Mercury Species in Environmental Samples Studied by Spectroscopic Methods

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Abstract

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In this thesis, attention is focused on the determination, stability, reaction, and chemical association of mercury species in various types of environmental samples. Capillary gas chromatography (GC) was coupled to a number of systems for mercury speciation analyses, including microwave-induced plasma atomic emission spectrometry (MIP-AES), inductively coupled plasma-mass spectrometry (ICP-MS), atomic absorption spectrometry (AAS) and mass spectrometry (MS). In addition, synchrotron-based mercury L_{III}-edge extended X-ray absorption fine structure (EXAFS) spectroscopy, sulphur *K*-edge X-ray absorption near edge structure (XANES) spectroscopy were used to explore the chemical association of monomethyl mercury (CH_3Hg (II)) in soil organic matter (SOM).

Reactions of mercury species in organic solutions were investigated to determine the stability of mercury species in hydrocarbon products, standards and reference materials. A simple and rapid sample preparation procedure including acid leaching and simultaneous ultrasonic-assisted *in situ* ethylation and extraction was developed for the determination of methyl mercury in biological samples. The efficiency of different solvent extraction and derivatisation methods for the determination of methyl mercury in soils was evaluated. It was found that the binding affinity of Cu^{2+} to SOM functional groups and affinity of Br^- for mercury ions are strong enough to extract in average 93% of the total mercury in the soil samples investigated. The results indicated that the extraction efficiency was related to the oxidation state of sulphur present in the soil. Reactions with Grignard reagent for the derivatisation of mercury species for GC separation were characterised. Evaluation of derivatisation efficiency and species transformation reactions allowed for efficient optimisation of methods to reduce analytical errors.

EXAFS analysis provided direct molecular level evidence for the bonding between mercury (in CH_3Hg (II)) and sulphur in the first shell at a CH_3Hg (II)/Org-S_{RED} ratio below 0.26 in SOM. Calculated surface complexation constants were in the range of $10^{16.3}$-$10^{16.7}$, that was very similar to stability constants for associations between methyl mercury and thiol groups (RSH) in well-defined organic compounds. Concentrations of CH_3Hg (II), Hg-tot and sulphur species were determined in soil, soil solutions and streams of a forested catchment. Higher ratios of CH_3Hg (II)/Hg-tot were found in stream bank soils and open mire peat soils, indicating that these sites provide favourable conditions for net methylation on a landscape level. Org-S_{RED} made up 50 to 78% of total S in all soil and stream organic samples examined. Model calculations showed that under oxic and slightly reduced conditions nearly all the Hg (II) and CH_3Hg (II) were complexed by Org-S_{RED} in soil, soil solution and stream water.

Key words: biological samples, derivatisation, EXAFS, GC-AAS, GC-ICP-MS, GC-MIP-AES, GC-MS, mercury species, methyl mercury, organic solution, soil solution, SOM, stream water, XANES.

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To My Grandfather's Mother
To My Family

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Appendix

Papers I-VI

This doctoral thesis is based on studies reported in the following papers, which will be referred to in the text by the corresponding Roman numerals.

I. Snell, J., J. Qian, M. Johansson, K. Smit and W. Frech. 1998. Stability and reactions of mercury species in organic solution. *Analyst 123*, 905-909.

II. Tu, Q., J. Qian and W. Frech. 2000. Rapid determination of methylmercury in biological materials by GC-MIP-AES or GC-ICP-MS following simultaneous ultrasonic-assisted *in situ* ethylation and solvent extraction. *Journal of Atomic Absorption Spectrometry 15*, 1583-1588.

III. Qian, J., U. Skyllberg, Q. Tu, W. F. Bleam and W. Frech. 2000. Efficiency of solvent extraction methods for the determination of methyl mercury in forest soils. *Fresenius Journal of Analytical Chemistry 367*, 467-473.

IV. Emteborg, H., J. Snell, J. Qian and W. Frech. 1999. Sources of systematic errors in mercury speciation using Grignard reagents and capillary gas chromatography coupled to atomic spectrometry. *Chemosphere 39*, 1137-1152.

V. Qian, J., U. Skyllberg, W. Frech, W. F. Bleam, P. R. Bloom and P.-E. Petit. Binding of methyl mercury to reduced sulphur groups in soil organic matter as determined by X-ray absorption spectroscopy and binding affinity studies. (Manuscript.)

VI. Qian, J., U. Skyllberg, W. Frech, K. Xia and W. F. Bleam. Distribution of mercury and organic sulphur species in soil, soil solution and stream of a forested catchment. (Manuscript.)

1. Introduction

1.1. Overview

1.1.1. Speciation

It is well recognised that knowledge about the toxicity, metabolism and bioavailability of metals depends not only on their total concentrations but also on their chemical forms and their bonding to natural components in the environment. Without the species information, data for total metal concentrations are at best insufficient for adequate risk assessment and in many cases misleading. In addition, the chemical form of a metal is important for transport of the species in environment compartments. The analysis of metal species has proved critical in assessing their direct influence on the environment, predicting their transport behaviour in several compartments and the approach to the decontamination of hazardous pollutant materials (Wilken, 1992; Lobinski, 1998; Uria and Sanz-Medel, 1998). Therefore the importance of analytical methods for speciation analysis has increased over the last decade. Examples of species analysis include the different oxidation states of elements, the nature of complexes of metals in surface water or in body fluids, or the analysis of organometals such as Hg-, Sn-, Pb-, As-, Se-organic compounds in the environment.

The term "speciation" is defined in many different ways depending on the background of the scientist defining it. Recent discussions at the International Union of Pure and Applied Chemistry (IUPAC) have clarified the scope of speciation, which covers the following concepts (Quevauviller, 2000):

- chemical species: specific form of an element defined according to its molecular, complex, electronic or nuclear structure;
- speciation analysis: measurement of the amount of one or more individual chemical species in a sample;
- speciation of an element: distribution of defined chemical species of an element in a system.

Today we are witnessing a growing interest in metal speciation. As an example of these investigations, speciation of mercury in samples of environmental origin is the subject of this study.

1.1.2. Environmental samples

Environment describes a living thing's surroundings, including but not limited to plants, animals, sediment, soil, water, air and human products. Samples from industrial and biological origins are actually environmental samples. But in most cases, samples from industrial sources such as gas condensate are called industrial samples and samples taken from plants and animals are specified as biological samples, while environmental samples normally refer to sediment, soil, water, air and so on which are from natural sources. In this thesis, environmental samples analysed for mercury species include synthetic gas condensate, lobster, fish, soil, soil solution, stream water and sediment.

1.1.3. Mercury in the environment

Mercury exists in three oxidation states in the environment: Hg^0 (metallic, 0); Hg_2^{2+} (mercurous, +I); and Hg^{2+} (mercuric, +II). Mercury is a rare element in the earth's crust and it has an average crustal abundance on earth of approximately 0.05-0.10 mg kg^{-1}, the majority of which occurs as the mineral cinnabar (Fergusson, 1990).

As a chemical element, mercury cannot be created or destroyed. The same amount has existed on the planet since the earth was formed. Mercury, however, can cycle in the environment as part of both natural and human (anthropogenic) activities. Natural sources and transport mechanisms include volcanic emissions, wind borne dust, geysers, thermal fluids and sea-spray. Recent estimates of global emissions of mercury to the atmosphere are highly variable, ranging from 2 000-3 000 tons per year to 6 000 tons per year, owing to the uncertainty about natural emission rates (Fergusson, 1990; Morita et al., 1998). Most of the mercury in water, soil, sediments or plants and animals is in the form of inorganic mercury and organic forms of mercury (e.g., methyl mercury). As it cycles between the atmosphere, land and water, mercury undergoes a series of complex biological, chemical and physical transformations, many of which are not completely understood. Humans, plants and animals are routinely exposed to mercury and accumulate it during this cycle, potentially resulting in a variety of ecological and human health impacts (Keating et al., 1997).

