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MICROCOLUMN FIELD SAMPLING AND FLOW INJECTION TECHNIQUES FOR MERCURY SPECIATION

by

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ABSTRACT

Mercury is one of the most toxic heavy metals, and many serious incidents have resulted from mercury poisoning. The methylation of mercury and it's amplification by marine life have aggravated this pollution problem. Studies over the last three decades have shown that the toxicity of mercury is related to chemical form. A basic aim of the research has been to devise new methodology for the measurement and speciation of mercury. Key points of the investigation reported were the literature review of methodologies and techniques for mercury speciation and the development of a novel manifold which incorporates microcolumns of sulphydryl cotton which have a relatively high affinity and selectivity for inorganic and / or organomercury, and to utilise a continuous flow procedure for mercury speciation based on flow injection - atomic fluorescence spectrometry. This new and novel system has been used for the determination and speciation of mercury in a variety of water samples. The other column packing materials, eg. xanthate cotton, activated alumina and 8-hydroxy-quiniline were also investigated.

A further aspect of element speciation concerns the development of a field sampling technique using sulphydryl cotton columns. Sample collection and preconcentration using microcolumns at the site of sampling was successfully performed. Preliminary experiments indicated that the field sampling technique in combination with FIA - AFS was a robust and potentially useful speciation tool. Field surveys on mercury distribution and speciation in the Manchester Ship Canal and the River Rother have been intensively carried out in collaboration with the National Rivers Authority (North West Region). The analytical data on different mercury species in waters of the Manchester Ship Canal are reported for the first time. A high correlation between organomercury and organolead in the Manchester Ship Canal is found and the related data have been assessed in order to clarify the possible origins for organomercury.

Related work concerning participation in interlaboratory studies is reported in the Appendices.

CONTENTS

CHAPTER 1

INTRODUCTION

1.1 Mercury in The Environment

1.1.1 Mercury Distribution in The Environment

Mercury is one of the most toxic elements, producing serious irreversible neurological damage, especially when in the methylmercury form. A number of very severe instances of mercury poisoning have been reported which suggest that organomercury compounds constitute what is probably the most dangerous category of chemical pollutant in the environment. The best known and most publicised case of mercury pollution has been related to mercury poisoning in Japan [1] as a result of consumption of high levels of methylmercury in foodstuffs. For this reason mercury speciation has been the subject of extensive studies in the last 40 years.

A major problem with mercury pollution is that mercury is ubiquitously distributed and may be found in trace amounts in all parts of the environment. Hence it is often difficult to distinguish between those which are derived from natural inputs and those which have an anthropogenic origin.

Table 1.1 Concentrations of mercury in the environment

Area or material	Concentration*
Atmosphere	0.5 - 50 ng m^{-3} (20 ng m^{-3} mean)
Rain water	0 - 200 ng l^{-1}
Fresh water	10 - 50 ng l^{-1} (uncontaminated) 150 - 700 ng l^{-1} (industrial area)
Ocean	0 - 270 ng l^{-1} (depends on depth) 600 ng l^{-1} (Minamata Bay)
Sediment	0.23 - 3.42 μg g^{-1} (dry weight)
Soils	10 - 2000 ng g^{-1}
Fish	0.02 - 0.65 μg g^{-1}# (wet weight)

* Data from Bowen, H. J. M, Environmental chemistry of the elements, Academic Press, London (1979).

Data from " Aquatic Environment Monitoring Report " No.26, 1988 - 89, by M.A.F.F. UK.

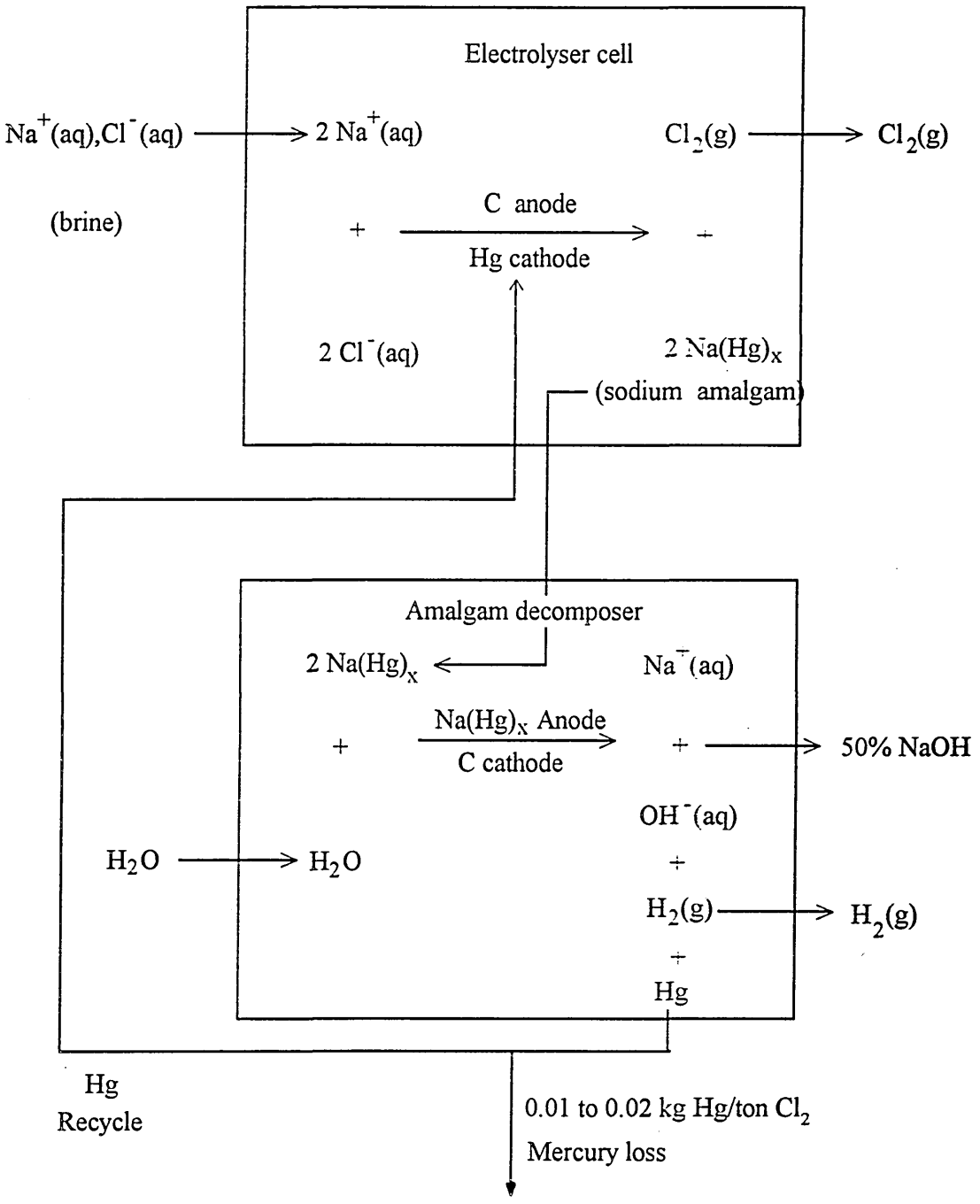
Mercury has a crustal abundance of 0.08 ppm, mainly associated with sulphur. The main ore is cinnabar, red hexagonal α-form mercuric sulphide (HgS), from which mercury may be extracted. Droplets of mercury are also sometimes found in the veins of cinnabar. Both elemental mercury and mercuric sulphide are extremely volatile. Thus

it is not surprising that traces of mercury appear everywhere and that, with a little help from man as well as natural processes, it cycles readily between soil, water, air, plants and animals. Representative concentrations of mercury in the environment are given in Table 1.1. The levels can vary considerably with geographical position and distance from pollution sources. Reports of mercury levels in top soils are extremely variable, ranging from 0.01 ppm to 2.0 ppm, and may be much higher around areas of industrial and agricultural usage.

The soluble mercury concentration in open sea waters is rarely above 0.3 $\mu g\ l^{-1}$ due to the dilution effects and absorption by sediments. In coastal areas which are subject to industrial waste contamination, mercury levels may exceed 3 $\mu g\ l^{-1}$. In U.K. coastal water, the level of mercury was reported around 0.04 $\mu g\ l^{-1}$ [2], and in the Irish sea levels ranging from 0.025 - 0.05 $\mu g\ l^{-1}$ were found [3]. Rain water and snow-melt water analysis have revealed figures around 0.011 - 0.428 $\mu g\ l^{-1}$ [4].

A major contributor to the high level of mercury in river water is the proximity of mining and mineralised areas. The water is often acidic in such locations due to the oxidation of sulphide from sulphide ores, which contributes to increased solubility of mercury species. Levels of mercury in fresh water were reported from around 0.02 -0.1 $\mu g\ l^{-1}$ [5].

High concentrations of mercury in the aquatic environment are almost invariably associated with chemical plants manufacturing chlorine, sodium hydroxide, and hydrogen. More than 25% of chlorine is produced in mercury cells, which operate as outlined schematically in Figure 1.1. In such cells nearly saturated brines are electrolysed to produce gaseous chlorine and hydrogen, leaving sodium hydroxide behind in solution. In order to prevent chlorine and hydrogen from recombining explosively the former gas is produced at a carbon anode in an electrolyser cell which uses liquid mercury as the cathode.Because sodium amalgam (a solution of sodium in



1982 EC directive for mercury discharge standard: 50 $\mu g\ l^{-1}$.

Figure 1.1 Schematic diagram of mercury cell for chloro-alkali production.

mercury) is relatively stable it forms at the cathode instead of gaseous hydrogen:

C anode: $Cl^-(aq) \text{ ----> } Cl_2(g) + e^-$ (1.1.1a)

Hg cathode: $Na^+(aq) + e^- + Hg \text{ ----> } Na(Hg)$ (1.1.1b)

(sodium amalgam)

$$Hg + Na^+(aq) + Cl^-(aq) \text{ ----> } Na(Hg) + Cl_2(g) \quad (1.1.1c)$$

The liquid amalgam is then allowed to flow into a decomposer where it becomes the anode. The sodium is oxidised back to Na^+ and hydrogen is released at a carbon cathode:

C cathode: $H_2O + e^- \text{ ------> } H_2(g) + OH^-(aq)$ (1.1.1.d)

Na(Hg) anode: $Na(Hg) \text{ -------> } Na^+(aq) + e^- + Hg$ (1.1.1e)

$$Na(Hg) + H_2O \text{ -------> } Na^+(aq) + OH^-(aq) + H_2(g) + Hg \quad (1.1.1f)$$

The mercury (except for a small fraction) is then recycled to the electrolyser and concentrated sodium hydroxide removed for sale. The 0.01 - 0.02 kg of mercury lost per tonne of chlorine seems a small quantity until one takes account of the fact that nearly 2500 tones of chlorine are produced in mercury cells each day. Treatment of waste from mercury cells with sulphur powder columns has been used to minimise mercury pollution, but a great quantity of mercury is still lost to the environment. It was assumed for a long time that because mercury sulphide is relatively inert and insoluble, it would settle rapidly to the bottoms of rivers and lakes and remain there. In fact most of it did just that, but many researchers discovered that certain anaerobic

bacteria are capable of decomposing and methylating insoluble mercury salts, converting approximately 1% of what is contained in the sediments to methylmercury, and dimethylmercury. This permits mercury to enter the water phase and the fresh water food chain and accounts for the high concentrations observed in fish [6,7].

Once the role of mercury cell chloro-alkali production in fresh water mercury pollution was recognised, steps to control discharges were rapidly taken.

Native mercury enters the atmosphere through the actions of volcanoes and earthquakes and evaporation from water and soil surfaces. The background level of mercury in the atmosphere under "normal" conditions has been variously estimated at less than 1 $\mu g\ m^{-3}$. However, localised natural deposits may increase this level as much as twenty fold.

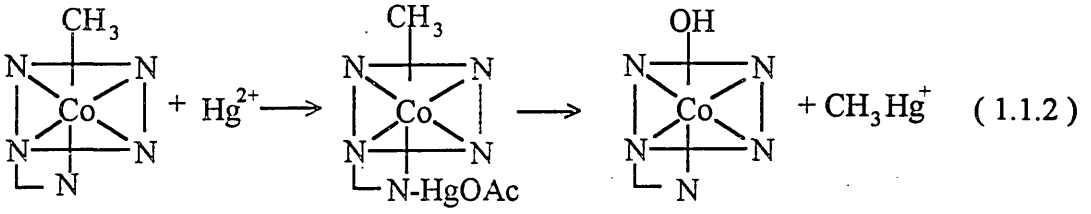
Man's industrial activities dramatically augment atmospheric levels, especially in enclosed areas. In electrical and chloro-alkali factories, the concentration of mercury in air may be in excess of 0.1 $mg\ m^{-3}$, and even higher closer to the source of emission.

Traces of mercury occur in all plants and animals so far examined. Plants may absorb and concentrate mercury from soil, even to the extent that droplets of the metal have been found in the capsules of chick - weed [8]. Levels of mercury in plant tissue were reported from 0.2 - 45 $mg\ kg^{-1}$ (dry weight) [9,10]. Animals, which are involved in long food chains, tend to accumulate a level of mercury far in excess of that found in their immediate environment. For examples, predatory birds, such as owls, eagles and falcons, are sometimes found to have greater than 100 $mg\ kg^{-1}$ of mercury in certain organs (especially the livers and kidneys). This mercury may have come from natural sources but it is more frequently from seed dressings, water pollutants, etc.

1.1.2 Biochemical Pathways of Mercury in The Environment.

It is apparent that, whilst inorganic forms of mercury are toxic to plants and animals, organic complexes may prove to be more toxic and a long term hazard in the environment.

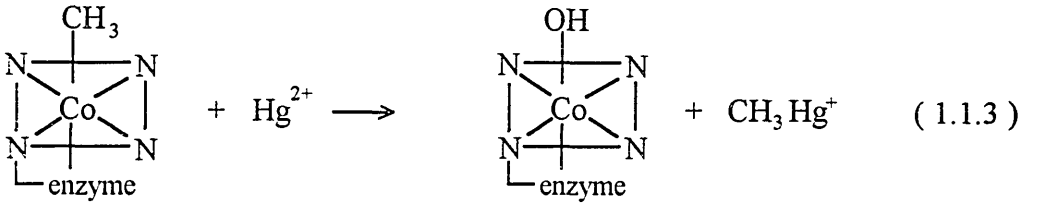
Mercury and its inorganic compounds undergo a variety of chemical and biological transformations. For example, it is now well known that methyl- and dimethylmercury are found in the aquatic environment due to the activities of free-living bacteria and fungi. This methylation process has also been observed to be mediated by enzyme systems in rotting fish and lake sediment, in addition to that caused anaerobically by the methanogenic fungus and members of the genus. It is suggested that this is a chemical process in which methylcobalamin is involved [11-13]. The methylation is



(1.1.2)

achieved by at least two biochemical pathways. One route is non-enzymatic under anaerobic conditions. The mercury (II) ion is methylated by methylcobalamin, a methyl derivative of Vitamin B12, formed by methane producing bacteria.

As the reaction requires anaerobic conditions and since in these conditions the main source of mercury is mercuric sulphide, it is unlikely that the reaction takes place to any great extent. The aerobic pathway occurs within cells, in which methionine is normally synthesised. This process is enzyme catalysed:



(1.1.3)

Both reactions are possible in the upper sediments, particularly sediments in suspension, where the outer surface is aerobic and the inner surface anaerobic. This is shown in Figure 1.2. The reactions are also favoured by low pH. Methylation may also occur for other species present in water, e.g. metal ions from elements such as Pb, Sn, Si, Tl, Pt.

It would appear that mercury in water sediment is in two forms. In the upper sediments the material is biochemically active, while in the lower (inorganic mineral layers) there is little or no activity, and weathering may be the only way the mercury is released again. The upper sediments are involved in the sorption of cations, and mercury (II), one of the poorly sorbed ions, is easily replaced by other cations.

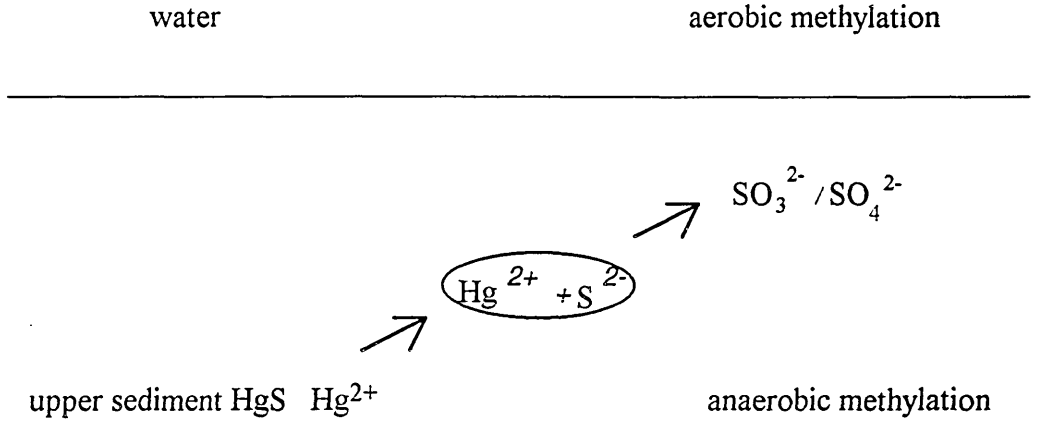


Figure 1.2 Methylation of Mercury

A series of chemical and biochemical reactions tend to lead mercury to the end products methylmercury and dimethylmercury, both of which are concentrated in fish, and so become involved in the food chain. Although not much methylmercury is produced in water sediments, it is bioamplified and since a great deal of mercury is present in water sediments one can expect its formation to continue for a long time.

1.1.3 The Use of Mercury in Industry

Mercury is used primarily in elemental form because it is the only unreactive metal that is a liquid at ordinary temperatures and hence can be used as a fluid conductor of electricity. The largest tonnage use of mercury in industry is in the Castner-Keller process for making chlorine and caustic soda by the electrolysis of brine, using a rocking mercury cathode. It is still very widely used; The products inevitably contain traces of mercury and, since they have so many outlets, have added to the mercury burden of the environment. (One of the main parts of this research was a field survey on distribution and speciation of mercury along Manchester Ship Canal; it is chlorine manufacture that is thought to have been responsible for the major inorganic mercury discharge into the canal for many years. This is reported in Chapter 4.)

Catalysts are employed in the manufacture of vinyl chloride, urethane plastics and acetaldehyde. Phenylmercuric acetate is a fungicide in paints. Many organomercury derivatives, some of whose structures are shown in Figure 1.3, have pharmaceutical applications and are often purposely dispersed in the environment the form of germicides, fungicides, bacteriocides and algicides. Such fungicides have been responsible for poisoning and are now banned by some countries.

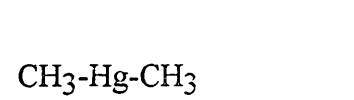
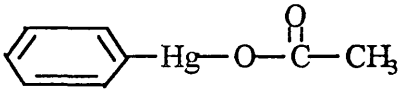
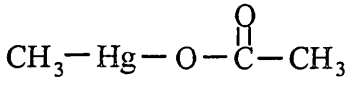
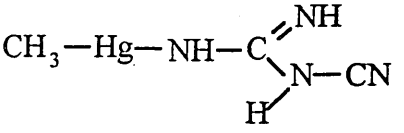
CH_3-HgCl	CH_3-Hg-OH
Methylmercuric chloride	Hydroxomethylmercury II
	
CH_3-Hg-CH_3	
Dimethylmercury II	Acetatophenylmercury II
CH_3-Hg-CN	CH_3CH_2-Hg-Cl
Methylmercuric nitrile	Ethylmercuric chloride
	
Methylmercuric acetate	Methylmercuric dicyandiamide

Figure 1.3 Structures of some organomercurial compounds released to the environment

1.1.4 The Chemistry of Mercury

Mercury has the electronic configuration [Xe] $4f^{14}5d^{10}6s^2$. It loses two electrons (but no more) to form dipositive ions. Since the values of standard electrode potentials for the couple Hg^{2+}/Hg and Hg_2^{2+}/Hg are so similar (+0.854 / +0.793) [14], essentially all oxidizing agents are able to oxidise mercury to mercury (I) and further to the mercury (II) state. Therefore many mercury (II) compounds may be also readily reduced to the Hg (I) or mercurous state. In Hg (I) compounds, two mercury atoms are associated to give Hg_2^{2+} ions. Such compounds are always diamagnetic, whereas an Hg^+ ion { $[Xe]4f^{14}5d^{10}6s^1$ } would be paramagnetic with one unpaired electron.

Mercury has so many peculiarities of it's own that it stands quite apart from the other metallic elements. The most important of these, though by no means the only one, is it's very high ionisation potential in the gaseous state. The values [15] for the first and second ionisation's of this element are:

	First	Second	
Mercury	10.43	18.75	eV

The value of 10.43 eV is higher than that for any cation-forming element except hydrogen (13.53), which explains it's strong tendency to form covalent rather than ionic bonds. Moreover it has a strong tendency to form covalent links with sulphur, nitrogen and chlorine, and to a lesser extent with chlorine, bromine, and iodine, but not with fluorine (owing to the weak acidity of hydrogen fluoride).

It is known that for mercury the s-p energy separation is relatively large, hence promotion to the valence state ($5d^{10}6s^{1}6p^{1}$) is difficult; This is no doubt associated with the relative inertness of mercury and also with its low electron affinity, as the added electron will occupy a p orbital. On the other hand, the small d-s separation for Hg^{2+} suggests that d-s mixing may occur, resulting in a distortion of the coordination arrangement [16]. This is why inorganic mercury ions often occur as various complex forms with different coordination numbers, and both inorganic and organo mercury species in natural and polluted waters normally coordinate to give anions. In the presence of chloride ions Hg (II) forms chloro-complex species (e.g. in sea waters). The donor atoms of the ligands are p-block elements, in particular S, N, O, and Cl in natural waters. These properties provide a theoretical basis of improving our understanding of states of different mercury species in waters.

The affinity of mercury for the -SH group is greater than that for any other single

ligand. Stability constants for some mercuric complexes are listed in Table 1.2. Reactions with these groups can result in different complexes depending on whether the metal is present as Hg^{2+} or $R\text{-}Hg^{+}$, whether the ligand is a mono- or di-thiol, and dependent on the relative concentrations of mercury and -SH groups. Importantly, complexes of the type $R\text{-}Hg^{+}$ bind sulphydryl group stoichiometrically [17], which is fundamental to specific and quantitative deposition/elution of mercury in this work.

Table 1.2 Stability constants of 1:1 mercuric complexes#

Ligand	Log
Cl^-	6.74
Br^-	9.05
I^-	12.87
OH^-	10.3
NH_3	8.8
Imidazole	3.57
Ethylenediamine	14.3
Cysteine (N-S)	45.4
Glycine (N-O)	10.3
Histidine	7

Data from Sillen K. G and Martell A. E, " Stability Constants of Metal - ion Complexes ", Chemical Society, London, 1964.

It was reported [18] that the hydrogen ion concentration can affect formation of the Hg-S complex, and that the presence of other ligands competing for mercury also affects the binding of mercury with -SH groups.

Most proteins contain various ligands for binding with mercury, including sulphydryl groups. There are also numerous enzymes that react with organic and inorganic mercurials with resultant changes either in spectral characteristics or in enzyme activity. In addition, most bacterial and plant proteases have -SH groups essential for proper functioning, and most are inactivated due to the formation of Hg-S bonds [19]. This loss of activity, however, is not due to actual loss of -SH groups but to a change in protein conformation [20].

Organomercury compounds are species in which there is bonding between mercury and organic carbon. Organomercury derivatives, some of which are shown in Figure 1.2 are typically covalent compounds. Mono-alkylmercurials are very important species in the light of environmental toxicology. Methylmercury behaves as a class b acceptor (a soft acid) and readily reacts with large sulphur donor atoms in amino acids. The nature of RHgX derivatives, depends markedly on the electro-negativity of halogen (X). Because of the substantial ionic character of the Hg-X bond, ionisation of RHgX is significant. Such derivatives tend to dissolve readily in polar solvents, such as water. The cation $MeHg^+$ has been likened to H^+; Also the degree of dissociation of MeHg-X in water is often similar to that of H-X [21]. This property is critical for the separation of MeHgCl from other mercury species by means of ion exchange.

It is quite well known that the methylation of mercury to CH_3Hg^+ occurs in the aquatic environment and is significant as regards Hg toxicity. The methylation has been widely reported to arise from biochemical pathways. Even so, there are just two classes of alkyl-bonded organomercury compounds, RHgX and R_2Hg that can be obtained by

simple preparative routes. There is however considerable diversity in the nature of the organic radical R. The mechanism of transfer of alkyl groups from various metals to mercury (II) salts has been reported by McAuliffe [17]. For example, transmetallations of mercury (II) salts with tetraalkyltins in methanol/water mixtures was shown and this is illustrated in the following reaction:

$$R_4Sn + HgX_2 \text{ -----> } RHgX + R_3SnX \quad (X = Cl, I) \qquad (1.1.4)$$

In work reported later in this thesis, a high degree of correlation between alkyllead and methylmercury distributions was observed from a field survey carried out in the Manchester Ship Canal. Presumably, a similar transmetallation between alkyllead and mercury (II) can occur; Both lead and tin belong to the same family in the periodic table and have a similar outer electronic structure. A detailed discussion of this topic is given in Chapter 4.

An important chemical reaction of organomercury is the replacement of mercury by halogens, which is termed halogeno-demercuration. All kinds of diorganomercurials and organo-mercurials react with chlorine, bromine, or iodine under a variety of generally mild conditions to yield alkyl halides and mercuric halides, as illustrated in equations (1.1.5) and (1.1.6):

$$R_2Hg + X_2 \text{ ----> } RX + RHgX \qquad (1.1.5)$$

$$RHgX + X_2 \text{ ----> } RX + HgX_2 \qquad (1.1.6)$$

A variety of other reagents have been used to bring about halogenodemercuration. These include Br_2, I_3^-, and ICl, cupric halides and cadmium halide [22]. Halogenodemercuration has been widely used as a selective digestion method for

converting organomercury into the inorganic form.

Diorganomercurials can also be decomposed by mineral acids, the process being termed acidolysis [22]:

$$R_2Hg + HX \text{ ----> } RH + RHgX \qquad (1.1.7)$$

$$RHgX + HX \text{ ----> } RH + HgX_2 \qquad (1.1.8)$$

Vigorous conditions are required for the second stage. This acidolysis, as a method of wet digestion, has been applied to the pretreatment of samples for total mercury determination by many workers.

The reduction of organomercury compounds is another important reaction which has been studied and applied to the cleavage of the Hg-C bond. Fortuitously, the reduction yields a hydrogeno-demercuration product and is carried out either with sodium amalgam, hydrazine or electrolytically [23]. However, metal hydrides are the best reagents for effecting reductive demercuration, the process being rapid and usually remarkably free of side reactions. Among the hydrides that have been used are lithium borohydride, lithium aluminium hydride, and sodium trimethoxyborohydride [24]. By far the most popular reagent, however, is sodium borohydride in aqueous sodium hydroxide. This was developed in 1966 by Bordwell and Douglass [25], who established the stoichiometry to be that shown in equation (1.1.9):

$$4RHgCl + 4OH^- + BH_4^- \text{ --> } 4RH + 4Hg + 4Cl^- + H_2BO_3^- + H_2O \qquad (1.1.9)$$

This reaction has been successfully used for organic synthesis and also applied to the conversion of organomercurials to inorganic mercury for speciation and determination [26 - 30].

1.1.5 General Problems of Mercury Pollution

Mercury is distributed widely in the earth's crust, in sea, ground and rainwaters, in plants and animals. Importantly, all phyla and species naturally contain trace levels, present either as inorganic or organomercury compounds, or both. It is known that mercury participates in the biological life cycle. The biological conversion of inorganic mercury into organomercury is particularly significant since extensive industrial and agricultural usage of mercurials affects and increases the distribution in specific regions.

The toxic effects of mercury depend, inter alia, on it's chemical form, the size of the dose, and the route of entry into the body. Metallic mercury can enter the body by ingestion, through the skin, and by injection. If swallowed it passes through the gastrointestinal tract without being absorbed and hence causes little or no adverse effects. Inhalation of mercury vapour can cause either acute or chronic effects. If metallic mercury is heated, it can liberate vapour of sufficient concentration to produce severe or even fatal effects. The classical manifestations in such instances are fine tremors, gingivitis and erethism.

Best known among inorganic mercury compounds for being toxic is mercuric chloride (corrosive sublimate). A gram or two taken by mouth will cause corrosion of the oesophagus and stomach, resulting in vomiting and diarrhoea as well as bleeding from the intestinal tract. When absorbed into the body, inorganic mercury tends to be deposited in the kidneys in higher concentrations than in any other organ and tissue [31]. Ordinarily these deposits have little or no pathological effect.

Organomercury compounds are very hazardous and their inhalation, ingestion or absorption via the skin leads rapidly to nervous system disorders. From a toxicological point of view it is necessary to distinguish between three classes of organo- mercurials: alkyl, aryl, and alkoxyalkyl. Of the three, alkyl-mercury derivatives present a special hazard because of the tendency to accumulate in the brain where they cause irreversible

damage. The most disastrous case of environmental mercury poisoning, and perhaps the most illustrative of the complicated ecological pathways of mercury, occurred about 40 years ago in the Japanese fishing port of Minamata [1]. A chemical company constructed a plastics plant which used organomercurials as catalysts and each year a small quantity of methylmercuric 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 Bay. By 1975, it was estimated that as many as 10,000 persons had been affected by Minamata disease, 703 seriously and permanently maimed and more than 100 killed, as well as the birth of numerous babies with congenital defects. In Japan a research institute has been established to study the health related effects of mercury poisoning [1].

The role and behaviour of methylmercury in aquatic ecosystems and particularly in food chains has been given considerable attention. A number of reports have revealed that the predominant form of mercury in aquatic life is methylmercury [32-36]. Methylmercury may be present not only as a result of man-made pollution but also from natural biological processes. An important aspect of the Minamata disease is the extent to which mercury becomes concentrated in fish relative to the waters of the Bay. Certain substances, of which methylmercuric chloride is an excellent example, tend to be concentrated along ecological food chains, by a process known as bioamplification. Any substance which is slightly more soluble in the tissues of simple organisms than in the surrounding water will be a more concentrated component of the diet of the more complex species which feed on the simple ones. Thus, at each higher tropic level in the food chain the toxin becomes more concentrated. Since humans are usually at the top of the food chain, high concentrations of poisonous substances may occur in human

diets, with tragic results such as Minamata disease.

Once in the human body organomercurials and divalent mercury both react with a large number of enzymes, usually inhibiting catalysis of essential metabolic reactions [37]. This apparently results from the strong bonds formed between the large Hg ions and sulphydryl group (-SH) on the amino acid cysteine in enzymes. The mercury-containing groups can disrupt the enzymes' structure [37], consequently impeding its catalytical function.

$$CH_3HgCl + Enzyme\text{-}SH \dashrightarrow CH_3Hg\text{-}S\text{-}Enzyme + H^+ + Cl^- \qquad (1.1.10)$$

The specificity and affinity of the sulphydryl group (-SH) for inorganic and organomercury species has been exploited in the work reported later in this thesis.

Fish in contact with water containing mercury at 0.01 $\mu g\ l^{-1}$ in solution and 300$\mu g\ kg^{-1}$ in sediments, have been found with concentration of 314 $\mu g\ kg^{-1}$ in their flesh, a 31,000 bioamplification (over the water levels). At Minamata, mercury levels in some fish attained 50 $\mu g\ g^{-1}$ (50 ppm) wet weight in contrast to other regions where levels around 0.2 $\mu g\ g^{-1}$ were common.

To conclude we can say that the toxicity and biological effects of mercury, in both its organic and inorganic forms, strongly depend on its chemical forms. Consequently, the ability to distinguish different forms of mercury in the hydrosphere gives an increasing awareness of its presence and distribution in the environment as well as its toxicological importance in terms of its environmental impact. The various methodologies and techniques used for mercury speciation have undergone rapid development in the last 30 years and will be discussed and evaluated in the next section.

1.2 Analytical Methods for Determination and Speciation of Mercury

Although numerous methodologies and techniques have been developed for mercury speciation, only a few are considered to be well suited to the determination of different mercury species present at ultra-trace concentrations ($\mu g\ l^{-1}$ - $ng\ l^{-1}$) in waters and other environmental samples. Such procedures should provide extremely high sensitivity so that pre-analysis manipulation of the sample is not necessary. In this section, the techniques offering the best combination of advantages for mercury speciation are classified and discussed, according to their modes of detection namely,

(1) Cold vapour - atomic absorption and atomic fluorescence spectrometry with various sample pretreatment procedures;

(2) GC and HPLC methods with solvent extraction;

(3) Atomic emission spectrometry and mass spectrometry with various separation and pretreatment processes;

(4) Hydride generation - cryogenic trapping with GC - GFAAS.

1.2.1 Cold Vapour - Atomic Absorption Spectrometry and Cold Vapour - Atomic Fluorescence Spectrometry With Various Sample Pretreatment Procedures

Cold vapour - atomic absorption spectrometry (CV-AAS) and cold vapour - atomic fluorescence spectrometry (CV-AFS) methods detect mercury in its elemental form. Therefore, the determination and speciation of mercury require decomposition of the organomercury species. Up to now, all decomposition methods have been either (a) two step processes (e.g. oxidation and reduction) or (b) a one step process (reduction). These are discussed separately.

Two Step Pretreatment

Of the numerous analytical techniques which has been used, CV-AAS which was

developed 2 decades ago [38,39] remains preminent, and constitutes the basis of official/reference methods [40-43]. In a typical determination, the inorganic mercury in the sample can be determined directly following reduction to elemental form. Total mercury can be determined by prior decomposition of all organomercury species into inorganic mercury followed by the use of reducing agent as before. Organomercury is determined by difference. It is apparent that the chemical pretreatment of samples for mercury speciation by CV-AAS or CV- AFS requires an oxidation and a reduction step.

The most widely used reducing agent is tin chloride in acidified media. Kimura and Miller [44] were the first to use the reaction between inorganic mercury (II) and tin (II), as a means of isolating the metal from it's matrix. Since then, numerous methods have adopted the same principle.

Another step in the pretreatment of samples containing organomercurials is the conversion of organo-bound mercury into the inorganic form. The very low concentration of different mercury species in natural waters makes it critical to achieve maximum conversion efficiency and to avoid any contamination. A wide variety of procedures have been employed for this purpose.

The earliest proposal, made by Armstrong et al. [45], was the use of ultra-violet radiation as a means of oxidising organic matter in sea water prior to the determination. In this case for the determination of total phosphorus. Soon after, ultra-violet irradiation coupled with CV-AAS was used by a number of workers for the determination of organomercury compounds and mercury speciation : in river waters by Goulden and Afghan [46], in sea waters by Fitzgerald et al.[47], in industrial waste water by Dujmovic and Winkler [48], and in natural water by Kiemeneij and his co-worker [49]. Determinations with and without irradiation made possible the separate measurement of total and inorganic mercury, respectively. Fitzgerald et al.

reported that complete photo-oxidation took at least 24 hours. Winkler and his co-worker completely decomposed a range of organomercury compounds in less than 10 minutes with either a 150 W medium- pressure or a 15 W low-pressure U.V source. Kiemeneij et al. found only 60% of methylmercuric chloride was oxidised in 20 minutes when using a pure mercury lamp, but 100% in 10 minutes with a zinc / cadmium / mercury lamp. Some detailed work on the efficiency of ultra-violet irradiation was carried out by the Mercury Analysis Working Party of the Bureau International Technique Du Chlore [50]. It reported that a range of organomercury compounds could be completely oxidised to the ionic state by UV irradiation in 15 minutes or less and that sample volumes up to 2 litres can be treated with no increase in irradiation time when using efficient stirring. However, some results showed that organic matter in waters seriously reduces the efficiency in which the organo-mercury compounds are destroyed. It was considered advisable, therefore, to restrict the UV irradiation method to drinking water and "clean" river water. It was also stated that care should be taken and that only water miscible organic solvents should be used to prepare the organomercury master calibration solutions.

In recent years few reports have been published concerning mercury speciation except for a study by Morita and Sugimoto [51], who used an automated flow injection (FI) system consisting of an on-line ultra-violet and acid digestion manifold. Organomercury compounds were decomposed in a flow system by first merging the sample stream with a stream of 0.5 M of sulphuric acid and then irradiating for 1.5 minutes with a UV lamp of 400 W prior to reduction and detection. The limit of detection was reported as 0.18 $\mu g\ l^{-1}$, and the sampling rate up to 20 samples per hour.

The decomposition of organomercury compounds under various stimuli and mechanisms has been widely studied [52, 53]. Over a quite long period, oxidation with powerful mineral acids and oxidants has been widely investigated. This has also

been the subject of an interlaboratory trial carried out jointly by the ASTM and the EPA of U.S [54,55]. It was generally agreed that oxidative conversion of all forms of organomercurials in waters to inorganic form is feasible prior to reduction to elemental mercury. A variety of strong acids (HCl, H_2SO_4, HNO_3), oxidants (H_2O_2, $KMnO_4$, $K_2Cr_2O_7$, $K_2S_2O_8$) and elevated temperatures have been used and officially recommended. As with conversion of organomercury into inorganic mercury, many workers in this area have favoured a number of different combinations. Early trials were done by Hatch et al. [39], who used CV-AAS detection following oxidation of organic matter with sulphuric acid and potassium permaganate. The limit of detection for total mercury in solution was reported as 1.0 $\mu g\ l^{-1}$. Omang [56] utilised a mixture of potassium permanganate and sulphuric acid, with overnight standing time. The oxidation procedure was thoroughly investigated by different workers [40,57-69], who tested various oxidative systems, as listed in the results presented in Table 1.3. Their results indicated that because of the presence of powerful oxidising reagents there is a high tolerance to organic pollutants and chloride. However, high blanks reduced the sensitivity of the method. Strict control of blanks was necessary for determinations at concentration of 1 $\mu g\ l^{-1}$ Hg and below.

Ping and Dasgupta [60] recently reported successful conversion using Fenton's reagent (Fe II + H_2O_2) for speciation of mercury in water and urine samples. Satisfactory recoveries were obtained for inorganic, methyl- and phenyl- mercury.

