-1}, 250 µl).

Chromium(VI) microcolumns. Preparation and elution/quantitation was identical to that for chromium(III).

Results and Discussion

Prior to undertaking stability studies and examining the effect of storage regimes on analyte recoveries it was

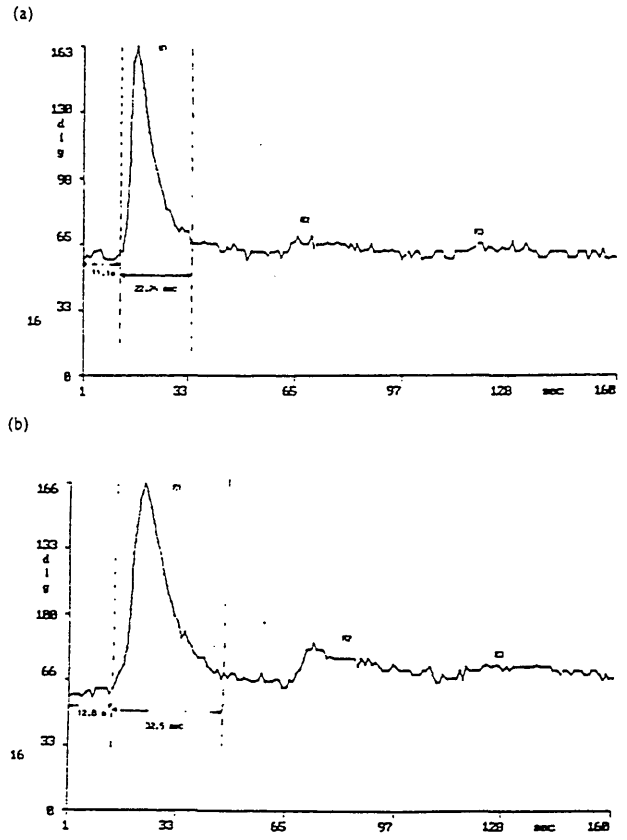
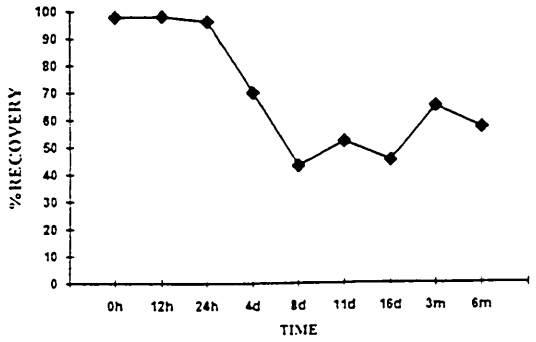


Figure 3. Typical emission-time response for an injection/elution process: (a) chromium(VI) (200 µg· L^{-1}, 250 µl, NH_4OH 2 mol· L^{-1}) and (b) chromium(III) (200 µg· L^{-1}, 250 µl, HNO_3 2 mol· L^{-1}).

necessary to first demonstrate that prepared microcolumns exhibited low column to column variability in terms of recovered chromium species. For this study 14 microcolumns were selected at random and seven were charged, in an off-line mode, with chromium(III) (200 µg· L^{-1}, 250 µl) and seven with chromium(VI) (200 µg· L^{-1}, 250 µl). The microcolumns were then inserted, one after the other, into the FI-ICP system for elution/quantitation. In a separate experiment chromium(III) solution (200 µg· L^{-1}, 250 µl) was processed on-line and the deposition/elution cycle was repeated seven times. Chromium(VI) solutions were similarly processed/measured. Typical ICP emission-time responses corresponding to elution of chromium(III) and chromium(VI) are given in (Figure 3 a, b). The analytical data of (Table 1) confirm a low column to column variability. On the basis of this finding it was considered appropriate to proceed with stability studies.