Research results indicate that human activities have altered the cycling of mercury and the amount of mercury mobilised and released into the biosphere has increased since the beginning of the industrial age (Mason et al., 1994; Jackson, 1997; Fitzgerald et al., 1998; 2000). Around 4 000 tons mercury per year was estimated to be released to the atmosphere by human activities (Mason et al., 1994). Mercury and its compounds have been extensively used in the production of electrical goods, catalysts, pulp and paper products, pigments, dental applications and pesticide formulations. About half of the anthropogenic input to the environment has come from the manufacturing of caustic soda and chlorine by the electrolysis of brine (Craig, 1986). Because of such a wide use and the volatility of some species, mercury is now a global pollutant that has been measured in the deep ocean, the atmosphere, Antarctica and the Arctic (Fergusson, 1990).

More recently, interest has grown about the biogeochemistry of mercury in the environment. Mercury in the atmosphere occurs almost exclusively as elemental mercury vapour (Hg^0). Hg^0 can circulate in the atmosphere for up to a year, and hence can be widely dispersed and transported thousands of miles from sources of emission (Keating et al., 1997). Oxidised forms such as Hg (II) and methyl mercury (I) typically constitute less than 2% of the total concentration in air (Fitzgerald, 1986; 1989). Inorganic mercury can be methylated to mono- and dimethyl mercury (in this thesis, methylmercury refers to monomethyl mercury if not stated otherwise). Laboratory and field studies have shown the methylation of Hg (II) by micro-organisms, but the contribution of abiotic processes has yet to be completely ascertained

(Compeau and Bartha, 1985; Weber, 1993; Hintelmann et al., 1995). Methylation occurs mainly in soils and sediments, under both aerobic and anaerobic conditions, and is favoured at neutral to acidic pH. The major factors affecting the process include temperature, sulphide concentration, organic and inorganic ligands, pH and redox conditions. A part of environmental mercury is bound to reduced inorganic sulphur, forming insoluble HgS, which may accumulate in sediments and reduced soils. Methyl mercury comprises approximately 0.1-1.5% of the total mercury in sediments and about 2% of the total mercury in seawater. In terrestrial ecosystems, some plants are known to concentrate Hg as less-toxic chemical forms such as elemental Hg or as HgS (Bloom and Effler, 1990; Downs et al., 1998; Morita et al., 1998).

1.1.4. Mercury in Sweden

Substantial amounts of mercury have been emitted to the atmosphere and water during the 1940s, 50s and 60s. The main sources were fungicides, the chloralkali industry, metal production and combustion of waste. More recently combustion of fossil fuels has been the major source and most mercury reaching Sweden is long-range transported through the atmosphere from central and western Europe. The atmosphere received about 20-30 tons per year and waters several tons per year in middle Sweden and along the coast of northern Sweden (Länsstyrelsen, 1993). As a result of the increased deposition of mercury during the 20th century, the amount of mercury in soil, water and lake sediment has increased by a factor of 4 to 7 in southern and middle parts of Sweden and by a factor of 2 to 3 in the north. In fish the mercury concentration has increased with a factor of 5 in many parts of the country. An accumulation of the highly toxic methyl mercury has led authorities to discourage people to eat fish from many lakes in Sweden. The Swedish Environmental Protection Agency has calculated that the concentration of mercury in fish in over 10 000 out of a total of about 83 000 lakes has Hg concentrations higher than 1 mg kg^{-1} and the concentration is still increasing in most of the lakes. The "health limit value" in Sweden is 0.5-1.0 mg Hg/kg in fish depending on fish species. For perch, pike, pikeperch, burbot, eel and halibut, the limit is 1.0 mg/kg. For all other species, the limit is 0.5 mg/kg (Johansson et al., 1991; Länsstyrelsen, 1993).

1.1.5. Toxicological effects and environmental impacts

One of the first scientifically documented incidents involving mercury pollution occurred in Minamata, Japan, and related to the accumulation of methyl mercury in fish and the subsequent poisoning of the local inhabitants (Kiyoura, 1964). This episode was a turning point for environmental levels of toxic metal species, because it was apparent that to provide a clear picture of toxicity, biogeochemistry and bio-accumulation, it is necessary to measure all the different physicochemical forms.

The contamination of the environment by mercury has been of great concern throughout the world for decades. For example, mercury poisoning, caused mainly by short-chain alkyl mercury compounds, was observed in marine life and humans at Minamata, Japan (Kiyoura, 1964), in marine birds in Sweden (Johnels

and Westermark, 1969) and in humans in Iraq (Morita et al., 1998). Mercury is a metal that has different toxicity for organic and inorganic forms. Data in both humans and experimental animals show that all forms of mercury (e.g., elemental, inorganic and methyl mercury) can produce adverse health effects at sufficiently high doses (Friberg and Vostal, 1972; Keating et al., 1997). Organic mercury compounds, of which monomethyl mercury is the most common, are of special concern because of their easy penetration of biological membranes, efficient bio-accumulation, high stability and long-term elimination from tissues (Westöö, 1973; Boudou and Ribeyre, 1985; Friberg et al., 1986; Bloom and Effler, 1990). Once present in the cell, mercury can interfere with a number of biochemical processes by binding to biomolecules containing thiol groups (Craig, 1986). The conditions for alkylation and de-alkylation of mercury are also very important because they determine both the immediate and the potential toxicity of an environmental sample.

Humans are most likely to be exposed to methyl mercury through fish consumption. Exposure may occur through other routes for different species of Hg as well (e.g., inhalation, drinking water and food sources other than fish, and dermal uptake through soil and water). In human, methyl mercury is known to be neurotoxic. The fetus is more sensitive to those effects than adults. Because of these risks, mercury and organic forms of mercury are included in the black list of compounds to be monitored in the framework of national and international regulations such as EC Directives (Quevauviller, 1996).

Fish eating birds and mammals are more highly exposed to mercury than any other known component of aquatic ecosystems. Adverse effects of mercury on fish, birds and mammals include death, reduced reproductive success, impaired growth and development, and behavioural abnormalities. Effects of mercury on plants include death and sublethal effects (Keating et al., 1997).

How much methyl mercury is harmful to humans? It is difficult to give an exact value because each individual is different. Recommended limits have been expressed in μg/kg body weight/day; concentrations of mercury in tissues such as blood and hair were published by WHO/IPCS in 1990. The WHO/IPCS estimated (1990) that a daily methyl mercury intake of 0.48 μg Hg/kg body weight will not cause any adverse effects to adults and that a methyl mercury intake of 3 to 7 μg/kg body weight/day would result in a <5% increase in the incidence of paresthesia in adults. In 1969, in response to the poisonings in Minamata Bay and Niigata, Japan, the U.S. proposed an administrative guideline of 0.5 μg/g for mercury in fish and shellfish moving in interstate commerce. This limit was converted to an action level in 1974 and increased to 1.0 μg/g in 1979 in recognition that exposure to mercury was less than originally considered. In 1984, the 1.0 μg/g action level was converted from a total mercury standard to one based on methyl mercury.

1.2. Mercury species in synthetic gas condensate

Mercury is present in natural gas and hydrocarbon products and must be monitored to satisfy environmental concern and to ensure the safe transport and efficient processing of these products. In addition to its poisoning of industrial catalysts, elemental mercury damages aluminium heat exchangers and pipelines by corrosion (Wilhelm and Bloom, 2000) and presents a health risk to engineers upon inspection and maintenance of mercury-contaminated equipment.