As discussed in section 1.1, both organomercury and diorgano-mercury compounds react with chlorine, bromine, or iodine under a variety of generally mild conditions to give cleavage of the Hg-C bond. The stability of bromine solution and excellent reaction efficiency for brominolyses makes bromine a preferred choice in analytical applications [61]. In this work an on - line brominolysis was used for decomposition of organomercury compounds.

Table 1.3 Various wet digestion procedures for mercury determination*

Wet digestion procedures	Measurement
Boiling with potassium persulphate in nitric acid	AFS
Boiling with potassium persulphate in nitric acid	AAS
Heating at 80°C for 9 h with potassium permanganate in sulphuric acid	AAS
Boiling with potassium persulphate	AAS
Hydrochloric acid and a bromide/bromate solution	AAS
Heated at 80°C for 4 h with potassium persulphate	AAS
Boiling with potassium permanganate in sulphuric acid	AAS

* Data from reference 58.

One Step Pretreatment

In contrast to the oxidation procedures, reductive decomposition of organo-mercury compounds occurs more readily, is simpler and more efficient with a single step to elemental mercury.

Amongst a few candidate reagents sodium borohydride is a preferred choice. Nevertheless, it has rarely been used in analytical procedures for mercury speciation as first reported in 1971 by Braman [26]. Shortly thereafter Sharma and Davis [62] and Taffatetti et al [63] reported the use of this reagent for conversion of organomercury in water and urine samples. Oda and Ingle [64] described methods using selective reduction with tin chloride and sodium borohydride for determinations of inorganic and total mercury, respectively. Other reductants such as tin chloride - cadmium chloride

[65], and sodium borohydride-cupric sulphate [66] have also been reported. This one step process is, to some extent, attractive because of it's simplicity and freedom of reagent blanks relative to the 2 step procedures. A one step procedure has been investigated recently in some detail [67,68].

1.2.2 Comparison Between Cold Vapour - Atomic Absorption Spectrometry and Cold Vapour - Atomic Fluorescence Spectrometry Techniques

The most convenient and widely used measurement techniques for the determination and speciation of mercury in environmental samples, especially in waters, are CV-AAS and CV-AFS utilising the 253.7 nm line.

The basic reaction for the spectroscopic transition of elemental mercury is illustrated as below:

$$Hg^{o} + hv \text{ ==== } Hg^{o*} \qquad (1.2.1)$$

where Hg^{o} is ground state mercury atom; Hg^{o*} is excited mercury atom; h is Plancks constant and v is frequency (hv is the photo energy). The ground state atom absorbs energy to yield the excited state which emits radiation through de-excitation. Atomic absorption can occur when radiation of a selected frequency passes through a vapour containing ground state atoms. Some of the radiation can be absorbed by excitation of the atoms, and the intensity of the radiation at a wavelength corresponding to the energy of the photon hv is decreased. If the concentration of R^{o} in the vapour is increased, the decrease in radiant energy will be greater. Since each species of atom can exist only in specific excited states, the photon energies required for each atomic species will be different. Only photons at the wavelengths corresponding to specific excitation states will be absorbed in each case. The magnitude of the atomic absorption

signal is directly related to the number of ground state atoms in the optical path of the spectrometry, and this is the basis of atomic absorption spectroscopy. The application of atomic absorption spectrometry to analytical chemistry began by Walsh [69] in 1955 and with the determination of mercury by Dean and his co-workers [70] in the 1960s'.

In the case of mercury, the excitation energy for mercury atoms using the 253.7 nm line is 4.88 ev [71]. The resonance line at 253.7 nm is the one most commonly used for the atomic absorption measurement [72]. A simple diagram expressing the spectroscopic transition process for mercury is shown in Figure 1.4.

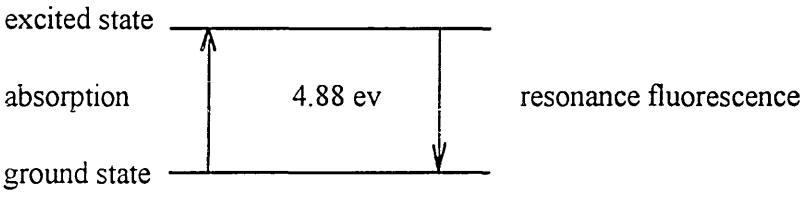


Figure 1.4 Diagrammatic representation of the atomic absorption and fluorescence of mercury

Atomic fluorescence is the result of the initial absorption of resonance radiation followed by re-emission at the same or some low frequency:

$$Hg^{o*} \text{ ----> } Hg^{o} + hv' \qquad (1.2.2)$$

In analytical atomic fluorescence the sample is reduced to an atomic vapour and then excited by radiant energy of a suitable wavelength. The excited atoms emit energy when they return to the lower excited state to the atomic ground state. The intensity of emitted fluorescence energy is measured and is a function of the concentration of the atoms in the sample. The fluorescence signal is usually observed at a right angle to the

exciting radiation to decrease the amount of scattered radiation from the excitation source entering the monochromator. This arrangement is particularly important in the case of resonance fluorescence when the excitation radiation is of the same wavelength as the fluorescence radiation. Mercury emission at 253.7 nm is due to resonance fluorescence [73] (also see Figure 1.4). The mercury atomic fluorescence analysis line is also 253.7 nm [72].

The spectral theory pertinent to atomic fluorescence as a viable spectrometric analytical procedure was given initially by Winefordner and Vickers [74]. A theoretical inference made by West [75] suggested that cold vapour atomic fluorescence spectrometry should be more sensitive and produce considerably less spectral interference from non-specific absorption compared with the corresponding atomic absorption spectrometry technique. This theoretical inference was later verified experimentally by Thompson and Godden [76], and a considerable number of applications have also been reported [77-79]. Today, although the most commonly used method for determination and speciation of mercury is cold vapour - atomic absorption spectrometry, there are several disadvantages associated with the use of atomic absorption spectrometry as the means of detection. There is a limited linear calibration range and spectral interference arising from non-specific background absorption [77]. Furthermore fluorescence measurements result in approximately one order of magnitude lower detection limits than those given by absorption measurements [72]. That is because fluorescence is measured relative to the background signal, whereas atomic absorption is measured as the difference between two relatively intense signals. In the former case, electronic amplification is a more effective procedure, and photon-counting detection can be used to advantage. Additionally, for the determination of mercury, the atomic fluorescence system is inexpensive and simple in both construction and operation [80].

1.2.3 Atomic Emission Spectrometry and Mass Spectrometry With Various Separation and Preconcentration Procedure

Atomic emission spectrometry (AES) and mass spectrometry (MS) are powerful analytical techniques, and have been actively researched in recent years. The coupling of chromatography and use of state-of-the-art excitation sources offer considerable potential for element speciation and determination, particularly where high sensitivity is required.

Atomic Emission Spectrometry

The earliest work in the application of AES to mercury speciation was done independently by Braman [26] and Bache et al. [81]. The former determined metallic, dimethyl- and total mercury in waters after sodium borohydride reduction and membrane probe diffusion prior to spectral emission detection. A lower limit of detection was reported down to 4 parts per trillion. Bache [81] applied coupled gas chromatography with microwave emission spectrometric method to the analysis of methyl mercury in fish samples. This method extended the pioneering work by Westoo [82], which consisted of extraction of a fish sample with hydrochloric acid, extraction of methylmercury into benzene, cleavage of Hg-S bond, isolation of methylmercury as hydroxide and finally reconversion to the chloride for gas chromatography separation. Five kinds of organomercuric compounds were tested, and the absolute detection limits reported for different organomercuric compounds were from 0.6 - 8.8 ng.

Much of the systematic work in mercury speciation using microwave emission spectrometry (MES) can be attributed to Giovanoli and Greenway [83] and Talmi [84]. They discussed the practical aspects of MES with direct alkaline digestion or benzene extraction prior to gas chromatography separation, simplification of analytical

procedures, enhancement of sensitivity, and a number of useful determinations for methyl- and dimethylmercury. The limit of detection for the analysis of 50 ml water samples was reported as 1 ng l^{-1}. Further developments include application of microwave induced plasma emission spectrometry with alkaline digestion to speciation of mercury in biological samples [85-88]. For example in 1983, Chiba and Fuwa [89] determined alkylmercury in sea water at the nanogram per litre level using atmospheric pressure helium microwave induced plasma emission spectrometry. In comparison with Talmi's work, the Fuwa study obtained a 500-fold sample preconcentration factor and reported the methylmercuric chloride in selected seawater as 2.03 ng l^{-1} with 89% recovery.

A semi-automated GC-MES system for speciation of mercury in biological samples has also been described by Decadt and Baeyens [90]. They proposed two procedures based on the quantitative formation of iodoacetic acid - organomercury complexes and selective extraction into a organic solvent. The limit of detection of methylmercury was 1.5 $\mu g\ l^{-1}$ of homogenate.

Krull and Bushee [91] were pioneers in the development of inductively coupled plasma - atomic emission spectrometry (ICP-AES) for mercury speciation. They demonstrated that high performance liquid chromatography (HPLC) can be used to separate different mercury species, and with a post-column cold vapour generation detection limits ranged from 32 to 62 $\mu g\ l^{-1}$ for four mercuric compounds.

Panoro and Erickson [92] used a direct current plasma -atomic emission spectrometer (DCP-AES) interfaced with GC to perform highly specific determinations of methylmercury in biological samples. Optimisation of the GC-DCP-AES interface resulted in a limit of detection of 38 $\mu g\ l^{-1}$. Most recently, a microwave plasma (MP) - AES coupled with capillary gas chromatography and an alternative current plasma (ACP) - AES detection for HPLC have been developed by Greenway and Barnett

[93]. Colon and Barry [94] combined an alternative current plasma (ACP) - atomic emission spectrometer (AES) with HPLC. Both groups obtained limits of detection down to ng l^{-1} level or lower for the determination of organomercurials in environmental samples.

Mass Spectrometry

Mass spectrometry (MS), which has been rapidly developed and widely used in the last 20 years, is mainly characterised by high speed, sensitivity and selectivity in the structural determination of organic compounds.

The first attempts at mercury speciation by gas chromatography - mass spectrometry were made by Jenson et al. [33] in 1969, and later by Westoo and his co-workers [95] using the isotope dilution technique. Isolation of different organomercury compounds by multi-extractions prior to gas chromatography separation and sensitive quantitation by isotope dilution - mass spectrometry detection were achieved. During the last 15 years, GC-MS has been widely utilised for determination and speciation of mercury in biological samples and in water samples [96-100]. This method offers some advantages over GC-AES procedures for mercury speciation, because of increased sensitivity and being less prone to interferences. However, the disadvantages of the method due to the limitation of preconcentration by extraction and the small quantity of sample used in GC (μl level), restricted it's application to the speciation of mercury in water samples. The limit of detection reported for organomercurials in aqueous solutions was at the 1 ppb level after solvent extraction into benzene and concentration of this extract to a few microlitres.

The high detection power of inductively coupled plasma mass spectrometry (ICP - MS) makes possible the determination and speciation of mercury in biological and water samples with the use of stable isotope dilution techniques. Key features of

interest for this technique are it's low detection limit, it's freedom from spectral interferences, and the possibility of isotope dilution analysis. Beauchemin et al. [101] have proposed a method which describes the extraction of organomercurials from biological matrices with the subsequent determination of mercury after sample introduction by the flow injection (FI) technique. A simple isotope dilution analysis was also reported. This method has been applied with excellent results to organomercurials in two marine biological standard reference materials.

Bushee [102] proposed a HPLC - ICP - MS system for speciation of mercury in tuna and in contact lens solutions. The limits of detection in the low $\mu g\ l^{-1}$ region were improved further by post-column cold-vapour generation. Spiked water samples were also used to validate the method. It is apparent that pretreatment procedures for ICP-MS detection to some extent complicate sample handling and thereby increase the problem of contamination. A further development of ICP-MS for mercury speciation in natural waters and reference sediments has recently been described by Haraldsson and his colleagues [103]. Cold vapour generation with sodium borohydride was integrated with the nebuliser flow and used to convert all organomercuric compounds into elemental form. The outlet tubing from the generation vessel was connected directly to the plasma torch. The method gave a limit of detection of 0.08 $ng\ l^{-1}$ with precision of 3% at the 1 $ng\ l^{-1}$ level.

1.2.4 Gas Chromatography and High Performance Liquid Chromatography With Solvent Extraction

The speciation of mercury in biological materials and water samples using gas chromatography (GC) and high performance liquid chromatography (HPLC) has been studied by a number of authors [104,105]. In this section, only GC-electron capture detection (ECD) and HPLC-electrochemical detection (ED), as conventional and

general GC and HPLC techniques, are discussed. Other combined techniques are covered in other sections according to their modes of detection.

Gas Chromatography - Electron Capture Detection

Since the pioneering achievement of Westoo [82,106,107] and of Sumino [108,109] in the 1960's, considerable efforts have been expended in the development of reliable, precise, and sensitive methods for the gas chromatographic determination of alkylmercurials, particularly in fish, other biological samples and in waters. The potential utility of some organomercury compounds for the determination at trace level of various inorganic species by means of GC after their conversion into organic derivatives has also been investigated. Most of the published methods involved extraction of organomercurials halides [33, 110-116] or other derivatives, e.g., dithizonates [117]. An extraction procedure is necessary to remove the sample matrix, which could otherwise poison the column. Matrix separation is achieved by adding to the organic phase a reagent such as cysteine [106-110], glutathione [111], or thiosulphate [112], which form strong water soluble alkylmercury complexes to effect extraction into the aqueous phase. Halide is then added to the aqueous phase, and the alkylmercury halides formed are back-extracted into an organic phase. Aliquots of this phase are finally injected into the gas chromatography system.

With this technique, Cappon and Smith [112] determined methylmercury in whole blood samples, while Watts and his co-workers [113] applied the same system to determination of methyl- and ethyl-mercury in fish samples with the same limit of detection of 1 ug kg^{-1} (wet weight).

Cappon and Smith [114] have further improved the GC-ECD approach. Methyl-, ethyl- and phenyl-mercury underwent extractions for separation and subsequent detection, and inorganic mercury was converted into methylmercury upon the reaction

with tetramethyltin, and isolated as organomercury species for further measurement. Limits of detection for different mercury species were reported at the 1 ng l^{-1} level or lower.

Since 1980, much work on GC-ECD for mercury speciation has been undertaken to improve sensitivity and to simplify the pretreatment procedures. Cappon and Smith [114] and Goolvard and Smith [115] have proposed an alkaline digestion method for pretreatment of biological samples instead of acidification and extraction. This method has, to some extent, simplified pretreatment procedures. A further study focusing on the behaviour of the GC column has been carried out by O'Reilly [116]. He reported that passivation treatment of the diethylene glycol succinate columns by lowering the column temperature to 115°C and injecting mercuric chloride solution (1 mg ml^{-1}) into the column, produced extraordinarily efficient columns, which was of significant benefit to chromatographic peak shape and sensitivity. The practical limit of quantitation was 2 - 5 $\mu g\ l^{-1}$ in the original samples. There was a relatively high freedom from interferences because of the high performance of the column. Afterwards, the US Food and Drug Administration (FDA) [117] organised an inter-laboratory study on the GC-ECD method for speciation of mercury in fish samples, which was collaboratively studied by Hight and Capar [118]. The limit of detection of mercury was reported at the level of 0.05 $\mu g\ g^{-1}$.

More recently the use of a high resolution capillary column was proposed by Brooks and Snowden [119], in order to overcome the limitation of conventional packed columns. These limitations are due in part to organomercuric halides exhibiting poor GC properties on packed columns, and losses of analyte which may readily occur particularly at low concentration levels [117].

GC-ECD has recently been developed [120] to meet the need to modify an official method adopted by AOAC in 1984 [121]. An improved sensitivity and modified

extraction procedures gave a more rapid analysis with an average recovery of 100.5%.

Filippelli [122] has reported an alternative extraction procedure based on the extraction of an organomercuric chloride by benzene, followed with re-extraction by a thiosulphate solution, and analysis by GC-ECD. The limit of detection was 2 $\mu g\ l^{-1}$ as Hg (II). There have been some recent application of GC-ECD for speciation of mercury in natural waters which merit attention. Because analysis of natural waters is more difficult than biological samples due to the extra phase-transfer and preconcentration required, Lee and Mowrer [123] used a capillary column - GC - ECD system following adsorbent preconcentration of the mercury species using sulphydryl cotton. A limit of detection down to the sub-ppt levels was achieved for lake and snow-melt water samples. Wang and Cui [124] determined trace methylmercury in sea water by GC-ECD with a multi-extraction procedure. A recent review of the application of GC-ECD for mercury speciation has been published by Horval et.al [125], in which modifications to organomercury extraction procedures are dealt with.

High Performance Liquid Chromatography with Electrochemical Detection

High performance liquid chromatography (HPLC) has been utilised for many years as a separation and analysis technique. The early work involved electrochemical detection [126,127]. Measurement was based on the separation of Hg^{2+}, $MeHg^{+}$, $EtHg^{+}$ and $PhHg^{+}$ on a packed column followed by detection using a platinum working electrode coated with a thin layer of mercury, in place of the normal dropping mercury electrode. Purification of the solvent was required to remove reducible species which gave rise to excessive background currents and increased detector noise. MacCrehan and Durst [128] subsequently utilised differential pulse electrochemical detection with the same HPLC parameters for the determination of $MeHg^{+}$, $EtHg^{+}$ and $PhHg^{+}$ in fish samples. In the early 1980's, MacCrehan [129] reported a fully

detailed optimisation procedure for his system and discussed the advantages of HPLC - differential pulse technique for detection of organomercuric cations. Holak [130] described a HPLC system with polarographic detection in dropping mercury and hanging mercury drop modes for the determination of organomercury compounds.

In recent years, electrochemical detection has rarely been coupled with HPLC for mercury speciation because of it's limitations. However, Evans and Mckee [131] reported their work using reductive electrochemical detection coupled with reversed - phase liquid chromatography to determine inorganic mercury and three organomercurials in spiked water samples. Limits of detection varied from 1 - 2 $\mu g\ l^{-1}$.

One of the main limitations of electrochemical measurement for HPLC is poor sensitivity. Furthermore a time consuming preconcentration step is required in many cases. Another important potential interference in electrochemical detection is the adsorption of organic matter on the electrode surface. An adsorbed layer of organic matter may hinder the diffusion of the active species, thus diminish or eliminate the diffusion current and cause a non-linear relationship between reduction current and concentration of active species [131].

1.2.5 Limitations in Speciation Measurement

Most of methods for mercury speciation can be divided broadly into two categories:

(i) those methods that are likely to determine the readily available inorganic mercury and total mercury after pretreatment of samples.

(ii) those methods, which in addition, are likely to determine inorganic and organo-mercury respectively as a direct approach for mercury speciation.

Inspection of various methods summarised in Figure 1.5 reveals the wide variety of techniques that have been employed for the speciation of mercury in waters and in biological samples.

All these techniques have several common disadvantages for trace mercury speciation. First of all, extraction and back extraction procedures are tedious and time consuming operations with the possibility of contamination; therefore a high degree of cleanliness for labware and laboratory environment is essential. The other general problem encountered in most trace mercury analysis methods occurs at the sampling stage and when addition of powerful acids or other chemicals to the samples is required to preserve/stabilise the analyte. There is no doubt that such procedures run the risk of destroying the natural state of the analyte. Also, analytical results may be subject to error at the various stages of the analytical method. There is unfortunately no universal panacea for these problems. There are, however, general guidelines which must be followed if reliable data are to be produced.

These guidelines are as follow: Whenever possible, manipulations in the field should be kept to a minimum, and the sample should be analysed as soon as is practicable after collection to keep the contact time with foreign surfaces short. If this is not feasible, then it may well be advantageous to consider preconcentration of the sample before storage. Obviously, all equipment should be scrupulously cleaned and conditioned prior to use and maintained thus between samples. All reagents should be of the highest quality available, and further purified if necessary to ensure that in the determination step the signal to blank ratio is large. Another major drawback of the above batch type operations is their "off - line" features which makes automation of analytical procedures more difficult.

The inability to measure the concentration of individual inorganic and organomercurials species is common to most mercury speciation techniques except gas chromatographic and HPLC methods. Normally, organomercury data is based on a difference between the total mercury and the inorganic fraction, and is quite inaccurate due to large dilution error.

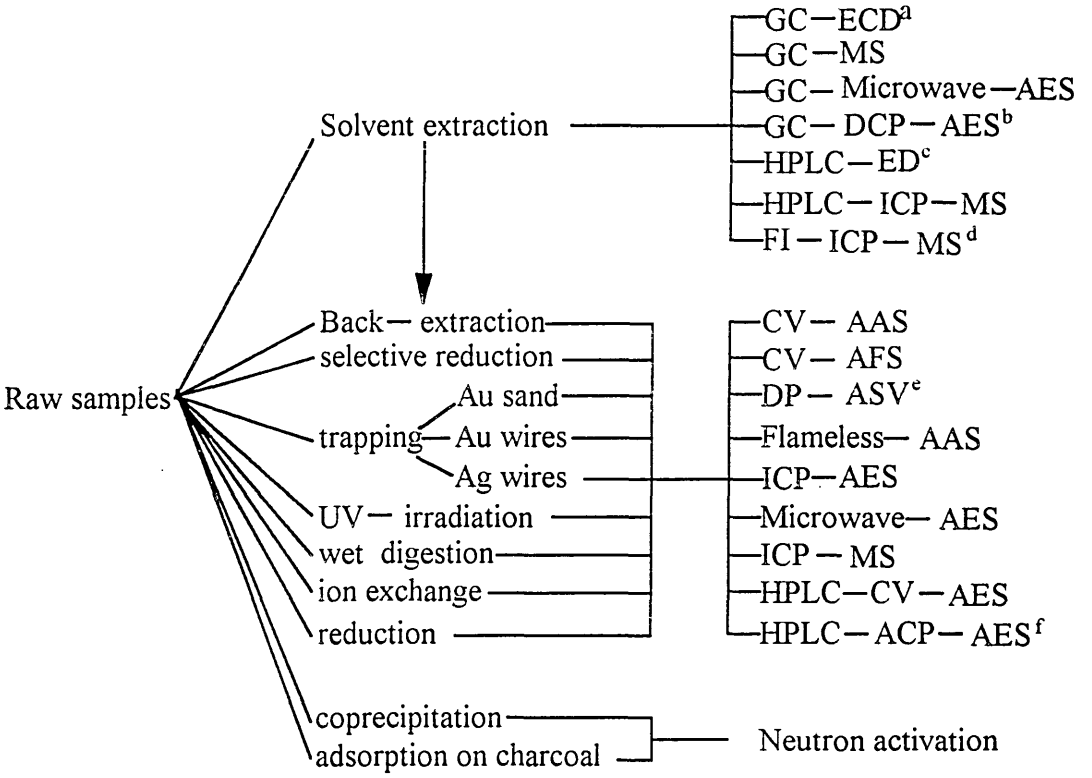
1.2.6 The Aims of The Present Work

The basic aim of the work reported in this thesis is to devise new methodology based on microcolumn techniques and flow injection analysis for the speciation of mercury in the environment. The approach should be simpler, more rapid, sensitive and more accurate than other procedures.

There are two major aspects to this work which involve the investigation of

1) a novel manifold employing microcolumns to achieve high selectivity, appropriate preconcentration, and stability of analyte species, for the analysis of a wide variety of waters.

2) a new concept of field sampling utilising microcolumns at the site of sampling, to evaluate the spatial distributions of inorganic and organomercury compounds in rivers.



a. GC - Electron Capture Detection;

b. GC - Direct Current Plasma - AES;

c. HPLC - Electrochemical Detection;

d. Flow Injection - ICP - MS;

e. Differential Pulse - Anodic Stripping Voltametry;

f. HPLC - Alternative Current Plasma - AES.

Figure 1.5 Methods for mercury speciation in waters and biological materials

CHAPTER 2

EXPERIMENTAL

2.1 Instrumentation

Flow injection - atomic fluorescence measurements were performed with a Merlin mercury detector (P S Analytical Ltd) equipped with a peristaltic pump (Ismatec) and a rotary injection valve (Omnichem). An on - line microcolumn packed with sulphydryl cotton fibre was used for separation and preconcentration of methylmercury.

A schematic diagram of the FI - CV - AFS system is shown in Figure 2.1. The system also included a mixing coil (4 meter in length) and a gas/liquid separator.

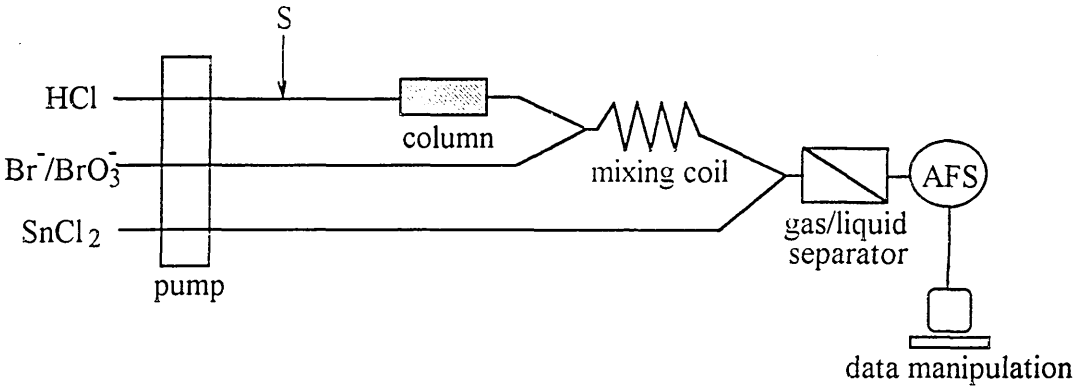


Figure 2.1. Flow injection - cold vapour - atomic fluorescence system incorporating microcolumn of sulphydryl cotton.

The operating conditions are presented in Table 2.1.

Table 2.1. Operating Conditions for Microcolumn - FI - AFS System

Flow injection manifold	
microcolumn length	50 mm (internal diameter: 1.5 mm)
mass of sulphydryl cotton	0.015 - 0.018 g
injection volume	0.5 ml
flow rate of streams	1.5 ml min^{-1}
mixing coil length	4 metres (0.8 mm, ID)
Merlin mercury detector	
source	low pressure mercury discharge lamp
analytical line	Hg 253.7 nm
sensitivity setting	2 x 1000
flow rate of sheath Argon	2 litres per minute
flow rate of sample aeration	2 litres per minute
integration time	136 seconds
Chart Recorder	
sensitivity	1 V
chart speed	10 mm min^{-1}

Flow injection analysis - inductively coupled plasma atomic emission spectrometry (FIA - ICP - AES) has also been used with other microcolumns packed with activated alumina or 8-hydroxyquinoline for separation and preconcentration of inorganic mercury. A schematic diagram of the FI - ICP - AES system is shown in Figure 2.2.

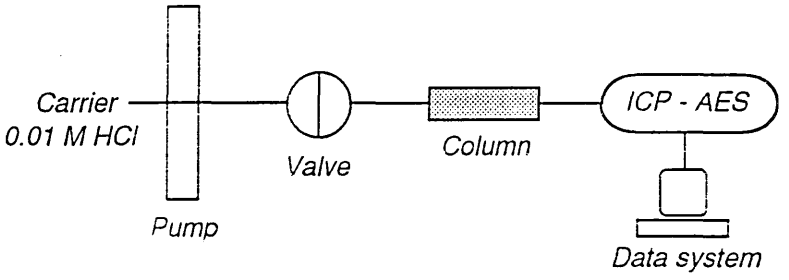


Figure 2.2 Manifold of flow injection - inductively coupled plasma - atomic emission spectrometry

The ICP spectrometer (Jarrell - Ash ICAP 9000) utilised the Hg 257.3 nm line. Typical plasma operating conditions are presented in Table 2.2.

Table 2.2. Typical Operating Conditions for FI - ICP - AES System

Plasma Operation Conditions:	
forward power:	1.1 kW
observation height	15 mm
coolant argon	20 l min^{-1}
nebuliser argon	1 l min^{-1}

Flow Injection Manifold:

microcolumn length	50 mm (internal diameter: 1.5 mm)
activated alumina	0.1 g
injection volume	0.5 ml
carrier stream flow rate	1.5 ml min^{-1}

2.2 Reagents

Tin chloride solution (3% m/V) in hydrochloric acid (15% V/V) was freshly prepared each day from tin chloride 2-hydrate salt(Spectrosol, BDH), hydrochloric acid (36 % m/m, Aristar, BDH) and high purity water. A potassium bromide/potassium bromate solution (0.5% + 0.14% m/V) was made by dissolving potassium bromide/potassium bromate salts (Analytical reagents, Fisons) in water. Hydrochloric acid solution (0.01 M) was prepared from hydrochloric acid (36% m/m, Aristar, BDH). 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.

Inorganic mercury standard solutions were prepared by dilution of commercial stock solution (Spectrosol, BDH, 1000 mg l^{-1}). Methylmercuric chloride stock solution (100 mg l^{-1}, as Hg) was prepared by dissolving methylmercuric chloride salt (0.125 g, Organics, BDH) with 25 ml of acetone (Analar, BDH) and then diluting to 1000 ml with Millipore water. Phenylmercury stock solution (10 mg l^{-1}, as Hg) was prepared by dissolving phenylmercuric acetate salt (0.0168 g, Organics, BDH) with 20 ml of glacial acetic acid (Analar, BDH) first and then diluting to 1000 ml with Millipore water. Methylmercuric chloride and phenylmercuric acetate standard solutions for calibration and synthetic samples were prepared by serial dilutions from

their corresponding stock solutions. Methylmercuric chloride and phenylmercuric acetate salts are highly toxic (they affect the central nervous system) and care was taken when handling solids and concentrated solutions (Wear gloves, do not re-use gloves, clean up all spillages as soon as possible).

All new batches of reagents were checked for mercury content prior to use.

2.3 Preparation of Sulphydryl Cotton Fibre (After reference [123])

Thioglycollic acid (50 ml GPR, 97% m/m, BDH), acetic anhydride (30 ml, 36% m/m, GPR, BDH), acetic acid (20 ml, 30% m/m, BDH)and sulphuric acid (0.15 ml, 96% m/m, GPR, BDH) 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 double - distilled 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.

An esterification reaction takes place between the hydroxy group on the cotton fibre and the carboxylic acid group in thioglycollic acid. The product is sulphydryl cotton fibre:

$$\underset{\text{cotton fibre}}{nC_6H_9O_4OH} + \underset{\substack{\text{thioglycollic}\\ \text{acid}}}{nHOOCCH_2SH} \Longrightarrow \underset{\text{sulphydryl cotton}}{nC_6H_9O_4O\overset{O}{\overset{\|}{C}}CH_2SH} + nH_2O \qquad (2.3.1)$$

In this way, a sulphydryl group is bonded to the fibre chain.The product is known as sulphydryl chelated ion exchanger fibre.

2.4 Preparation of Xanthate Cotton Fibre

30 g of commercial degreased cotton wool was firstly torn into small pieces, allowed to soak thoroughly in a 15 - 20% sodium hydroxide solution for 5 minutes, and then leached with water to remove sodium dioxide. The leached cotton wool was then immediately added to carbon disulphide, soaked for 20 minutes, and leached with water. Finally, a large volume of double - distilled water was used to rinse the cotton until the pH of the leach liquor was between 7 and 8. The cotton was finally placed in an oven (38°C) to dry. The xanthate cotton fibre was stored in a screw-capped brown bottle in a cold room.

The synthesis of xanthate cotton fibre required two steps. The first step is represented by equation 2.4.1 The product is alkaline cotton cellulose. The second reaction was also an esterification reaction involving the hydroxy group of cellulose and carbon disulphide (also called sulphydryl anhydride).

$$\underset{\text{cotton fibre}}{n(C_6H_9O_4OH)} + nNaOH \;\text{===>}\; \underset{\substack{\text{alkaline}\\ \text{cotton cellulose}}}{nC_6H_9O_4ONa} + nH_2O \qquad (2.4.1)$$

$$n(C_6H_9O_4ONa) + nCS_2 \Longrightarrow \underset{\text{xanthate cotton fibre}}{nC_6H_9O_4OC(=S)SNa} \qquad (2.4.2)$$

2.5 Preparation of Microcolumns With Different Packing Materials

Sulphydryl Cotton Microcolumns 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 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 flow injection system.

Xanthate Cotton Microcolumns were made in the same way as that for sulphydryl cotton microcolumns.

Activated Alumina Microcolumns- were made by using commercially available activated alumina (Brockman Grade 1, basic form, mesh size 150 μm, BDH) for column packing. Some particulate alumina was added into a small beaker containing double - distilled water to produce a slurry. Moistened and suspended alumina particulates were easily transferred into PTFE tubing (60 mm x 1.5 mm), using a syringe (2 ml capacity) to suck the slurry into the column, and packed evenly along 50 mm length of the column. Two small pieces of sponge were placed at both ends of the column to retain the packing. The other two small tubes (2 mm x 0.8 mm) were then fitted at both ends of the column to allow connection to the flow injection system (a 60 mm column packed to a length of 50 mm required approximately 0.1 g dry weight of alumina particulate).

The 8-hydroxyquinoline microcolumns- were prepared by packing commercially available silica immobilised 8-hydroxyquinoline (Pierce Chemical Co. Ltd) in accordance with the same procedure as that for activated alumina columns.

2.6 Practical Details

2.6.1 Sample Collection

It has been stressed by many different workers that sample collection is possibly the most important step in trace analysis [132-135]. It is therefore essential that sampling be performed with an awareness of all possible sources of contamination and changes that would occur. Ideally it should be possible to circumvent the many problems associated with sample collection by the use of *in situ* measurement methods. In the present work, investigations on mercury speciation have focussed on surface waters, so a polypropylene bucket was selected. Before use this bucket was carefully washed with

sulphuric acid/dichromate solution and water and soaked for several days with 4% v/v HCl to remove surface contamination. Careful rinsing with distilled water followed by several sample aliquots was carried out prior to sample collection. When sampling, it is necessary to avoid surface oil slicks, floating or suspended rubbish.

2.6.2 Sample Pretreatment

Since there is often a delay between the times of collection and analysis, as practised in official methods for the determination of mercury [121,136,137], the preservation and storage of the sample after collection must be followed to prevent contamination and storage losses during this interval. This is especially important where chemical speciation of trace metals is being studied because transformation of chemical species can occur during storage.

For filtration of water samples a wide variety of membrane filters are now commercially available. These are made from cellulose, cellulose esters, Teflon, nylon, polycarbonate, polyvinylchloride, polyamide, glass fibres and silver foil. For trace metal analysis of water samples, polycarbonate or cellulose ester filters are commonly used. In this study, Minisart filters (Versapor, supported acrylic copolymer) from Sartorius (Belmont, UK) have been used. This work only required 200 ml of sample volume, so careful washing of the filters and syringes (60 ml capacity) was carried out. This involved passing 20 ml of the eluent (3 M HCl) followed by sample solution three times (20 ml each) prior to collection of the filtrate has been undertaken to minimise contamination. The filters and syringes were discarded after use.

2.6.3 Sample Storage

In this work the author used borosilicate glass flasks to collect water samples. All flasks were pretreated with acid (4% v/v HCl solution) for several days. The flasks

were then well-rinsed with Millipore water and 0.5 ml of nitric acid (70% m/m, Aristar, BDH) added as a stabilising agent. All pre-cleaned flasks were stoppered tightly and taken to the site for sample collection. The flasks for preparation of standards were precleaned with the same batch of acid and Millipore water. A separate analysis of each batch of the nitric acid was always carried out prior to using the acid.

For mercury speciation studies, the addition of any powerful mineral acid or oxidant will clearly destroy the natural state of mercury species. For this work acidification of water samples with dilute nitric acid (0.5%) has been employed, and the speciation studies were performed immediately on receipt of the samples in the laboratory. Because sulphydryl cotton columns have a maximum absorption efficiency and selectivity for organomercuric ions when the sample pH is 3, acidified sample solutions were adjusted to pH 3 by adding dropwise dilute ammonium hydroxide solution (0.5 M , Aristar, BDH).

2.6.4 Analysis Procedures

a) Microcolumn - FI - AFS Manifold

The manifold, a three line system, is shown in Figure 2.1. Pure argon for aeration of elemental mercury and for the sheath gas to the Merlin detector was supplied from a cylinder and the Argon flow rate was measured and regulated by a rotameter. The argon passed into the air/liquid separator which contained entrained standard or sample solution introduced by the continually flowing streams. The carrier (0.01 M HCl), the oxidant (0.5% m/V KBr + 0.14% m/V $KBrO_3$) and the reductant (3% m/V $SnCl_2$ in 15% HCl, w/v) streams were pumped by a Ismatec Mini-s peristaltic pump using Watson Marlow number12 pump tubes to maintain the correct flow rate in each feed line. PTFE tubing (ID. 0.8 mm) was used as transmission tubing. A mixing coil of PTFE tubing (4 m x 0.8 mm) was also used to ensure significant conversion of any

organomercury to the inorganic mercury. Injection of standards, samples and eluent was accomplished by an Omnifit rotary valve with a 500 μl capacity sample loop. The valve was operated manually. The standard and sample streams enter a glass air/ liquid separator for extraction of elemental mercury. Mercury signals are measured by a Merlin atomic fluorescence detector using the 253.7 nm line. The signals from this are recorded with a Hitachi 056 strip chart recorder, and processed by standard software routines.

b) Cleaning of Microcolumn - FI - AFS System

The new commercially available glass air/liquid separator was cleaned by soaking in hydrochloric acid (36% m/m) overnight, and then rinsing with double - distilled water before use. The Teflon beakers (25 ml capacity), used as containers for standards, samples and eluent, were kept in an HCl/HNO_3 bath and rinsed thoroughly with Millipore water immediately prior to use. The FI-AFS system was set up without insertion of the microcolumn, and cleaned by firstly pumping 5 M hydrochloric acid solution through the three feed tubes for 20 minutes and then pumping Millipore water for 10 minutes. The microcolumn was placed in the system, and the three streams (the carrier, the oxidant and reductant) pumped into the system according to the diagram shown in Figure 2.1. A stable baseline was observed after a few minutes. The pre-cleaned PTFE beaker and the disposable syringe (2 ml capacity) were used to inject the eluent (3 M HCl for sulphydryl cotton columns) through the valve into the sample loop. At this time the valve was in load position. The valve was then turned to the inject position and simultaneously the data acquisition routine initiated. This procedure was repeated three times to minimise the contamination of mercury from the freshly-made sulphydryl cotton columns.

c) The Procedure for Mercury Speciation

The procedure for mercury speciation is as follows : The carrier, oxidant and

reductant streams were continuously pumped at a flow rate of 1.5 ml min^{-1}. After injection of water sample (0.5 ml), immediate breakthrough of inorganic mercury occurred indicating little retention on the sulphydryl cotton microcolumn. Inorganic mercury is transferred into the separator, where reduction into elemental form occurs. The mercury vapour is then detected by the CV-AFS system. Hydrochloric acid (3 M) is then injected to elute retained methylmercury, the species first being oxidised by Br^-/BrO_3^- solution and then converted to elemental mercury as before. In this way, a rapid sequential monitoring of methylmercury and inorganic mercury is achieved.