Alumina microcolumns (n=108) were charged with chromium (acidic alumina: chromium(VI) 200 µg· L^{-1}, 250 µl; basic alumina: chromium(III) 200 µg· L^{-1}, 250 µl) and then stored at 4°C until analysis. The charged microcolumns were then inserted into the FI system at

(a)



(b)

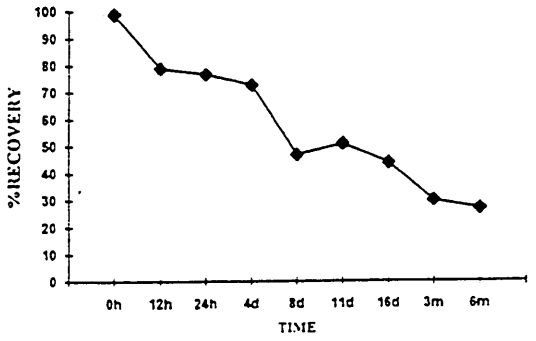


Figure 4. Stability study for (a) chromium(VI) and (b) chromium(III).

appropriate time intervals and subjected to elution/quantitation. From the analytical data presented in (Figure 4a,b) there would seem to be a progressive decline in analyte recovery with time over a 16 day period. For the first 24 hours analyte loss in the case of chromium(VI) Figure 4a) was marginal but within 8 days recoveries for both chromium(VI) (Figure 4a) and chromium(III) Figure 4b) were of the order 47±3% and 47±4% respectively. Generally between 8 days and six months recovery values were relatively constant for chromium(VI) (40 - 60%) whereas there was a progressive decline for chromium(III). A possible explanation for reduced recovery is a gradual and increasing loss of analyte to residual fluid in the microcolumns (0.02 mol· L^{-1} NH_4OH or 0.02 mol· L^{-1} HNO_3). Another possibility is the incomplete removal of analyte during the elution step. Further experiments concerning variation in storage conditions were next attempted in order to gain insight into the loss mechanisms.

Alumina microcolumns (n=108) were charged as before and then subjected to 3 different storage regimes before analysis: (1) storage at 4°C as before, (2) storage at -70°C and (3) oven drying at 65°C (overnight) and subsequent storage in desiccator at room temperature. Aged microcolumns were then inserted into the FI system at appropriate time intervals and subjected to elution (for chromium(III), 250 µl, HNO_3 2 mol· L^{-1}; for chromium(VI), 250 µl, NH_4OH 2 mol· L^{-1}). The results for the analysis performed over an 8 day period, are summarised in (Table 2). It can be seen that for both chromium forms and for all storage conditions there still remains considerable signal loss. The magnitude of analyte recoveries for the experiments performed at 4°C (for both chromium(VI) and chromium(III), of the order 57±3% and 54±3%, respectively) correlate well with the data of (Figure 4a,b). Storage of microcolumns at -70°C tended to yield relatively constant recoveries within the 8 day period for both chromium(VI) (47±3% - 54±1%) and chromium(III) (49±5% - 62±3%). Oven drying and subsequent storage in a desiccator also resulted in relatively constant but low recoveries: chromium(III) (21±1% - 27±1%) and chromium(VI) (44±3% - 51±1%).

On the basis of the above results it would seen that for aged microcolumns the strength of bonding between chromium species and the alumina structure altered with

able 1. Precision (n=7) for determination of chromium species y FI-ICP-AES.

	Without alumina microcolumn		With 1 alumina microcolumn		With 7 alumina microcolumn	
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
X	2283	2427	2310	2336	2290	2346
S	26	35	61	57	72	78
CV%	1.20	1.50	2.60	2.40	3.20	3.30

Table 2. Analyte recovery (%) for chromium species under various storage regimes (n=3).

		24 hours	48	4 hours	8 hours
Cr(III)	I	79±4	77±1	73±3	54±3
	II	61±6	62±5	62±3	49±5
	III	26±4	27±1	25±1	21±1
Cr(VI)	I	98±5	97±2	73±5	57±3
	II	47±3	50±4	54±1	50±2
	III	44±3	49±3	51±1	21±2

I. Storage at 4°C. II. Storage at -70°C. III. Oven drying (70°) and storage in desiccator. See experimental for full details.

storage time and thus in order to achieve effective elution more vigorous conditions for the elution step are needed. This was confirmed by subjecting aged columns to conventional hot plate digestion with nitric acid. Recoveries for chromium(III) were 101±1% and 96±5% for fresh and aged microcolumns, respectively and for chromium(VI) were 99±5% and 98±5%.