The majority of mercury in natural gas is in its elemental form (Hammer et al., 1996; Wilhelm and Bloom, 2000). Condensates are expected to reflect the mercury species composition of the gas, with elemental mercury being the dominant form while organic mercury species, particularly dimethyl mercury, are also present in smaller amounts (Snell et al., 1996). Other studies suggested the presence of trace amounts of monomethyl mercury, ethyl mercury and organic species containing sulphur (Tao et al., 1998; Wilhelm and Bloom, 2000).

The different chemical and physical properties of the species must be considered in the evaluation of potential health effects and in the design of systems to remove mercury from natural gas. A sample may have a long journey from sampling location to the analytical laboratory. To be able to estimate the contribution of storage conditions to the error of measurement, it is necessary to obtain data on the stability of mercury species. In addition, there is a need from industry for reference materials containing trace levels of mercury species to control the accuracy of the determination of mercury species. For the preparation of such a reference material, it is essential to know the stability of mercury species and possible mechanisms of their loss.

1.3. Mercury species in biological materials

Mercury accumulates in an organism when the rate of uptake exceeds the rate of elimination. Mercury accumulates most efficiently in the aquatic food web. Predatory organisms at the top of the food web generally have higher mercury concentrations. Although all forms of mercury can accumulate to some degree, methyl mercury accumulates to a greater extent than other forms of mercury. Inorganic mercury can also be absorbed but is generally taken up at a slower rate with lower efficiency than methyl mercury (Fisher et al., 1995; Wang et al., 1998; Bowles et al., 2001; Lawrence and Mason, 2001). Elimination of methyl mercury takes place very slowly resulting in tissue half-lives ranging from months to years. Elimination of methyl mercury from fish is so slow that long-term reductions of mercury concentrations in fish are mainly due to growth of the fish and subsequent dilution.

The analytical techniques most frequently applied for the determination of mercury species generally involve a chromatographic system for separation, combined with element specific detection (Quimby and Sullivan, 1990; Qvarnström et al., 2000). In addition, multi-element detection capabilities can be realised through coupling of chromatography to MIP-AES and ICP-MS (Bulska et al., 1991;

Emteborg et al., 1994; Tu et al., 2000). Despite the excellent sensitivity and selectivity of the above-mentioned techniques, several limitations, related to tedious sample pre-treatment steps, remain. The sample preparation protocols for the biological materials are often composed of three different steps: sample leaching/decomposition/distillation, extraction of mercury species into an organic solvent, and derivatisation. In general, these sample preparation steps make procedures time-consuming and can give rise to losses of analyte, contamination and/or species transformation. In order to minimise these disadvantages during analysis, simpler and more rapid sample preparation procedures need to be developed.

1.4. Mercury species in soils, soil solutions and streams

Soils may contain various species of mercury, i.e. organic mercurials, inorganic ions and elemental mercury, as a result of deposition and/or microbial and chemical activity (Lee et al., 1994; Fitzgerald et al., 1998). More than 98% of mercury in soil is inorganic, originating from geological sources and from atmospheric deposition (Davis et al., 1997; Morita et al., 1998). Mercury in soil is subjected to a variety of chemical and biological reactions, determining concentrations and composition of species and their complexes. These reactions depend on soil conditions such as redox potential, dissolved and solid phase organic substances, soil pH, mineral content and composition, temperature and moisture content (Revis et al., 1990; Warfvinge, 1997). Mercury in soil is effectively bound to soil organic matter (SOM) pertaining to both aqueous and solid phases in the upper soil horizons. Mercury concentrations reaching streams are therefore highest during high-flow events when dissolved mercury-SOM complexes are being transported through near-surface soil layers (Bishop et al., 1995). According to Wilken (1992), most of the water soluble mercury species in soil are complexed, often in a colloidal form, by SOM. The molecular weight is higher than 500 and the particles are smaller than 1.2 μm in grain size.

The risk of Hg in soil is related to exposure pathways between soil and humans. The mercury concentration in soil has increased with at least a factor of 5 during the 20th century (Revis et al., 1990; Canady et al., 1997; Davis et al., 1997). Exposure pathways include Hg contamination of aquatic organisms, edible crops, drinking water and air. Human exposure may also occur as a result of direct ingestion of contaminated soil (Ford and Gruba, 1984; Revis et al., 1990).

Mercury deposited in the form of Hg^{2+} may either be strongly bound to SOM by reduced sulphur groups (Xia et al., 1999; Skyllberg et al., 2000) or undergo transformation to CH_3Hg^+ as a result of methylation processes. The opposite process, i.e. demethylation, also takes place in soils and therefore the size of the "pool" of methyl mercury is dynamic and sensitive for changes in the environment. Methylation is known to be enhanced in environments with periodically changing redox conditions, such as reservoirs (Morrison et al., 1994) and wetlands (Hurley et al., 1995; St. Louis et al., 1996). Little is known about factors affecting demethylation rates in soils and waters (Zillioux et al., 1993). The type of chemical bonding of CH_3Hg^+ and Hg^{2+} to SOM should be of central importance for both methylation

and demethylation processes. It has been shown that the bonds through which methyl mercury associates with well-defined organic compounds are important for its stability and reactivity. Also the bioavailability and detoxification of CH_3Hg^+ are affected by the identity of binding ligand and the coordination number in organic substances (Miller et al., 1989; Moore et al., 1990; Barone et al., 1995). Thus, the binding strength of methyl mercury to soil organic functional groups is important for understanding the cycling of methyl mercury in the environment.

Mercury speciation analysis in soil samples is important for understanding the biogeochemistry and physiological processes leading to an accumulation of mercury in organisms at higher trophic levels (Revis et al., 1990; Zillioux et al., 1993). Despite this demand for information we have a limited knowledge in this field. Mercury speciation analysis of soil is for several reasons a difficult task. Natural organic ligands binding mercury are very heterogeneous and therefore their structure and association to mercury is difficult to determine. These ligands originate from different sources and are in various states of decomposition. Moreover, methods have to be optimised for the very low concentrations of mercury species in soil samples in order to guarantee accuracy and precision (Horvat et al., 1993; Vazquez et al., 1997). Natural levels of methyl mercury are in the lower ppb range and no certified reference material is yet available for soil. As a substitute, certified sediment reference materials are used for quality assurance. The majority of soil data so far reported have been obtained by various distillation (Horvat et al., 1993; Lee et al., 1994; Bloom et al., 1997) or solvent extraction procedures (Rogers, 1977; Hintelmann et al., 1995; Bloom et al., 1997). Little evidence is available showing the accuracy of these results. In order to obtain accurate results sources of systematic errors such as unintended abiotic methylation of inorganic mercury during sample work-up, interferences caused by co-elution reaction by-products obtained when GC separation is used, and chemical interferences caused by halogens need to be investigated and thereby corrected for (Talmi and Mesmer, 1975; Horvat et al., 1993; Emteborg et al., 1996; Bloom et al., 1999 Quevauviller and Horvat, 1999).

2. Objectives

The objectives of my research were:

a) to improve and validate different spectroscopic methods to determine the concentration of mercury species in industrial, biological, soil and other environmental samples;

b) to study the stability and reaction of mercury species in synthetic gas condensate;

c) to investigate the distribution and chemical association of mercury species in soil, soil solution and stream.