2.7 Field Surveys in the Manchester Ship Canal

As the second part of the research the field sampling technique using sulphydryl cotton microcolumns has been applied to the field surveys on speciation and distribution of mercury in the Manchester Ship Canal and the River Rother. In this section, the practical details in field surveys and analytical procedures are fully reported.

2.7.1 Cruise Details and Sample Stations

Two surveys were undertaken for the field sampling of methylmercury (using the microcolumn technique) and bulk water sampling for inorganic mercury (in microcolumn effluents) and total mercury determinations. These cruises were organised by Dr. Peter Jones of the National Rivers Authority, North West Region - Warrington) who made the arrangements for sampling taking account of the location of industrial discharge, possible mercury dispersion and the movement of tides. The distributions of salinity, total mercury in sediments and pre-laid tracer (fluorescence dye) were also carried out. The data on salinity and total mercury in sediments are also reported and discussed in this chapter.

Details of the individual cruises are given below:

(1) First cruise (15/10/91)

The primary purpose of this cruise was to make a detailed survey of the salinity, temperature, pH and to take routine samples as part of the regular monitoring programme of the National Rivers Authority (North West Region). Sediment samples were collected for the analysis of various metal species. Total mercury in sediment samples was also measured by the National Rivers Authority who generously made data available for the author to discuss in the thesis. The whole survey was completed without difficulty. Weather conditions during the cruise were good. The stations at which water samples were collected are shown in Figure 2.3. The Manchester Ship Canal is filled by the River Irwell and the River Medlock which flow through Manchester. The River Mersey and the canal separate at Bollin point and run side by side to Eastham where the canal enters the estuary (station 1). In the polluted region between Warrington and Runcorn, fresh water and sea water converge due to tidal movement. The entry point to the canal for the River Weaver is close to station 6.

(2) Second cruise (30/10/91)

During this cruise, water samples were collected for methylmercury using the microcolumn field sampling method, and for the inorganic mercury fraction using precleaned flasks to collect effluents from microcolumns. Samples for total mercury analysis were collected according to the AOAC method [136]. The sampling stations selected were the same as those used on first cruise (see Figure 2.3).

Because the whole distance of the cruise was short (about 32 km), weather factors did not change significantly during the cruise. At each station water samples were collected and the salinity monitored by determining chloride contents and converting into salinity, conversion rate is 1.806. The conductivity in the surface waters were determined using a portable conductivity meter. The turbidity in surface waters was

very high with visible particulate matter arising from the frequent passage of large barges.

2.7.2 Field Sampling Kit

The sampling kit consisted of an on-line filter (0.45 um,Anachem), a sulphydryl cotton microcolumn (5 cm x 1.5 mm) which had been pretreated by twice passing through 2 ml of 3 M hydrochloric acid solution (Aristar, BDH) followed with twice passing through 2 ml of water using a syringe (60 ml, capacity). This is shown in Figure 2.4. Filtration was used to remove particulate matter.

Sample solution (1 - 2 l) was adjusted on collection to pH 3 - 3.5 by adding dropwise a small amount of concentrated nitric acid (Aristar, BDH) and measured using a portable pH meter. A plastic syringe (60 ml, capacity) was quickly filled and rinsed with the sample solution, connected with a on - line filter (0.45 μm) and then the sample passed through the filter. This syringe and filter were accurately filled with a specified volume of sample solution (typically 10 ml), and then sequentially fitted up with the rinsed-filter and a pretreated microcolumn. The sample solution was passed through the filter and the microcolumn by syringe action. Meanwhile, the column effluent was collected in a pre-cleaned 50 ml flask, which contained 0.5 ml of nitric acid (Aristar, BDH), for determination of inorganic mercury.

The microcolumns were then disconnected, placed in a light tight box and returned to the laboratory. At each sampling station, an additional 200 ml of water was collected with a pre-cleaned flask for determination of total mercury by the batch method (oxidation - flow injection - cold vapour - atomic fluorescence spectrometry).

For each sample, three microcolumns were used to generate three data points (methylmercury fraction). A flow rate of 4 ml min^{-1} was maintained as far as possible.

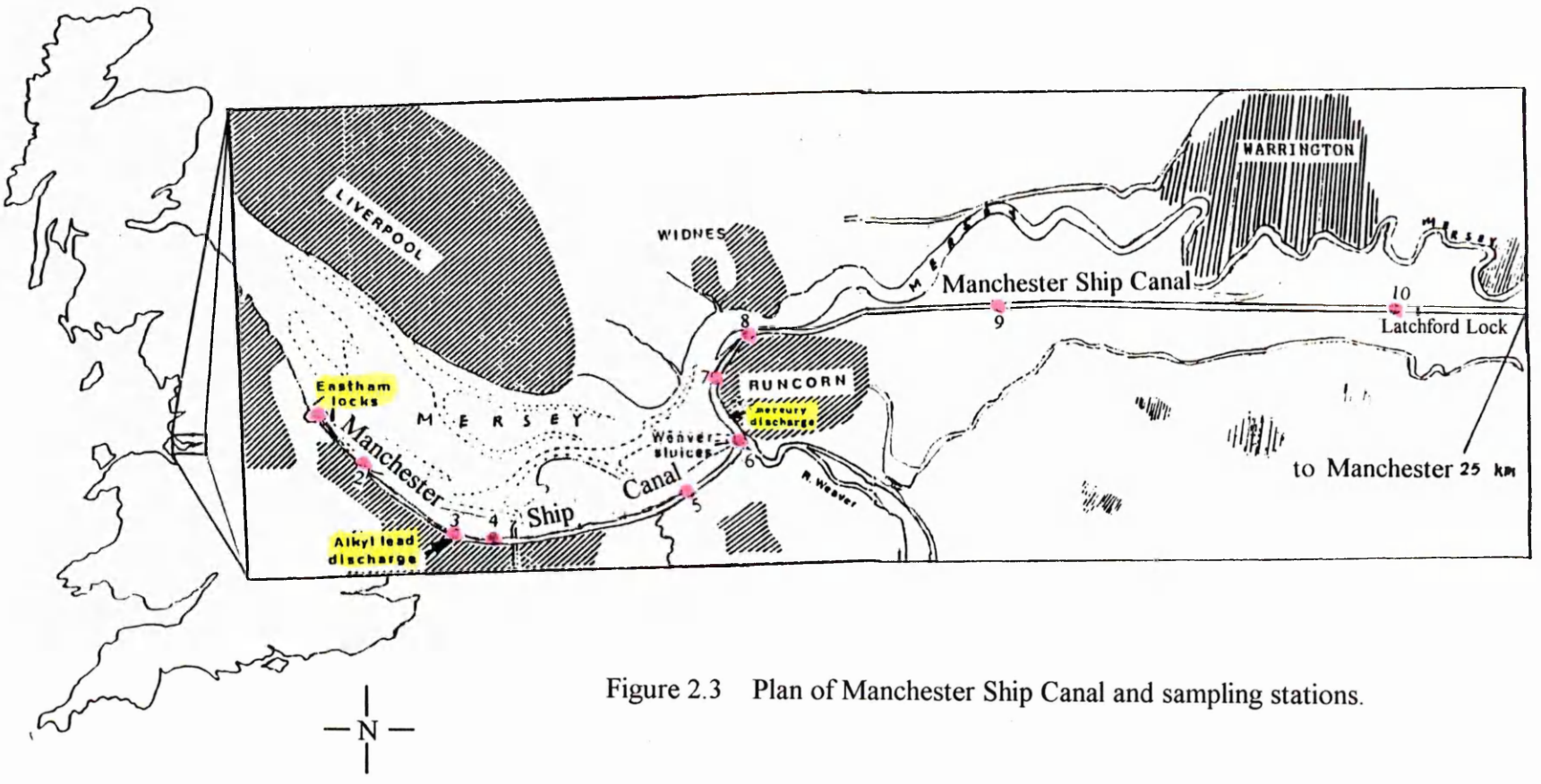


Figure 2.3 Plan of Manchester Ship Canal and sampling stations.

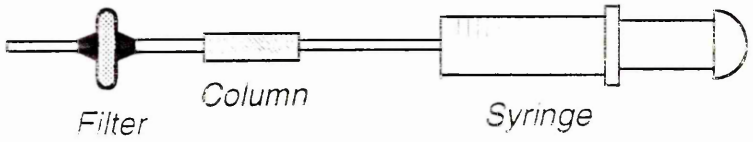


Figure 2.4 Field Sampling Kit

Fresh syringes and filters were used to assemble field sampling kits at all times. Figure 2.5 shows view of field sampling in the Manchester ship Canal.

Figure 2.5 View of field sampling in the Manchester Ship Canal

2.8 Analysis Procedure for Survey Work

Determination of mercury were based on the following classifications:

(1) dissolved organomercury (corresponding to fraction retained on microcolumn after sample filtration, 0.45 μm); (2) dissolved inorganic mercury (corresponding to fraction in effluent after filtration and microcolumn processing); (3) dissolved total mercury (corresponding to fraction in raw water after filtration and acidification); (4) unfilterred total mercury (corresponding to fraction subject to acidification and oxidation using Bromide/Bromate solution).

2.8.1 Determination of Organomercury

The determination of organomercury collected on microcolumns was carried out using flow injection - cold vapour - atomic fluorescence spectrometry, which was set up in the National Rivers Authority laboratory. The manifold (illustrated in Figure 2.1.) was cleaned and rinsed by pumping first 5 M hydrochloric acid solution for 20 minutes and deionised water for 10 minutes. Then the reductant ($SnCl_2$), the oxidant (Br^-/BrO_3^-) and the carrier (acidified deionised water) streams were continuously pumped at a flow rate of 1.5 ml min^{-1}. The flow rate of pure argon was 2 l min^{-1} for aeration and 2 l min^{-1} for the cell sheath. When a smooth baseline was observed, the system was ready for calibration and sample analysis.

Three standard solutions of concentrations (as Hg II) of 0.00, 0.10 and 0.20 μg l^{-1} were used for instrument calibration.

A pre-cleaned glass beaker (500 ml) was filled with about 400 ml deionised water, and the pH adjusted with nitric acid (Aristar, BDH) within the range 3 - 3.5 for the preparation of methylmercuric chloride standard solutions.

Standard solutions were prepared from a 100 μg Hgl^{-i} methylmercuric chloride

solution (100 μg l^{-1}) using 100 ml pre-cleaned flasks and a Finnipipette (40 - 200 μl, capacity). The freshly made acidified water was used for dilution to the mark. Three field sampling kits were prepared and pretreated as described in the section 2.7.2 and sampled with standard solutions to keep the calibration procedure the same as the field sampling procedure.

Standard software routines (Touchstone) were used to process signals and calculate results automatically. Peak height and peak area methods of measurement were available. In this work the peak area method was selected because of its high measurement precision. TouchStone does not extrapolate the calibration curve so the top standard concentration must be higher than the expected sample concentrations.

Measurements were made by first switching off the feed pump and inserting the sampled-column between the valve and the mixing coil. The pump was switched on and kept running. When a smooth baseline was observed after a few minutes 2 ml (actually only 0.5 ml remained in sample loop) of hydrochloric acid (0.5 ml, 3.0 M) was injected into system and the valve turned to effect the elution of retained methylmercury species. The oxidant was used to convert organomercury into inorganic mercury and downstream merging with tin chloride generated elemental mercury for detection by cold vapour - atomic fluorescence spectrometry. For the blank test, the above procedure was repeated using unsampled microcolumns made from the same batch of sulphydryl cotton and pretreated with the same batch of hydrochloric acid solutions as the sampled columns.

2.8.2 Determination of Inorganic Mercury

The determination of inorganic mercury in water samples (collected microcolumn effluent in flasks) was performed by utilising a simplified FI-CV-AFS system, shown in Figure 2.6. The on-line oxidation step is not required, and was omitted.

The manifold consisted of a peristaltic pump (Ismatec), a rotary injection valve (Omnichem) and a gas/liquid separator. Hydrochloric acid solution (0.01 M) served as the carrier stream for both standards and samples. Tin chloride solution (3% m/V $SnCl_2$ in 15% v/v HCl) was used as a reductant to convert inorganic mercury into elemental form. Before calibration and sample analysis, a hydrochloric acid solution (5M) was pumped into the system for 20 minutes followed with deionised water for 10 minutes to clean the manifold. Afterwards, the reductant ($SnCl_2$) and the carrier (0.01 M HCl) streams were continuously pumped into the system at a constant flow

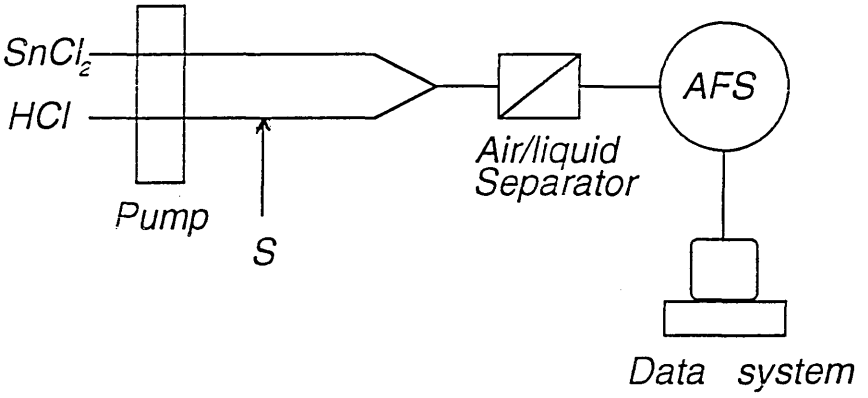


Figure 2.6 FI-CV-AFS system for determination of inorganic and total mercury. Tin chloride solution (3% m/V in 15% v/v HCl) as reductant and hydrochloric acid (0.01 M) as carrier.

rate (1.5 ml min^{-1}). The flow rates of aeration argon and cell sheath argon were 2 l min^{-1} and 2 l min^{-1}, respectively. A smooth baseline indicated that the system was ready for calibration and sample analysis.

The flasks used for preparation of standard solutions were soaked overnight in hydrochloric acid solution (4% V/V), and immediately rinsed with plenty of deionised

water before use. The flasks also contained 1% V/V nitric acid (Aristar, BDH) as preservative for inorganic mercury standard solutions.

Mercury stock solution (2 ml, 1000 mg l^{-1}, spectroscopic, BDH) was added to a pre-cleaned volumetric flask (200 ml) and diluted to the mark with deionised water. The concentration of this solution was 10 mg l^{-1}. The mercury standard solution (10 mg l^{-1} , 2 ml) was added to a pre-cleaned flask (200 ml) and made up to the mark with deionised water. The concentration of this medium standard solution was 100 μg l^{-1}.

The medium standard solution (0.0, 1.0 and 2.0 ml) was added to three pre-cleaned volumetric flasks (100 ml), respectively, and each made up to the mark with 1% V/V nitric acid solution. These calibration solutions have concentrations (as Hg II) of 0.00, 1.0 and 2.0 μg l^{-1}.

At the beginning of the calibration procedure, the injection valve is in the load mode, and the first standard (0.0 μg Hgl^{-1}) is injected into the sampling loop (0.5 ml, capacity), while the carrier is being continuously pumped. The valve is then turned to the injection mode, and the carrier routed through the loop to deliver the standard to the system. Downstream inorganic mercury merges with tin chloride solution and is converted to the elemental form for subsequent detection. For the remaining standards the same procedures are performed in order to minimise any memory effect.

Duplicate measurements were performed for each sample, and the raw fluorescence intensities were processed by Touchstone software and sample concentration values calculated.

2.8.3 Procedure for Determination of Dissolved Total Mercury

Water samples were filtered and acidified with nitric acid immediately on arrival at the laboratory.

The determination of total mercury in water samples was carried out using a

pretreatment procedure prior to FI-AFS detection. The pretreatment procedure was performed according to the AOAC standard method [136]. This involved adding hydrochloric acid (5 ml of 3.0 M, Aristar, BDH) to a precleaned flask (100 ml) followed by the water sample which was added to the flask 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 inverted several times to thoroughly mix the solution. The reaction flask was left to stand for at least 1 hour to allow oxidation to proceed. The total mercury in water samples was determined using the FI-CV-AFS system shown in Figure 2.6.

The procedure for calibration and measurement of samples for dissolved total mercury was the same as that for inorganic mercury, described in section 2.8.2.

2.9 Field Survey in The River Rother

2.9.1 Survey details and sampling positions (23/3/1992)

The River Rother arises near Clay Cross to the south of Chesterfield in Derbyshire. From its source it flows northward for a distance of some 51 km, joining the River Don in Rotherham. The contributory population of the thirty-nine sewage treatment works in the area is about 326 000 and there are about 130 permitted trade discharges to the river. Trade effluents are largely associated with coal mining, coke and chemicals, steel works, metal finishing and engineering. Abandoned mines cause serious pollution from ocherous mine water mostly in minor tributaries and for which no remedy is obtainable at present. The most crucial trade discharges are from three coking plants and a chemicals complex.

A long history of industrial discharges and drainage from a number of sewage treatment works has resulted in the quality of water in the River Rother to be classified for a number of years at grade four, and as one of the most seriously polluted stretches

of river in Britain, as defined by National Water Council criteria [138]. The evidence of discharge of mercury-containing waste water into the River Rother from a chemicals complex and an indication of elevated concentration of methylmercury species in this river was presented in a preliminary study [139], this study was a continuation of that work. The main objective was to investigate the speciation and distribution of mercury in the river. Seven sampling positions were used along the river stream between the Chesterfield and The Rother Valley Country Park. A sketch map of the area (Figure 2.7) shows the extent of gross pollution and locations of the major polluting discharges and sampling positions. There is a bridge over the river at each sampling position, which is convenient to obtain the water samples from the middle site of the river using a plastic bucket with a long rope. The survey was organised specially for this study.

The temperature of the surface water was measured using a thermometer (Model 2000, Jencons). The sample pH was determined using a portable pH meter. Because drainage from sewage treatment works constitutes a considerable input to the river system, the colour and turbidity were quite strong, particularly in downstream water.

2.9.2 Analysis Procedure

Field sampling, collection of columns' effluent and river water samples were carried out as described in section 2.7.2. At each sampling position, five replicate microcolumns were sampled for the determination of organomercury. The effluent for two columns was used for the determination of dissolved inorganic mercury, and one bottle of water (200 ml) was collected for analysis of total mercury. It was noted that the on-line filters were readily blocked by the high content of particulate matter when sampling was performed at positions 1, 2 and 3. This could only be overcomed by changing the filter each time it blocked. The drainage and ditch entry points close to

these sampling sites stirred the sediments and enhanced the mobility of particulate matter.

The determination of mercury species was performed at Sheffield Hallam University according to the procedures as previously described in the section 2.8. For each sample, five replicate samples columns were inserted, in turn, into the flow injection - cold vapour - atomic fluorescence system according to the sampling order. As the number of sampling syringes (60 ml, capacity) was limited, small volume syringes (2 ml, capacity) were used for organomercury calibration in the laboratory.

Two calibration standards and a blank were prepared (0.00, 0.50 and 1.00 $\mu g\ l^{-1}$). The sample volume for standards was 2.0 ml, but for field sampling the volume for organomercury was 10 ml. The dilution factor used for the calculation is 5. When sampled microcolumns were eluted, the fluorescence responses of sample No 2 were found to be higher than that of the top standard, so concentrations of samples No 2 were calculated by extrapolation of the calibration curve which was within the linear range (see section 3.2.2).

For the measurements of inorganic and total mercury, the calibration standards were selected as 0.00, 2.50 and 5.00 $\mu g\ l^{-1}$. Previous work reported that the total mercury concentration (filtered) in this river was 0.264 $\mu g\ Hgl^{-1}$ with an unfiltered fraction of up to 6.0 $\mu g\ Hgl^{-1}$. Inorganic mercury and total mercury (as Hg II) were determined by injecting 0.5 ml sample into the system and comparing the fluorescence signal with those obtained for standards of inorganic mercury in aqueous solutions.

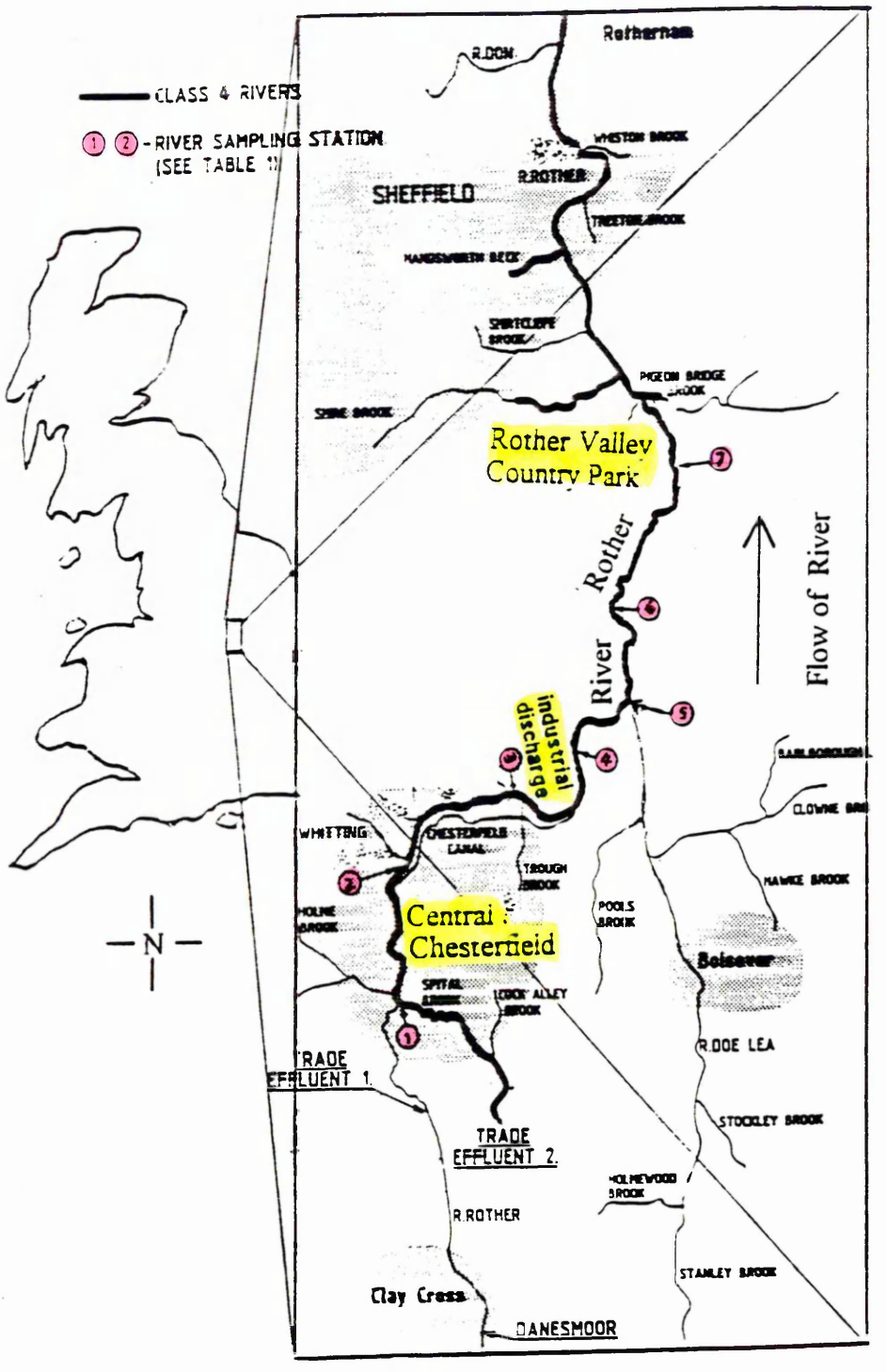


Figure 2.7 Plan of the selected section of the River Rother and Sampling Stations, " o ".

CHAPTER 3

MERCURY SPECIATION BASED ON MICROCOLUMN SEPARATION AND FLOW INJECTION ANALYSIS

3.1 Introduction

Flow injection analysis is a powerful analytical technique, and the range of its applications has increased rapidly since the first reported studies in 1975 [140]. Early work concentrated on the increase of sampling rate and reproducibility of measurements. But most of the recent work has investigated it's use as a sample handling and pretreatment technique. The key to utilising a flow injection manifold for speciation studies lies in the ability to undertake on - line manipulation. Flow injection systems can be configured to perform a variety of chemical / physical operations in order to achieve species separation and, if necessary, analyte preconcentration. The time scale for such pretreatments is short and this is attractive in terms of conserving the natural speciation state of the analyte. In combination with atomic spectrometry as the means of determination, the flow injection approach would seem to offer

considerable potential. It would allow much information to be gained about trace mercury levels from both natural geochemical weathering and industrial pollution and their effects on biological systems.

The last decade has seen the development of microcolumn flow injection analysis techniques. These have been applied to on-line analyte enrichment, speciation, matrix removal and modification by many authors. On-line flow injection analysis systems incorporating ion - exchange and/or solvent extraction were reported by Olsen *et al* [141] and Kamson and Townshend [142]. Davies et al. [143] reported the separation of phosphorus from a matrix using an activated alumina column and measurement by a colorimetric method. The principle of the differential deposition/elution was based on the differing affinity of the ion exchanger for specific analytes. With the advent of flow injection analysis, there have been a lot achievements in developing on-line sample preconcentration procedures with microcolumn technique followed by spectroscopic detection in an attempt to not only enhance sensitivity but to reduce analysis time. Olsen et al. [141] used a microcolumn of Chelex 100 for the concentration of heavy metals in polluted sea water prior to determination by flame atomic absorption spectrometry. In the studies by Fang and co-workers [144,145] the combination of flow injection-microcolumn system with electrothermal atomisation - atomic absorption spectrometry resulted in improved analytical capabilities. Chelex 100, resin 122 and immobilised 8-hydroxyquiniline were used as column packing material. Many studies on the microcolumn - flow injection technique for element speciation have been intensively carried out by Mcleod and co-workers [146-153]. For example, microcolumns packed with the acidic alumina, which acts as an ion-exchange medium, were used in flow injection analysis - inductively coupled plasma atomic emission spectrometry (FIA - ICP - AES) and applied to the determination of phosphorus in steel [146] and to the speciation of Cr(III) - Cr(VI) in natural waters

[148,149]. A microcolumn of acidic alumina has also been used in the flow injection manifold to preconcentrate sulphate [150] and other oxyanions [151] prior ICP-AES detections. Zhang and Mcleod [152] showed that a flow injection system incorporating a basic alumina microcolumn could be used in conjunction with flame atomic absorption spectrometry for the preconcentration and determination of lead in drinking waters. The limit of detection was 0.36 $\mu g\ l^{-1}$.

In this chapter a range of microcolumn packing materials are investigated in order to compare their selectivity, preconcentration efficiency amd freedom from interferences. As discussed in the Chapter 1, the affinity of mercury for -SH group is greater than for any other single ligand, more importantly, complexes of organomercury ions bind sulphydryl groups stoichiometrically. In the present work, a high selectivity and efficiency obtained with sulphydryl cotton microcolumn is of great analytical interest because it permits the separation and preconcentration of trace amounts of organomercury from inorganic mercury and matrix. The optimised system is applied to the speciation and determination of mercury in a variety of waters. Measurements are made using cold vapour - atomic fluorescence spectrometry. Initial results using sulphydryl cotton packed microcolumns have been published [153].

3.2 Results and Discussion

3.2.1 Performance of Sulphydryl cotton microcolumns

The relatively high affinity of the sulphydryl group for mercury has been discussed in Chapter 1, and it is also known that high toxicity of organomercury species also results from the higher affinity of sulphydryl-containing amino acid (eg. cysteine) for organomercury than for inorganic mercury. This prompted us to investigate the possibility of preparing and utilising sulphydryl cotton microcolumns in FI systems to achieve a selective separation and preconcentration for different mercury species. The

deposition/elution process for methylmercury would proceed in the flow injection system according to the scheme:

$$C_6H_9O_4\overset{O}{\overset{\|}{C}}CH_2SH + CH_3HgCl \underset{\text{Elution}}{\overset{\text{Deposition}}{\Longleftrightarrow}} C_6H_9O_4\overset{O}{\overset{\|}{C}}CH_2SHgCH_3 + HCl \qquad (3.2.1)$$

In order to study relative affinities of different mercury species, synthetic standard solutions of methylmercury and inorganic mercury were injected into the flow injection system while monitoring fluorescence as a function of time. The fluorescence-response curves given in Figure 3.1a clearly indicate that inorganic mercury was not retained on the microcolumn since immediately after sample injection a breakthrough signal is evident. In contrast methylmercury underwent deposition, and a subsequent injection of eluent (3 M HCl) was required to effect elution. Thus, in the case of a solution containing a mixture of inorganic mercury and methylmercury, it is possible to achieve a rapid sequential monitoring capability for the two species. Transient signals due to methylmercury are shown in Figure 3.1b.

3.2.2 Method Development Based on Sulphydryl Cotton Microcolumn-FI-AFS Technique

The application of the microcolumn - flow injection technique to element speciation has developed rapidly due to its enrichment capability and simplicity. Typical fluorescence responses for methylmercury and inorganic mercury for the measurement sequence are given in Figure 3.2., which shows that the separation and sequential measurements for both mercury species has been achieved. The ratio of peak height for methylmercury to inorganic mercury is about 50% and this suggests a relatively

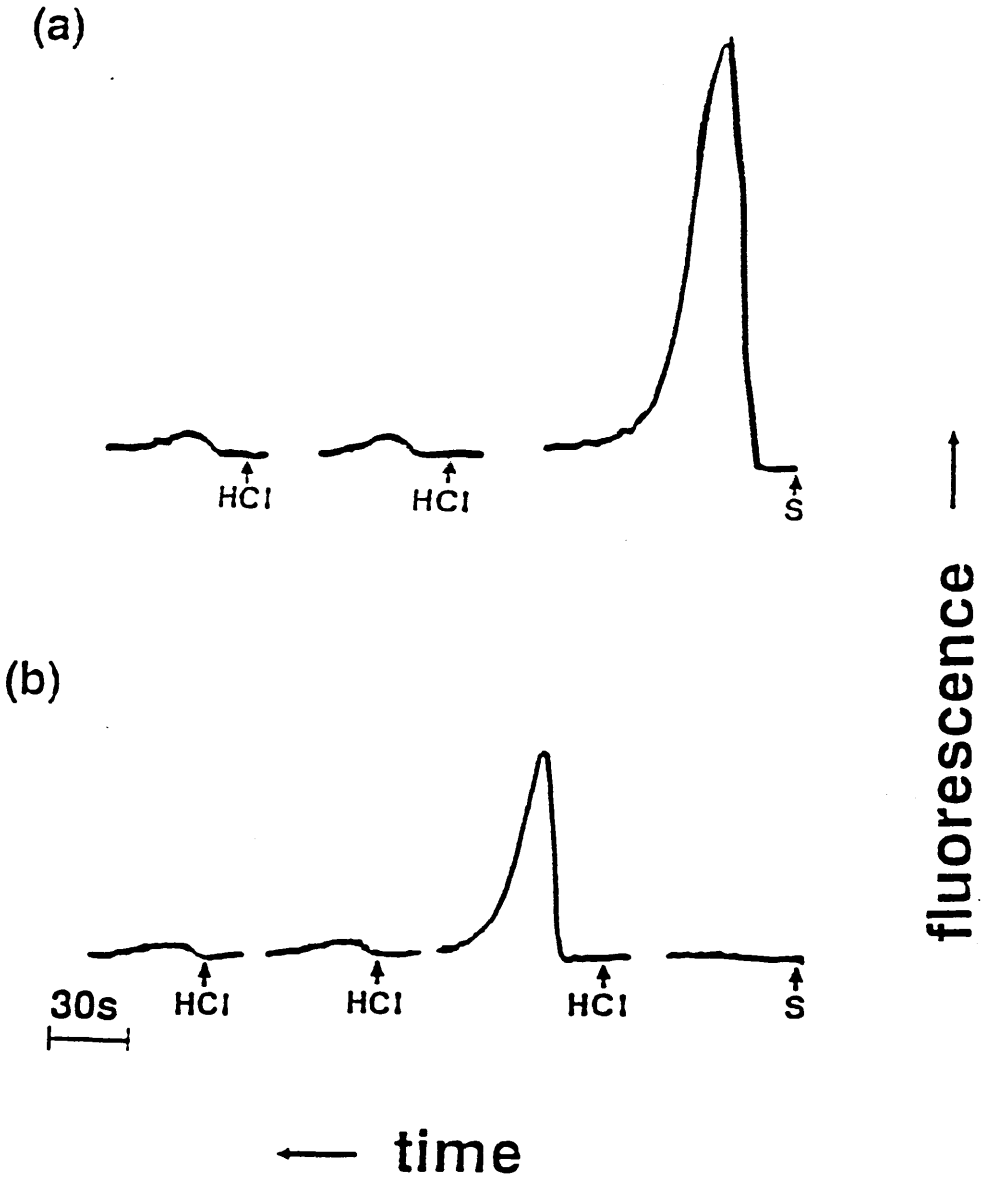


Figure 3.1 Fluorescence versus time responses for (a) inorganic mercury (0.5 ml, 2.0 µg Hgl^{-1}) and (b) injection/elution of methylmercury chloride (0.5 ml, 2.0 µg Hgl^{-1}).
S = sample; time = 0s

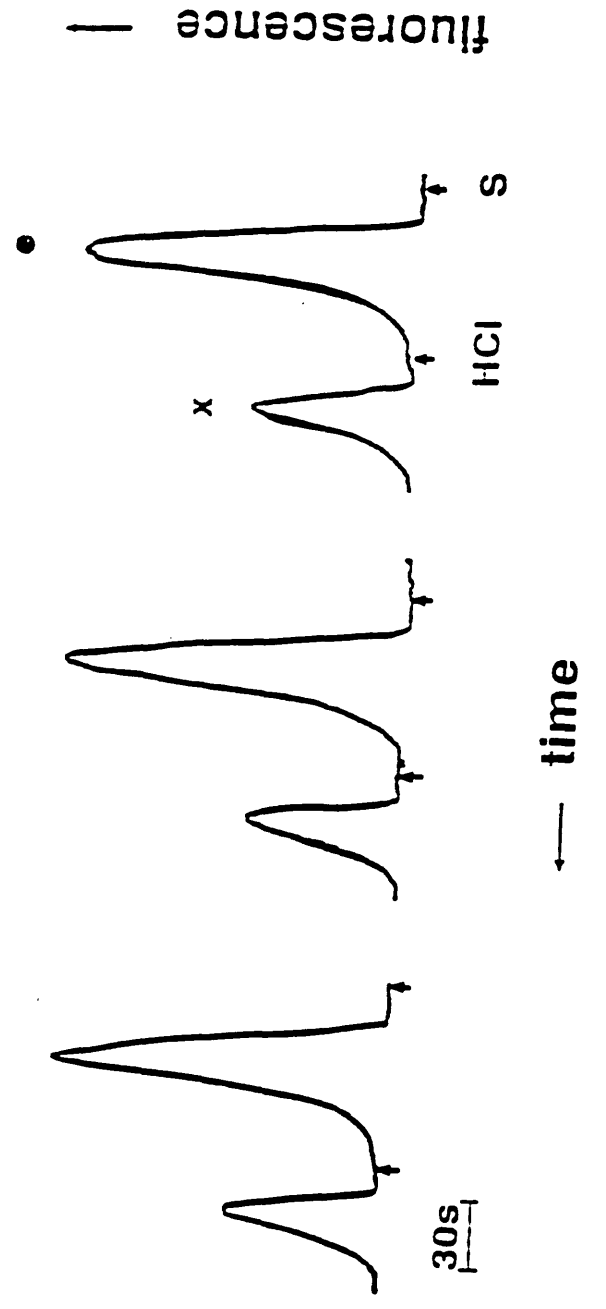


Figure 3.2. Fluorescence - time responses (in triplicate) for injection/elution of mixed standard solution - 0.5 ml methylmercuric chloride (2.0 μg Hgl^{-1}) / mercuric nitrate (2.0 μg Hgl^{-1}).

• inorganic mercury ×methylmercury.

poor oxidation/conversion efficiency. To check the efficiency of deposition and elution for methylmercury, the ratio of eluted responses of methylmercury and breakthrough responses of inorganic mercury was examined and compared with the same solutions measured by the batch operation method using flow injection - cold vapour - atomic fluorescence spectrometry as shown in Figure 2.5. For methylmercury species, batch operation using bromination was performed for convertion of methylmercury into inorganic mercury prior to measurements. It was found that under the specified experimental conditions (sample pH 3.5; concentration of tin chloride solution 3% m/V; mixing coil length 1 meter) a relatively low efficiency (53%) was noted for the methylmercury species relative to that for inorganic mercury. Therefore the method required further development, and various parameters which affect the methylmercury deposition/elution needed to be studied with a view to system optimisation. Therefore standard inorganic and methylmercuric chloride solutions were prepared, and fluorescence responses measured for variation in key system parameters such as sample pH, the length of microcolumn, eluent volume, mixing coil length, the concentration of tin chloride solution and the effect of interference on deposition and elution for methylmercury and inorganic mercury.

a) Effect of Sample pH

The acidity of samples and standard solutions was found to change the affinity of sulphydryl cotton for different mercury species and to influence overall efficiency of the operation in terms of deposition and elution.. Standard solutions of methylmercuric chloride (pH 2-9) were subjected to the operating procedure given in Chapter 2. It was found that methylmercuric chloride underwent deposition /elution in a reproducible manner over a wide pH range. Only at pH 2 or lower was analyte breakthrough found to be significant. As noted previously, in contrast to results for

methylmercury, most of the inorganic mercury was not retained on the microcolumns and only small amounts underwent deposition (less than 5%). No eluted signals of retained inorganic mercury were recorded on injection of hydrochloric acid (3 M). Reproducible responses for both inorganic and methylmercury were obtained. The effect of sample pH on deposition/elution is shown in Figure 3.3. At sample pH 3, the maximum recovery of methylmercury and optimum stability of this trace species is achieved. Meanwhile, most of the inorganic mercury passes through the microcolumn and underwent the subsequent detection.