In a further attempt to improve recovery under continous flow conditions a microwave reactor was incorporated into the FI system as shown in (Figure 2) (KOH 2 mol· L^{-1} was used as eluent for chromium (VI) while HNO_3 4 mol· L^{-1}, for chromium (III)). Alumina microcolumns (n=90) were charged as before (acidic alumina: chromium (VI) 200 µg· L^{-1}, 250 l; basic alumina: chromium (III) 200 µg· L^{-1}, 250 µl) and after 1 month storage were inserted into the FI system for elution/quantitation. Elution response was studied as a function of microwave power (0, 10, 20 and 30%) and length of the heating coil (0.4, 1 and 3 m). The results are summarised in (Table 3). It can be seen that for both chromium forms 100% analyte recovery can be obtained but there is dependency on microwave power and length of the heating coil. Analyte recoveries for the experiments performed in the absence of microwave power (for both chromium(VI) and chromium (III) were of the order 55±12% and 23±3%, respectively) and again were similar to the data of (Figure 4a,b). An increase in microwave power to 10% for chromium(VI) and to 30% for chromium(III), (the length of the heating coil for both species was independently 1 or 3) resulted in analyte recoveries of 106±7% - 108±8% for chromium(VI) and 107±15% and 110±3% for chromium(III). Aged microcolumns (6 months) were also studied at 30% power and with 3 m of heating coil. Relatively poorer recoveries of 90±8% for chromium(VI) and 80±10% for chromium(III) were obtained.

Table 3. Analyte recovery (%) for chromium species with on-line microwave digestion

		0% Power	10% Power	20% Power	30% Power
Cr(III)	I	23±3	43±5	35±2	47±5
	II	23±3	82±7	88±9	107±15
	III	23±4	82±14	88±9	110±3
Cr(VI)	I	55±12	49±6	67±4	86±2
	II	55±12	108±8	98±12	102±14
	III	55±12	106±7	-	-

I. Heating coil: 0.4 m. II. Heating coil: 1 m and III. Heating coil: 3 m. See experimental for full details.

Conclusions

Studies have demonstrated that, for alumina microcolumns charged with chromium(III) and chromium(VI), there is a difference in binding strength between freshly prepared and aged microcolumns. As a result, unless vigorous elution conditions are established (e.g. microwave heating) under FI conditions, analyte recoveries will be incomplete. Prospects for developing alumina microcolumns, in their present format, as a new candidate reference material are therefore compromised unless elution is performed in a batch/off-line manner so that 100% elution efficiency is achieved. Further work aimed at modifying/deactivating the alumina structure could prove useful for FI measurements as would be investigation of alternative column packings.

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Mercury Species Immobilized on Sulphydryl Cotton: a New Candidate Reference Material for Mercury Speciation

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Abstract. A batch of sulphydryl cotton microcolumns was prepared and charged with a mixed Hg standard solution (methyl-, ethyl- and inorganic Hg, 10 $\mu g\ l^{-1}$ as Hg, 3 ml) and stored at 4°C in a light-tight box. At regular time intervals over a 4 month period microcolumns were removed and Hg species were quantified by gas chromatography microwave-induced plasma atomic emission spectrometry (after elution, extraction and derivatization steps). It was found that analyte recoveries for methyl- and inorganic Hg were quantitative over the 4 month period while ethyl-Hg species appeared to be stable for up to 2 months.

Key words: mercury speciation, candidate reference material, flow injection, atomic fluorescence spectrometry, gas chromatography microwave-induced plasma atomic emission spectrometry.

Over the last decade significant advances in methodology for the determination and speciation of Hg in environmental systems have been reported [1–3]. In the author's laboratory, for instance, work has focused on the use of flow injection (FI) systems incorporating microcolumns of sulphydryl cotton fibre (SCF) to effect on-line sample preconcentration prior to quantification by cold vapour atomic fluorescence spectrometry (CV-AFS) and gas chromatography microwave-induced plasma atomic emission spectrometry (GC-MIP-AES) [4, 5]. In the case of natural waters the same microcolumn may be used in the field to collect and immobilize Hg species. Microcolumns with retained analytes are subsequently returned to the laboratory for final treatment and analysis. Based on these activities an integrated analytical scheme for determination and speciation of Hg in waters has been devised [6]. Besides using microcolumns of SCF as an aid to sample processing and sampling, microcolumns charged with analyte could serve as external calibrants and, furthermore, if species integrity was maintained, a new class of reference material (RM) could be developed.