The detailed objectives for each attached paper are listed as following:

I. Determination of the stabilities of elemental mercury, mercury (II) chloride, methyl mercury chloride and dimethyl mercury in synthetic gas condensate (paper **I**);

II. Development and validation of a simplified and rapid procedure for mercury speciation in biological materials based on derivatisation in aqueous phase in the presence of an organic solvent (paper **II**);

III. Development and validation of solvent extraction methods for the determination of methyl mercury in soils (paper **III**);

IV. Investigation of spectral and chemical interferences that occurred when using Grignard reagents for derivatisation of mercury species before capillary gas chromatography separation and atomic spectrometry determination (paper **IV**);

V. Exploration of the methyl mercury chemical binding structure and binding affinity in soil organic matter (paper **V**);

VI. Distribution of CH_3Hg (II) and Hg (II) in soils, soil solutions and streams of a forested catchment; identification of compartments and positions with favourable conditions for net methylation; determination of the concentration of organic sulphur species and model calculation of the speciation of CH_3Hg (II) and Hg (II) in soils, soil solutions and streams (paper **VI**).

3. Methods

Methods used for quantifying metal species and for establishing their chemical associations can be grouped into direct and indirect analytical approaches as well as chemical equilibrium and reaction modelling. Direct and indirect analytical methods can produce separate observations that can be combined to reveal the speciation of metals in environmental samples. Once total metal concentrations and possibly some major species have been determined, components, chemical equilibrium and reaction have been specified, modelling may be used to give a more complete picture of all possible species and their concentrations.

There are two ways for modelling (Mattigod, 1995). The commonly used method is the equilibrium constant approach, that uses the mass action principle which relates the activities of free metal, free ligand, and the metal-ligand complex. The mass action relationships are linked with mass balance equations, resulting in a set of linear equations. The equilibrium concentration of each component is obtained by solving the set of linear equations. The second way is the free energy minimisation

technique in which the chemical potential of each species is combined with the mass balance equation for each component. The equilibrium composition is calculated by minimising the free energy of the system. This approach is less common because the chemical potentials for a number of species are not as reliable as the equilibrium constants and also not as readily available (Nordstrom and Ball, 1984). Modelling is a very useful approach for an approximation to metal speciation in real samples (e.g., Harris, 1992; Qian et al., 1998). It is probably the only way to obtain quantified information of some species (e.g., Hg^{2+}, CH_3Hg^+) which are many orders of magnitude lower than detection limits of available analytical methods. There are limitations as well. In complex environmental matrices, we have limited knowledge regarding all the ligands able to interact, the corresponding constants of interaction, and the data for kinetics involved. Therefore the scope of this approach is restricted to well-known systems and the results should always be treated with care in real-life situations.

Direct analytical methods include techniques such as solid nuclear magnetic resonance, X-ray absorption spectroscopy (XAS) for non-crystallised material and X-ray diffraction for structural determination of crystallised material. Direct methods would be ideally suited for the determination of mercury species in various environmental samples because, in principle, they provide means to determine *in situ* a given species in a complex matrix without any pre-treatment. In this way disturbance of equilibria and/or mercury species composition could be avoided. Unfortunately, one of the major disadvantages of these methods is their low sensitivities and high detection limits (normally higher than ppm level). Despite this limitation, XAS techniques can still be useful because they are sensitive to the local structure of solid amorphous materials such as soil.

Indirect methods are taking the lead of mercury speciation analysis in environmental samples. These methods are, in most cases, based on a succession of operational steps and involve various types of couplings among separation tools and detectors. First, the mercury species are transferred from the solid matrix into a simpler solution, either directly or after further treatments such as derivatisation or purification. This step should be performed in such a way that mercury compounds are separated from the interfering matrix without losses, contamination, or change of speciation and with a minimum of introduced interferences. Distillation, solvent extraction, alkaline digestion, acid leaching and supercritical fluid extraction are some of the commonly used separation methods (Bayona and Cai, 1994; Quevauviller and Morabito, 2000). Subsequently, mercury speciation analysis is normally based on a combination of a separation technique (gas-, liquid-, or supercritical fluid chromatography or capillary electrophoresis) and element-specific detection. The reported detection systems include atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), Fourier transform infrared (FTIR), microwave induced plasma- or inductively coupled plasma atomic emission spectrometry (MIP-AES or ICP-AES), mass spectrometry (MS) or inductively coupled plasma mass spectrometry (ICP-MS). In some cases, non-specific detection has been used

but these methods normally do not provide detection limits low enough for the analysis of "real" samples. The choice of the hyphenated techniques depends primarily on the research objective. Among these techniques, GC-MIP-AES, GC-ICP-MS and GC-AAS were selected for our experiments due to their low detection limits, good reproducibility and high selectivity. In addition, GC-MS can provide identification and quantification of a broad range of unknown compounds. This makes this technique unique and we therefore used it in this study as well.

The different indirect and direct methods used in this thesis are briefly described below.

3.1. GC-MIP-AES

Traditionally, the most widely used methods for the determination of methyl mercury are based on packed column GC with detection by an electron capture detector (ECD) (Westöö, 1966). However, poor reproducibility of the packed column was observed (Hight and Corcoran, 1987; Emteborg et al., 1993; Quevauviller et al., 1996) and it was necessary to condition the column using a number of injections of mercury chloride to maintain column performance (Hight and Corcoran, 1987). In addition, these methods suffer from the non-selective detector (ECDs are not selective for mercury but rather for the halide moiety in organomercury chlorides). Recent improvements include new separation techniques using capillary column GC and element-specific techniques for detection, e.g., AAS, MIP-AES and ICP-MS, to obtain higher sensitivity and selectivity. Since methyl mercury compounds present in the environment are mostly polar or ionic, derivatisation of the analyte is recommended for GC work, i.e., the transformation into species suitable for the chromatographic processes. Derivatisation must result in volatile and thermally stable compounds for better chromatographic separation. The most common derivatisation procedures used include Grignard reagent alkylation (Bulska et al., 1991; Emteborg et al., 1993; Minganti et al., 1995), aqueous phase ethylation using sodium tetraethylborate ($NaBEt_4$) and hydride generation using sodium borohydride ($NaBH_4$) (Bloom, 1989).

In this work, the determination of methyl mercury was based on butylation with a Grignard reagent or aqueous phase ethylation, followed by GC-MIP-AES, as described previously (Bulska et al., 1991; Emteborg et al., 1993; Snell et al., 1996; Tu et al., 2000).