Figure 3.3 clearly indicates that sample pH (from pH 2 to 9) affects the behaviour of deposition and elution of methylmercury, in particular, at sample pH lower than pH 4. It can be assumed that sample pH functions mainly by dominating the states of mercury species and competitive adsorption on the active sites of sulphydryl cotton by hydrogen ions. The high concentration of hydrogen ions would competitively occupy the sulphydryl groups and decrease the efficiency of deposition for methylmercury, but in turn, high concentration of hydrogen ions would break the mercury-sulphur bond and increase the efficiency of elution for methylmercury. In this work, hydrochloric acid solution (3 M) is used as eluent to release the retained methylmercuric ions from sulphydryl cotton column.

The experimental data also indicated that sample pH did not significantly affect the behaviour of inorganic mercury ions. For pH 2 - 9, when inorganic mercury standard solutions were injected, most of the species passed through the microcolumn. The breakthrough responses were found to be slighly lower than the eluted responses of methylmercury. Repeated injections of hydrochloric acid also showed that no elution signal for inorganic mercury were observed, which implies that a small amount of complexed mercury was retained on the microcolumn and was not eluted by injection of 3 M hydrochloric acid solution. It has been reported [154-156] that the Hg^{2+} ion

hydrolyse to $HgOH^+$ and $Hg(OH)_2$ in the pH range 2-6, as shown by the distribution diagram Figure 3.4.

It is also noted that in Figure 3.1 and 3.2 inorganic mercury breakthrough signals are much higher than methylmercury signals even though equivalent concentrations of mercury were examined. The result implies a relatively inefficient oxidation reaction, that is conversion of methylmercury to inorganic mercury. Further studies using a 4 meter mixing coil gave improved signals for methylmercury (87%, ratio of methylmercury to inorganic mercury; this is to be further discussed in section e).

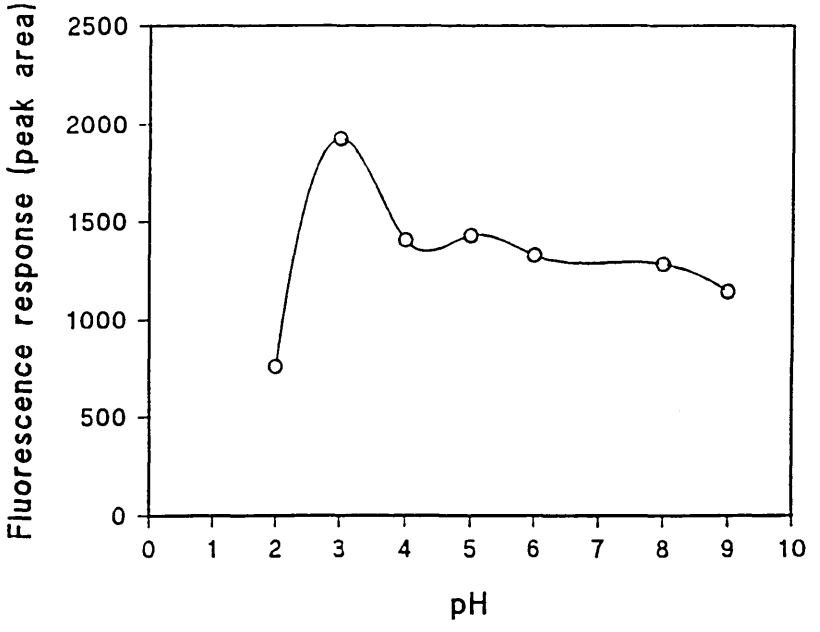


Figure 3.3 Effect of sample pH on analytical responses from standard solutions
o : eluted responses from methylmercury chloride.
(2.0 μg Hgl^{-1}, 0.5 ml).

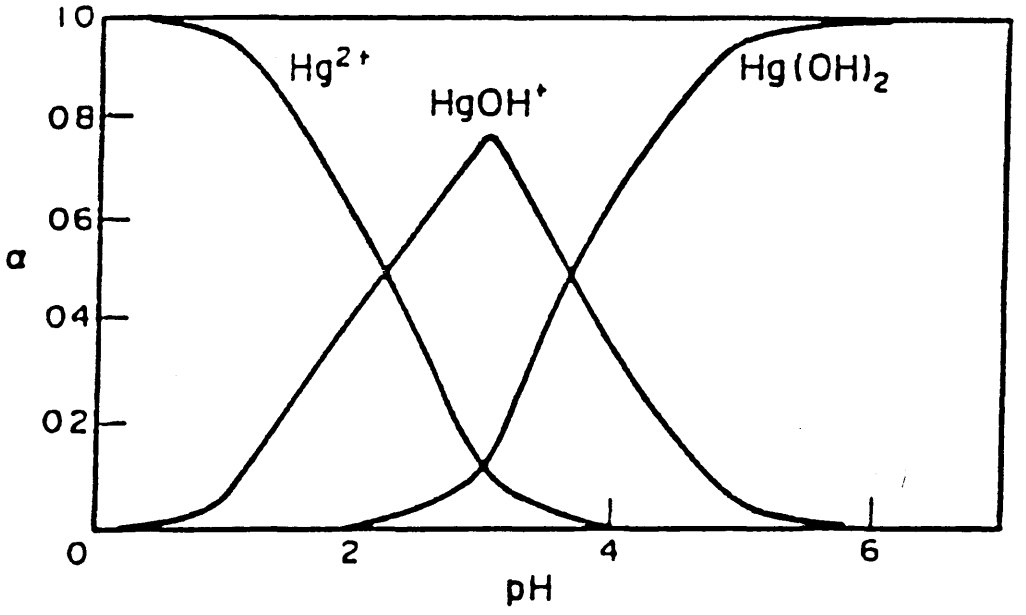


Figure 3.4 Mercury (II) - hydroxy species distribution diagram. Data from references [154 - 156].

The dominant inorganic species in fresh water is $Hg(OH)_2$, which forms significantly in solution at and higher than pH 4. According to the following reaction [157]:

$$2(C_6H_9O_4\overset{O}{\overset{\|}{C}}CH_2SH) + M^{2+} \Longleftrightarrow (C_6H_9O_4\overset{O}{\overset{\|}{C}}CH_2S)_2M + 2H^+ \qquad (3.2.5)$$

where M^{2+} is divalent metal ion. Of the three inorganic mercury species found in fresh water, only the Hg^{2+} ion should be deposited on the sulphydryl cotton columns. At pH 2 or lower, more than half of the inorganic mercury species appears as Hg^{2+}.

However, relatively high concentration of hydrogen ions would have a more competitive affinity for $-S^-$ sites than Hg^{2+} because -SH group is much weaker acid than the $(-S)_2Hg$ group. So in this pH range, the inorganic mercury passes straight through the columns. At pH 3 or higher, a small amount of inorganic mercury appears as Hg^{2+}, and the results of pH study showed a small amount of inorganic mercury deposited on the columns. At pH 5 or higher, the increased concentration of $Hg(OH)_2$ caused the losses of inorganic mercury species due to the adsorption of this species onto the foreign surface, particularly at the $\mu g\ l^{-1}$ or lower levels. A slight reduction in inorganic mercury breakthrough signals were found in the pH range 5 to 9.

According to the following reaction:

$$C_6H_9O_4\overset{O}{\overset{\|}{C}}CH_2SH + CH_3HgCl \Longleftrightarrow C_6H_9O_4\overset{O}{\overset{\|}{C}}CH_2SHgCH_3 + HCl \qquad (3.2.6)$$

$MeHg^+$ can be retained on the sulphydryl cotton. The main species in fresh water is likely to be CH_3HgOH, whereas in seawater it is CH_3HgCl. It was shown in Chapter 1, that methylmercury tends to ionise into $MeHg^+$ and X^- due to the considerable ionic character of the Hg-X bond, and the ionisation to $MeHg^+$ and X^- was pronounced, particularly in acidified medium. It can be inferred from the results shown in Figure 3.3 that methylmercury species are likely to be of the $MeHg^+$ form at low pH 2-4 and readily deposit on the sulphydryl cotton columns. Between pH 4 and 9, significant decreases in methylmercury elution signals were also observed, which suggests that some of the methylmercury occurred as MeHgOH, and may not retained on the sulphydryl cotton column. Further experiment may be suggested to determine the effluent from the sulphydryl cotton column after the immediate injection of methylmercuric chloride solution in high pH matrix. This would confirm breakthrough of methylmercuric hydroxide (MeHgOH) from the sulphydryl cotton microcolumn.

Further work was therefore performed with samples and standards solutions at pH 3.

During the course of this work it was evident that from pH 3 to 4, the affinity of sulphydryl cotton for inorganic mercury varied from batch to batch. It can be inferred from the equation (3.2.5) that if inorganic mercury ions are adsorbed by sulphydryl cotton, one inorganic mercury ion would "occupy" two active sites ($-S^-$) on the absorbent. According to data of Wang [157], for the preparation of sulphydryl cotton, the experimental conditions, particularly reagents quality, strongly affect the distribution of sulphydryl groups and exchange capacity of this absorbent. Thereafter,the stereo structure and distribution of sulphydryl groups would clearly affect the deposition of divalent metal ions. Hence in order to minimise errors which would result from these change the same microcolumn was used for both standards and samples.

b) Effect of Microcolumn Length

In order to test the efficiency of deposition/elution, and optimise microcolumn performance for separation and preconcentration, various lengths of microcolumn were tested. Methylmercuric chloride standard solution, 1.0 μg Hgl^{-1} (pH 3), and an injection volume of 0.5 ml for both samples and eluent was used. The flow rates of these three streams were 1.5 ml min^{-1}. The flow rates of aeration argon and the sheath argon were both 2 l min^{-1}. In order to evaluate the behaviour of microcolumns and ensure consistent performance between columns, various column lengths were used. First a 60 mm microcolumn was used, then 10 mm was removed at the outlet end giving a microcolumn of 50 mm for use in the next experiment, and so on. The inlet end of the microcolumn was always retained and set up in the same stream direction. The results show that the absorption of methylmercuric ions with the sulphydryl group proceeds very quickly, with more than 93% of $MeHg^+$ ions retained on the first

centimetre of the microcolumn. Figure 3.5 illustrates the effect of microcolumn length on fluorescence response of methylmercuric chloride along a microcolumn. With a 50 mm microcolumn, the highest efficiency deposition and elution for methylmercuric chloride was obtained. In this work, the recommended microcolumn length was 60 mm (packing length, 50 mm).

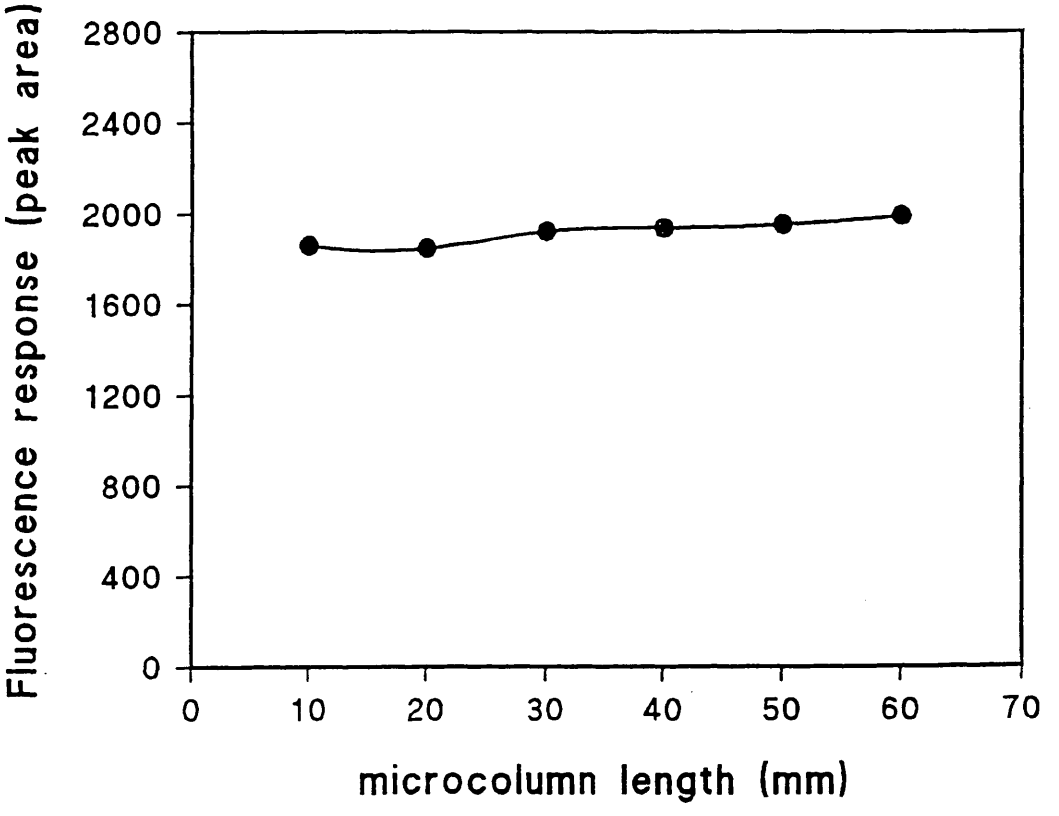


Figure 3.5 Effect of microcolumn length on analytical responses from methylmercuric chloride solutions (0.5 ml, 2.0 μg Hgl^{-1}) .

c) Effect of Tin Chloride Concentration

Tin chloride solution was used as the reducing agent for conversion of inorganic mercury to the elemental form. The reagent concentration undoubtedly affected the rate of reduction and conversion efficiency. Additionally, tin chloride served another purpose: the removal of free bromine which interferes with mercury atomic fluorescence detection [137]. Hydroxylamine is widely used for the removal of excess bromine in batch operating methods of mercury determination. Differing concentrations of tin chloride solutions in the same hydrochloric acid medium (15% HCl v/v) were prepared and purified by blowing pure argon into the freshly-made solutions for half hour before use. The effect of varing the concentration of tin chloride solutions on the responses of methylmercuric chloride (1.0 $\mu g\ l^{-1}$, pH 3) is shown in Figure 3.6 using the system described in section 2.1: the flow rate of carrier (0.01 M HCl) and oxidant (KBr 0.5% m/V + $KBrO_3$ 0.14%) streams were 1.5 ml min^{-1}. The different concentrations of tin chloride solution (from 1-10% m/V) were used at the flow rate of 1.5 ml min^{-1}. When the standard solutions of methylmercuric chloride (0.5 ml, 2.0 ug Hgl^{-1}) were injected into the system, methylmercuric ions were retained on the sulphydryl cotton microcolumn and then eluted with hydrochloric acid solution (3.0 M). The fluorescence responses were recorded. The Figure 3.6 provides an evaluation of the performance of the system using different concentrations of tin chloride solutions. Maximum sensitivity was obtained by using a $SnCl_2$ concentration of 5% w/V, in 15% HCl V/V, at a flow rate of 1.5 ml min^{-1}. The concentration of 5 % (m/V) was subsequently adopted for further investigations.

d) Effect of Eluent Volume

The effect of eluent volume on methylmercury elution signals was investigated. Various volumes of eluent (3 M HCl), from 0.25 ml to 1.0 ml, were tested using 0.25,

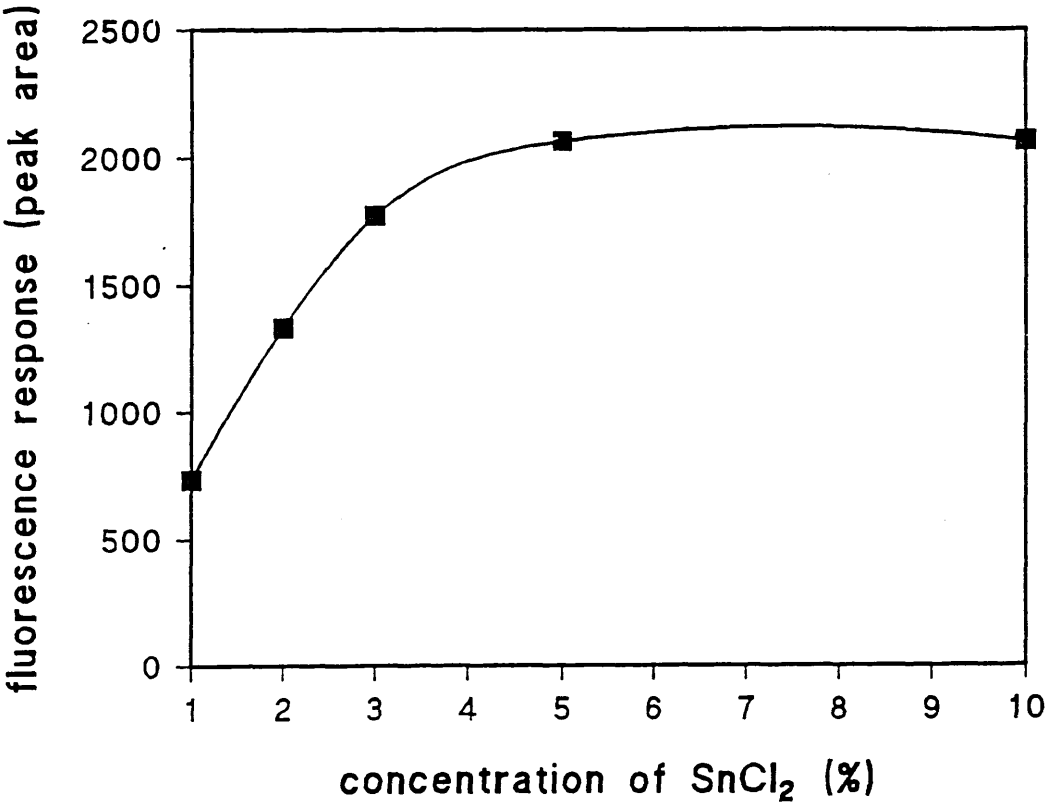


Figure 3.6 Effect of concentration of tin chloride solution on analytical response from methylmercuric chloride solutions (0.5 ml, 2.0 μg Hgl^{-1}).

0.50 and 1.0 ml sampling loops. To avoid contamination due to the changes of sampling loops and ensure the same volumes of methylmercury standard solution (2.0 μg l^{-1}) were employed in deposition, a fixed volume of sample (0.50 ml) was studied by pumping the standard solution into the flow injection system at the flow rate of 1.0 ml min^{-1} for exactly 30 seconds before the elution sequence. The results shown in Table 3.1 indicate that the smaller volume of eluent (0.25 ml) yielded a signal strength of about 93% compared with the results using larger volumes (0.5 and 1.0 ml). Repeat injections (typically 3 times) were required to remove residual analyte before sampling again. A large volume of eluent (1.0 ml) gave maximum elution efficiency, and no residual signals were observed in the subsequent elutions. It was found however, that 1.0 ml of eluent produced elution signals with significant tailing.

A 0.5 ml eluent volume was therefore selected for subsequent work.

Table 3.1 Effect of Eluent Volume on Fluorescence Responses

Volume of eluent (ml)	Methylmercuric chloride concentration ($\mu g\ Hgl^{-1}$)		
	nominal value	found value	
		1st elution	2nd elution
0.25	2.0	1.86	0.14
0.50	2.0	2.01	< 0.006
1.0	2.0	2.01	< 0.006

e) Effect of Mixing Coil Length

In Chapter 1 it was explained that, in current official methods employing batch operation, organomercury is oxidised to inorganic mercury by adding bromide/bromate solution to the acidified water samples and leaving the reaction mixture for at least one hour.

In this work, an on-line oxidation was attempted: a mixing coil of 1 meter being used to facilitate the reaction for conversion of methylmercuric chloride into the inorganic form. It was suspected that oxidation efficiency might be enhanced by utilising a mixing coil of extended length. Therefore mixing coils of various length were evaluated using a mixed standard solution of 2.0 $\mu g\ l^{-1}$ mercury nitrate (as Hg II) and 2.0 $\mu g\ l^{-1}$ of methylmercuric chloride (as Hg II). The results given in Figure 3.7

indicate that a minimum length of 3 metres is desirable for optimum performance. The maximum signal strength was obtained with lengths over three metres. The longer mixing coils gave more opportunity for the oxidation reaction to proceed. It also shows that the variation of mixing coil length does not affect peak shape if the volume of eluent was fixed. A volume of 0.5 ml is used. It has also been noted that mixing coil length did not affect the inorganic mercury breakthrough signals. In this work, the mixing coil selected was 4 metres (I.D. 0.8 mm) in length, which gave a residence time of 1 minute. This result also implies that the standing time for oxidation in the conventional method can be reduced.

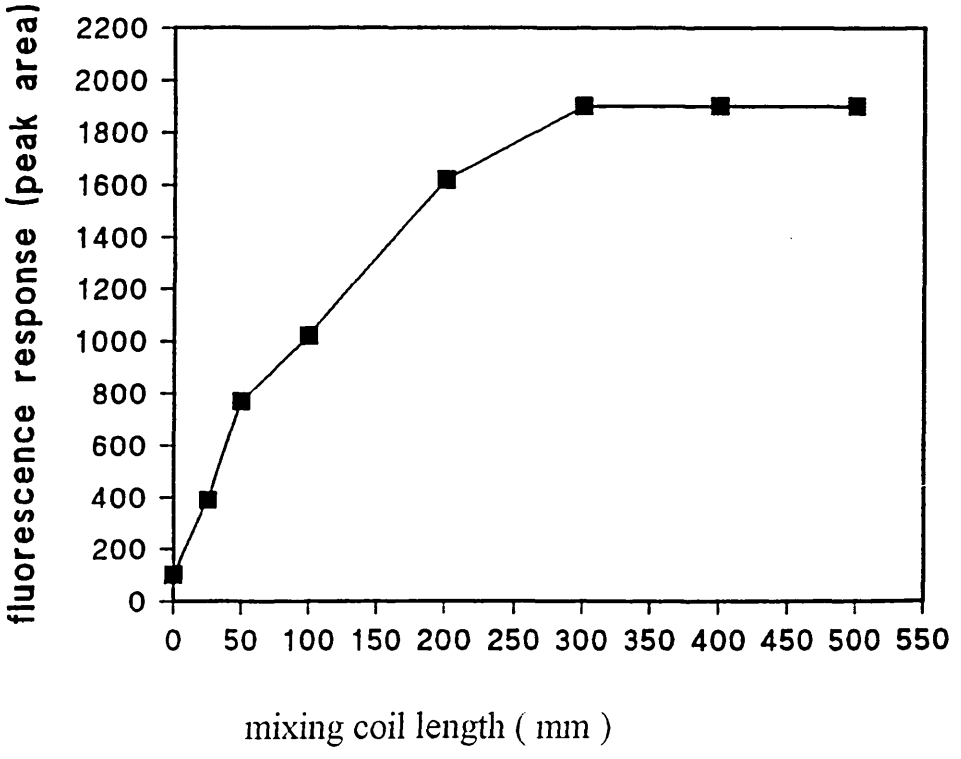


Figure 3.7 Effect of mixing coil length on analytical response from methylmercuric chloride solutions (0.5 ml, 2.0 μg Hgl^{-1}).

f) Effect of Concentration of Chloride on Fluorescence Responses of Both Methylmercuric Chloride and Inorganic Mercury

The concentration of chloride in standards and in water samples will change the behaviour of inorganic and methylmercuric chloride in solution, so as to possibly alter uptake behaviour on the microcolumn. Therefore, the effect of various concentration of chloride (as the sodium salt) on overall efficiency of deposition/elution for both inorganic and methylmercury species has been investigated. Separate solutions of mercuric nitrate (2.0 $\mu g\ l^{-1}$, as Hg II) and methylmercuric chloride (2.0 $\mu g\ l^{-1}$, as Hg II) were prepared and spiked with different volumes of a sodium chloride solution (Analar, BDH). The concentration range of chloride was from 200 to 5000 mg l^{-1}, which are higher than typical levels of chloride in river waters (10 - 50 mg l^{-1}) and lower than in sea waters (15000 - 19000 mg l^{-1}). The results shown in Figure 3.8 indicate that at pH 3 and a chloride concentration in the range 200 to 8000 mg l^{-1}, fluorescence signals were fairly constant except at very high chloride ion concentrations. But when chloride ion content was higher than 8000 mg l^{-1} the fluorescence responses declined. In order to evaluate whether the chloride content affected either the deposition or the elution processes or the fluorescence detection, the same FI-AFS system (but without microcolumn) was used, and a set of inorganic mercury standard solutions (2.0 $\mu g\ l^{-1}$, as Hg II), each containing a different chloride content, were prepared and tested. The results presented in Figure 3.9 show that when 0.50 ml aliquots of the above standards solution were injected into the system, inorganic mercury values tended to decline as they did when using the microcolumn. This indicated that the presence of chloride ions in mercury solutions would affect mercury fluorescence responses, particularly when the chloride content was high. Inorganic mercury (II) species as a function of chloride concentration is discussed in order to clarify the effect of chloride content on mercury fluorescence response. It has

been reported by different workers [158-160] that at a concentration of chloride of 3.5μg l^{-1} the species $HgCl^{+}$ is significant, and for chloride concentration from 0.035 to 350 mg l^{-1} $HgCl_2$ is the dominant species, and $HgCl_3^-$ peaks at a chloride ion concentration of 3550 mg l^{-1}. At the concentration of chloride ion in sea water (around 18000 mg l^{-1}) $HgCl_4^{2-}$ is probably the main species. By comparison of Figure 3.8 with Figure 3.9, it can be inferred that high concentrations of chloride ion did not affect the deposition/elution of both methylmercuric chloride and inorganic mercury on sulphydryl cotton column. The significant decrease in mercury fluorescence responses might be quite possibly due to the formation of mercury-chloro complexes ($HgCl_3^-$ and $HgCl_4^{2-}$), such mercury- chloro complexes, which have high coordination number and high stability [158,159], impede the reduction efficiency for conversion of inorganic mercury into elemental form. Hence it is proposed that mercury standards spiked with chloride ions are required when high content chloride-containing waters are analysed, eg. determination of mercury in brine and some sea water samples.

g) Interference Test

The effect of the presence of large number of other metal ions on methylmercuric chloride retention/elution behaviour and its fluorescence responses was examined. A standard solution of MeHgCl (2.0 μg l^{-1} as Hg), a tap water spiked with methylmercuric chloride standard solution (2.0 μg l^{-1} as Hg) and a methylmercuric chloride standard solution (2.0 μg l^{-1} as Hg) were spiked with a multi-element solution (Mg II, 20 mg l^{-1}; Fe III, 20 mg l^{-1}; Mn II, 4 mg l^{-1}; Pb II, 4 mg l^{-1}; Cu II, 4 mg l^{-1}; Zn II, 20 mg l^{-1}; Cd II, 4 mg l^{-1}; Al III, 20 mg l^{-1}; Cr III, 4 mg l^{-1} and As III, 4 mg l^{-1}). The three solutions adjusted to pH 3 were then injected, separately, into the microcolumn - flow injection - atomic fluorescence spectrometry system followed by injection of eluent (3 M HCl). The fluorescence intensities corresponding

to breakthrough and elution signals were recorded and the concentration for each solution was calculated. The results presented in Table 3.2 indicate that there was no significant variations between the measured values of methylmercuric chloride in these solutions.

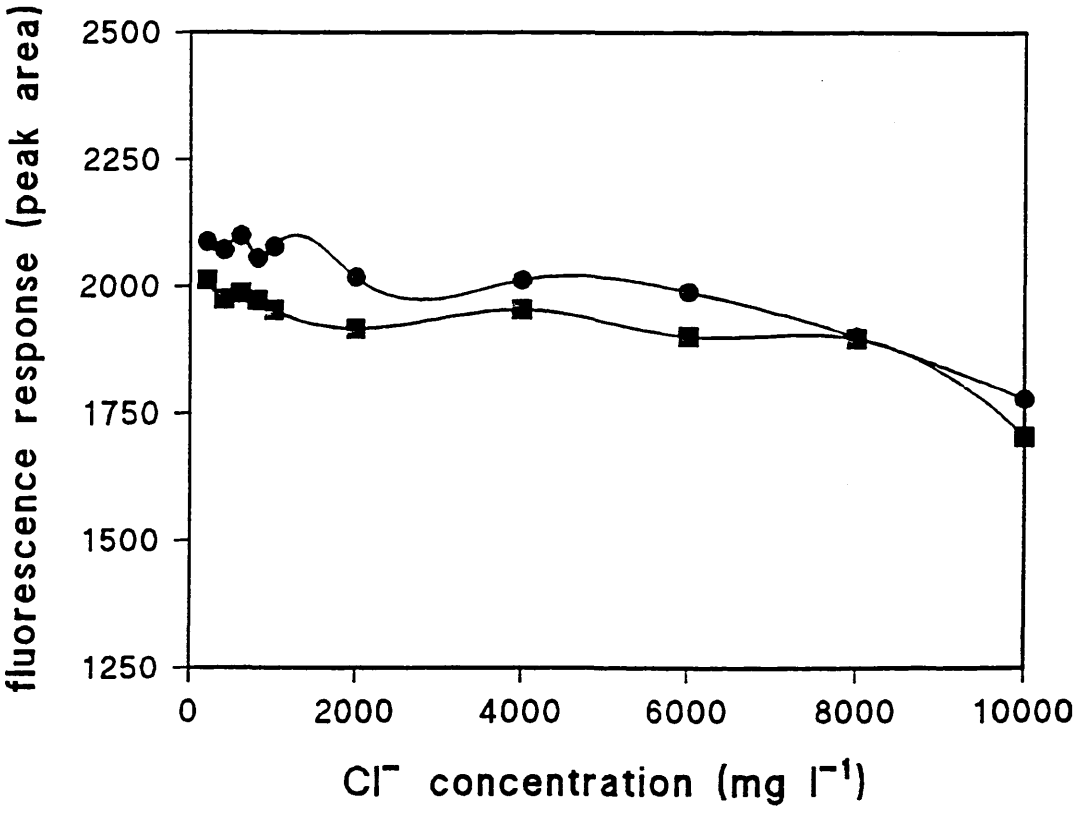


Figure 3.8 Effect of chloride concentration on fluorescence responses methylmercuric chloride solutions (0.5 ml, 2.0 μg Hgl^{-1}) and inorganic mercury solutions (0.5 ml, 2.0 μg Hgl^{-1}). With the use of microcolumn.

● inorganic mercury; ■ methylmercury.

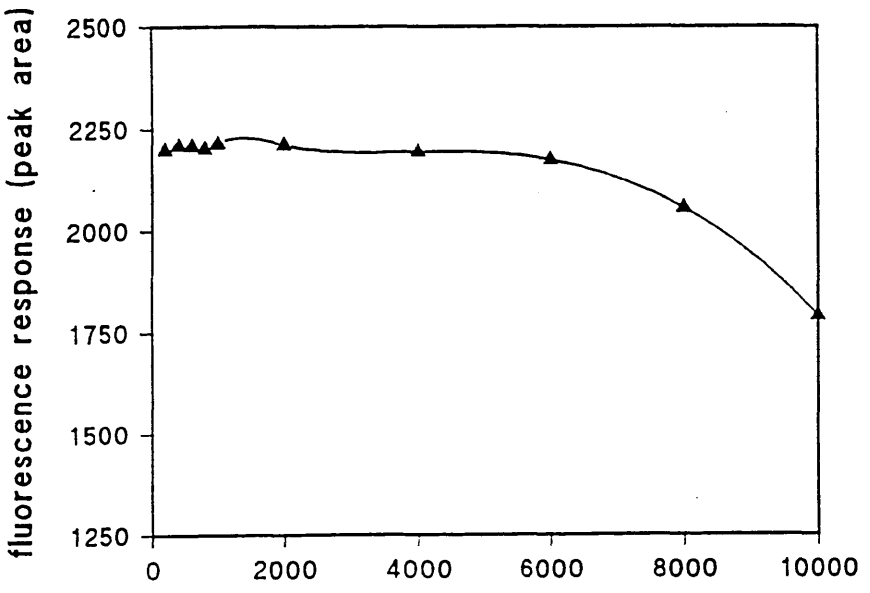


Figure 3.9 Effect of chloride concentration on fluorescence response of inorganic mercury (0.5 ml, 2.0 μg Hgl^{-1}), without use of microcolumn.

Table 3.2 Analysed values for methylmercuric chloride in standard solutions and spiked samples

	Concentration of methylmercuric chloride (μg Hgl^{-1})	
Standard solution*	2.08	2.17
Spiked tap water*	1.99	2.14
Standard solution-multielement spike*#	2.16	2.15

* Concentration of CH_3HgCl, 2.0 μg Hgl^{-1};

See section 3.2.2 (g) for composition.

i) Analytical Enrichment

One of the important features of the microcolumn technique is the on-line analyte preconcentration capability. The results obtained for the trace enrichment of methylmercuric chloride as a result of processing large sample volumes were very significant.

Dramatic improvements in signal intensity were obtained on increasing sample volumes from 1 ml up to 5 ml as shown in Figure 3.10. This procedure used 1.0 ml, 2.0 ml, 3.0 ml and 5.0 ml of a standard solution of methylmercuric chloride (0.2μg Hgl^{-1}), which was pumped through the microcolumn of sulphydryl cotton, at a flow rate of 1.5 ml min^{-1}, followed by elution (0.5 ml, 3 M HCl). Thus, analyte enrichment with nominal preconcentration factors from 2 to 10 were achieved if it was assumed that a 100% of recovery was obtained (see Figure 3.10). In order to estimate the exchange capacity of sulphydryl cotton columns, a standard solution of methylmercuric chloride (10 mg Hgl^{-1}, 0.5 ml) was injected onto a microcolumn with 0.015 g of sulphydryl cotton. No breakthrough signals were observed at flow rate of 2.0 ml min^{-1}, which gave the dynamic exchange capacity of sulphydryl cotton for methylmercuric chloride as 0.0002 mM/0.1 g. For the analysis of samples containing concentrations at the ng l^{-1} to μg l^{-1} level, this exchange capacity is deemed high enough for quantitative adsorption of methylmercuric chloride.

j) Calibration and Limit of Detection

A calibration curve for methylmercuric chloride was obtained by measuring a set of aqueous methylmercuric chloride solutions (10.0 ml of 50 - 200 ng l^{-1} at pH 3). The graph presented in Figure 3.11. is linear with a correlation coefficient of 0.9997. The calibration was also carried out using standard solutions with a concentration range of 1.0 - 10.0μg l^{-1} (correlation coefficient, 0.9996). The limit of detection for methyl-

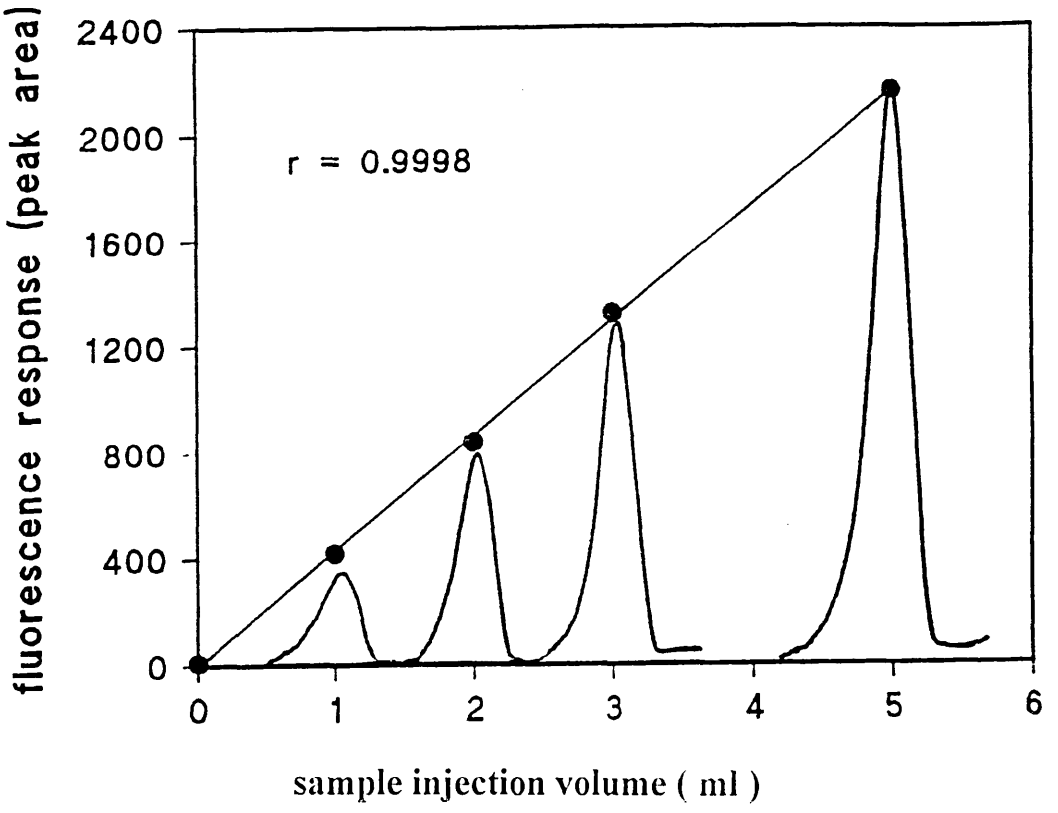


Figure 3.10 Effect of sample injection volume on analytical response. Methylmercuric chloride (0.2 μg Hgl^{-1}).

mercuric chloride was calculated as 6 ng Hgl^{-1} of methylmercuric chloride from 10 replicate measurements of the method blank (hydrochloric acid, 3 M). For inorganic mercury ($HgNO_3$) the limit of detection was calculated as 1 ng Hgl^{-1} from 10 replicate measurements of the method blank (Millipore water). It was also found that commercially available hydrochloric acid (Aristar, BDH) gave a major contribution to the blank signal, and was responsible for the relatively high limit of detection of methylmercuric chloride. Lower limits of detection can be achieved by repurifying the hydrochloric acid. Blank variation due to batch to batch difference in hydrochloric acid quality were also found to control detection capability during the course of analysis.