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In order to validate new methods and check their reliability it is essential to use RMs certified for elemental and species composition. In the case of natural waters few RMs are available for Hg speciation measurement due to the problems associated with production of natural materials and maintaining Hg species stability. Lansens et al. undertook stability tests on Hg standard solutions and recommended that samples be prepared in PTFE containers and stored in a refrigerator at 4 °C [7, 8]. Glass volumetric flasks need a pre-treatment with HNO_3. Stability studies on seawater conflict with previous investigations in that conversion of methyl-Hg chloride to inorganic Hg and adsorption to container walls have been reported [8]. Adsorption losses were rapid for polyethylene vessels and hence glass was recommended as the preferred container material. It was also suggested that samples, after collection, be stored in brown bottles because light may promote the conversion of methyl-Hg to inorganic Hg. Given the problems associated with maintaining species stability in aqueous media the possibility of achieving stabilization through immobilization on a solid support was considered an interesting research topic. The present study, therefore, examines the stability of Hg species immobilized on SCF. A batch of microcolumns (30) was prepared and enriched with methyl-, ethyl- and inorganic Hg species. The microcolumns were then analyzed over a 4 month period using GC-MIP-AES in order to assess stability of the immobilized Hg species.

Experimental

Reagents and Materials

The SCF adsorbent was produced by introducing the sulphydryl functional group in natural cotton fibres according to the procedure of Lee et al. [2]. Standard solutions of inorganic Hg were prepared by appropriate dilution of stock solution (1000 mg l^{-1} of $Hg(NO_3)_2$ in 1% v/v HNO_3) with high purity water (Millipore, Molsheim, France). The organo-Hg stock solutions (1000 mg l^{-1} of RHgCl, R=CH_3 and C_2H_5) were prepared by dissolving the compounds in acetone (1 ml, Aristar, Merck, Darmstadt, Germany) and standard solutions were prepared by appropriate dilution with high purity water (Millipore). HCl (0.01 *M* and 3 *M*) was prepared from concentrated reagents (Aristar, Merck). Tin chloride solution (3% m/v) in HCl (15% v/v) was prepared daily from $SnCl_2 \cdot 2H_2O$ (Spectrosol, Merck), HCl (36% m/m; Aristar, Merck) and high purity water. A KBr-$KBrO_3$ solution (0.5% + 0.14% m/v) was made by dissolving respective reagents (Analytical grade, Fisons, Loughborough, UK) in water. A buffer solution of NaOH (2.7 *M*) and CH_3COONa (3 *M*) was prepared by dissolving the compounds in high purity water (Millipore). Sodium tetraphenylborate (5% solution in Millipore water) was freshly prepared a few minutes before use.

Preparation of Enriched Microcolumns

Microcolumns were prepared by packing PTFE tubes (5 cm × 1.5 mm id) with SCF (~ 0.015 g). Columns were then inserted, one at a time, into an FI system and conditioned by passing eluent (HCl 3 *M*, 3 injections of 0.5 ml) and then carrier solution (HCl 0.01 *M*, 2 ml). A mixed Hg standard solution (10 µg l^{-1} methyl-, ethyl- and inorganic Hg; 3 ml) was adjusted to pH 3.5 by dropwise addition of concentrated HNO_3 and then injected into a single line FI system to charge the microcolumn. A fresh column was then inserted and the process repeated until loading of the 30 columns was complete. Microcolumns were placed in polyethylene bags in a light-tight box and stored at 4°C in a refrigerator until analysis by GC-MIP-AES. In a separate experiment, to check column-to-column variability, 10