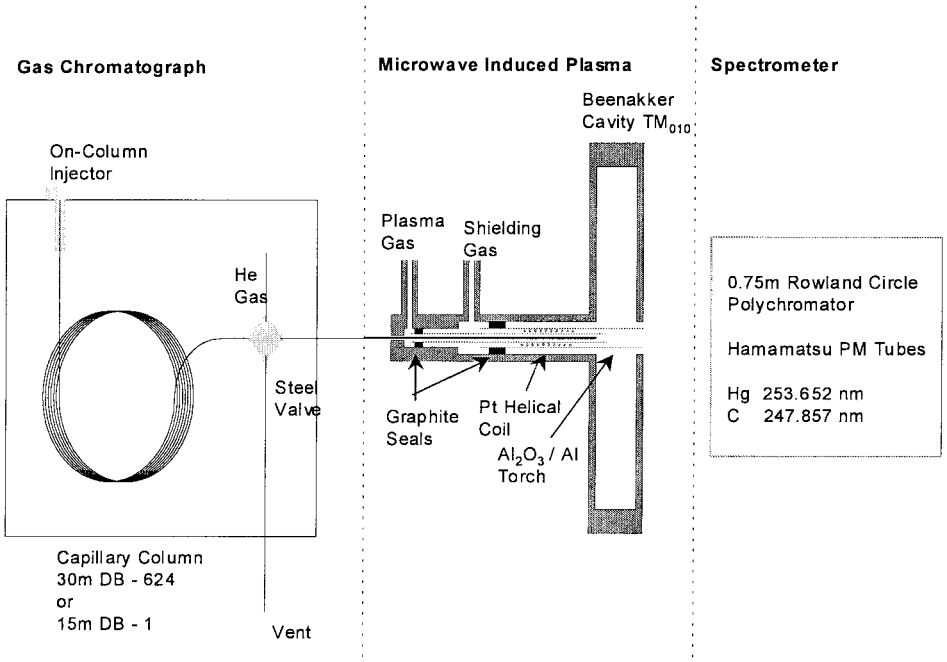

Fig. 1. *Simplified diagram of the GC-MIP-AES system.*

In order to separate different mercury species, two kinds of fused silica bonded capillary GC columns (DB-1, 15 m x 0.53 mm, 1.5 μm film thickness; DB-624, 30 m x 0.53 mm, 3 μm film thickness) were used. Poly dimethylsiloxane was used as DB-1 stationary phase while the more polar poly methylphenolsiloxane was used for the DB-624 stationary phase. Both columns were able to efficiently separate the components of interest.

MIPs use the microwave region electromagnetic radiation (2 450 MHz normally) to produce a hot, partially ionised gas, which forms the plasma. One of the frequently used MIPs is generated at atmospheric pressure in a Beenakker cavity (Beenakker, 1976; Ingle and Crouch, 1988). The electrons in the plasma gas start to oscillate in the electric component of a standing electromagnetic wave. When they have reached sufficient kinetic energy the support gas is ionised by collisions and plasma is formed. The absolute detection limit of MIP-AES for mercury is between 0.1 and 0.4 pg Hg (Quimby and Sullivan, 1990; Emteborg et al., 1995), which makes it one of the most sensitive methods for the detection of mercury. However, due to the low power input to the MIP, it cannot readily volatilise and atomise large amounts of sample which means that larger amounts of sample constituents will decrease the excitation potential or extinguishing the plasma (Ingle and Crouch, 1988). Therefore, the solvent peak eluting from the GC must be vented (Quimby and Sullivan, 1990; Sullivan and Quimby, 1990). Helium was chosen in our experiments both as a mobile phase for the GC and as plasma gas for the MIP.

AES deals with the radiative relaxation of atoms (i.e., by emitting photons of a characteristic wavelengths) following non-radiational excitation. In our work the 253.652 nm mercury line was used corresponding to the transition from the 6^3P_1 to the 6^1S_0 ground state.

Details of this work can be found in papers **I-IV**.

3.2. GC-ICP-MS

The GC separation is described in 3.1. The inductively coupled plasma (ICP) is an electrodeless discharge in a gas at atmospheric pressure, maintained by energy coupled to it from a radio frequency (RF) generator. The RF current supplied from the generator produces a magnetic field that varies in time at the generator frequency, usually 27 or 40 MHz in the systems used for ICP-MS. The discharge is usually initiated in a cold torch by a spark from a Tesla coil, which provides free electrons to couple with the magnetic field. The electrons oscillate around the magnetic field and the electrical energy supplied to the RF coil is converted into kinetic energy of electrons. At atmospheric pressure the free electron path before collision with an argon atom, to which its energy is transferred, is only about 10^{-3} mm, and thus the plasma is heated, forming a bright discharge.

ICP-MS distinguishes itself from the other spectrometers such as MIP-AES in that it provides isotope-specific information. This can be used for the study of the possible transformation of mercury species during sample pre-treatment and for standardisation (Hintelmann et al., 1997). The plasma gas is normally argon, the gas flow is much greater, at 15 l min^{-1}, and the applied RF power is also much greater at around 1 200-1 600 W. As a direct consequence, the plasma tolerated the introduction of higher masses of organic materials than MIP sources. Therefore there is no need for a solvent vent valve when GC is coupled to the ICP-MS, as the solvent caused only a brief disturbance that did not coincide with signals of interest. This indicated that there were no significant changes in ionisation potential or thermal temperature of the plasma caused by co-eluting matrix components. The GC-ICP-MS method was used in the work described in paper **II**.

3.3. GC-AAS

The GC separation is described in 3.1. If mercury atoms in a cell are irradiated with light originating from a line source (normally a mercury hallow cathode lamp), emission lines from the lamp might be absorbed by the atoms thereby decreasing the light intensity detected. This technique, atomic absorption spectrometry, is very sensitive, selective, simple and widely used especially for the determination of metals. Normally the atoms are generated in a flame or in an electrically heated graphite furnace.

In this work (paper **IV**), a gas chromatography – quartz furnace atomic absorption spectrometry (GC-QTAAS) system was used. The instrumentation was described elsewhere (Emteborg et al., 1996). Briefly, the analytical column was a 30 m long

0.53 mm i.d. megabore column coated with a 3 μm DB-624 film (J&W Scientific). A deactivated fused silica capillary was fed from the GC through a transfer line, held at 100°C using a resistance wire, to a 12 cm long pyrolysis unit. The pyrolysis unit was an electrically heated quartz furnace, and was kept at 800°C for destruction of the derivatised mercury species. Following pyrolysis, the mercury atoms were swept to an AAS-detector, which was a modified flow injection mercury system (Perkin-Elmer). The largest fraction of the light was directed through a quartz plate set at a 45 degree angle to a solar blind photo tube, sensitive in the far UV, used for detection of the absorption occurring at the 184.957 nm line. This line was found to be more than five times more sensitive than the conventional 253.652 nm line (Hoffmann et al., 1979). The rest of the light (about 4%) was reflected to another phototube for measuring the absorption on the 253.652 nm line for mercury. The dynamic range of both analysis lines provides a linear response over more than three orders of magnitude.

3.4. GC-MS

GC-MS makes an effective combination for chemical analysis. Components in the samples are separated using gas chromatography and then are shattered into mass fragments by either electron ionisation (EI) or chemical ionisation (CI). The mass fragments are separated according to their mass-to-charge ratio by a mass filter, which is one of the most commonly used. The resulting mass-to-charge pattern is the mass spectrum for that particular constituent. GC-MS can provide identification and quantitation of a broad range of unknown materials, both pure materials and mixtures, based on mass spectral and chromatographic data. It can also provide molecular weights and chemical structure information.

EI is the most commonly used method of ionisation, and a great number of organic compounds are amenable to EI. It was also used in our experiments. To give an EI spectrum, the compound must be volatile. Specifically, it must have a vapour pressure of at least 10^{-6} torr. It can be heated up to 400°C to achieve the necessary pressure. Ions are formed when a 70 eV beam of electrons hits the sample molecules in the gas phase. This gives the sample molecules an excess of energy such that many fragmented ions are formed. These ions can be useful in determining the structure of the molecule. Since samples must usually be heated, thermally labile samples are difficult to analyse, although it is possible to cool the ion source from the usual 200°C to about 50°C. Unfortunately, some compounds will fragment completely and not give molecular ions. The GC-MS technique has been applied for the determination of mercury species in environmental samples (Cai and Bayona, 1995). Our research related to this technique can be found in paper **I**.

3.5. XAS

X-ray spectroscopy, like optical spectroscopy, is based upon measurement of emission, absorption, scattering, fluorescence and diffraction of electromagnetic radiation. When a beam of X-rays is passed through a thin layer of matter, its intensity or power is generally diminished as a consequence of absorption and

scattering. The effect of scattering for all but the lightest elements is ordinarily small and can be neglected or corrected in those wavelength regions where appreciable absorption occurs.