3.2.3 Determination and Speciation of Mercury in Water Samples

The FI-AFS method was used for the analysis of a tap water collected in the laboratory. An acidified tap water (4.5 ml, pH = 3.5) was pumped into the system for 3 minutes at the flow rate of 1.5 ml min^{-1}. Inorganic mercury was unretained and monitored by the fluorescence detector. Subsequent injection of 3.0 M hydrochloric acid eluted the retained methylmercuric ions for further reaction and determination. The values found for methylmercuric chloride were 46 ng Hgl^{-1} and for inorganic mercury 69 ng Hgl^{-1}. These results were consistent with the estimated values of mercury in fresh water (150 - 700 ng l^{-1}, see Chapter 1). The percentage of methylmercury in this tap water is however relatively high (40% of total mercury) and thus merits further investigation.

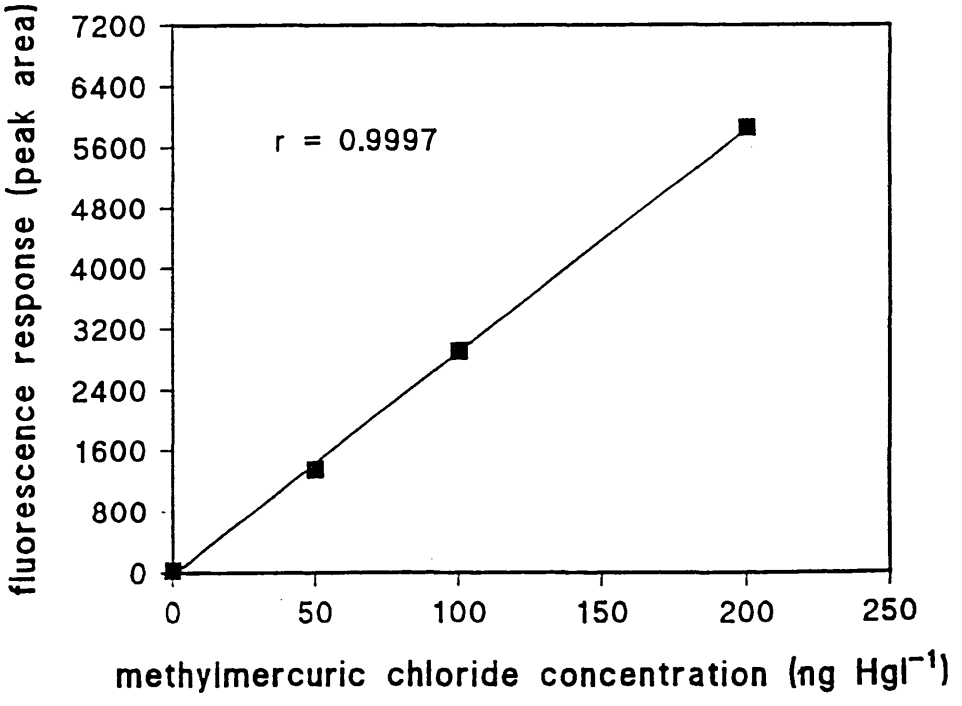


Figure 3.11 Calibration curve for methylmercury measured with concentration range from 50 to 200 ng Hgl^{-1}.(Sampling volume: 10ml)

Table 3.3 Analytical values for methylmercuric chloride and total mercury in waters (filtered)

Results of replicate samples	Concentration μg Hgl^{-1}			
	tap water	clean river water	clean river water	polluted river water
	0.013	0.005	0.010	0.017
	0.013	0.008	0.012	0.017
	0.014	0.006	0.013	0.016
	0.012	0.006	0.011	0.018
	0.013	0.006	0.012	0.017
mean	0.013	0.006	0.012	0.017
s.d	0.0007	0.0012	0.0011	0.0007
RSD%	5	20	9	4

results of spiked water samples

amount added ($\mu g\ l^{-1}$)	0.020	0.020	0.010	0.010
amount found ($\mu g\ l^{-1}$)	0.018	0.025	0.011	0.010
recovery (%)	90	125	110	100
total mercury ($\mu g\ l^{-1}$)	0.053	0.040	0.081	0.412

Some river waters were analysed and analytical precision was also evaluated by replicate analysis. Sample volumes of 2 ml for calibration standards and 20 ml for water samples and spiked water samples were employed. The results shown in Table 3.3 indicate that the precision of the replicate sample analysis was acceptable for ultra-trace analyte (4 - 20%, RSD), and that for high concentrations was more favourable than for low concentrations.

3.2.4 Deposition/Elution Characteristics of Phenylmercuric Acetate

Microcolumns of sulphydryl cotton have been successfully used for separation and preconcentration of methylmercuric chloride from inorganic mercury and the matrix. In order to extend the application of this technique and clarify the performance of sulphydryl cotton microcolumns for other organo- mercury species, ethyl- and phenyl-mercury should be investigated. In this work, only phenylmercuric acetate has been tested since ethylmercury was not readily available. The results indicated that sulphydryl cotton columns have a high affinity for phenylmercuric ions, with a good performance for deposition and elution, which means not only methylmercury but other organomercury species, eg. phenylmercury, possiblly ethylmercury can also be separated and preconcentrated by sulphydryl cotton microcolumn.

The experimental procedures used for preconcentration and determination of phenylmercuric acetate were the same as those for methylmercuric chloride. The same system has been employed with all optimised parameters.

The results, shown in Table 3.4, indicate that sulphydryl cotton microcolumns give a high affinity for phenylmercuric ions and very satisfactory performance for deposition/elution. Under the recommended conditions, the values of phenylmercuric acetate using the microcolumn technique were about 99% of the values obtained using a batch method. This shows that efficiency of the on-line bromination procedure was

high enough to completely convert phenyl-mercuric ions into the inorganic mercury.

The calibration was carried out using phenyl-mercuric acetate standard solutions (concentration range 0.00 - 10.0 $\mu g\ l^{-1}$, as Hg II). The results in Figure 3.12 show that very good linearity for fluorescence response versus concentration. As discussed one of important features of the microcolumn technique is its ability for on-line analyte preconcentration. The trace enrichment of phenylmercuric acetate using large sample volumes is critical to the success of this technique, which was based on the principle that fluorescence response is proportional to the sample volume. The results given in Figure 3.13 show dramatic improvement in signal intensities in going from sample volumes of 0.5 ml to 5.0 ml. It also shows that sulphydryl cotton microcolumns are capable of preconcentrating phenylmercuric ions from aqueous solutions under certain conditions. Additionally it can be concluded, since ethylmercury has similar structural and chemical properties to those of methylmercury, that sulphydryl cotton microcolumns can also be used for separation and preconcentration for ethylmercury. As a result of the studies the mercury fraction determined by the microcolumn technique should be referred to as organomercury and not methylmercury.

3.3 Studies with Other Column Packings

3.3.1 Xanthate Cotton Microcolumns

Structurally, xanthate cotton fibre ($C_6H_9O_4O\overset{S}{\overset{\|}{C}}SNa$) has the same donor atom ($-S^-$) as sulphydryl cotton fibre, and may be expected to chemically bond with mercury species and many other heavy metal ions. Some workers have reported using xanthate cotton fibre or xanthate wood wool as an effective absorbent to recover heavy metal ions from industrial waste waters, especially for the collection of Cd (II) and Au (III) [161]. Some results indicated that the reversibility of deposition/elution for heavy metal ions was better than for sulphydryl cotton fibre.

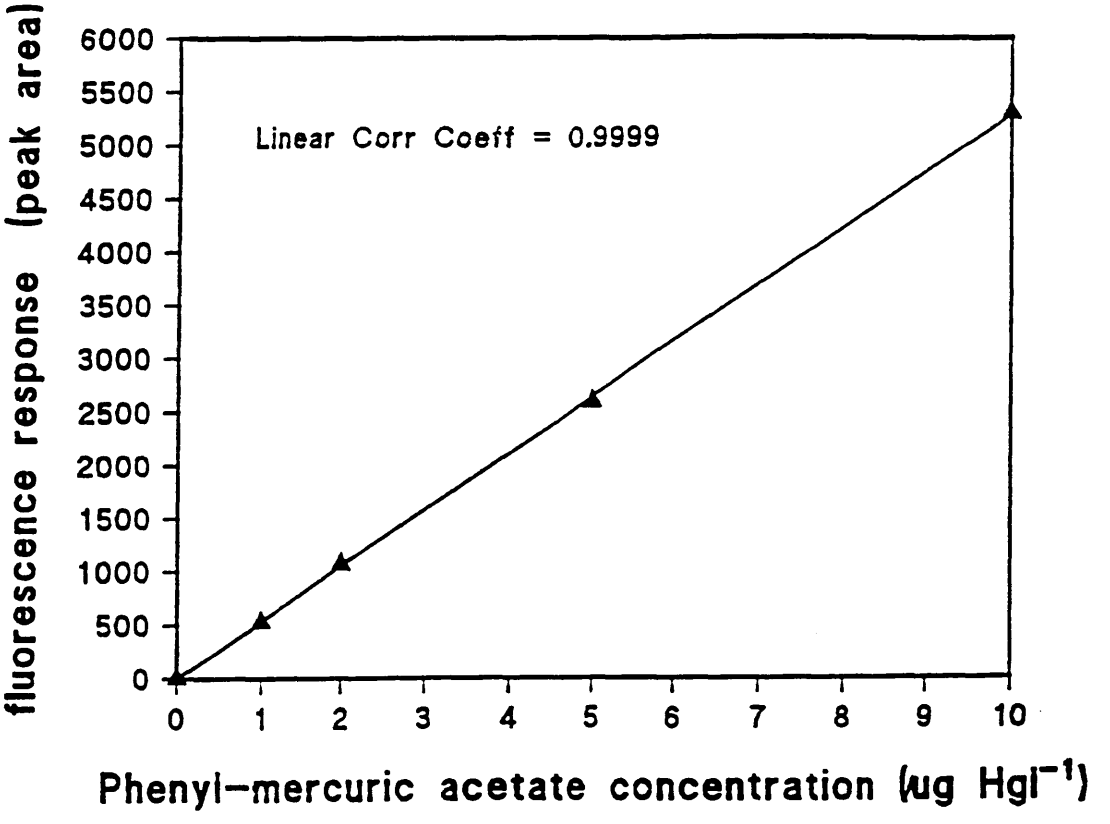


Figure 3.12 Calibration curve for phenylmercuric acetate using microcolumn-FI-AFS system. Concentration range from 1 to 10 μg Hgl^{-1}.

Table 3.4 Comparison of phenylmercury fluorescence responses using microcolumn technique and batch operation

(8 replicate measurements)

	microcolumn technique	batch method*
1	3939	3716
2	3684	3546
3	3715	3900
4	3657	3823
5	3705	3786
6	3688	3823
7	3856	3805
8	3827	3972
mean	3759	3796
s.d	101.6	126.7
RSD%	2.7	3.3

phenylmercuric acetate standard solution, 2.0 μg Hgl^{-1};

* phenylmercuric acetate + Br^-/BrO_3^- + HCl.

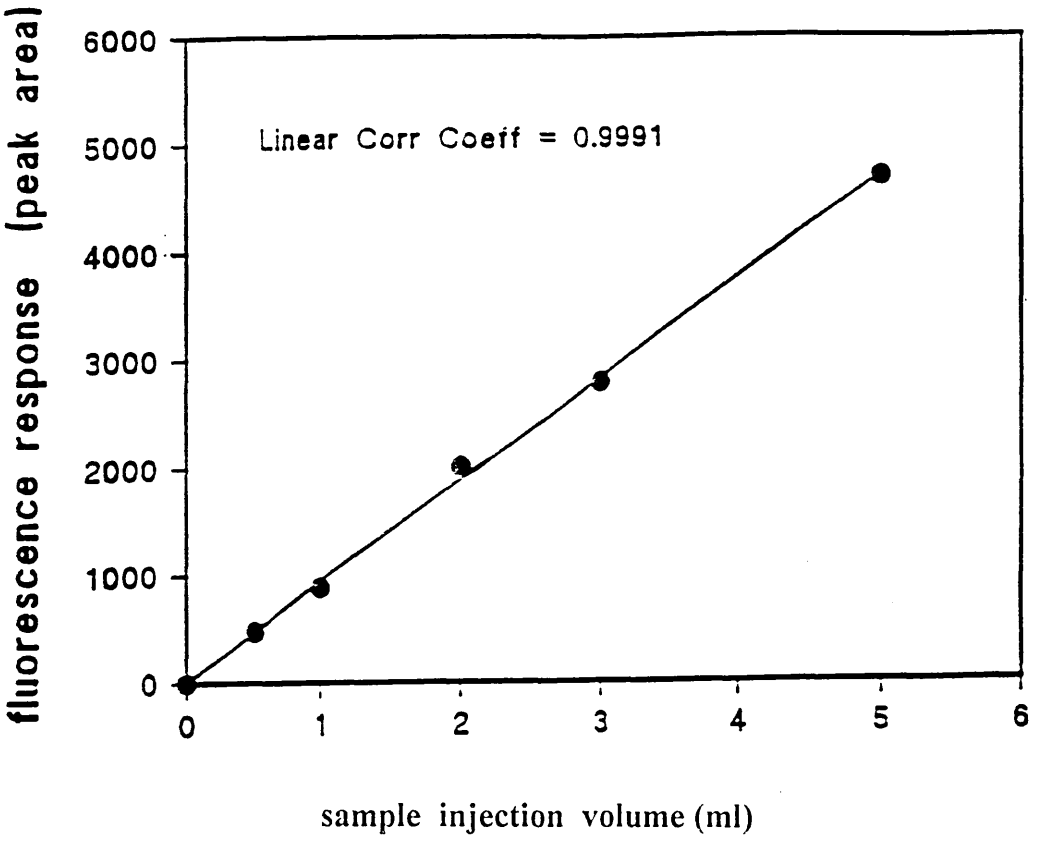


Figure 3.13 Correlation curve between fluorescence signals and sample volume (phenylmercuric acetate, 0.5 μg Hgl^{-1}).

Preliminary studies have been undertaken using xanthate cotton microcolumns for mercury speciation. The results indicate that methylmercury was retained on the column, and most of inorganic mercury was not retained when the experimental conditions were the same as those used for sulphydryl cotton microcolumns. A problem noted however was that this xanthate cotton exhibited severe memory effect for the elution of methylmercury. Small elution signals of inorganic mercury were also observed. Typical responses for $MeHg^{+}$ (2.0 μg l^{-1}, as Hg II) and inorganic mercury (2.0 μg l^{-1}, as Hg II) are given in Figure 3.14a and b. It is evident that significant residual signals occur for both inorganic mercury and methylmercuric chloride. The exchange reaction for methylmercuric chloride is given by equation 3.3.1:

$$C_6H_9O_4O\overset{S}{\overset{\|}{C}}SNa + CH_3Hg^+ \Longleftrightarrow C_6H_9O_4O\overset{S}{\overset{\|}{C}}SHgCH_3 + Na^+ \qquad (3.3.1)$$

In comparison with the product which obtained using sulphydryl cotton, $C_6H_9O_4O\overset{O}{\overset{\|}{C}}CH_2SHgCH_3$, it is clear that creation of the conjugate ($C_6H_9O_4\,O\overset{S}{\overset{\|}{C}}-S^{\ominus}$) makes the donor sulphur atom more negative than that in sulphydryl cotton product, to give a stronger S-Hg bond making elution more difficult. Under present conditions, xanthate cotton appears unsuitable for separation of different mercury species.

3.3.2 Activated Alumina Microcolumns

Activated alumina can be considered as an amphoteric ion exchanger, and this can be represented by the dissociation equilibria show below:

$$Al-O-H \overset{+H^+}{\Longleftrightarrow} Al\cdots O-H\cdots H^+ \qquad (3.3.2)$$

$$Al-O-H \overset{+OH^-}{\Longleftrightarrow} Al-O\cdots H\cdots OH^- \qquad (3.3.3)$$

The amphoteric character and the ability to exhibit ion exchange characteristics has also been widely recognised in the past. It has other desirable properties. For example, it has good resistance towards strong oxidizing and reducing agents. It shows favourable ion exchange selectivity, and undergoes little swelling or shrinking in water or solutions containing electrolyte and organic modifier.

It's particular properties can be easily modified by washing with either acid or base solutions. When neutral alumina is washed with a sodium hydroxide solution, the

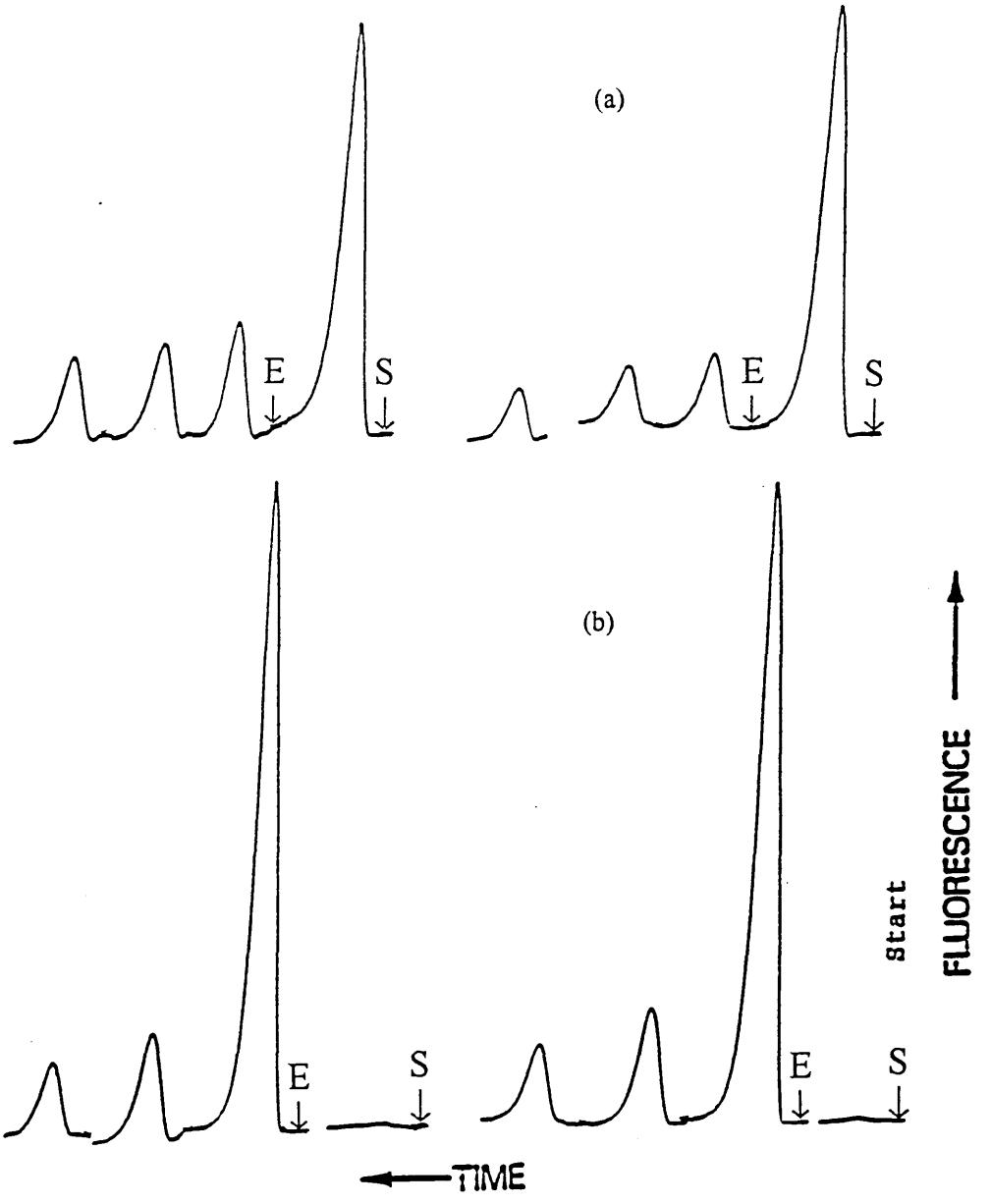


Figure 3.14 Typical responses of methylmercury and inorganic mercury from xanthate cotton column

(a) breakthrough and elution signals of inorganic mercury ($2.0\ \mu g\ Hgl^{-1}$ of standard and 3 M HCl of eluent);

(b) breakthrough and elution signals of methylmercury ($2.0\ \mu g\ Hgl^{-1}$ of standard and 3 M HCl as eluent)

chemisorbed protons will be neutralised and replaced by more loosely bound sodium ions which can then exchange with other cations. If alkaline alumina is washed with hydrochloric acid, the proton of the acid produces two effects. The desorption of hydroxyl groups, which are then replaced by chloride ions, gives rise to changes in the anion exchange properties of acidic alumina. The protons also replace the sodium cations attached to oxygen atoms. These properties are demonstrated in Figure 3.15.

Due to the amphoteric property of activated alumina dilute aqueous alkali (0.02 M NH_4OH recommended) can convert alumina into the alkaline form which has cation exchange properties, and dilute acid solution (0.02 M HCl recommended) converts it into the acidic form which has anion exchange properties. The function required for the retention of inorganic mercury ion is obviously that the activated alumina must be performing as a cation exchanger. In the on-line system of the present work, this is controlled by pumping an alkaline solution (0.02 M NH_4OH) through the activated alumina microcolumn. While activated alumina is maintained in its basic form it displays a high affinity for cationic species and it's microcolumn can be used for retention of inorganic mercury. Because basic alumina columns require alkaline and acidic solutions as carrier and eluent, in the FI-AFS system, the neutralisation process which occurs when the alkaline and acidic streams are merged produces air bubbles which severely interfere with the mercury fluorescence.

Inductively coupled plasma - atomic emission spectrometry was therefore used as detector. The microcolumn-FI-ICP-AES system is shown in Figure 2.2. Conversion of inorganic mercury into elemental form using tin chloride solution was not required using this combined technique.

Because the adsorption properties of activated alumina depend significantly on pH, the effect of sample pH was tested. The results showed that basic alumina has a relatively high affinity for inorganic mercury over a wide range of solution pH

(pH 2 -11), in contrast to acidic alumina columns. This suggests that for the solutions examined inorganic mercuric ions exist in cationic form (Hg^{2+}, $HgCl^{+}$, $HgOH^{+}$ or hydrate mercury dihydroxide $HgOH_2$ nH_2O), and only a small amount of mercury exists in anion form ($[HgCl_n^{-(n-2)}$ or $[HgOH_n^{-(n-2)}]$, $n > 2$). The results shown in Figure 3.16 and Figure 3.17 indicate that basic alumina has a high affinity for inorganic mercury, and it's affinity rises with the increase of sample pH. This is not surprising since H^{+} will compete for active sites of alumina in low pH media. On the contrary, acidic alumina had a very poor affinity for inorganic mercury, with very small elution signals being observed. It had been assumed before the experiments were carried out that addition of more chloride ion into sample solution would convert free inorganic

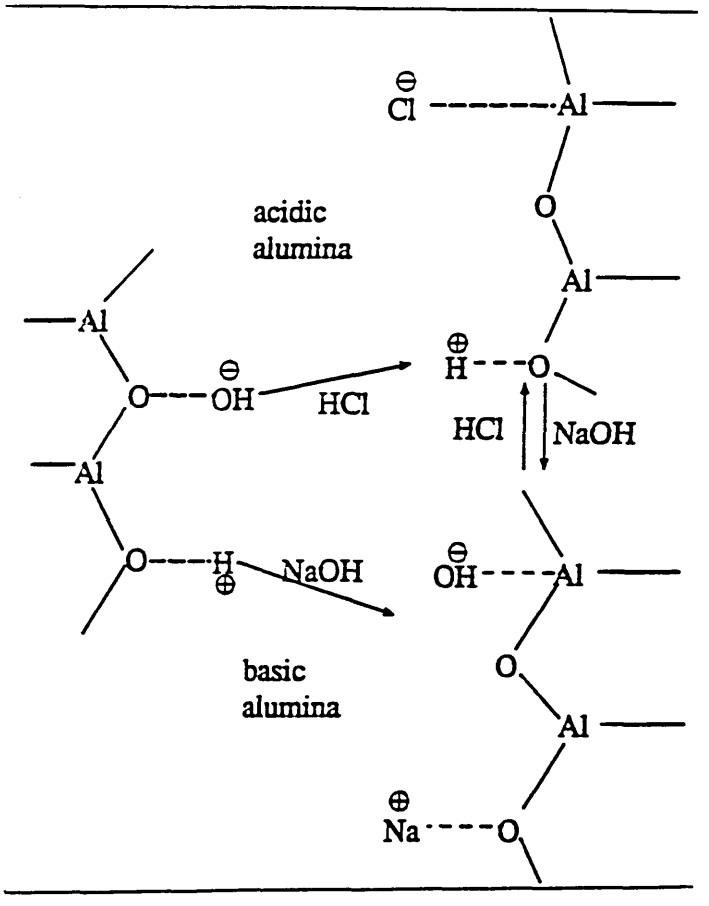


Figure 3.15 Surface behaviour of alumina in basic and acidic media.

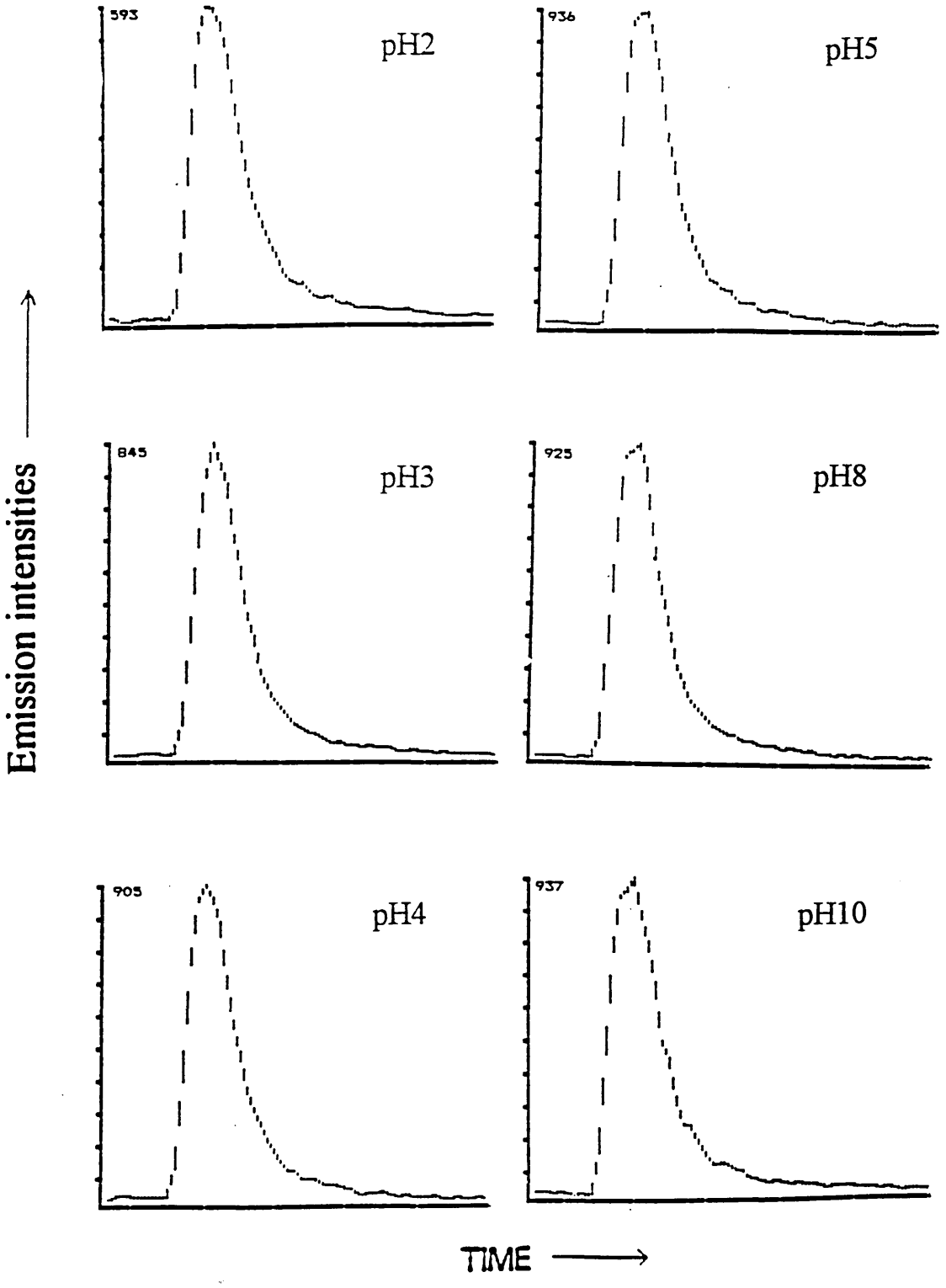


Figure 3.16 Effect of sample pH on Emission - time response for inorganic mercury: Sample: 0.5 ml, mercuric nitrate 2.0 mg Hgl^{-1}; basic alumina column.

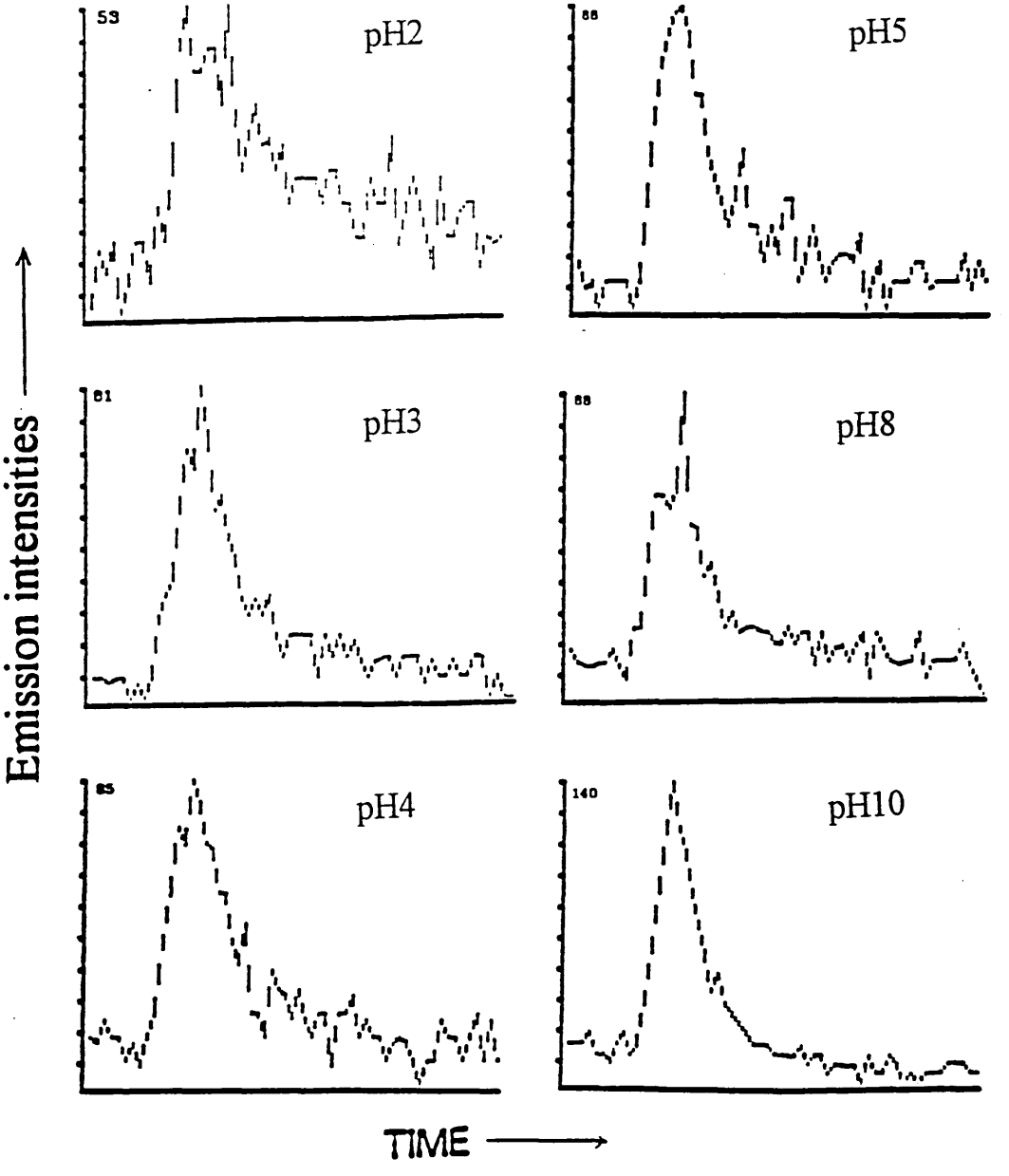


Figure 3.17 Effect of sample pH on Emission - time response doe inorganic mercury: Sample: 0.5 ml, mercuric nitrate 2.0 mg Hgl^{-1}; acidic alumina column.

mercuric ions into mercuric chloride complex ions negatively charged which would help inorganic mercury retention on acidic alumina columns. Unfortunately, extra addition of Cl^- caused total breakthrough of inorganic mercury without any retention.

It is concluded that basic alumina is effective for separation and enrichment of inorganic mercuric ions. But the microcolumn can not be used and combined with CV - AFS in this study, since the mixing of alkaline carrier with acidified tin chloride produces air bubbles which interfere with fluorescence detection.

3.3.3 8-Hydroxyquinoline Microcolumns

The chemical structure of 8-hydroxyquinoline (Oxine) indicates that it is an almost universal complexing agent (see Figure 3.18). It reacts with many metal ions to give water-insoluble precipitates. The complexes can be decomposed with strong acids and the oxine liberated in an amount equivalent to the metal ions may be determined by many analytical methods [162].

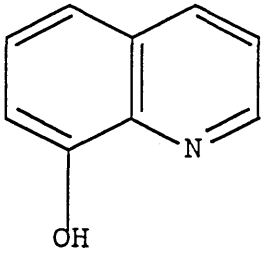


Figure 3.18 Structure of 8-hydroxyquinoline

Oxine (HA) gives chelated complexes of composition MA_2 with a series of divalent metal ions, MA_3 with trivalent metal ions, and MA_4 with thorium and zirconium; all are insoluble in water. Although oxine reacts with many metal ions, it's

reactions can be made selective in many case by appropriate choice of the reaction conditions. Normally, it is suitable for the selective separation and determination of some metal ions in the presence of phosphate, tartrate, oxalate, F^-, EDTA or malonate as masking ligands [162].

The oxine columns also require both alkaline (0.02 M NH_4OH) and acidic solutions (0.25 M HCl) as carrier and eluent, respectively. If atomic fluorescence spectrometry is used as a detector, neutralisation due to the mixing of alkaline and acidic solutions produces air bubbles which interfere with fluorescence detection. Thus like basic alumina microcolumns, oxine columns have been incorporated with FI-ICP-AES for investigation on mercury speciation. The FI-ICP-AES system is shown in Figure 2.2.

Firstly, the effects of sample pH on the intensity of signals from inorganic mercury were tested by using a series of mercuric nitrate standard solutions (pH 2-11; 1.0 mg Hgl^{-1}, used in preliminary work). Synthetic solutions of mercuric nitrate (250 µl) were injected into the carrier stream (flow rate, 1.5 ml min^{-1}). The same volumes of eluent were injected to decompose retained mercuric ions. The results show that elution intensities for the mercuric ion decreased significantly with an increase of sample pH. Another important aspect was that even small concentration of chloride ion in the sample causes severe break through and no retention of inorganic mercury on the microcolumn. These can be explained based on Burger's report [162] that the optimum pH range for complex formation is dependent upon the stabilities of the oxinate complexes. No stability data for mercuric oxinate complex was given by Burger, but the results in this work show that the stability of mercuric oxinate complex decreases with the increase of pH of mercuric nitrate solutions. It can be explained that at high pH, the relatively high tendency of formation of mercuric-hydroxide complex would decrease the stability of mercuric oxinate complex. The presence of chloride

ions in mercuric nitrate solution results in the formation of mercuric-chloro complexes,which might impede the formation of mercuric oxinate complexes. Additionally, the results of interference tests indicated that among 10 metal ions (Ca, Mg, Fe, Mn, Pb, Cu, Zn, Cd, Al and Cr), Fe and Cu severely interfered with inorganic mercury retention on the oxine column at sample pH 2. The use of F^- and EDTA as masking agents could still not eliminate or alleviate this interference. This is also consistent with Burger's report [162] that among these metals, only Fe (III) and Cu (II) can react with oxine and give stable oxinate complexes at sample pH 2. Since natural waters normally contain chloride ions and many other metal ions (such as Fe and Cu), oxine microcolumns are not suitable for the retention of trace mercury in natural waters. Typical responses of inorganic mercury retained on 8-hydroxyquinoline columns are given in Figure 3.19.

3.4 Conclusions

A novel and robust microcolumn technique coupled with flow injection - atomic fluorescence detection has been developed and applied to differentiate between inorganic and methylmercury. Prior to creation of the microcolumn - FI - AFS manifold for mercury speciation several microcolumn packing materials were prepared and their analytical characteristics studied. The results indicated that xanthate cotton fibre has high affinity for methylmercuric ions, similar to those of sulphydryl cotton fibre. However, significant memory effects were found, and the residual methylmercuric ions and inorganic mercuric ions could only be released after more than 5 injections with 3.0 M HCl. The high residual signals affected the reproducibility and prolonged operating times. An increase in eluent concentration did not raise elution efficiency. Even so, xanthate cotton fibre is proposed as having analytical potential for mercury speciation, if memory effects could be eliminated by using more powerful

eluents. Dilute acid solutions containing CNS^- may be the best candidates for this purpose. The stability constant of the mercury complex with this commonly used masking agent is less than that with sulphydryl chelating agents (Hg-SCN, $\log K_1$ 17.26; and Hg-S, $\log K_1$ 45.4) [163,164], but relatively high concentration of SCN^- may shift the equilibrium towards the formation of Hg-SCN [165]. 8-hydroxyquinoline (Oxine), a popular chelating agent, has been tested. The results indicated that inorganic mercuric ions could be retained on the oxine column and eluted quantitatively with 0.25 M HNO_3. But interference from trace amounts of Cl^- and mg l^{-1} levels of Fe (III) and Cu (II) prevented the total retention of inorganic mercury ions due to the formation of non-retained mercuric-chloro complex ions and competitive adsorption by Fe (III) and Cu (II) ions. The common masking agents, EDTA and F^-, did not eliminate the interference from Fe (III) and Cu (II). The fact that chloride is always found and iron and copper sometimes occur in natural waters, implies that oxine is not suitable for this work.