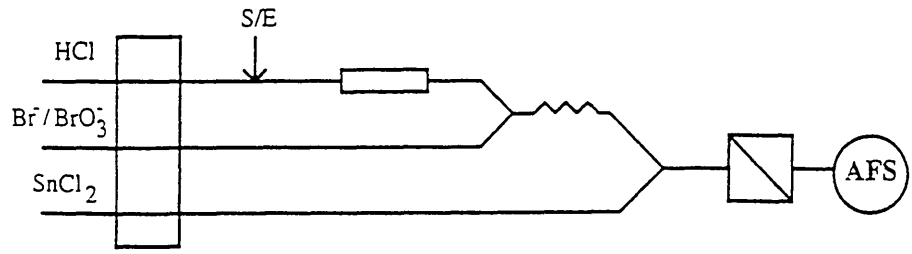

Fig. 1. FI system incorporating microcolumn of SCF for determination of methyl-Hg (see text for operating parameters)

microcolumns were charged with methyl-Hg chloride (10 μg l^{-1}, 0.5 ml) and then analyzed using FI-AFS. For comparison purposes, a single microcolumn was used for processing of the methyl-Hg standard solution in an identical manner to the above.

Instrumentation and Measurements

Flow injection – atomic fluorescence spectrometry: Main components of the FI-AFS system, illustrated in Fig. 1, were a peristaltic pump (Ismatec, London, UK), rotary injection valve (Omnifit, London, UK) and atomic fluorescence detector incorporating gas-liquid separator (Merlin, P. S. Analytical, Sevenoaks, UK). Enriched microcolumns were inserted into the FI-AFS system for elution and quantitative analysis. The methyl-Hg, after elution (with 250 μl of HCl 3 *M*) from the microcolumn, was oxidized to inorganic Hg by a Br^--BrO_3^- solution and then reduced to elemental Hg by $SnCl_2$ reagent. Prior to use, the FI system was cleaned and rinsed by pumping HCl solution (5 *M*) for 25 min and Millipore water for 10 min. The reductant ($SnCl_2$), oxidant (Br^--BrO_3^-) and carrier (0.01 *M* HCl) streams were continuously pumped, each at a flow rate of 1.5 ml min^{-1}. When a smooth baseline was observed, the FI system was ready for calibration and sample analysis.

Transient signals were registered on a chart recorder (Hitachi 056, Tokyo, Japan) and quantitative measurements were based on evaluation of peak area using standard instrument software (Merlin, P.S. Analytical).

Gas chromatography – microwave induced plasma – atomic emission spectroscopy: Enriched microcolumns were subjected to elution, derivatization and extration prior to quantification of Hg species by GC-MIP-AES. Retained Hg species were eluted with 0.5 ml of HCl 3 *M* with an elution flow-rate of 1 ml min^{-1}. The eluate (0.5 ml) was collected in a glass vial (5 ml capacity) containing 1 ml of high purity water (Millipore). To the glass vial containing eluate and 1 ml of water, buffer solution (NaOH–CH_3COONa, 380 μl, 2.7–3 *M*) was added to obtain a pH of 2. Then Na tetraphenylborate (120 μl, 5% m/v) and n-hexane (1 ml) were added and the tube shaken for 30 min using an automatic shaking machine (Gallenkamp, UK). After centrifugation (2000 rpm) for approximately 5 min (MSE, Mistral 200, Fisons Scientific Equipment, UK) the organic phase was withdrawn using a glass pipette and placed in a screw-capped glass vial (2 ml) ready for automatic injection to the GC apparatus. For instrument calibration the mixed Hg standard solution was subjected to microcolumn processing and derivatization in the same way as for samples.

Extracts were analyzed by GC-MIP-AES (HP 5890 Series GC equipment, Palo Alto, CA, USA). The analytical column was a non-polar column (25 m length × 0.23 mm i.d. × 0.17 μm film thickness HP-1), with He as carrier gas at 2.5 ml min^{-1} (linear velocity 43 cm s^{-1}). Splitless injection was employed (purge time 60 s) and during elution of the solvent (5 min) the column outlet was vented to prevent the plasma from being extinguished. An auxiliary He flow (30 ml min^{-1}) was directed to the plasma. After the solvent peak had passed, the valve was switched, thereby re-routing the column effluent to the plasma through a fused-silica capillary transfer line.

Atomic emission for Hg was monitored at 253.6 nm using the diode array spectrometer (HP 5921A). Standard software routines were used for evaluation of chromatographic peaks. Unless stated otherwise peak height was used for quantification. Operating conditions for the GC-MIP-AES were similar to those of a previous study [5].