Absorption of a X-ray quantum can cause ejection of one of the innermost electrons from an atom and the consequent production of an excited ion. The highest probability for absorption arises when the energy of the quantum is exactly equal to the energy required to remove the electron just to the periphery of the atom. As shown in Fig. 2, the absorption coefficient increases abruptly when certain critical electron energy is reached. This discontinuity is called the absorption edge. *K*- and L-edge refers to an excitation of electrons in the innermost and next innermost shell, respectively. The edge energy depends on the electron energy level in atoms and is also characteristic of each element. The absorption of X-rays is normally recorded as a function of the photon energy (Fig. 2). The development of synchrotron radiation facilities has greatly enhanced the use of XAS techniques because of improved possibilities offered by the fine and very intense beam in terms of energy resolution and intensity.

Two regions are usually defined in XAS, corresponding to different physical phenomena (Teo, 1986): extended X-ray absorption fine structure (EXAFS) spectroscopy and X-ray absorption near-edge structure (XANES) spectroscopy.

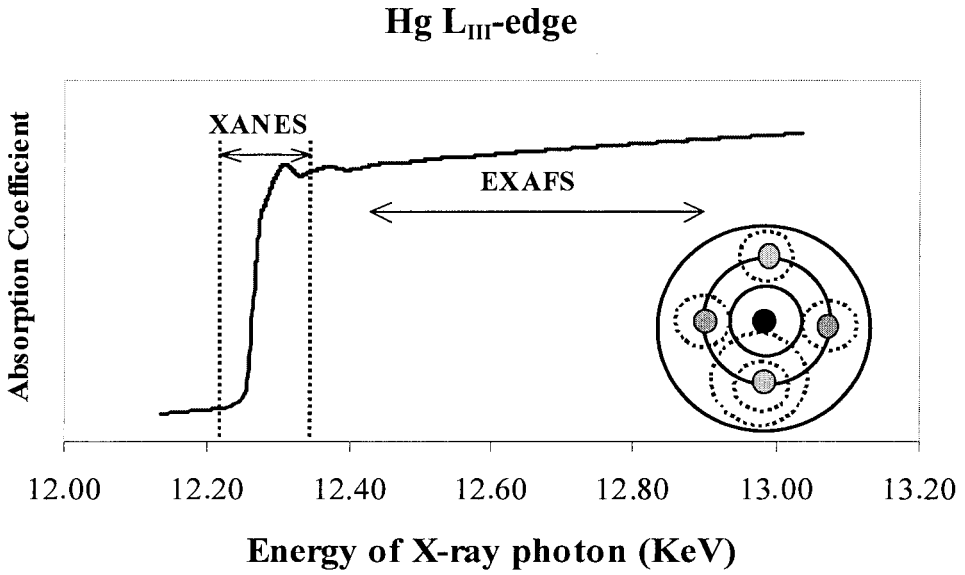

Fig 2. *Principles of XAS: absorption coefficient as a function of energy, example of the Hg L_{III}-edge. Insert right: interaction of the outgoing wave with the backscattering atoms.*

3.5.1. EXAFS

EXAFS reflects the fine structure in the X-ray absorption coefficient starting somewhat past the absorption edge and extending typically up to 1 000 eV. The EXAFS is a result of back scattered photoelectrons. Therefore it does not occur for isolated atoms, such as in the gaseous phase, but appears when atoms are in a condensed state. X-ray absorption in the photon range up to 40 keV, the range of most importance for EXAFS, is dominated by complete absorption of the photon, transferring its energy to excite a photoelectron and leaving behind a core hole in the atom. When the excited photoelectron has about 15 eV or greater kinetic energy, the energy is large compared with its interaction energy with the surrounding atoms (around 3 eV). Then the interaction with the surrounding medium can be treated as a perturbation about an isolated atom. The EXAFS is a direct consequence of the wave nature of the photoelectron. The distance between the centre atom and backscattering atoms will determine how the phase varies with the wavelength of the photoelectron. The variation of the backscattering amplitude as a function of the photoelectron energy depends on the type of backscattering atoms. Thus, EXAFS contains information on the atomic surroundings of the centre atom such as bond distance, number of atoms in the binding shells and atomic number of backscattering atoms. More detailed theory behind EXAFS and its interpretation can be found in Teo (1986) and Koningsberger (1988).

We used synchrotron-based Hg L_{III}-edge EXAFS to identify the groups in SOM that bind methyl mercury and to determine the coordination chemistry of this association (paper **V**).

3.5.2. XANES

XANES applies to the energy region just below the absorption edge, up to 50 eV past the edge.

The peak position in this region depends on the oxidation state of the central atom, atoms surrounding it and their symmetry. The peak position can vary by as much as 10 eV from the tabulated value. A detailed study can provide useful information about the geometry and the oxidation states of the atoms. Lytle (1984) can be consulted for detailed theory about XANES.

Synchrotron based sulphur *K*-edge XANES was used in papers **III**, **V** and **VI** to identify the sulphur oxidation states in SOM. The procedure of Xia et al. (1998) was followed. The spectra were fitted using a series of Gaussian peaks, representing the s→p transitions, and arctangent step functions, representing the transition of ejected photoelectrons to the continuum. The energy positions of the peaks were used to identify the oxidation states of sulphur and the corrected peak area was used to determine the relative quantities of each major S form in the sample.

3.6. Solvent extraction and derivatisation methods

3.6.1. Biological samples

Sample preparation protocols for biological materials are often composed of three different steps: sample leaching/decomposition/distillation, extraction of mercury species into an organic solvent, and derivatisation. The first step normally includes acid leaching, alkaline digestion and distillation.

In the well-known acid hydrolysis method for biological materials proposed by Westöö (1966), samples are typically homogenised in aqueous solution. Methyl mercury is released from proteins using hydrochloric acid and enriched in an organic solvent (benzene or toluene). This is followed by a cleanup step in which cystein is used to complex and concentrate CH_3Hg^+ before back-extracted into benzene. Many methods used to release mercury species from the binding sites of environmental sample matrix are variations of Westöö's extraction method following the steps of acid leaching followed by formation of a suitable mercury complex which is transferred into an organic solvent. Methods based on alkaline digestion or distillation are more time-consuming than acid leaching. In addition, these methods have been shown to occasionally overestimate the methyl mercury concentration owing to the risk of unintended methylation of inorganic mercury during sample preparation (Hintelmann, 1997).

After extraction, derivatisation with appropriate Grignard reagents in organic media is a well-established method used to convert the mercury species into their corresponding non-polar, dialkyl analogues and therefore improve performance during GC separation. However, since Grignard alkyaltion has to be carried out in an organic medium, species of interest must be transferred from an aqueous phase to an organic phase, thereby making sample preparation more time-consuming. With sodium tetraethylborate, ethylation takes place in an aqueous phase, which makes it possible to simplify the pre-treatment procedure by combining of derivatisation and extraction in one step. In this study, the ethylation and solvent extraction were carried out simultaneously in an ultrasonic field. This procedure minimises the disadvantages of conventional extraction procedures in terms of time, extraction efficiency and solvent consumption by facilitating and accelerating processes during the pre-treatment of biological samples. Details of this method can be found in paper **II**.