Both basic and acidic alumina columns have been used effectively for on line preconcentration/separation, as discussed earlier. In the present work, the results show that only basic alumina has a high affinity for inorganic mercury ions over a wide range of solution pH (from pH 2 to 10), this is because only basic alumina can function as a cation exchanger for the adsorption of inorganic mercury ions. The results also show the the basic alumina microcolumns facilites the quantitative deposition and elution for inorganic mercury ions. However the basic alumina microcolumn requires alkaline solutions as carrier (0.02 M of NH_4OH was used in this work), and acidified tin chloride solution is nomally used for the conversion of inorganic mercury ions into elemental form for cold vapour - atomic fluorescence detection. The resultant neutralisation, due to the merging of alkaline and acidic streams, can produce air bubbles and severely interfere with fluorescence measurements. So the basic alumina

microcolumn is not suitable to be incorporated with cold vapour - atomic fluorescence spectrometer. In this work, it was successfully used for separation and preconcentration of inorganic mercury ions in synthetic solutions and determination by flow injection - inductively coupled plasma - atomic emission spectrometry system.

Sulphydryl cotton microcolumns with cold vapour - flow injection - atomic fluorescence spectrometry has proved an excellent tool for the determination of trace levels of different mercury species. The determination of very low levels of inorganic mercury in natural waters can be achieved by direct detection. Methylmercury species (normally 2 orders of magnitude less concentrated than inorganic mercury) can be separated, preconcentrated and determined using the same experimental set-up. As a new approach towards mercury speciation, the microcolumn technique was proposed to perform microcolumn sample processing in the field is discussed in Chapter 4.

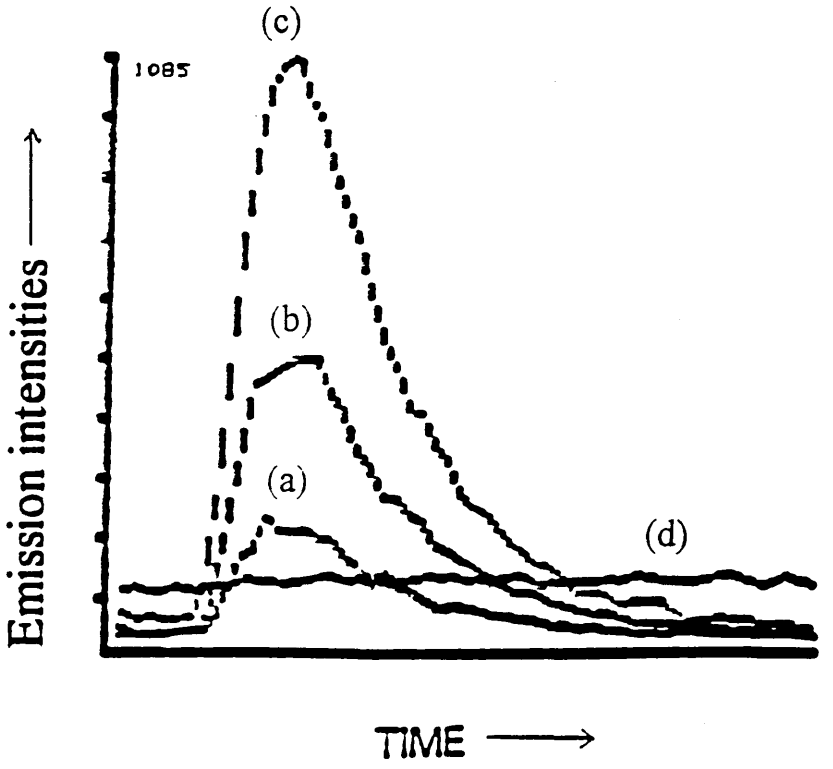


Figure 3.19 Emission intensities of inorganic mercury against concentration (20, 50, 100 μg Hgl^{-1}) by oxine microcolumn FI-ICP-AES system: (a) 20 μg Hgl^{-1} inorganic mercury; (b) 50 μg Hgl^{-1} inorganic mercury; (c) 100 μg Hgl^{-1} inorganic mercury; (d) 2000 μg Hgl^{-1} inorganic mercury by direct injection without preconcentration.

CHAPTER 4

DEVELOPMENT OF MICROCOLUMN - FIELD SAMPLING TECHNIQUE FOR MERCURY SPECIATION

4.1 Field Sampling

It is also well known that reliable trace element speciation data depend not only on the use of sensitive and accurate analytical methods, but also on the availability of reliable sampling and storage techniques which ensure the trace amount and state of trace element have not altered in the time between sampling and laboratory measurement. Sampling is one of the first difficulties encountered in the speciation studies for trace metals, particularly for mercury.

With reference to mercury in natural waters, reliable speciation data are scarce. Two main reasons are the extremely low concentration of individual species (ng l^{-1} level) and the acute problem with sampling and storage which aim to ensure the natural speciation state is maintained until analysis is performed. Many studies have indicated that the true concentration of different mercury species in natural waters are very low, necessitating substantial preservation and preconcentration before analysis.

As is often practised in official monitoring programmes for mercury, speciation data are actually destroyed at the sampling site (addition of powerful oxidants to sample) and only total mercury data are reported [136,137,166]. Another problem, according to Cragin [167] and Jacgues and Fabrice [168], is that water samples can be contaminated by trace mercury species during sampling and subsequent storage. Too little attention is directed to these matters. Standard sampling and storage procedures take water samples in plastic or glass containers and then transfer the samples to another container for subsequent analysis. Studies on trace mercury speciation have shown that plastic or glass containers can both absorb trace mercury ions from the sample and/or contribute mercury to solution by surface dissolution [168], which cause contamination and tremendous changes in sample composition. The analyst can often only report results obtained on the particular test specimen at the moment of analysis, which may not provide the desired information.

In order to overcome the problems of sampling and storage on the integrity of the sample, the sample would best be maintained at the same dynamic equilibrium conditions from which it was taken. This is virtually impossible when water samples are collected and transported to the laboratory for subsequent analysis. Essentially, the only solution to the problems from sampling and storage is to make an **in situ** analysis without removing or perturbing the sample from its environment. **In situ** analysis has the further advantage of virtually instantaneous turnaround of results. Thus, if anything unusual is found at a particular location, further investigations and clarifications can be performed immediately and thoroughly. Various **in situ** assay methods have been developed by using specified sensor detection, such as pH, dissolved oxygen and pre-laid fluorescent tracers for specific purpose. Few **in situ** methods for the determination and speciation of trace metals have been attempted, because the most widely used methods generaly utilise sophisticated laboratory instrumentations. For example,

atomic absorption spectrometry, atomic emission and mass spectrometry, anodic stripping voltammetry and neutron activation analysis. Therefore, a system for complete **in situ** analysis of trace element presents many problems, especially expense, which make it difficult at present for the application to trace metal speciation. An alternative approach to overcoming these limitations and improving analytical results is the development of field sampling techniques to remove these sampling and human errors. Paulsen and co-workers [169] specified the criteria for developing field sampling techniques. They are summarised below:

1. The system must be sampled in a manner that renderes it physically and chemically inert;

2. The sample must be in a form to be analysed, preferably with one step with no chemical preservative and preparative steps involved;

3. The analysis method must be one that is potentially applicable for shipboard or field use in order to get rapid analytical results;

4. The analytical method must provide high quality resolution for simultaneous identification of different element species;

5. Samples must occupy a small space for convenient storage.

Much of the systematic work in field sampling techniques can be attributed to Mark and his colleagues [169 - 172], who constructed an electrodeposition field sampling device. It consisted of a submersible, self-contained potentiostat, power supply, reference electrode, and working electrode. Metals (Au, Ag, Cu, Co and Mn) are electrodeposited on the modified pyrolytic graphite working electrode which can then be removed from the sample at the surface and stored. The metal film can be either analysed directly by x-ray fluorescence spectrometry or emission spectrometry, or it can be dissolved and analysed by atomic absorption spectrometry. The electrode deposition method had two disadvantages: the metals which can be sampled are

limited, and the reproducibility of data is poor due to the complicated electrolysis process and matrixes.

Another field sampling technique using ion exchange membranes was reported by the same authors [173, 174]. Semi-permeable ion exchange membranes (for cations and anions respectively) have been used for simple direct sampling to separate and preconcentrate analytes, which can be directly analysed for trace element speciation using neutron activation analysis. But this membrane technique has limitations because of the long time required to reach the distribution equilibrium of even sufficient concentrations of trace elements in the membrane matrix. Other problems included interferences, such as the adsorption of organics, which affected the sensitivity of the ion exchange. Bauman and Weetall [175] described the preparation of diazo-ligands such as dithizone and hydroxyquinoline coupled to benzidine-carboxymethylcelloluse. These ligand-cellulose products were used to collect trace metals from sea water directly. Hughes and co-workers [176], and Vernon and Eccles [177] have reviewed this area and summarised the numerous facets of research concerning this broad and important subject.

Sugawara and co-workers [178] subsequently reported the preparation and properties of controlled pore glass-8-hydroxyquinoline (CPG-8-HOQ) and its applications to preconcentration of iron and copper in distilled-deionised water. This has potential as a field sampling method for trace metals. Recently, Kohata and his colleagues [179] developed a field sampling technique, which used a small GF/C filter (Whatman International, Maidstone, UK) to collect the trace photosynthetic pigments from large volume of sea water sample (from 2 to 6 litre), followed with the extraction and the speciation by high performance liquid chromatography. Kolotyrkina and Tsysin [180] proposed to use an ion exchange microcolumn for sampling and preconcentrating trace manganese. Shipboard flow injection method with a catalytic

spectrophotometer allowed the determination of manganese in the concentration range 20 $\mu g\ l^{-1}$ to 10 $ng\ l^{-1}$ in deep sea water samples.

It can be concluded from the above review that the development of a field sampling techniques could solve the inherent problems encountered in the determination and speciation of trace elements in natural waters. The preconcentration and separation using ion exchangers or chelates which occur during sampling allow for the simple and rapid analysis of samples by widely used measurement techniques.

In the last decade, the flow injection technique has proven to be an invaluable analytical tool in the versatile sample handling and pretreatment procedures. One of the major benefits of the flow injection technique as compared with other types of automated chemiscal systems is its capability of performing sample pretreatment functions on-line, which including solvent extraction, ion exchange, redox reaction and if necessary, analyte preconcentration. These primary factors of this technique are critical for the speciation and determination of metal elements in environmental samples.

In order to determine ultratrace amounts of different metal species in complex matrices by instrumental analysis, a separation and preconcentration technique is frequently required. The separation procedure eliminates sample matrix components that might interfere with the subsequent detection, whereas the preconcentration technique concentrates the analytes of interest from a large volume of sample solution. The microcolumn technique has great potential for the preconcentration of trace metals in waters. The analytical advantages of coupling microcolumn techniques with a flow injection system provides rapid on-line separation and preconcentration and an ability to have high sample throughput and reduced risk of contamination. Also the system of microcolumn - flow injection incorporated with various instrumental analysis has increasingly been applied to the speciation and determination of heavy metals in water

samples. As reported in Chapter 3, a novel FI manifold with a microcolumn of sulphydryl cotton has been combined with atomic fluorescence spectrometry for the speciation of trace mercury in natural waters.

In the work reported in this Chapter, field sampling in combination with FI-AFS is developed for mercury speciation in river systems.. The procedure involves sample collection and preconcentration using microcolumns at the site of sampling. Sulphydryl cotton fibre offers the possibility of developing a microcolumn technique for use in field sampling for mercury speciation. During this research synthetic solutions, and river water samples have been examined in the laboratory to develop a reliable method. Field surveys in the Manchester Ship Canal and the River Rother (South Yorkshire / Derbyshire) were then carried out.

Although it is generally accepted that methylation occurs in the aquatic environment, many workers have been unable to find even trace amounts of methylmercury in a wide variety of naturally occurring sediments and waters. The Manchester Ship Canal, as a main channel and wastewater discharging course, has a long history of industrial pollution. Mercury and organolead are often the two main heavy metal pollutants due to industrial discharges. Many studies on pollution sources, health impact and abatement of lead have been carried out [181]. But at the beginning of this work, little data for concentrations of organomercury in this canal was available - the only official monitoring information was for total mercury [182]. Furthermore, the area has been used for the sea-dispersal of large quantities of sewage sludge (known to contain relatively high levels of mercury) [183]. Very large quantities of contaminated dredging spoil enter the River Mersey and also approach the Canal [184,185], as well as the substantial volume of industrial wastes [186]. It has been assumed that the predominant mercury species discharged into the Manchester Ship Canal is inorganic mercury from a chloro-alkali plant. As discussed in Chapter 1, it is

generally believed that bacteria produce methylmercury found in river and lake sediments through the methylation of inorganic mercury (Hg^{2+}). Because of the serious public health implication of elevated levels of methylmercury in aquatic environments, it is important to understand the mercury cycle in the Canal system. Because no data on this most toxic mercury species were available, the mechanisms of the transport of mercury in the canal was unknown. In this chapter, evidence of presence of methylmercury and its distribution are reported for the first time.

The existing mercury cells in chemical plants located on the bank of the Manchester Ship Canal, have been equipped with units for retrieving mercury on the basis of its density. In some cases this additional recycling of mercury has proved economically as well as environmentally beneficial.

Unfortunately, the prevention of further mercury discharges now makes only a small contribution to the abatement problem. Ninety-nine percent of all mercury discharged is still present in the bottom sediments downstream from chloro-alkali plants, and it has been reported [182] that the Manchester Ship Canal was grossly polluted for mercury due to a chloro-alkali plant at Runcorn. In this study, concentrations of mercury in sediments as high as 124 $\mu g\ g^{-1}$ has been detected [182]. If methylation processes continue to convert these mercury deposits to methylmercurials, it may take several hundred years until they are depleted. In the meantime, the movement of tides and water traffic continue to dredge up the mercury-containing sediments and increase mercury concentrations in the water phase, at least temporarily. The aim of this work is to investigate the application of the microcolumn technique to field sampling for speciation and determination of different mercury species in river waters and to clarify the source of organomercurials in monitored waterways.

4.2 Results and Discussion - Manchester Ship Canal

4.2.1 General Features

The pH, dissolved oxygen and content of nutrients (N and P) in waters are three important indices that can be used to indicate the general degree of water pollution. The parameters significantly influence the chemical state of metal pollutants, redox potential of the waters and the origin of some of organometallic compounds of elements such as Hg, Sn, Pb, As, Se and Ge in the hydrosphere. In this work, these indices were measured and found common to the main stream system of the Manchester Ship Canal. For most of the sampling sites in this study there are routinely measured data available from the National Rivers Authority. The pH of the water was slightly alkaline within the range 7.2 to 7.5. The lowest concentration of dissolved oxygen (DO) was found at station 10 (Latchford Lock, Warrington central). The low value (0.88 mg l^{-1}) is related to the discharge of domestic sewage from a residential area. However, at station 5 which is about 18 km from station 10, the highest dissolved oxygen concentration was found and this is quite possibly due to the entry of fresh water into the canal from the River Weaver (see Figure 2.6.). The distribution profile of dissolved oxygen in the canal is shown in Figure 4.1.

Two kinds of nutrients (nitrogen and phosphorus) were determined according to their different species (NH_4^+-N, NO_3^--N, NO_2^--N and PO_4^{-3}-P), and distributions of these nutrients in the canal are shown in Figure 4.2.. It was found that NO_3^--N and NO_2^--N showed a similar distribution pattern to each other and no significant change in this selected area. The highest concentration of NH_4^+-N is found around station 5, which is the result of waste water discharge from a fertilizer manufacturer. The salinity (as chloride concentration) reached a maximum value at station 1 (Eastham Lock, Runcorn), due mainly to a mixing of canal water with sea water at this site. Then salinity decreased rapidly, the minimum concentration being found at station 10

(Latchford Locks, Warrington Central). This is obviously due to the simple dilution of sea water with canal water which is sufficient to account for the downstream gradients.

The whole length of the canal investigated was subject to some tidal flow depending upon the opening of relevant lock gates.

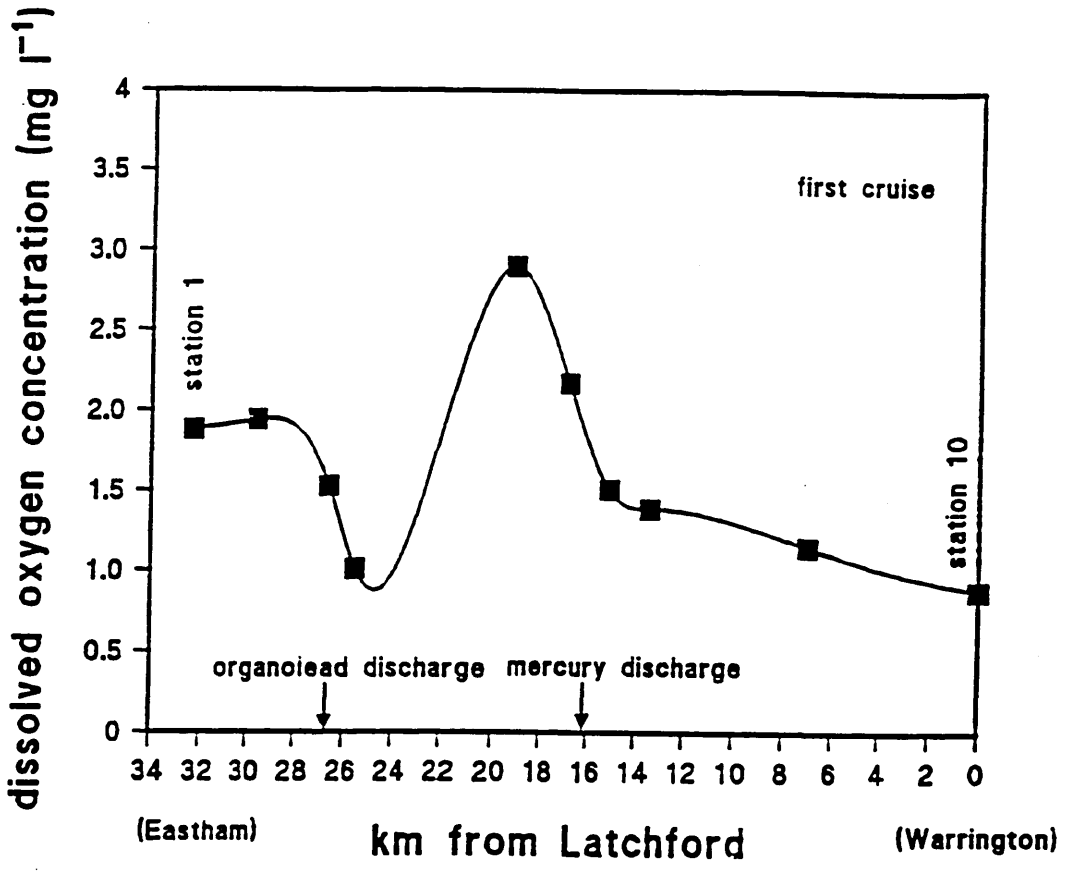


Figure 4.1 Distribution profile of dissolved oxygen in surface waters along the Manchester Ship Canal

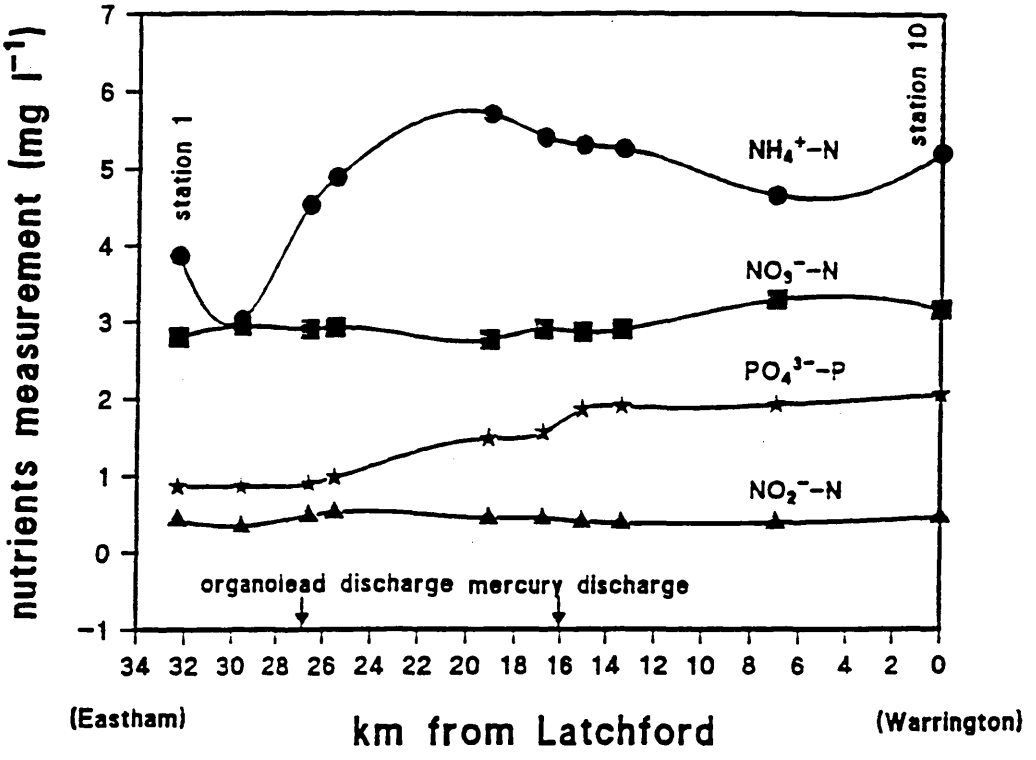


Figure 4.2 Distribution profiles of NH_4^+-N, NO_3^--N, NO_2^--N and PO_4^{3-}-P in surface water of Manchester Ship Canal.

4.2.2 Speciation and Distribution of Mercury in the Surface Water

Before the cruises commenced, several checks were made on the accuracy and reproducibility of organomercury determinations in standards and real samples. The data shown in Table 4.1 indicate that reproducibility strongly depends on the concentration of analyte in the samples and on the sampling itself. As expected, higher organomercury concentrations can be determined with higher precision than lower concentrations. Determination of organomercury in artificial solutions was more reproducible than the determination in natural water samples, probably due to sample matrix effects on the deposition/elution of organomercury onto the microcolumn and

on fluorescence detection. In certain samples, mainly those heavily polluted with organic matter, even filtration did not prevent the fluctuation of replicate measurements. Consequently, the uncertainties calculated for analysis of a single sample may not strictly be transferable to another sample. Nevertheless, the precision of the results was generally better than 10% at the lowest organomercury concentrations determined.

On the first cruise, only organomercury species were monitored. The data are presented in Table 4.2, and the distribution is shown graphically in Figure 4.3. The concentration of organomercury was highest at stations 1, 2, 3 and 7, with very low values (< 0.006 $\mu g\ Hgl^{-1}$) at the remaining stations. Although the occasional navigation of large barges can stir the bulk water and increase the water mobility between surface and bottom phases, it is known that the Eastham lock gates are opened infrequently. Hence it is not surprising that this distribution pattern of organomercury indicates that methylmercury formation takes place in limited regions only.

For the second cruise, concentrations of organo-, inorganic and total mercury were determined. The data are presented in Table 4.3. The distribution of organomercury is shown in Figure 4.4 and of inorganic and total mercury in Figure 4.5. The high organomercury concentration at station 3 (0.058 $\mu g\ l^{-1}$) was even greater more than that observed in the first cruise (0.026 $\mu g\ l^{-1}$). The similar distributions of inorganic and total mercury together with their high values (mainly greater than 0.2 $\mu g\ l^{-1}$) in the canal water indicate that the predominant mercury species in this sub-ecosystem is inorganic mercury. This distribution pattern also shows the diffusion of inorganic mercury from the discharge point to ambient areas is due to the movement of large barges.

Table 4.1 Analytical data for standard solutions
(MeHgCl) using field sampling technique ($\mu g\ Hgl^{-1}$)

Standard 1

replicate No	1	2	3	4	5	6	7	8	9	10
gross fl. intensity	3186	3389	3184	3173	3063	3218	3130	3324	3252	3148
blank intensity	----------112.9----------									
net fl. intensity	3073	3276	3071	3060	2950	3105	3017	3211	3139	3035
concentration	0.871	0.929	0.871	0.868	0.836	0.880	0.855	0.875	0.890	0.860
dilution factor	----------15----------									
final value	0.058	0.062	0.058	0.058	0.056	0.059	0.057	0.058	0.059	0.057
mean $\mu g\ l^{-1}$	----------0.058----------									
S.D	----------0.0016----------									
RSD%	----------2.8----------									
nominal value	----------0.060----------									

Standard 2

replicate No	1	2	3	4	5	6	7	8	9	10
gross fl. intensity	1548	1481	1739	1376	1508	1479	1405	1555	1470	1569
blank intensity	----------112.9----------									
net fl. intensity	1435	1368	1626	1263	1395	1366	1292	1442	1357	1456
concentration	0.403	0.384	0.458	0.354	0.392	0.383	0.362	0.405	0.381	0.409
dilution factor	----------15----------									
final values	0.027	0.026	0.030	0.024	0.026	0.026	0.024	0.027	0.025	0.027
mean $\mu g\ l^{-1}$	---------- 0.026----------									
S.D	----------0.0018----------									
RSD%	----------6.9----------									
nominal value	----------0.030----------									

(Table 4.2.1 continued)

Standard 3

replicate No	1	2	3	4	5	6	7	8*	9	10
gross fl. intensity	499	483	484	462	580	470	481	315	496	519
blank intensity	----------	----------	----------	----------	112.9	----------	----------	----------	----------	----------
net fl. intensity	386	370	371	349	467	357	368	208	383	406
concentration	0.103	0.098	0.099	0.092	0.126	0.095	0.098	0.050	0.102	0.109
dilut. factor	----------	----------	----------	----------	15	----------	----------	----------	----------	----------
final value	0.007	0.007	0.007	0.006	0.008	0.006	0.007	0.003	0.007	0.007
mean (ug l^{-1})	----------	----------	----------	----------	0.007	----------	----------	----------	----------	----------
S.D	----------	----------	----------	----------	0.006	----------	----------	----------	----------	----------
RSD%	----------	----------	----------	----------	8.6	----------	----------	----------	----------	----------
nominal value	----------	----------	----------	----------	0.010	----------	----------	----------	----------	----------

* fluorescence signal with Aristar "*" was deducted due to delayed scanning and the result is rejected.

4.2.3 Distributions of Total Mercury and Other Metals in Sediments

Total mercury concentrations in sediments are typically two and three orders of magnitude higher than their concentrations in waters, since sediments act as a trap for trace metal elements [5]. Data of total mercury concentrations in sediments, therefore, should offer a reasonable picture of the degree of mercury pollution in the aquatic environment and evidence for the methylation process, which is responsible for formation of organomercury, particularly methylmercury in the water systems. The total mercury concentrations in surface sediments are summarised in Table 4.4 and the distribution profile is shown in Figure 4.6.

Table 4.2 Analytical data for organomercury in surface water of the Manchester Ship Canal (first cruise)

Sample No	Fluorescence signals peak area	peak height	Concentration (μg Hgl^{-1}) analysed values	mean
1a	307	10	0.010	
1b	404	10	0.013	0.013
1c	464	12	0.015	
2a	753	23	0.025	
2b	751	24	0.025	0.025
2c*	-	-	--	
3a	719	17	0.024	
3b	839	21	0.028	0.026
3c*	-	-	--	
4a	101	3	<0.006	
4b	87	2	<0.006	<0.006
4c	0	1	<0.006	
5a	93	3	<0.006	
5b	101	2	<0.006	<0.006
5c	33	2	<0.006	
6a	27	1	<0.006	
6b	70	2	<0.006	<0.006
6c	0	1	<0.006	
7a	271	7	0.009	
7b	313	7	0.010	0.009
7c	241	5	0.008	
8a	106	3	<0.006	
8b	30	2	<0.006	<0.006
8c	0	1	<0.006	
9a	111	2	<0.006	
9b	140	3	<0.006	<0.006
9c	151	3	<0.006	
10a	103	3	<0.006	
10b	180	4	<0.006	<0.006
10c	90	2	<0.006	
Microcolumn blank				
a	0	0	<0.006	
b	40	1	<0.006	
c	0	1	<0.006	

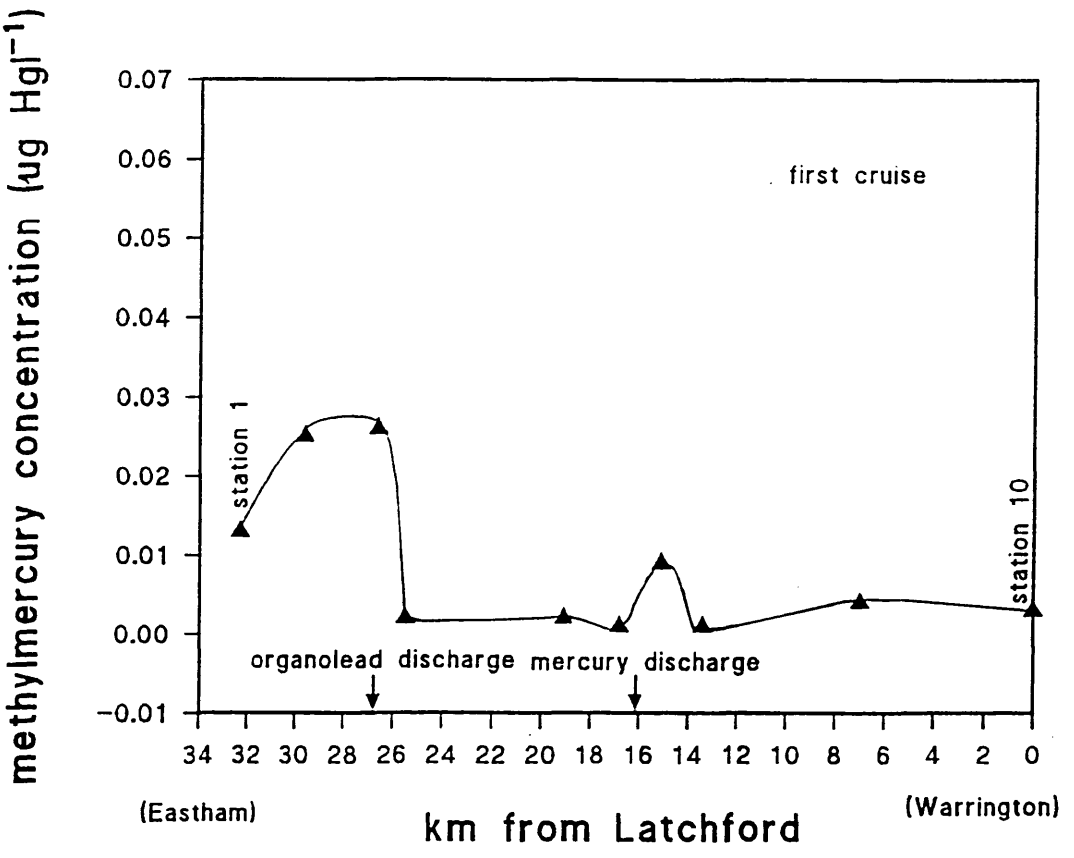


Figure 4.3 Distribution profile of organomercury in surface water of Manchester Ship Canal (first cruise).

Table 4.3 Analytical data for determinations of organo-, inorganic and total mercury in surface water (second cruise)

Sampling sites	Concentration (μg Hgl^{-1})		
	Organomercury[1]	Inorganic Mercury[2]	total mercury[3]
1. Eastham Lock	0.009	0.325	0.329
2. Fishers Wharf	0.022	0.320	0.471
3. Stanlow Wharf	0.058	0.530	0.561
4. Stanlow Point	0.035	0.250	0.423
5. Frodsham Marshes	<0.006	0.140	0.201
6. Weaver Confluence	<0.006	0.495	0.517
7. Weston Point Dock	<0.006	0.270	0.448
8. Runcorn Docks	0.006	0.265	0.416
9. Keckwick	<0.006	0.245	0.205
10. Latchford Locks	<0.006	0.045	0.038

Note: 1. data represent mean values of three separate columns;

2. data represent mean values of four measurements from two duplicate samples;

3. data represent mean values of duplicate measurements for a single sample.

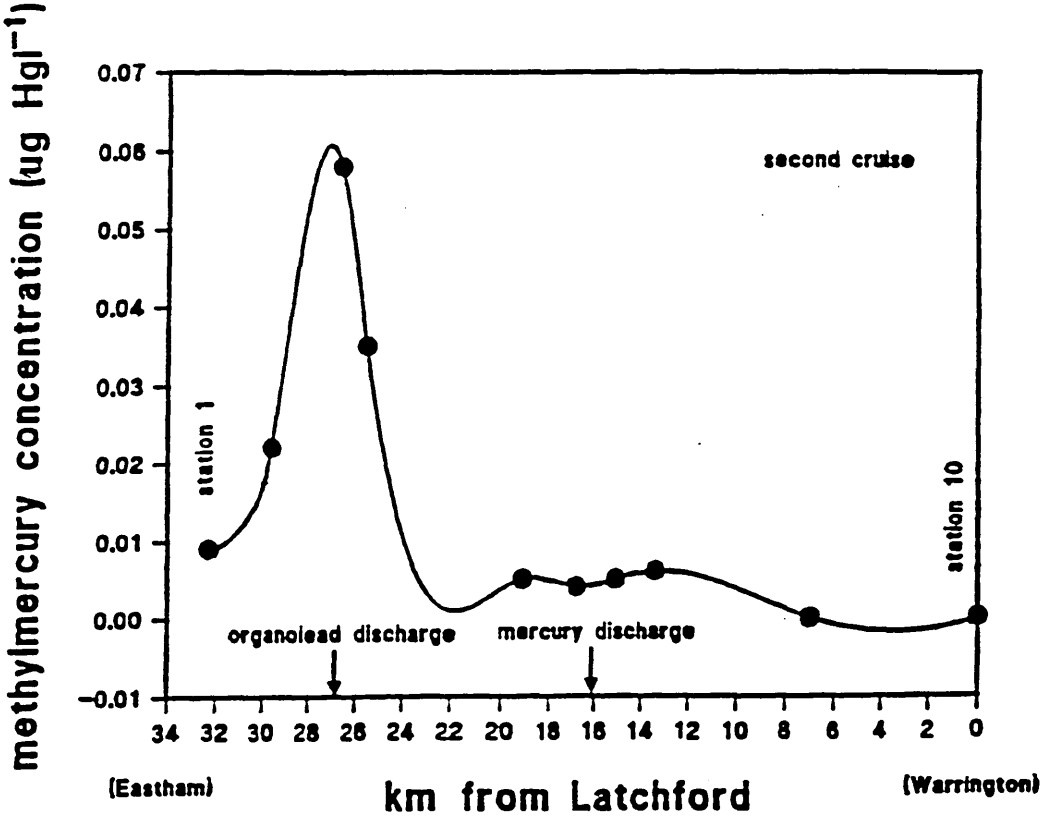


Figure 4.4 Distribution profile of organomercury in surface water along the Manchester Ship Canal (second cruise).

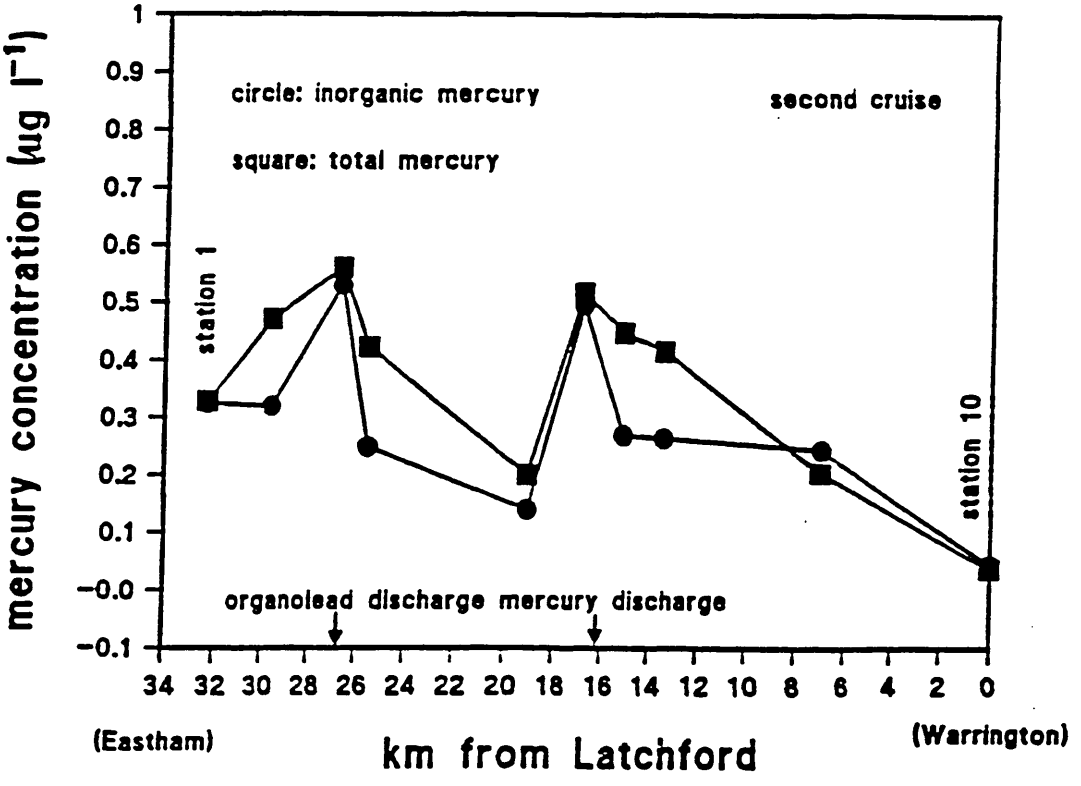


Figure 4.5 Distribution profiles of dissolved inorganic and total mercury along the Manchester Ship Canal (second cruise).

• dissolved inorganic mercury; ■ total dissolved mercury.

Table 4.4 Analytical data for total mercury in sediments

Stations	km from Latchford Locks	Lab SPT No.	Total mercury mg $Hgkg^{-1}$ (dry weight)
1. Eastham Locks	32.3	903870	3.40
2. Fishers Wharf	29.6	903865	8.10
3. Stanlow Wharf	26.6	903858	23.4
4. Stanlow Point	25.5	903850	11.0
5. Frodsham Marshes	19.1	903830	12.2
6. Weaver Confluence	16.8	903265	33.8
7. Weston Point Dock	15.1	903264	124.0
8. Runcorn Docks	13.4	903263	92.2
9. Keckwick	7.0	903249	37.0
10. Latchford Locks	0.0	903240	13.6

Data for other elements in the sediment samples are presented in Table 4.5 and their distribution profiles shown in Figure 4.7, to facilitate a better appraisal of mercury results. Data on lead will be presented and discussed in an independent section because of its close correlation with methylation of mercury in this work.