Results and Discussion

A major aim of the study was to ascertain whether immobilization of Hg species on a solid support would provide an effective means for stabilization of Hg species at the trace level and thus offer a possibility of developing a new class of RM. Prior to commencement of stability studies an initial experiment to check column to column variability was performed. Microcolumns of SCF were enriched with methyl-Hg chloride (10 μg l^{-1}; 0.5 ml) and then analyzed by FI-AFS. The AFS method offers good precision and hence any significant intercolumn variability should be readily detected [4]. For comparison purposes a single microcolumn was charged with the same standard solution and analysis was repeated 10 times to allow calculation and comparison of respective precisions. The two data sets shown in Fig. 2 (a, 10 microcolumns: RSD, 0.4%; b, 1 microcolumn: RSD, 0.3%) testify to the high precision of the FI-AFS method and to the fact that prepared microcolumns were homogeneous and contained essentially identical quantities of analyte.

For stability studies a batch of SCF microcolumns was prepared and stored at 4°C in a light-tight box. At fixed time intervals (1 h, 1 week, 2 months and 4 months) microcolumns were removed and Hg species were quantified by GC-MIP-AES according to the above described procedure. Unlike the FI-AFS procedure the GC method does provide quantification of individual organic compounds; however, method precision is typically around 5% RSD for methyl-, 10% RSD for ethyl- and 12% RSD for inorganic Hg [5].

At each time interval methyl-, ethyl- and inorganic Hg were determined by subjecting microcolumns to the GC-MIP-AES procedure. For each sample, automatic injection (1 μl) was performed in triplicate. Thus, a total of 9 measurements for each species were acquired. Analyte signal intensities (peak height) were referred to

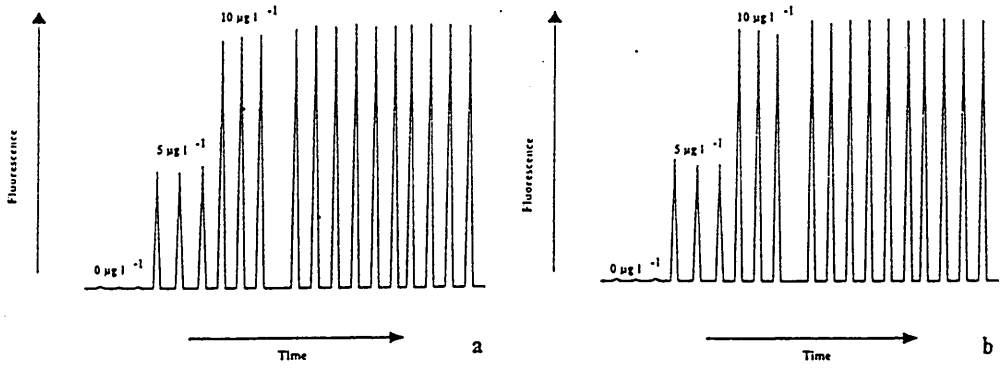

Fig. 2. Typical FI-CV-AFS transient signals (fluorescence vs. time) for a replicate analysis (10 microcolumns) and b repeat analysis (single column) (10 μg Hg l^{-1} as methyl-Hg chloride, 0.5 ml). Each figure includes calibration standards

emission intensitites derived from freshly prepared mixed Hg standard solution in order to calculate recoveries. The standard solution was processed three times and, for each extract, automatic injection was performed in triplicate again to give a total of 9 measurements (for each species). Typical GC responses for fresh and aged (2 months) microcolumns are presented in Fig. 3, while recovery data are summarized in Table 1. It is clear that analyte recoveries are essentially quantitative throughout the 4-months study, the only exception being ethyl-Hg chloride. While ethyl-Hg species appear to be stable for up to 2 months, poor recovery data were obtained for 4 months (recovery, 7%). This result is difficult to interpret and further work is needed to clarify this point.