3.6.2. Soil and sediment samples

The pre-treatment steps for biological, soil and sediment samples are basically the same. As organomercurials are not found in the structure of mineralogical matter, but only bind to surfaces of sediment and soil particles, the complete dissolution of the soil sample prior to analysis is often not considered necessary. However, for soil and sediments, the efficiency of acid leaching extraction methods may be reduced owing to incomplete penetration of the solvents into the solid matrix (Horvat et al., 1994; Emteborg, 1996). It has also been reported that methyl mercury

compounds can be completely released from sediments by alkaline digestion or distillation (Horvat et al., 1993). The alkaline digestion process seems to release CH_3Hg (II) quantitatively but serious matrix interference could result if elevated sulphide concentrations are present in the aliquot from the alkaline digestate in a direct ethylation procedure. The distillation technique has been reported giving high recovery for both biological and sediment samples. However, under certain conditions this technique may give rise to artefactual formation of methyl mercury in sediments if high concentrations of inorganic Hg (II) are present (Bloom et al., 1997; Hintelmann et al., 1997). A recent communication (Quevauviller and Horvat, 1999), has addressed the particular concerns with regard to the certification of a sediment CRM (CRM 580 from the former BCR) and indicated that further studies with both enriched methyl mercury and inorganic mercury isotopes were necessary to confirm whether there was a systematic bias using methods involving distillation. This debate highlights the importance of accurate mercury speciation in environmental samples using currently available methods and emphasizes the need to develop quantitative extraction methods without a risk of inter-conversion of mercury species present in the original sample.

In paper III of this study, $Cu(NO_3)_2$ and KBr were used to extract mercury from soil. We used these compounds because Cu^{2+} binds very strongly to thiol groups in SOM and Br^- has a high affinity for mercury and can compete with SOM ligands. Two derivatisation methods were applied. One was based on extraction of mercury species into toluene, pre-concentration by evaporation and butylation of CH_3Hg^+ with a Grignard reagent. With the other, CH_3Hg (II) was extracted into dichloromethane and back-extracted into water followed by *in situ* ethylation and collection of ethylated mercury derivatives on Tenax. Both methods were employed for comparison purposes.

3.7. Halide competitive complexation method

In paper **V**, we took advantage of the strong complexation between methyl mercury and halides for the determination of the binding strength of methyl mercury to SOM. We calculated the free ion concentration of methyl mercury by using tabulated stability constants for halides. The concentration of reduced organic sulphur groups (RSH) was determined by sulphur *K*-edge XANES by the method of Xia et al. (1998) and the average dissociation constant of thiol (Hilton et al., 1975) was used to calculate the concentration of dissociated ligand (RS^-). The surface complexation constant was calculated by equation (1).

$$K_{CH_3HgSR} = [CH_3HgSR] / [RS^-][CH_3Hg^+] \qquad \text{(Eq. 1)}$$

In this equation K_{CH_3HgSR} is the conditional formation constant for the methyl mercury – reduced organic sulphur complex CH_3HgSR.

4. Major results and conclusions

4.1. Stability and reactions of mercury species in synthetic gas condensate

To test the stability of four selected species in hydrocarbon condensate solution, standards were prepared containing trace amounts of elemental mercury, monomethyl mercury chloride, dimethyl mercury and mercuric chloride. The results show that significant losses of mercury species from solution can occur by two pathways: by adsorption on the container wall and by reactions forming mercury (I) compounds.

The standards were stored in glass and high-density polyethylene (HDPE) containers, which were commonly used to store gas condensate samples. It was found that different materials interact to different extents with the Hg species, the rates of loss of both elemental mercury and $HgCl_2$ showed a large surface dependence. Both species were removed from solution faster with HDPE containers and there was a difference in the rate of loss between species, with Hg^0 being lost at a significantly higher rate.

There is no doubt that the container material can affect species loss by different adsorption mechanisms. However, the highest rates of loss noted for $HgCl_2$ in the presence of Hg^0 are due to the formation of Hg_2Cl_2 (equation 2).

$$HgCl_{2(sol.)} + Hg^0_{(sol.)} \rightarrow Hg_2Cl_{2(s)} \downarrow \qquad \text{(Eq. 2)}$$

Hg_2Cl_2 is relatively insoluble in organic solvents and forms colloids that precipitate and readily adsorb on the container walls. This compound could be suspended in the solvent by ultrasound treatment of the container and detected and identified after derivatisation by GC-MIP-AES and GC-MS. In contrast to $HgCl_2$, CH_3HgCl is stable even in the presence of Hg^0. This may be explained by the predicted instability of methylated mercury (I) compounds compared with mercury (I) halides reported previously (Schwerdtfeger et al., 1993).

The results suggest that Hg speciation analyses in hydrocarbon solutions may be hindered by the formation of Hg_2^{2+}, which is not available for specific determination because of its precipitation. Therefore, the production of a stable hydrocarbon reference material, useful for comparative measurements and contains both Hg^0 and Hg^{2+} might only be possible after the addition of a reagent to inhibit reactions shown in equation 2. Real condensate samples should be analysed as soon as possible after collection but may contain Hg_2^{2+} precipitates, which could lead to the erroneous determination of total Hg. Ultrasonic treatment of the sample container prior to the determination of total Hg is likely to increase the recovery.

4.2. Rapid determination of methyl mercury in biological materials by GC-MIP-AES or GC-ICP-MS following simultaneous ultrasonic-assisted *in situ* ethylation and solvent extraction

A simple and rapid sample pre-treatment procedure was developed for the determination of methyl mercury in biological materials. The procedure is based on acid leaching (5 min) of sample materials followed by simultaneous *in situ* derivatisation and extraction (40 min) in the presence of sodium tetraethylborate and nonane, buffered at pH 7.0, in an ultrasonic field.

Incipient methyl mercury was efficiently leached (>90%), the ethylation and extraction to the organic phase was almost quantitative. Detection limits (as Hg), based on three times the standard deviation of a standard solution, were 4.4 ng g^{-1} for GC-MIP-AES and 2.6 ng g^{-1} for GC-ICP-MS. No artefact formation of methyl mercury during sample pre-treatment was observed following the addition of a $^{201}Hg^{2+}$ isotope standard. With this procedure, the number of handling steps, the sample preparation and analysis time, as well as potential sources of analytical errors were reduced. The method was validated by the analysis of three biological certified reference materials and applied to the determination of methyl mercury in a fish sample. The amount of ethylation reagent needed per sample increases the costs for reagents slightly and also the mercury concentration from the reagent blanks. It might be feasible to use this method for other element species as well.

4.3. Solvent extraction methods for the determination of methyl mercury in soils

The binding affinity of Cu^{2+} to soil organic matter functional groups and the affinity of Br^- for mercury ions were strong enough to extract in average 93% of the mercury in the organic soil samples investigated. Since inorganic Hg is much more strongly bound to soil organic matter than methyl mercury (Skyllberg et al., 2000; paper **III**), the good extraction efficiency for inorganic Hg suggests that soil adsorbed methyl mercury was quantitatively extracted.

For IAEA 356 sediment certified reference material, mercury was less efficiently extracted and determined CH_3Hg^+ concentrations were below the certified value. Because of the controversy concerning artefact formation of CH_3Hg^+ the values of certified reference materials are at the moment questioned. We obtained values for IAEA 356 (3.22±0.17 ng g^{-1}) significantly lower than the certified value (5.46±0.71 ng g^{-1}) and closer to the values reported by Hintelmann et al. (1997) (3.27±0.57 ng g^{-1}) and Bloom et al. (1997) (4.814±0.649 ng g^{-1}). Partly, the lower values using extraction techniques may be due to a strong bonding between methyl mercury and inorganic sulphide surfaces. Using XANES we found that 55% of total sulphur of the IAEA 356 was in the form of inorganic sulphides.

In conclusion the solvent extraction method proved to be relatively simple, showed acceptable reproducibility and low detection limits for natural samples. Accuracy was established by recovery tests, measuring reference materials and using two

different analytical methods. In a future study it would be interesting to relate the extraction of inorganic and methyl mercury to the amount and species of sulphur present in various types of soil samples.