It can be seen from Figure 4.6 and 4.7 that the distribution pattern of total mercury in the sediments is dissimilar to that for the other metallic elements which have been measured. The six metals, mercury, copper, cadmium, chromium and nickel, have high

concentration at stations 8, 9 and 10. The concentrations of the six metals are lowest at station 1, due to the effect of dilution from the relatively unpolluted sea water. It is also noted from Figure 4.7 that the concentrations of these six metals have low values at station 5, this is assumed to be due to the effect of dilution from the River Weaver.

Table 4.5 Analytical data for total metals in sediments

(mg kg^{-1}, dry weight)

Stations	km from Latchford Locks	Cu	Zn	Cd	Cr	Ni
1. Eastham Locks	32.3	104	608	1.91	81.4	29.2
2. Fishers Wharf	29.6	150	952	2.20	124	48.0
3. Stanlow Wharf	26.6	139	821	1.85	117	49.1
4. Stanlow Point	25.5	215	1070	4.30	128	55.3
5. Frodsham Marshes	19.1	102	450	1.54	103	40.3
6. Weaver Confluence	16.8	129	509	2.21	132	44.4
7. Weston Point Dock	15.1	232	1090	4.86	175	62.0
8. Runcorn Docks	13.4	406	1270	5.55	462	189
9. Keckwick	7.0	203	790	4.04	248	52.9
10. Latchford Locks	0.0	269	1200	5.43	245	53.5

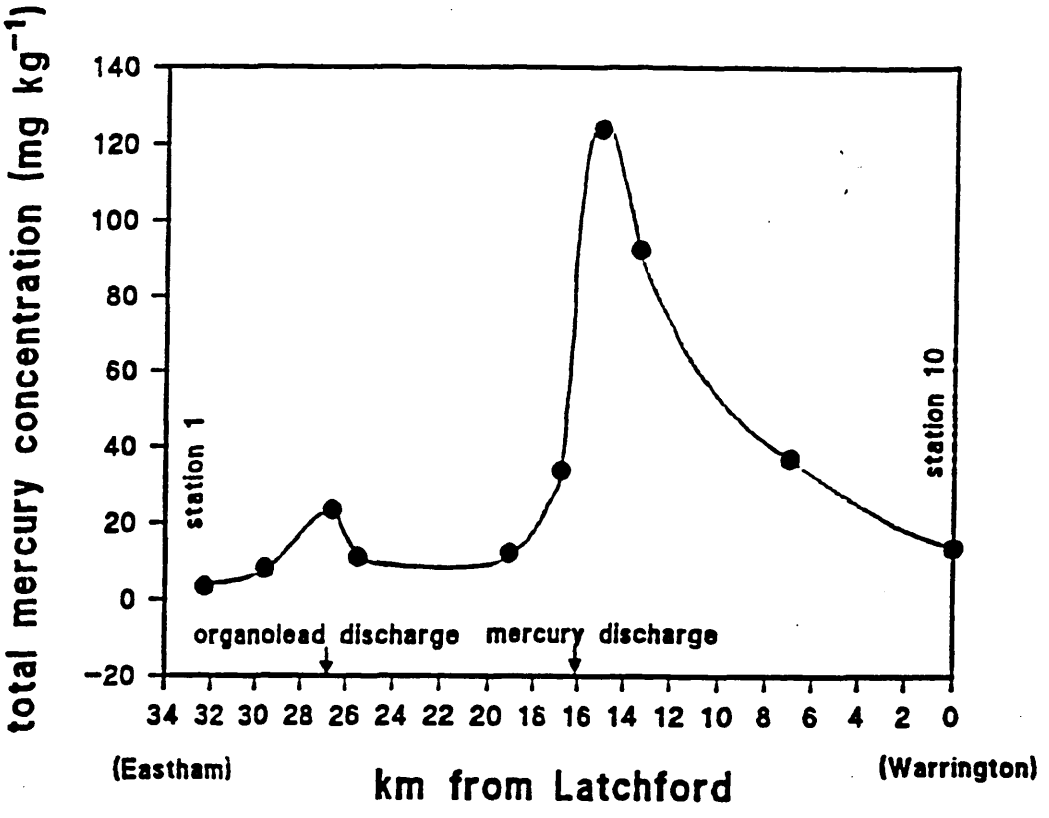


Figure 4.6 Distribution profile of total mercury in sediments of Manchester Ship Canal.

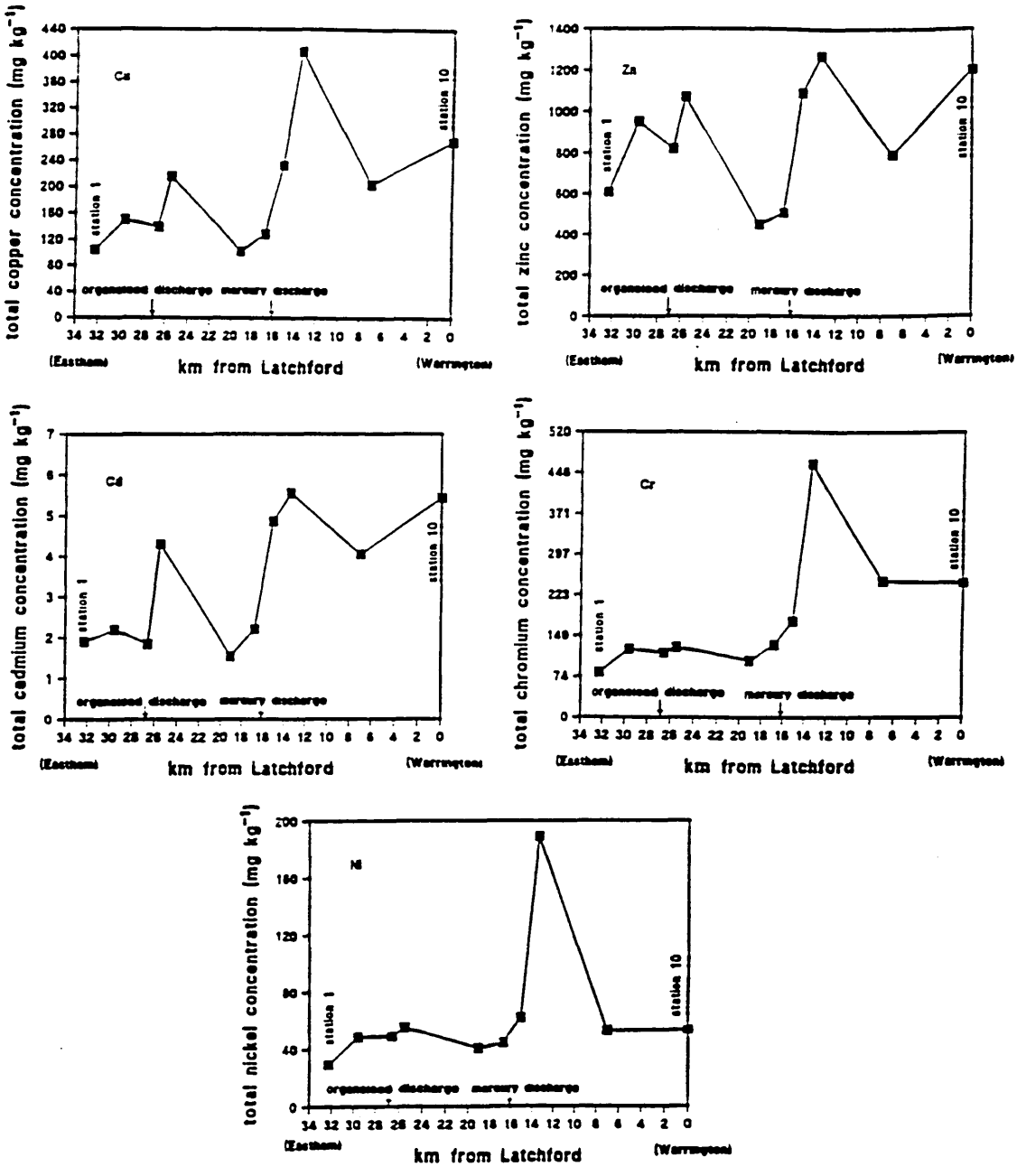


Figure 4.7 Distribution profiles of elements in sediments in the Manchester Ship Canal

4.2.4 Distributions of Lead In Water and Sediment

Lead is one of the pollutants that attract interest because of its widespread use and high toxicity. Organolead compounds are generally more toxic than inorganic lead compounds with the tetraalkyl derivatives being the most toxic form. Tetramethyllead and tetraethyllead have been used as antiknock additives in gasoline since 1923 and have stimulated much public attention due to their environmental impact in the last twenty years. Much studies on methylation of lead by anaerobic microorganisms has been revealed by different authors as a result of lead biotransformation in the environment [187-190]. In the Manchester Ship Canal and the River Mersey, enormous amounts of organolead in water (up to 1000 $\mu g\ l^{-1}$) were detected ten years ago [181]. This was attributed to tetraethyllead discharged from a manufacturing process at Ellesmere port, which is close to sampling station 3. During the last decade, intensive and effective measures have been adopted to limit this pollution. But there is still a considerable amount of alkyllead discharged into the Manchester Ship Canal [182], which causes elevated levels of organolead in this waterway.

In this work, a parallel survey on the total lead in sediments was carried out at the same time as the survey of total mercury (unfortunately no parallel surveys on alkyllead in water and speciation data on lead in sediments are available). The National Rivers Authority generously provided their routine monitoring data on alkyllead in waters and have allowed the author to quote and discuss their data in this thesis. These data were collected in the same season, and valid comparision between them should be possible. The concentrations of organolead in water samples are presented in Table 4.6. The determination of organolead was carried out by polarographic technique. The distributions of alkyllead in water in different months are shown and overlaid in Figure 4.8. The concentrations of total lead and its distribution in sediments are presented in

Table 4.7 and Figure 4.9. From these data, it is therefore possible to describe the present state of dispersion and precipitation of organolead pollution at each sampling site. It can be seen in Figure 4.8 and Figure 4.9 that organolead in the canal water and total lead in sediment are present in the highest concentrations around sampling station 3, which is very close to an organo-lead discharge point. This distribution pattern also indicates that the accumulation and precipitation of organolead should reach its highest level in the same region.

4.2.5 Correlation between Organomercury and Organolead in the Manchester Ship Canal

The data on alkyl-lead in waters and total lead in sediments cannot be compared directly because only data on different species is available. However it can be inferred that the proportion of organolead in sediments to total lead in sediments is large, because it is known that trialkyl-lead is the main species from the industrial discharge, and relatively high concentration of organo-lead were detected in the water phase. Hence, the data on total lead concentration are used to represent organolead in sediments for the ensuring discussion. A comparison of distributions of organo-mercury, alkyllead and total lead in different phases of the Manchester Ship Canal can be made from Figure 4.4, 4.8 and 4.9. It is quite clear that organomercury in water has a quite similar distribution pattern with those of organolead and total lead. Up to now, the pathways for the biological conversion of mercury are well understood. It can be assumed that if the biological process which functions in sediments were mianly responsible for the formation of organomercury in the Manchester Ship Canal, the distribution pattern of organomercury would be different, with the maximum values around station 7, because the highest concentration of total mercury in sediments was found there. Furthermore, the activity of microorganisms in sediments in the eastern

Table 4.6 Analytical data for organolead in water of the Manchester Ship Canal

Stations	km from Latchford Locks	Organolead ($\mu g\ l^{-1}$) June	July	December
1. Eastham Locks	32.3	28.3	27.0	21.0
2. Fishers Wharf	29.6	30.9	36.0	30.5
3. Stanlow Wharf	26.6	33.1	68.5	36.5
4. Stanlow Point	25.5	22.8	15.5	21.5
5. Frodsham Marshes	19.1	8.3	5.5	4.05
6. Weaver Confluence	16.8	2.6	4.4	3.30
7. Weston Point Dock	15.1	3.9	3.35	3.90
8. Runcorn Docks	13.4	3.0	1.30	2.95
9. Keckwick	7.0	1.8	0.80	3.15
10. Latchford Locks	0.0	<0.5	<0.5	1.10

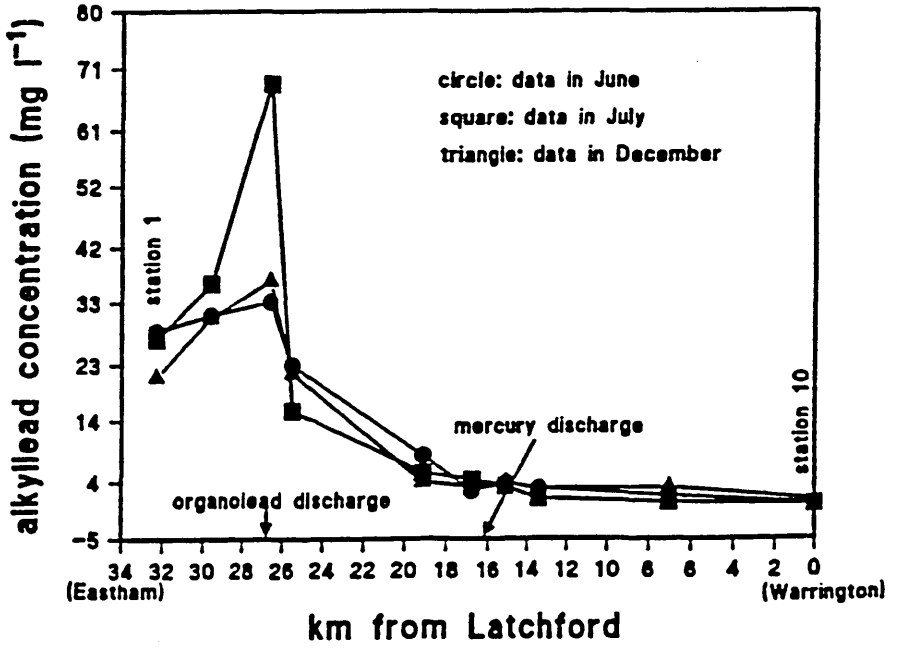


Figure 4.8 Distribution profiles of organolead in water along the Manchester Ship Canal.

Table 4.7 Analytical data for total lead in sediments of Manchester Ship Canal.

Stations	km from Latchford Locks	Total lead (dry weight, mg kg^{-1})
1. Eastham Locks	32.3	171.0
2. Fishers Wharf	29.6	508.0
3. Stanlow Wharf	26.6	2190
4. Stanlow Point	25.5	1770
5. Frodsham Marshes	19.1	242.0
6. Weaver Confluence	16.8	468.0
7. Weston Point Dock	15.1	359.0
8. Runcorn Docks	13.4	459.0
9. Keckwick	7.0	257.0
10. Latchford Locks	0.0	369.0

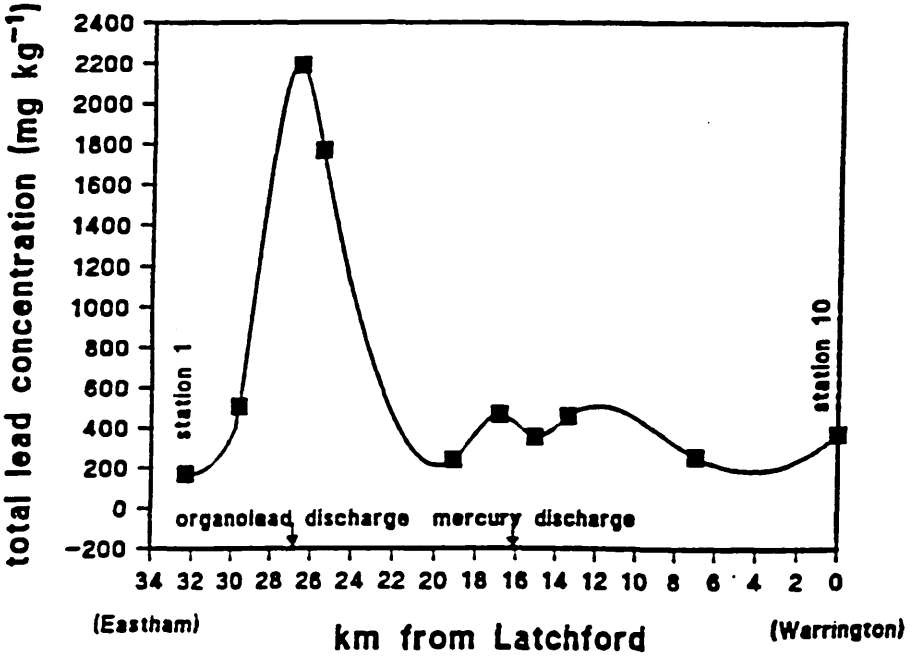


Figure 4.9 Distribution profiles of total lead in sediment of the Manchester Ship Canal

part (in the region of station 7) should be higher than that in the western part (in the region of station 3), since most of the trade waste from sewage works and domestic waste pipes enter the Manchester Ship Canal in the eastern part. Furthermore, contaminated sediments in the western part can be diluted by uncontaminated sea water sediment during tidal movements. The actual results show a substantial maximum concentration of organomercury in the region of station 3 require an alternative explanation.

A high correlation (see Figures 4.4, 4.8 and 4.9) between the distributions of organomercury, organolead in surface water and total lead in sediment of the Manchester Ship Canal has been observed. These results imply the possibility of methylation of inorganic mercury through a chemical or bioorganism route in order to transfer alkyl groups from organolead to inorganic mercury. Although such a chemical reaction and experimental results have not so far been reported, two similar alkyl-transfer reactions have been proposed [17]. For the synthesis of RHgX and R_2Hg, the compounds can be derived through the alkyl group transfer, by simple preparative routes that permit great diversity in the nature of the organic radical R.. The mechanism of transfer of an alkyl group from various metals to Hg (II) salts was also reported and thoroughly examined by McAuliffe [17]. For example, transmetallation of Hg (II) salts with tetraalkyltin in methanol/water mixture was expressed as below:

$$R_4Sn + HgX_2 \text{ -----> } RHgX + R_3SnX \quad (X = Cl, I) \qquad (4.2.2)$$

The same reaction can take place for alkyl groups of a wide range of metals, besides tin, eg., germanium [191], gold [192], chromium [193], manganese [194], iron [195] and cobalt [196]. As tin, germanium and lead are elements in the same group, they have similar outer electronic structure and some similar chemical properties.

Presumably, a similar transmetallation between alkyllead and Hg (II) may proceed and be responsible for the source of alkylmercury in the Manchester Ship Canal. Further research involving a simulation study in the laboratory may confirm the above as a potential process of substitution of alkyl- groups from alkyllead to inorganic mercury. As a simulation a large tank containing raw sediment and water obtained from sampling station 3 at the Manchester Ship Canal could be used, and an attempt made to maintain the same temperature as in the field. After spiking inorganic mercury into the system ($\mu g\ Hgl^{-1}$ level), samples would be analysed for determination of organomercury at regular intervals (daily basis).

4.3 Results and Discussion - The River Rother

4.3.1 General features

The River Rother, like any urban river, receives pollution from several sources: the sewage treatment works discharging industrial and domestic sewage effluents; direct discharges of industrial effluent and storm-sewage overflows from sewers. Effluent discharges to the river include sewage effluent from thirty-nine sewage treatment works. Among the direct industrial discharges, the first significant one received by the river is from a chemical complex at Staveley, which discharges poor quality effluent containing significant amounts of inorganic mercury and ammonia [138]. A long history of serious pollution has made the River Rother one of the worst of rivers in South Yorkshire and even in Britain. Previous work has reported the presence of a relatively high concentration of organically bound mercury in the River Rother [138].

The objective of the work reported here is to obtain speciation information on mercury, using the field sampling technique, in a similar manner reported for the Manchester Ship Canal exercise.

The River Rother flows through the centre of Chesterfield to Rother Valley

Country Park in a West-East direction (see Figure 2.8.). A temperature profile showed that the surface water layer, 0.5 meter thick, was 7.2°C. with an average pH of 8.2 (8.1 - 8.3). Figure 4.10.and 4.11 show the distribution profiles for pH and temperature in this river. The relative high alkalinity of the river water might result in rapid precipitation of mercury species, since heavy metals are more soluble at low pH. Acidification on the other hand is expected to enhance the mobility of mercury species [197,198].

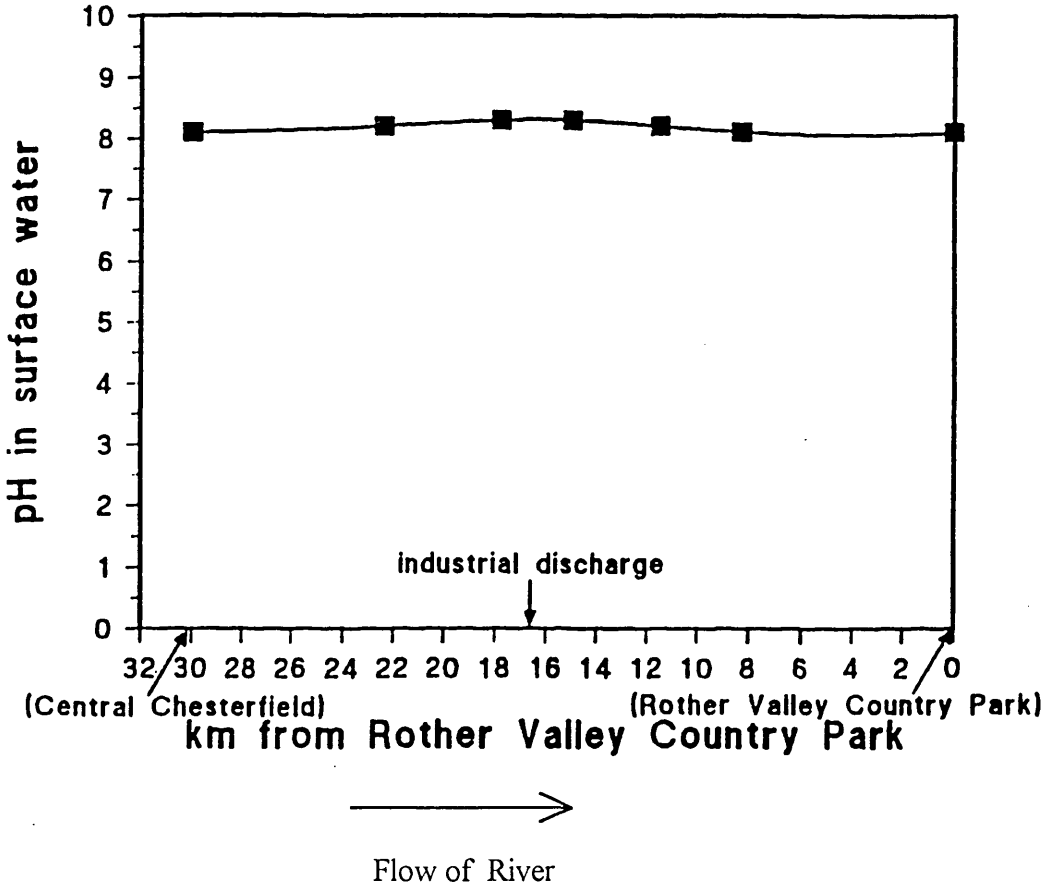


Figure 4.10 Distribution profile of sample pH in the River Rother between Chesterfield and Rother Valley Country Park.

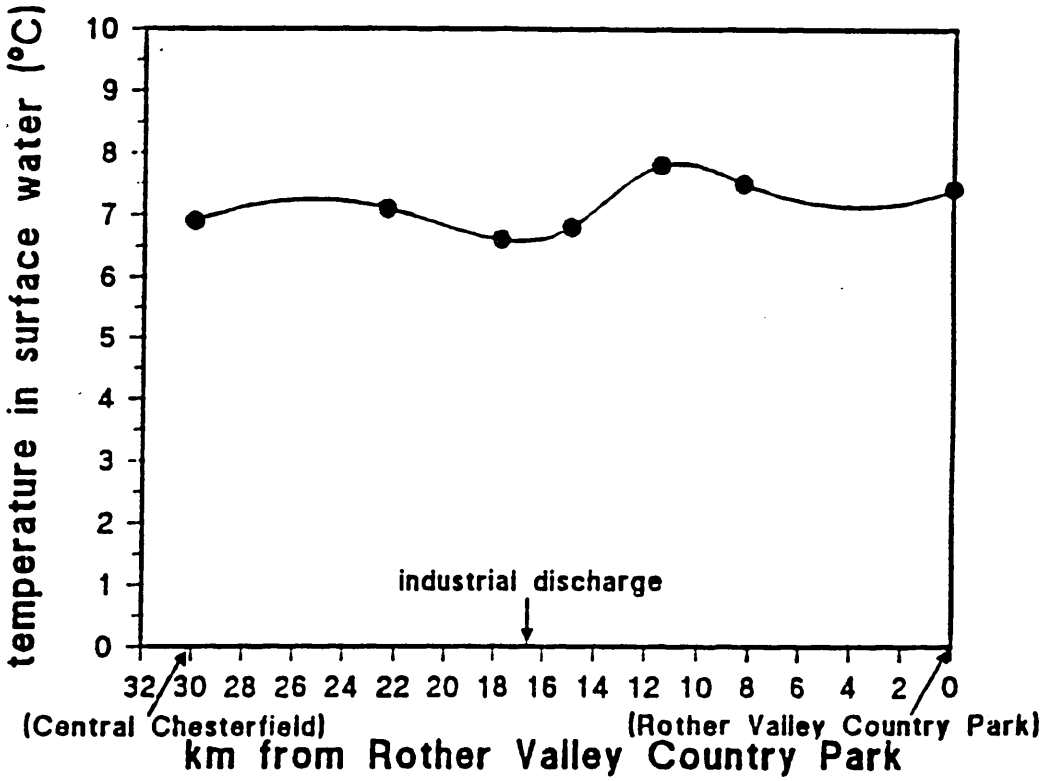


Figure 4.12 Distribution profile of temperature in surface water of the River Rother.

4.3.2 Distribution of organomercurials

To check the precision of the whole analytical procedure, five replicate samples were collected at each sampling station using the field sampling technique. The estimated standard deviations for samples from seven sampling stations are given in Table 4.8. It can be seen that the concentrations of methylmercury ranged from 0.210

(at the first and last stations) to 1.701 μg Hgl^{-1} (at station 3). The standard deviations were not correlated with the concentration as expected. However it is probable that the high content of particulate matter in particular sampling sites (in close proximity to drainage or ditch points) readily blocked the on-line filters and altered the sampling rate, thereafter affecting the deposition efficiency of organomercury on the microcolumns.

The distribution profile of methylmercury in the River Rother is shown in Figure 4.13. The highest concentration (1.73 μg Hgl^{-1}) was recorded from station 3. Similar low concentration (0.25 μg l^{-1}) of methylmercury was detected at station 1 and 7. The results for stations 3 ,4 and 5, which are in the vicinity of a industrial discharge point, show an interesting difference: a distribution pattern which appears to be U-shaped. It is clear that a very localised area of relatively high mercury levels has been observed. The amount of mercury transferred into this river will depend on the rate of river effluent flow, on the concentration of inorganic mercury in the water phase, in particulate matter and in the sediments. We have no concentration data of inorganic mercury in particulate matter and in the sediments of the River Rother, but the figures presented in Table 4.9 suggest that mercury (both methyl- and inorganic species), is retained very firmly by the sediment. A full survey is needed to monitor different mercury species in different phases (Water, particulate and sediment) in order to clarify the methylmercuric chloride distribution pattern and its origin.

4.3.3 Distributions of Inorganic and Total Mercury

The concentrations of inorganic and total mercury (dissolved and unfiltered) are also presented in Table 4.10. The distribution profiles for these three fractions are shown in Figure 4.14.. From Table 4.10, it can be seen that the concentrations values of inorganic mercury in surface water were mostly below 0.3 μg Hgl^{-1}. At both end

sites (station 1, 2 and 7), inorganic mercury was not detected (concentration less than 0.001 $\mu g\ l^{-1}$). The precision of inorganic mercury measurements ranged from 13 - 25%, RSD. This is explained by the fact that the concentrations of inorganic mercury in the waters were at about the same level as that of the blank (0.29 $\mu g\ Hgl^{-1}$) and were thus subject to great uncertainty.

Table 4.10 also lists the concentrations of total dissolved mercury and unfilterred total mercury measured in the River Rother water. It can be seen that there is a significant difference between these two fractions: The concentrations of unfilterred total mercury is much higher than that of total dissolved mercury in the monitored region of the River Rother except for the two end sites (station 1 and 7). However, in this case interpretation of the causes of such differences, particularly in relation to the presence of large amounts of colloidal and particulate matter in waters, is constrained by the fact that speciation data associated with colloidal and particulate matters are not available. A detailed speciation study on mercury in solid phases (colloidal, particulate and sediment) would be useful to clarify this difference.

It is clear that the distribution patterns of total mercury from indirect measurement (organomercury plus inorganic mercury) and direct measurement (obtained by experiment) for total dissolved mercury in water along selected sections of the River Rother have high correlation (correlation coefficient of 0.998). Moreover, all values of total dissolved mercury by the direct method are higher than those from the indirect method. This implies either a slightly low deposition efficiency for organomercury or a lower on-line oxidation efficiency compared to the batch oxidation procedure is occurring. The concentrations of total mercury (unfiltered) are much higher than the corresponding dissolved fraction. The total mercury (unfiltered) distribution pattern is similar to that of dissolved inorganic mercury, with the highest value being recorded at station 4.

Table 4.10 Analytical data for different mercury species in surface water of River Rother

Stations	Concentration (μg Hgl^{-1})							
	1	2	3	4	5	mean	SD	RSD%
Methylmercury[a]								
1	0.206	0.195	0.257	0.224	0.169	0.210	0.033	15.7
2	0.327	0.370	0.300	0.396	0.371	0.353	0.039	10.9
3	1.730	1.859	1.699	1.975	1.262	1.701	0.27	15.9
4	0.545	0.518	0.483	0.523	0.492	0.512	0.025	4.9
5	0.832	0.872	0.870	0.939	0.757	0.854	0.067	7.8
6	0.398	0.367	0.385	0.318	0.326	0.359	0.035	9.9
7	0.226	0.195	0.210	-	-	0.210	0.016	7.4
Inorganic Mercury[b] (μg l^{-1})								
1	0.007	<0.001	<0.001	<0.001		<0.001	-	-
2	<0.000	<0.001	<0.001	<0.001		<0.001	-	-
3	0.093	0.133	0.121	0.133		0.120	0.019	15.7
4	0.242	0.286	0.301	0.424		0.313	0.078	25.0
5	0.250	0.316	0.312	0.345		0.306	0.040	13.0
6	0.249	0.250	0.192	0.179		0.218	0.037	17.1
7	<0.001	<0.001	<0.001	<0.001		<0.001	-	-

(Table 4.3.1 continued)

	1	2	mean
Total mercury (dissolved)[c] (μg l^{-1})			
1	0.353	0.357	0.355
2	0.407	0.386	0.397
3	1.920	1.891	1.906
4	0.891	0.901	0.896
5	1.219	1.305	1.262
6	0.661	0.623	0.642
7	0.303	0.296	0.300
Total mercury (unfiltered)[c,d] (μg l^{-1})			
1	0.370	0.260	0.310
2	1.160	1.150	1.155
3	3.010	3.220	3.115
4	4.390	4.530	4.461
5	4.090	3.970	4.030
6	3.300	3.300	3.300
7	0.310	0.310	0.310

a. five replicate microcolumns were sampled for each station;
b. duplicate effluents were collected for each station and duplicate measurements were made for each bottle;
c. single bottle of water sample was collected for each station and duplicate measurements were made for each bottle;
d. raw water samples were pretreated by acidification and bromination without filtration.

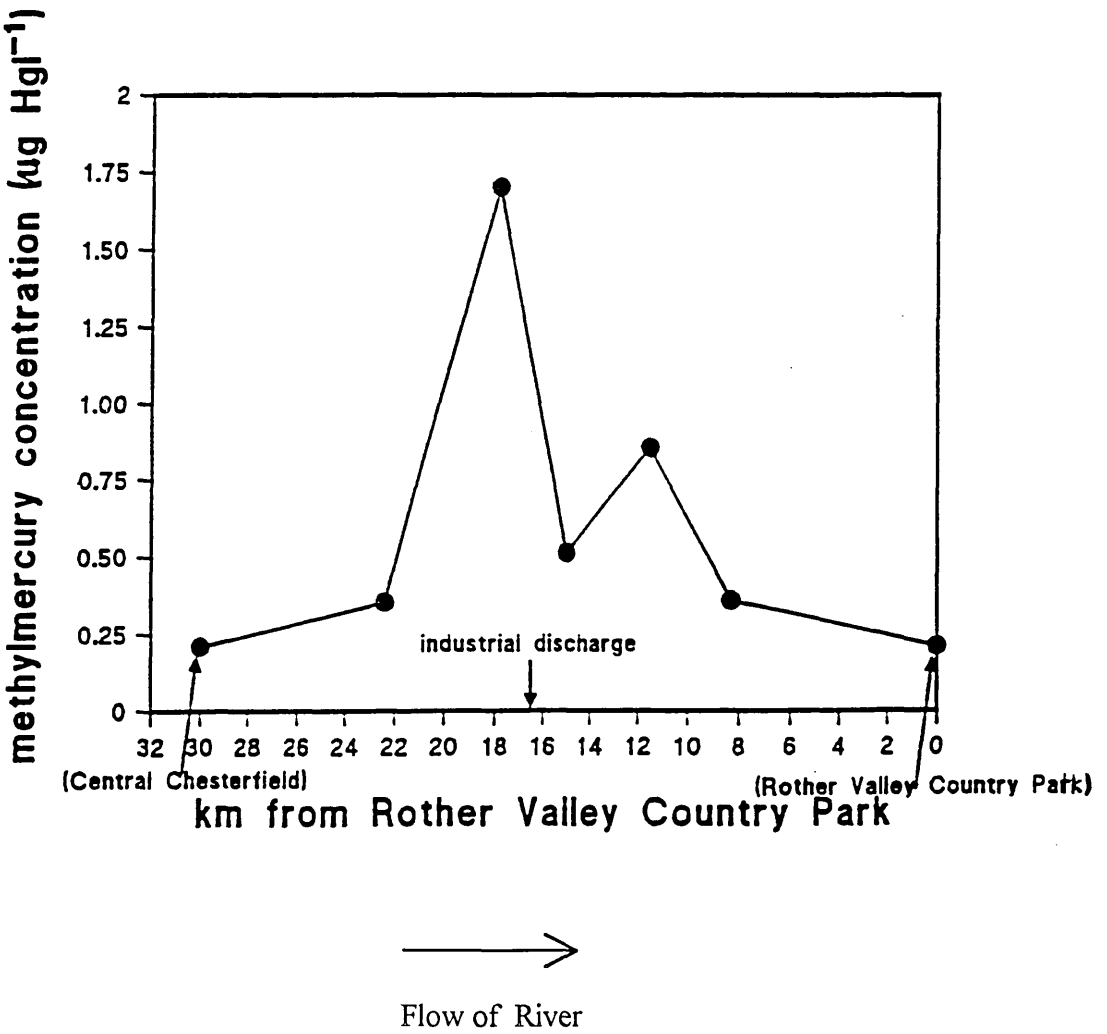
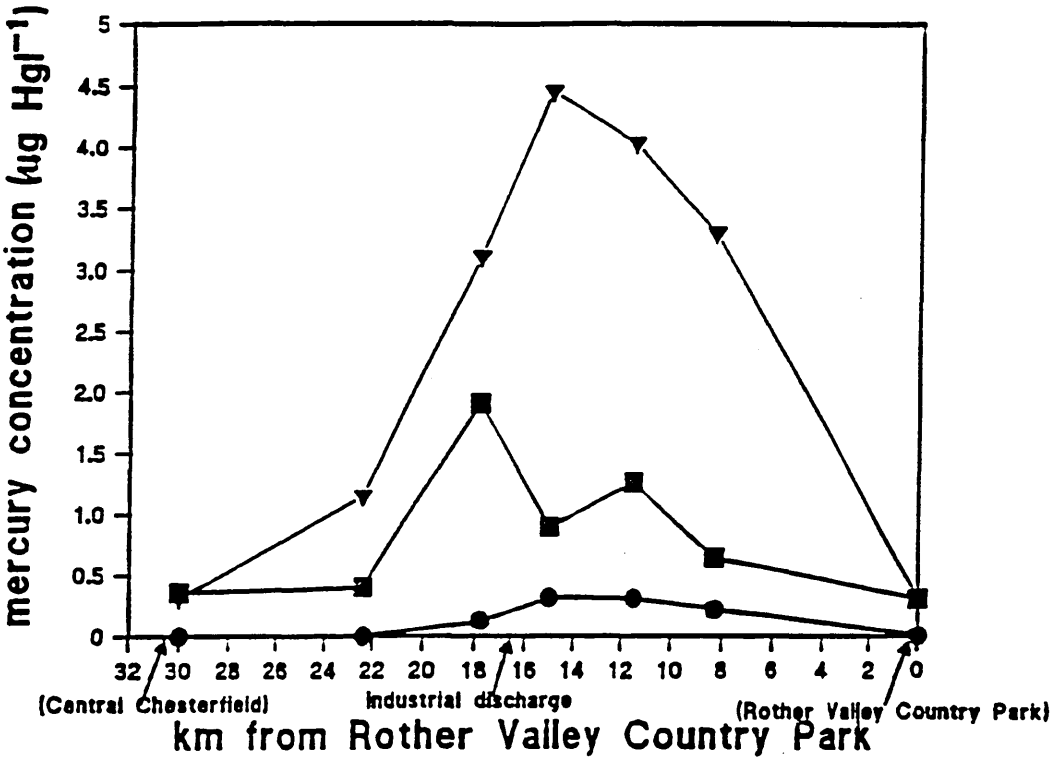


Figure 4.13 Distribution profile of organomercurials in water along the monitored section of the River Rother.