Based on this short study it is clear that immobilization of Hg species (methyl-Hg chloride and inorganic Hg) on SCF provides an effective means for analyte stabilization and preservation. The possibility, therefore, exists to develop new RMs and calibrants based on immobilization technology. Microcolumn sample processing is seen as an increasingly important trend in ultratrace analysis methodologies and hence new demands for RMs prepared in column format can be anticipated. As

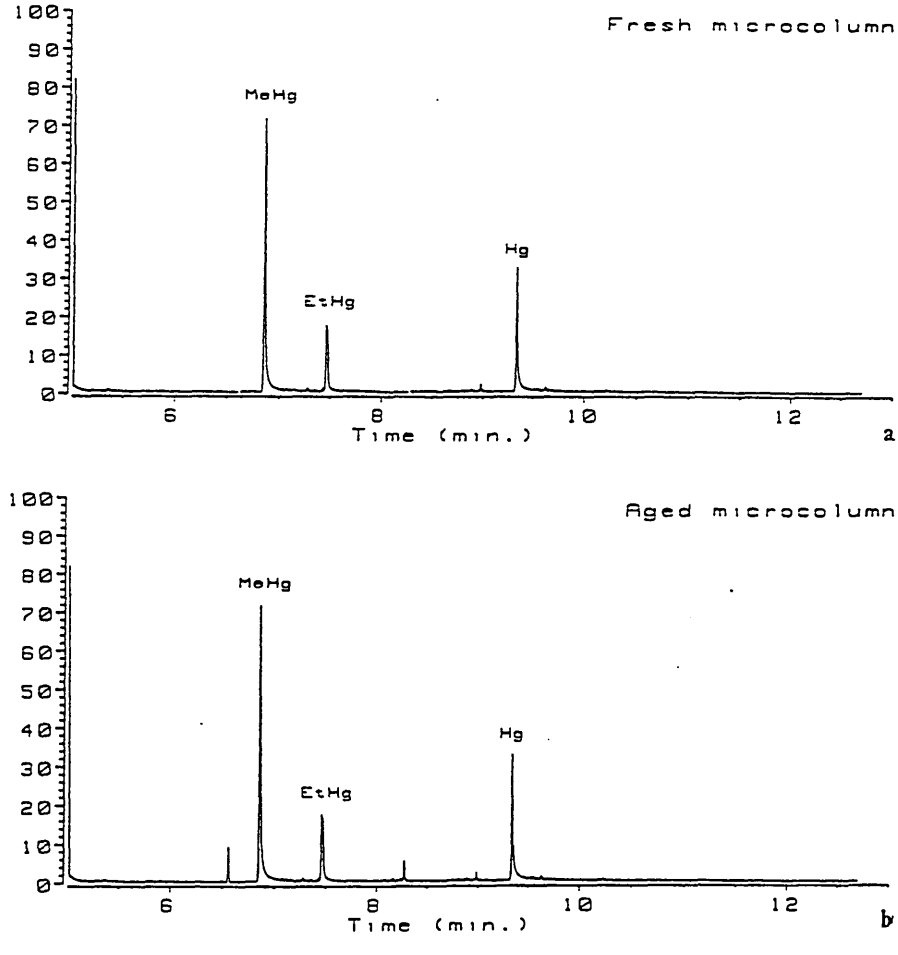

Fig. 3a,b. Typical CG-MIP-AES response for fresh (a) and aged (b) microcolumns over a 2 month period (10 μg Hg l^{-1} as methyl-, ethyl- and inorganic Hg; 3 ml)

Table 1. Analyte recoveries ($x \pm 2s$) as function of storage time for Hg species (10 μg Hg l^{-1}, methyl-, ethyl- and inorganic Hg; 3 ml) immobilized on SCF

Hg species	1 hour	1 week	2 months	4 months
Methyl-Hg	100 ± 8	103 ± 10	100 ± 6	96 ± 6
Ethyl-Hg	100 ± 12	100 ± 8	97 ± 6	7 ± 1
Inorganic	99 ± 12	99 ± 6	97 ± 8	102 ± 10

shown in the present study column format is versatile and practically useful in that it is compatible with both on-line FI methodology and also with classical/batch operations. For follow-up work it is planned to circulate enriched microcolumns (low level and high level) to specialist laboratories and subject samples to rigorous interlaboratory study. At the same time alternative solid support media will be tested.

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