4.4. Spectral and chemical interferences associated with derivatisation by Grignard reagents

Spectral and chemical interferences might occur when using Grignard reagents for derivatisation of mercury species. There are:

- Spectral interferences caused by co-eluting reaction by-products obtained during derivatisation and appearing when using GC-MIP-AES or GC-QTAAS.

  Alkylation of mercury species using butylmagnesium chloride in tetrahydrofuran (THF) is straightforward provided that the mercury species are extracted into an organic solvent such as hexane or toluene. However, there is a risk for co-elution of interfering by-products if the separation capacity of the analytical column is insufficient. This might cause changes in the background emission or absorption. To avoid these interferences the use of pentylmagnesium bromide and hexylmagnesium bromide was investigated. These Grignard reagents showed poor derivatisation efficiencies and species stability. Maybe due to the effect of bromide on the stability of the derivatised mercury compound.

- Transalkylation reactions during derivatisation in the reaction vessel.

  Replacement of indigenous methyl groups with butyl groups was found to take place during derivatisation with butylmagnesium chloride after prolonged contact of monomethyl and dimethyl mercury with the Grignard reagent. The degree of methyl group replacement appeared to be proportional to the reaction time and therefore it has to be optimised.

- Chemical interference caused by some halogens during derivatisation or on the separation column.

  Iodide and bromide at concentrations of the order of 1 mg l^{-1} were found to de-alkylate organic mercury species when present in an organic matrix. In addition, if these halides were not completely removed from the GC column under a normal chromatographic run, they reacted with derivatised mercury species in subsequently injected samples. Organomercury iodide or bromide compounds were formed and identified by GC-MS. Possibly these mercury-halide complexes resulted in an underestimation of mercury concentrations.

It is clear from the above comments that systematic errors may occur in the steps prior to determination, e.g., in the derivatisation step. Precautions must be taken when developing methods for new applications in order to obtain accurate results. It will be necessary to perform studies of yields for different steps in the analytical procedure before their full utilisation in practice becomes possible.

4.5. Binding of methyl mercury to reduced sulphur groups in soil organic matter

We combined synchrotron-based Hg L_{III}-edge EXAFS, sulphur *K*-edge XANES and desorption studies to determine the coordination, geometry and strength of methyl mercury bonding in soil organic matter.

With sulphur *K*-edge XANES, the reduced organic sulphur (Org-S_{RED}) were determined in a low sulphur organic soil and in gently extracted potentially soluble organic substances from this soil and from a high sulphur fen peat. Samples were added methyl mercury to yield various CH_3Hg (II)/Org-S_{RED} ratios. Subsequent EXAFS analysis provided direct molecular level evidence for the bonding between mercury (in CH_3Hg (II)) and sulphur in the first shell. This was achieved at a CH_3Hg (II)/Org-S_{RED} ratio between 0.01 and 0.26. The Hg-C bond (in CH_3Hg (II)) was on average 2.05±0.02 Å and the Hg-S bond on average 2.32±0.01 Å. At CH_3Hg (II)/Org-S_{RED} ratios of 0.72 and 0.83, oxygen and/or nitrogen ligands were quantitatively more important than reduced S groups in the first coordination shell of Hg. Both organic soils and organic substances extracted from organic soils gave similar EXAFS results.

A method based on competitive complexation (ligand exchange) of methyl mercury by bromide and iodide was used to determine the average binding strength of native concentrations of methyl mercury in organic soils. Based on data on Org-S_{RED} concentrations, surface complexation constants calculated using equation (1) were in the range of $10^{16.3}$-$10^{16.7}$ for a thiol model site having an acidity similar to mercaptoacetic acid. These constants compare favourably with similarly defined constants for methyl mercury thiol associations in well-defined organic substances. This strongly indicates that methyl mercury associates with reduced organic sulphur groups in soil organic matter.

Since in most soils Org-S_{RED} are in large excess compared to CH_3Hg (II) or even total Hg, we can conclude that CH_3Hg (II) prefer high-affinity sites (Org-S_{RED}), whereas oxygen or nitrogen groups do not take part in the binding of CH_3Hg (II) except in severely contaminated soils.

4.6. Distribution of mercury and organic sulphur species in soil, soil solution and stream of a forested catchment

Concentrations of CH_3Hg (II), Hg-tot and sulphur species were determined in soils, soil solutions and streams of a small (50 ha) forested catchment in northern Sweden, during snow free season in 1998 and 1999. Regression analysis showed that both CH_3Hg (II) in soil solution and soil were significantly positively correlated with Hg-tot in soil solution. This may suggest that Hg-tot in soil solution is more available for methylation processes than Hg-tot in soil. In soil the percentage contribution from CH_3Hg (II) to Hg-tot decreased from relatively high values in the peat soils of the stream bank (1.2-17.2%) to lower values in mineral soils and peat soils 20 m away from the streams (0.4-0.8%). This may indicate that conditions for a net methylation of Hg-tot are most favourable in stream bank soils.

We successfully estimated Org-S_{RED} concentrations in soil, soil extract and flocculates of organic substances from streams by using S *K*-edge XANES. The proportion of Org-S_{RED} in our samples (between 50 and 78% of total S) is higher than previously reported literature values from similar types of samples. The ratio of CH_3Hg (II) to organic C or Org-S_{RED} were higher in soil solution and stream than in soil. This may indicate that the solid phase of the soil has a lower density of sites binding CH_3Hg^+ and Hg^{2+} per organic C than soil solution, and per organic C and Org-S_{RED} than stream. Another possibility is that chemical equilibrium was not completely attained with the solid phase of the soil.

Model calculations showed that under oxic conditions nearly 100% of Hg (II) and CH_3Hg (II) were complexed by Org-S_{RED} at soil surfaces, in soil solution and in the stream water. At pH 5, concentrations of CH_3Hg^+ and Hg^{2+} free ions were on the order of 10^{-18} and 10^{-32} M, respectively, in both soil solution and stream. Model calculations also showed that inorganic bi-sulphide complexes may contribute to an overall solubility of CH_3Hg (II) when concentrations of reduced inorganic S (II) exceed 10^{-9} M.

5. Implications and future studies

Our study showed that species transformation reactions and interactions with container walls affected mercury species stability in synthetic gas condensate. Further investigations are needed to find the ways to block the reactions and interactions in order to be able to produce a stable hydrocarbon reference material.

For the determination of methyl mercury in biological samples, although we have developed a relatively simple and rapid sample preparation procedure, it is still labour intensive. It is necessary to develop simpler and more efficient methodologies that can be used for routine analyses.

Studies of concentrations of naturally bound methyl mercury in soils have proven to be a difficult task. The main reasons for this are:

1) The very low concentrations of mercury in most soils, putting a strong demand on analytical techniques.
2) The very strong bonding between mercury species and soil organic mater complicating extraction procedures.
3) The heterogenic soil matrix limits the use of analytical techniques.

Furthermore, a soil reference material with a certified methyl mercury value is urgently needed for quality control.

X-ray absorption spectroscopy would be more useful if its sensitivity could be improved making it possible to study the bonding of methyl mercury at naturally occurring concentrations in natural organic matter of soils and streams. Furthermore,

binding affinity studies of soils of different origin, e.g., soils formed under reduced conditions, combined with EXAFS and XANES studies would be very useful in order to investigate whether our findings can be generalised also to other types of soils.

More research is needed for a better understanding of the transportation and distribution of mercury species in the environment in a dynamic point of view.

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