Flow of River

Figure 4.14 Distribution profiles of inorganic and total mercury in surface water of the River Rother

▼ unfiltered total mercury

■ dissolved total mercury

● dissolved inorganic mercury

4.4 Conclusion

On the basis of these surveys, it may be concluded that

- The different mercury species were successfully determined in the Manchester Ship Canal and the River Rother. Industrial discharges make the major contribution to the elevated concentrations of mercury species in these two waterways;

- The concentration of organomercurials in the River Rother generally higher than that in the Manchester Ship Canal, which suggests higher bioavailability of mercury in the River Rother. The large amounts of trade effluent discharged into this river and the distribution pattern of different mercury species suggest that a bioorganic methylation process is operating for which a high methylmercurial compound production is expected;

- In the Manchester Ship Canal, a high degree of correlation between distributions of organomercurials and alkyl-lead in this waterway may suggest a possible methylation of inorganic mercury through the transfer of alkyl- groups from alkyl-lead to inorganic mercuric ions. Future work would be needed to confirm this hypothesis.

- In general, about 57 - 89% of the total dissolved mercury in the River Rother water samples was in an organic form. The organomercury distribution pattern is quite similar to that of total dissolved mercury, which suggests that the organo- mercury functions for the mobility of mercury within this monitored region of this river;

- In the River Rother, the ratio of dissolved total mercury and the unfiltered total fraction varied significantly from 19 to 100%. The highest values for this ratio (dissolved / unfiltered = 1) were recorded at Stations 1 and 7 (see Figure 2.8.1), where the lowest values of organo-, inorganic and total mercury were obtained. This implies that at both ends of the sampling sites (Central Chesterfield and Rother Valley

Country Park), most of the mercury (organo- and inorganic fraction) is in the dissolved fraction. At the remaining sites, about 39 to 81% (corresponding to the dissolved / unfiltered = 61 - 19%) mercury was associated with colloidal and particulate matter, due to the convergence of industrial and municipal effluents, with the latter containing a high content of particulate matter.

- Details of the nutrient loadings processes and redox potential in water and in sediment, and other biochemical interactions in the Manchester Ship Canal and the River Rother are inadequately understood at present. The analytical technologies reported in this thesis should enable an improved understanding of chemical and biological mechanisms for the methylation of mercury to be gained and thus give better insight into future management of these and similar water courses.

CHAPTER 5

GENERAL CONCLUSION AND RECOMMENDATION

Two separate but related investigations involving a combined system of microcolumn technique - flow injection - atomic fluorescence spectrometry are reported in this thesis.

The first is the development and evaluation of a novel manifold made up of a sulphydryl cotton microcolumn coupled with a flow injection system, using atomic fluorescence spectrometric detection, for rapid sequential monitoring of inorganic mercuric ions and methylmercuric chloride in synthetic solutions and in natural waters.

The use of other microcolumn packing materials, such as activated alumina, silica immobilised 8-hydroxyquinoline and xanthate cotton were also investigated. Because of the lack of specificity and limitations to separation and preconcentration of the different mercury species, these were not developed further.

The use of sulphydryl cotton, which has a relatively high affinity for organomercuric ions, incorporated with flow injection - atomic fluorescence spectrometry, has been

extensively investigated. This system is readily applicable to the speciation of trace mercury in natural waters. The limits of detection for inorganic mercury and methylmercuric chloride were 1 ng Hgl^{-1} and 6 ng Hgl^{-1}, respectively. The precision of this method is of the order of 5 - 20% at the 5 - 100 ng l^{-1} level.The determination of inorganic mercury is achieved directly using the FI-AFS system. The sulphydryl cotton microcolumn quantitatively retains the organomercuric ions and subsequent injection of hydrochloric acid (5 M) enables complete elution followed by on-line oxidation and detection using the same FI-AFS system. In this way both inorganic and organomercury are determined in a single sample of waters.

In the second part of the investigation, microcolumn technology was transferred to the field and surveys of the distribution and speciation of mercury in surface waters of the Manchester Ship Canal and the River Rother undertaken. This was the first time in which the mercury speciation in these two seriously polluted waterways in Britain has been measured. High degrees of correlation between dissolved organomercurials and alkyllead in surface water (with a correlation coefficient $\gamma_{water} = 0.8474$) and dissolved organomercurials and alkyllead in sediment (with a correlation coefficient $\gamma_{sediment} = 0.9619$) of the Manchester Ship Canal were found. It is suggested that transfer of alkyl groups from alkyl-lead to inorganic mercuric ions may be responsible for the production of organomercury compounds in this waterway. For the River Rother, the accumulated distribution patterns of organomercurials, inorganic and total dissolved mercury indicated that the direct discharge of inorganic mercury into this waterway is from a chemical plant. The very high quantity of organisms in the River Rother is due to the effluent discharges from tens of sewage treatment works. There organisms are likely to be responsible for the methylation of inorganic mercuric ions and hence the presence of significantly elevated organomercurial concentration in the River Rother (0.21 - 1.7 $\mu g\ Hgl^{-1}$). Organomercury concentrations were typicaly 30

times that of the Manchester Ship Canal (0.006 - 0.058 μg Hgl^{-1}).

In general this new approach, using the microcolumn technique of field sampling for element speciation, is most suited to surface water environments. For analytical chemistry this is an important field of research, because of

- the low concentrations of species relative to the total amount of an element;
- the difficulties of contamination and analyte loss at such low concentrations;
- the rapid interchange of species under natural conditions and for the conditions employed in sampling and storage;
- the interactions between different species and their matrices, which require highly sophisticated analytical methods.

With the field sampling technique coupled to the FIA system, toxicity and environmental behaviour of trace mercury can be monitored and assessed. It is hoped, however, that the experimental results may be useful to researchers clarifying the origins of organomercurials and mechanism of methylation of mercury in the hydrosphere. Compared with other trace element speciation methods, the field sampling technique coupled with flow injection - atomic fluorescence spectrometry is convenient, very sensitive and relatively precise. Reliable river quality information is essential to enable any river management authority to carry out its duties, particularly for the River Rother, since the Rother Valley Country Park has been developed as an area for outdoor recreation including water sports. As a stretch of the River Rother, the water quality in the Rother Valley Country Park must meet the river quality standards. Reliable mercury speciation data would no doubt help any improvement planning for the water quality in the Rother Valley Country Park and the River Rother.

Future research based on the results and conclusions of the work are projected:

- Extension of the established procedure is required in order to achieve separation and preconcentration of inorganic mercuric ions by microcolumn technique with on-line

and sequential features, and it's determination by cold vapour - atomic fluorescence spectrometry. For this purpose, many ion - exchanger are possible candidates. Amberlite IRA-400 [199], Chelex-100 [200] and Deacidite FF-IP [201] were reported to have been successfully used for proconcentration and separation of inorganic mercury from natural waters. These ion-exchangers require dilute acid solutions as carriers and eluent, which might be incorporated with the model system created for preconcentration and determination of methylmercury, in the hope that a novel technique for both inorganic and methylmercury species may be developed.

- Application of the sulphydryl cotton microcolumn to the analysis of multi-elements may be suggested. Wu's work [202] has opened the way to using sulphydryl cotton as an absorbent to separate and preconcentrate additional elements such as Cu, Pb, Cd, Zn, Ni and Co etc. It is hoped that gradient elutions using different concentrations of acid solutions and detection by inductively coupled plasma - atomic emission spectrometry or mass spectrometry may be developed as a novel tool for speciation and determination of multi-elements.

Because the data are based on only limited surveys, the concentrations of different mercury species in surface water of the Manchester Ship Canal must be interpreted with caution. A long-term monitoring programme at selected sites in this waterway might be beneficial to obtain more information about the natural and anthropogenic flux of mercury in this system. Future research must also include studies on mercury speciation and distribution in sediments and a simulation study in the laboratory may confirm the process of substitution of methyl- and/or ethyl groups from alkyl-lead to inorganic mercuric ions, which may be responsible for the origin of organomercurials in the Manchester Ship Canal.

APPENDICES

The development and improvement of analytical procedures for environmental analyses are paramount. This has encouraged the Community Bureau of Reference (BCR) of the Commission of the European Communities to support a series of projects and coordinate internationally funded research. In the last years, many projects have successfully been concluded (eg. trace metals in sediments, plants, soils, biological materials and sludges, etc.). With the increasing interest in speciation of trace elements new projects eg. on mercury, tin, arsenic, lead and aluminium, have been started or are in a preliminary phase. The working parties of the concerted action and workshops have been organised by the Community Bureau Reference, which serves to promote regular exchanges of information on relevant research carry out the interlaboratory comparison among member states participating in the improvement of the quality of measurements.

As a participating laboratory, many contacts have been made by this University research group in last three years. In following Appendices, two separate works related to the determination of mercury in fish [203] and in sea water are presented.

APPENDIX I.

SIMPLE OXIDATIVE PRETREATMENT FOR THE DETERMINATION OF ORGANOMERCURY IN TOLUENE EXTRACTS

This section describes a simple oxidative procedure which has been developed and published for the direct determination of organomercury in toluene extracts [204]. Such a procedure would be utilised for fish tissue analysis. Oxidative pretreatment is performed directly in a volumetric flask and avoids the need for back extraction and phase separation. Bromine is known to have favourable partition characteristics in toluene and on this basis it was thought that efficient oxidation of organomercury compounds might occur in toluene.

Experimental

Reagents

Inorganic mercury working solutions were prepared by dilutions of 1000 mg l^{-1} commercial stock solution (Spectrosol, BDH). Methylmercury stock solution (100 mg l^{-1}, as Hg) was prepared by dissolving 0.1250 g of methylmercuric chloride salt

(Organics, BDH) with 25 ml of acetone (Analar, BDH) and then diluting with deionised water to 1000 ml. Inorganic and methylmercury standard solutions (practically 0.000 - 10.00 μg l^{-1}) for calibration were freshly prepared from serial dilution of the above stock solutions. A 5% (m/V) tin chloride solution (in 15% HCl v/v) was freshly prepared from tin chloride 2-hydrate salt (Spectrosol, BDH), concentrated hydrochloric acid (Aristar, BDH) and deionized water. A bromide/bromate solution was made by dissolving potassium bromide/potassium bromate salts (AR, Fisons) with deionized water ($KBr/KBrO_3$, 2.5% + 0.7%, w/v). A hydrochloric acid solution (0.01 M) was prepared from concentrated hydrochloric acid (Aristar, BDH). A synthetic methylmercury extract was prepared from the methylmercuric chloride salt (Organics, BDH) and purified high purity toluene. The commercial high purity toluene (Aristar, BDH), was found to be contaminated with inorganic mercury. Purified toluene was therefore prepared by extracting the toluene with a 0.05% of EDTA solution (Analar, BDH). All glass flasks, bulb pipettes and PTFE beakers were soaked with hydrochloric acid (4% v/v) and thoroughly rinsed with deionized water before use.

Instrument

The two line flow injection-atomic fluorescence spectrometer system shown in Figure A. consisted of a peristaltic pump (Ismatec, minis), rotary injection valve (Ominifit), gas - liquid separator (P S Analytical), and an atomic fluorescence detector (Merlin, P S Analytical) which monitors the 253.7 nm line. The flow rate for the carrier (0.01 M HCl) and reductant streams (5% $SnCl_2$ in 15% HCl) is 4.0 ml min^{-1}.

A centrifuge (UV, international Equipment Co., Needham Heighte), centrifuge tubes with glass caps (50 ml, capacity) and transfer pipette (Pasteur - type) were used for phase - transfer of methylmercury from fish samples to the organic solvent phase.

All glassware was carefully washed with detergent and rinsed thoroughly with tap water followed by distilled water. It was then placed in a 4% hydrochloric acid bath (Analar, BDH) for 48 hours. The pre-cleaned glassware was rinsed three times with acetone (Analar, BDH) and three times with toluene (Analar, BDH) and dried under a clean hood before use.

The instrumentation parameters were the same as those previously described in section 2.6.4.

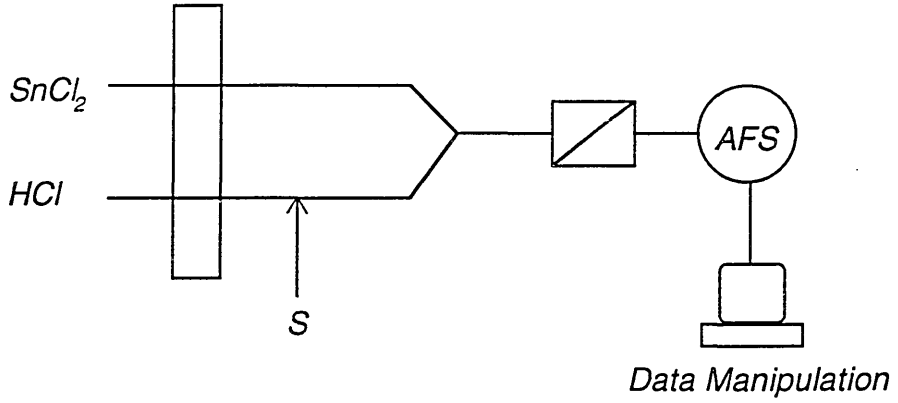


Figure A. Manifold of The Flow Injection - Atomic Fluorescence Spectrometry System for the Analysis in Toluene Extracts for Methylmercury.

Sample Treatment and Measurement

Owing to the high dilution factor (1:100) during the sample pretreatment, and the extremely low mercury concentration in final sample solutions, it is of paramount importance that the pretreatment procedure does not contribute greatly to the blank level. In this work, the quantity of toluene used for analyte extraction was relatively large, and the inorganic mercury level in this reagent was found to be relatively high (2 mg l^{-1}) [205]. For a 1 g fish sample, the blank level obtained would thus

be higher than the endogeous methylmercury concentration in final sample solutions. In order to minimise contamination from toluene, purification was carried out by adding 10 ml of 0.05% EDTA solution into a 100 ml pre-cleaned separating funnel which contained 50 ml of toluene (Aristar, BDH). The funnel was shaked vigorously by hand for one minute, and the EDTA solution layer discarded. The procedure was repeated to ensure no inorganic mercury remained in the toluene layer. Purified toluene was placed in a pre-cleaned flask for use.

Fish powder reference material was used for the routine method development. The sample preparation was performed by the modified AOAC official method [206]. In order to save time and conserve reagents, the extraction was carried out with the following modification: the bottle of fish sample was vigorously shaked for 2 minutes for homogenisation prior to weighing the sample. The method blanks were tested by taking empty centrifuge tubes through all the operation steps. Accurately weighed homogenised fish powder (1 g) was placed in a 50 ml centrifuge tube and 2.5 ml (1:1) hydrochloric acid solution added. Methylmercuric chloride was extracted by adding 20 ml purified toluene, and then gently shaking the tube for 2 minutes by hand. The cap was loosened and the tube centrifuged for five minutes at 2000 rpm. The toluene layer was carefully transferred to a 50 ml pre-cleaned flask using a dropping pipette. The walls of the centrifuge tube were rinsed with 2 ml of toluene. The extraction step was repeated. Both extracts were combined, diluted to 50 ml with purified toluene, stoppered, and mixed well for subsequent retreatment.

Oxidative pretreatment was carried out by adding a single 1 ml aliquot (MeHgCl in toluene) and 2.5 ml of the bromination solution to a glass volumetric flask (100 ml) containing deionized water and 5 ml of hydrochloric acid (Aristar, BDH).The sample solution was then diluted to the mark with deionized water. The flasks were gently inverted three times and allowed to stand for 20 minutes. The procedure was repeated

after a further 20 minutes. and again after another 20 minutes. After a total time of one hour the mercury in the aqueous phase was measured using flow injection - atomic fluorescence spectrometry. Optimum oxidation efficiency requires the bromine colour not to fade within a one hour standing time, prior to measurement.

The aqueous standard solutions of inorganic mercury (0.00 -10.0 μg Hgl^{-1}) were prepared and 0.5 ml of each standard was injected into the FI-CV-AFS system for calibration. An aliquot (0.5 ml) of pretreated sample solution was then injected into the carrier stream. The amplified signals were recorded by a strip-chart recorder (Hitach, 056) and standard software routines used for calibration and calculation.

Results and Discussion

Assessment of Performance

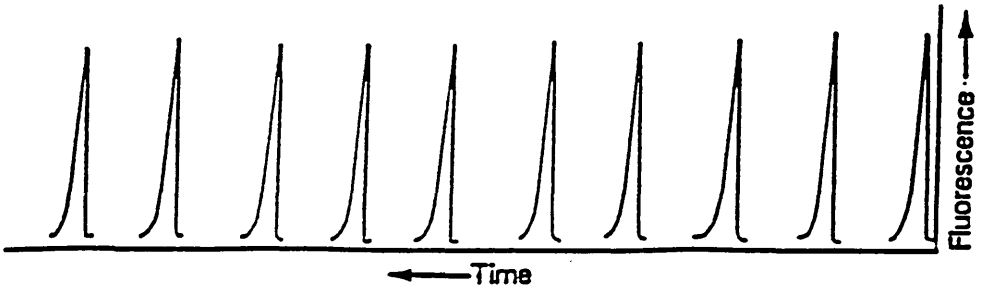
In assessing the usefulness of this new method it was assumed that there were two reactions taking place within the two-phase system: oxidation of organomercury and bromination of toluene. The latter is a side-reaction which consumes bromine and could suppress the oxidation reaction. This was confirmed by the observation of a rapid decoloration of bromine. Further tests showed that gentle shaking could minimise the side-reaction. Initial experiments also indicated that oxidative pretreatment of toluene samples was successful with intense and reproducible signals being registered using FI-CV-AFS. The fluorescence signals given in figure B correspond to processing and measurement of a standard solution of MeHgCl (2.5μg l^{-1}) in toluene. The upper signals represent replicate measurements (n = 10) of a single processed sample whereas the lower correspond to measurements (n = 10) performed on 5 separate samples. It is clear from the results that the method is reproducible although some deterioration in precision occurred for replicate analysis of separate samples (% RSD upper, 2.6% ; % RSD lower, 4.1%). As a check on recovery the oxidative procedure was performed

in triplicate for standard solutions of methylmercuric chloride in toluene (2.0 $\mu g\ l^{-1}$, as Hg) and for inorganic mercury in aqueous solution (2.0 $\mu g\ l^{-1}$). The results summarised in table A. confirm efficient oxidative pretreatment, with signal responses being independent of chemical form. This finding has a important consequence for calibration, since it permits the use of either aqueous or non aqueous synthetic standard solutions. Typical calibration data for the concentration range 0.5 - 10.00 $\mu g\ l^{-1}$ are presented in Figure C.

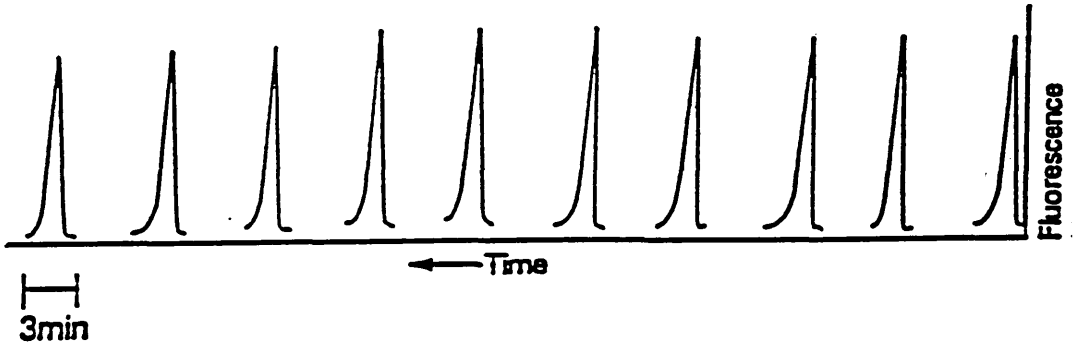
Sample Analysis

The determination of methylmercury in a biological reference material (DORM-1) was the important step to assess the new pretreatment method. DORM-1 is available form the National Research Council of Canada, with a certified content of 0.731 ± 0.06 mg Hg kg^{-1}. For the methylmercury determination in this material, five replicate samples were taken and pretreated with this new method. The results show there is no significant difference in the results at the 95% confidence level (see table B.), as the methyl-mercury content of the fish sample was found to be 0.769 ± 0.05 mg $Hgkg^{-1}$, which was in fairly close agreement to the certified value of 0.731 ± 0.06 mg $Hgkg^{-1}$.

Candidate reference materials issued by the Community Bureau of Reference of (EC) have also been analysed by the proposed method.



(a) Fluorescence signals showing replicate measurement of a single processed sample (MeHgCl 2.5 μg l^{-1}) % RSD = 2.6



(b) Fluorescence signals corresponding to measurements performed on 5 separate samples (MeHgCl 2.5 μg l^{-1}) % RSD = 4.1

Figure B. (a) and (b) Fluorescence signals of Pretreated Methylmercury Toluene Extracts

Table A. Analyte Recovery For Analysis of Standard Solutions (Hg 2.0 $\mu g\ l^{-1}$)

	Aqueous Standard		Toluene Standard*	
	Analysed Value# ($\mu g\ l^{-1}$ Hg)	% recovery	Analysed Value# ($\mu g\ l^{-1}$ Hg)	% Recovery
Run 1	1.98	99	2.09	105
Run 2	2.15	108	2.01	100
Run 3	2.06	103	2.00	100
Run 4	2.09	105	2.08	104
Run 5	2.02	101	2.12	106
Mean Value	2.05±0.07		2.06±0.05	
Mean Recovery		103		103

* Each value, mean of 2 injections; uncertainties, ± 1 standard deviation;
# prepared as 2.5 $\mu g\ l^{-1}$ MeHgCl.

Table B. Analytical Data for Dorm-1 Reference Material Methylmercury mg $Hgkg^{-1}$

Reference Material	this method#	Certified value
1	0.786	
2	0.793	
3	0.681	
4	0.812	
5	0.773	
mean value	0.769±0.05	0.731±0.06

Each value is the mean of two injections;
uncertainties, ± 1 standard deviation.

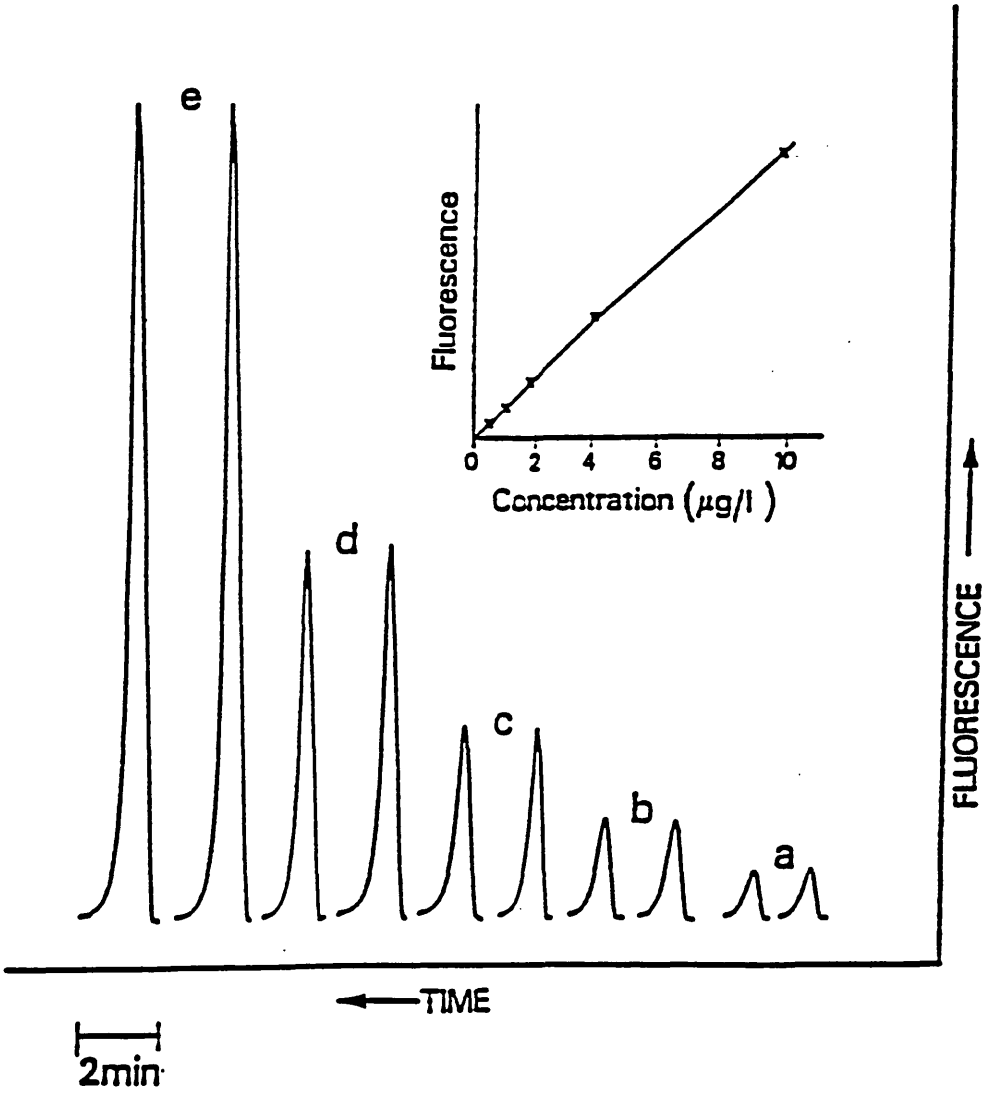


Figure C. Calibration curve for methylmercury in toluene extracts within the concentration range from 0.5 - 10.0 µg l^{-1}.

APPENDIX II

DETERMINATION OF INORGANIC MERCURY IN SEA WATER: ASSESSMENT OF HOMOGENEITY OF BCR SAMPLES

As part of an interlaboratory study organised by the Community Bureau of reference (BCR) of European Community, the determination of mercury at the ng l^{-1} level in 17 bottles of sea water was performed in an attempt to assess sample homogeneity [207]. A combined system of gold amalgam preconcentration - continuous flow - cold vapour - atomic fluorescence spectrometry was assembled and successfully used for the analysis of these sea water samples. The work reported refers to methodology and performance for synthetic standard solutions. For reasons of confidentiality data for sea water are excluded.

Experimental

Instrumentation and Reagents

The instrumentation used consisted of a peristaltic pump (Ismatec, mini-s), gold amalgam preconcentration unit (Galahad, P S Analytical) and an atomic fluorescence

detector (Merlin, P S Analytical). The wavelength for measurement was 253.7 nm. A schematic diagram of the AF system is shown in figure D. The system based on atomic fluorescence measurement was operated in a normal analytical laboratory and, to minimise dust, was enclosed by a protective covering of polyethylene sheet. Experimental parameters were as follows:

Manifold: flow rate of carrier (Millipore water) and reductant
streams (3% $SnCl_2$ in 15% hydrochloric acid): 3.5 ml min^{-1};
flow rate of aeration argon: 2 l min^{-1};

Gold Amalgam Unit: flush time: 30 seconds;
vaporisation time: 20 seconds;
cooling time: 2 minutes;
flow rate of cooling argon: 2.5 l min^{-1}.

The unit was operated manually.

Atomic Fluorescence Detector:
sensitivity range: 1000 x 2.

All acids were high purity grade (Aristar, BDH). Inorganic mercury working standards (in 1% v/v nitric acid) were freshly prepared by serial dilution from a commercial stock solution (1000 mg l^{-1}). A tin chloride solution (3% w/v) was prepared daily by dissolving the reagent in hydrochloric acid (15% v/v, Aristar, BDH) solution. A hydrochloric solution (5 M) was prepared by dilution of high purity acid (Aristar, BDH). Two separate argon lines for mercury aeration and gold amalgam cooling were utilised; the former was purified with an on-line charcoal column.

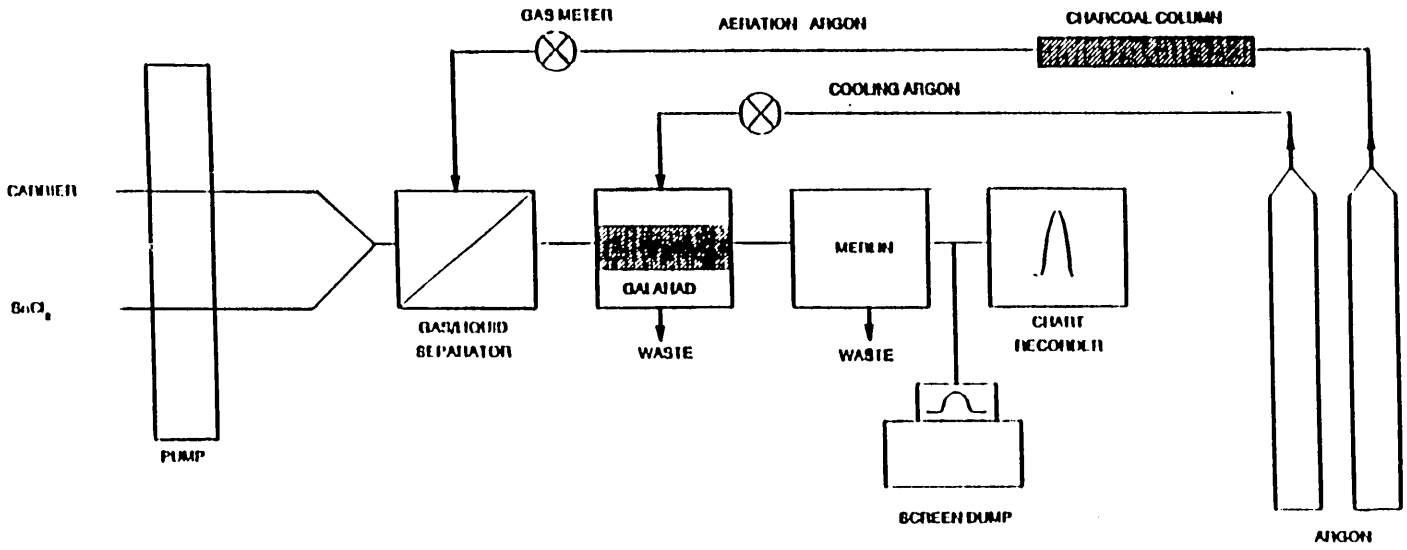


Figure D. AFS system for Ultratrace Determination of Mercury in Sea Water Samples.

Analytical Procedure

The AFS system was first precleaned by pumping hydrochloric acid (5 M) for 10 minutes. Purified argon was allowed to flow through the gas/liquid separator, the gold amalgam unit and the AF detector for 20 minutes before analysis. Nitric acid solution (1% v/v) as carrier, and tin chloride solution as reductant, were then pumped as shown in figure D. After pumping for two minutes, the pump was stopped and the heating cycle of the gold amalgam unit commenced. This procedure was repeated twice.

The first calibration standard was then introduced into the carrier line for one minute and the pump was stopped. The heating cycle was initiated to vaporise mercury although the signal was not recorded. The pump was started again and the standard was pumped for precisely two minutes (corresponding to a sample volume of 7 ml). On stopping the pump and after a delay time of 30 seconds, the heating cycle was initiated. System software was activated to display the transient fluorescence signal which was also registered on a chart recorder. The total time for analysis of a single sample was 5 minutes. Exactly the same procedure was performed for other standards and sea water samples.

Analytical Performance

A calibration graph based on the measurement of the three standard solutions (0.00, 5.00 and 10 ng l^{-1} Hg in 1% nitric acid) was prepared. The analytical curve, presented in Figure E, did not pass through the origin due to trace contamination in the low standard (Millipore water + 1% HNO_3 = 2 ng l^{-1} Hg) Transient signal response for the standard solutions are given in Figure F. and short term precision, as indicated in Table C., for the 5 ng l^{-1} standard was good.

Experiments were performed to test the reproducibility of thr blank signals and to establish the lower limit for quantitative determination. Typical signal responses and

Table C Replicate analysis of Standard Solution (5 ng l^{-1})

replicate No	1	2	3	4	5	X	S	RSD
concentration (ng l^{-1})	5.02	4.87	5.11	4.98	4.93	4.98	0.09	1.8

Table D Replicate analysis of Standard solution (0 ng l^{-1})

replicate No	1	2	3	4	5	6	7	X	S	RSD
concentration (ng l^{-1})	2.93	2.99	2.98	2.86	2.93	2.85	2.75	2.90	0.085	2.9

analytical values are given in Figure G(a) and Table C for replicate injections of the low standard (Millipore water +1% HNO_3; 0.0 ng l^{-1} Hg). Signal responses were significantly less for (i) processing of Millipore water alone (i.e. HNO_3 not added): the blank signals and to establish the lower limit for quantitative determination. Typical signal responses and analytical values are given in Figure G(a). and Table D for replicate injections of the low standard (Millipore water + 1% HNO_3; 0 ng l^{-1} Hg). Signal responses were significantly less for $X = 1.49 \pm 0.16$ ng l^{-1} (see Figure G(b) and for (ii) measurements without pumping solution (i.e. signals reflect contribution from argon gas and residual system contamination): $X = 0.97 \pm 0.13$ ng l^{-1} (See Figure G c). On the basis of the above data the lower limit for quantitative determination was taken as 1 ng l^{-1}. (For completeness of the experiment calibration standards are also given in Figure G(d).

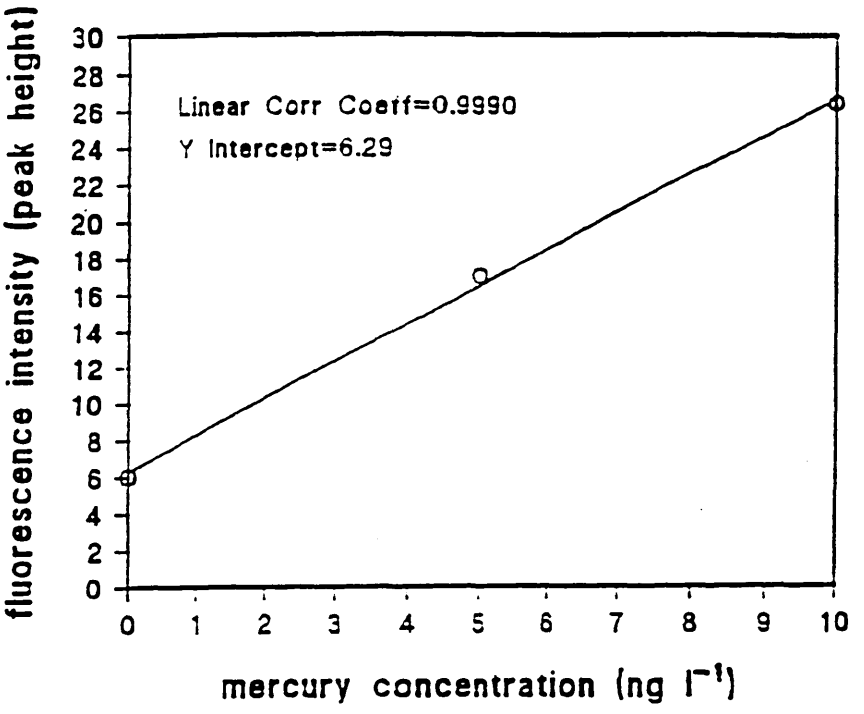


Figure E. Inorganic Mercury Calibration Graph

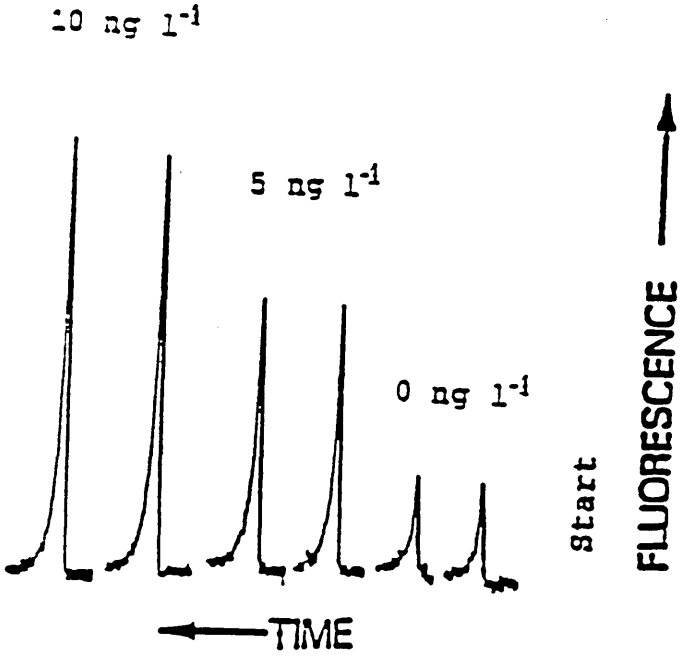


Figure F. Fluorescence via time response for standard solutions

Analysis of Sea Water Samples

Procedure

The sea water samples (20 bottles) were stored in a cool room (4°C) on receipt. The day before analysis, bottles were moved to the analytical laboratory (room temperature, 10°C) and left standing overnight. At this point it was noted that 3 bottles had been smashed. The integrity of the remaining 17 bottles including seals of the plastic bags were intact.

At commencement of analysis, after establishing the calibration graph, the bottle were opened under the polyethylene protective cover and tubing of the inlet manifold directly introduced to the sample bottle in order to withdraw precisely 7 ml of sample. Cross contamination was avoided by adhering to the procedure specified in Chapter 2.

Analysis was carried out on two separate days. For Run 1 (16 January 1992),calibration standards were recorded and then each bottle of sea water was sequentially analysed, interdispersed with a 5 ng l^{-1} standard. The total analysis time was 7 hours.

For Run 2 (23 January 1992), the different bottles were analysed in a sequential manner (1 measurement/sample) and this was followed by 17 replicate analysis of a single sample bottle. A 5 ng l^{-1} standard was analysed at the beginning and end of the analysis.

Results and Discussion

For reasons of confidentiality, analysed values for sea water are not reported. Method sensitivity however was sufficient to quantify mercury at the low ppt level in sea water and as a result homogeneity testing was successfully performed.

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STUDY PROGRAMME

As part of the research programme the author has:

Attended selected lecture courses and departmental research meetings.

Presented work at research group meetings.

Presented work at:

i) The Royal Society of Chemistry, R & D Research Meeting held in Dublin, March, 1989.

ii) The Euro-Analysis VII Conference held in Vienna, August, 1990.

iii) The Royal Society of Chemistry, R & D Research Meeting held in Runcorn, May, 1991.

iv) The Royal Society of Chemistry, R & D Research Meeting held in Aberdeen, July, 1992.

Demonstrated on BSc Analytical and Instrumentation courses (1989 - 1992).

Completed work at the laboratories of the B.G.S., Wallingford, National River Authority, North West Region, Warrington, and Yorkshire Water, Sheffield.

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