# Thiols, sulfide, and speciation with trace metals in seawater

*Electroanalytical studies and speciation modelling of thiols and sulfide with trace metals in seawater*

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Laboratory of Oceanography
Department of Earth Sciences
University of Liverpool
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Abstract

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R. Al-farawati

In this work the development of a flow-system, speciation of sulfide in seawater, and the distribution of thiols in coastal waters of the western North Sea, English Channel, and the water column of the Black Sea are presented.

During preliminary measurements on free sulfide in seawater using cathodic stripping voltammetry (CSV) and a hanging mercury drop electrode (HMDE), the response of free sulfide (peak height) measured by batch-mode voltammetry was shown to decrease with time. For this reason, a flow system was used to determine sulfide in seawater using detection by voltammetry with an HMDE. The flow-system consists of a personal computer, an Autolab voltammeter, a home-made flow-cell, an in-line purging system and a pump. The measurements of sulfide by the flow-system resulted in a stable peak of sulfide. The precision of the flow-cell was 2.8%, and a detection limit of 0.5 nM at a deposition time of 60 s was obtained. Comparison of sulfide measurements using the flow-cell and the conventional (batch) cell revealed that the formation of insoluble mercuric sulfide salt is the prime caused for the removal of sulfide in batch-cell. Interference from thiol compounds was investigated and it is shown that thiols produce a peak at, or very close to, the sulfide peak.

The conditional stability constants of metal-sulfide complexes were determined in pH 8 sea water at various salinities, by flow-analysis with detection by cathodic stripping voltammetry (FA-CSV). Two methods were used; ligand competition, and detection of the free sulfide concentration. Comparison of the two methods showed good agreement. The stability constants of copper and silver sulfide complexes were much higher than those of the other metal sulfide complexes. The high stability of the copper sulfide complexes and the similarity to Ag(I) suggest that the copper could be complexed by sulfide as Cu(I).

The flow-system was automated to study the spatial distribution of thiols in the coastal water of the western North Sea and the English Channel. The thiol concentrations ranged between 0.70 to 3.60 nM. Thiols were also determined in the water column of the Black Sea using batch-mode voltammetry. The thiol concentrations in the upper water column were similar to those in the North Sea The production of thiols in the North Sea and the Black Sea were shown to associate with the activity of marine phytoplankton. Anoxic conditions of the deep water of the Black Sea resulted in thiols concentrations much higher than in the surface (up to 9 μM), possibly due to interaction of sulfide with organic matter. Titration experiments using ligand competition technique indicated that thiols are powerful complexing ligands to copper. Calculation shows that the speciation of sulfide in seawater appears to depend on the ratio of copper to sulfide, and on the concentration of organic copper complexing ligands.

# Contents

# Chapter 1

## Introduction

## 1.1 General introduction

Oceanography is a multi-disciplinary science which has been defined as " the science of the oceans, their interaction with the atmosphere above and with the underlying sea- floor sediments and oceanic crust, their chemical and biological components, their physical properties and motion, their geology, their creation, past history, and development, their present state, and their future" (Summerhayes and Thorpe, 1996). Many of the problems addressed by oceanographers are interdisciplinary, so their solution demands a breadth of knowledge that crosses conventional scientific boundaries and requires multi-disciplinary team collaboration. Oceanography uses a range of facilities of all these basic sciences, such as advanced computers for modelling and advanced analytical instruments of chemists. This thesis is an example of this multi-disciplinarity, with topics ranging from analytical chemistry and chemical oceanography to computers.

The main aim of this research is the metal speciation with, and distribution of, sulfur-containing compounds (i.e. sulfide and thiol) in seawater. The speciation of sulfide has become a subject of increasing importance in recent years. The importance originates from the known existence of sulfide at pico to nanomolar levels in seawater, and its

importance as complexing ligand, competing with other inorganic, and natural organic, ligands for trace metals in seawater (Chapter 3). This study confirms previous data showing that thiols are present in seawater from oxic and anoxic environments. Marine micro-organisms are likely to be the major factor that control their distribution in seawater (Chapter 4).

### 1.2 Cathodic stripping voltammetry (CSV)

The improvements in sensitivity of analytical techniques since the mid-1970s have increased our knowledge in the distribution of various elements and compounds in seawater, thus improving our understanding of their biogeochemical cycles .

Voltammetry and polarography are methods for the determination of reducible or oxidisable substances in solution. In voltammetry, a stationary, hanging mercury drop electrode (HMDE) is used whereas a dropping mercury electrode (DME) is used in polarography. Voltammetry and polarography are based on a current-voltage relationship at a polarised electrode (Skoog et al., 1992).

Stripping voltammetry is a type of voltammetry that combines a preconcentration stage with the end-determination. The analyte is first collected at a constant electrode potential during which either a process of electrolysis or of adsorption takes place. After an accurately measured period of time, the electrolysis is discontinued and the analyte is redissolved or stripped from the electrode during the potential scan. Stripping methods are of prime importance because the concentrating aspect of the electrolysis permits the determination of minute amounts of the analyte with reasonable accuracy. The technique is therefore attractive as it is accurate, precise, easily automated and has low detection limits ($10^{-10}$-$10^{-11}$ M).

Two kinds of stripping voltammetry can be used: cathodic stripping voltammetry (CSV) and anodic stripping voltammetry (ASV). Stripping voltammetry is called anodic or cathodic depending on the direction of the current through the circuitry; a negative or oxidation current is called anodic, and a positive or reduction current is called cathodic (van den Berg, 1989). The scan direction is towards positive potentials in ASV, and towards negative potentials in CSV.

The application of ASV is limited to metals which can be reduced to the metallic state at potentials within the stability boundaries of water and mercury (between 0 and -1.5 V), and which at the same time are soluble in mercury.

In CSV the deposition step differs from that in ASV as the metals are chelated with a suitable complexing agent and then adsorbed or deposited rather than plated directly on the HMDE (van den Berg, 1989). CSV procedures have been shown to be powerful tools to study the distribution and speciation of metals (e.g. van den Berg, 1986; van den Berg and Nimmo, 1987; Abollino et al., 1991; van den Berg and Khan, 1991; Donat and van den Berg, 1992). Using these techniques the investigator can determine trace levels of the analyte without prior sample preconcentration.

CSV is not limited to amalgam-forming metals as is ASV. Also, the application of CSV is not limited to metal determination but it is also used for the determination of other inorganic and organic compounds.

The polarographic behaviour of sulfide ion has been investigated by (Turner et al., 1975). The electrode process is a two electron oxidation of mercury and the formation of the insoluble HgS.

$$S^{2-} + Hg \Leftrightarrow HgS + 2e^-$$

The electrode reaction is applicable even when the solution species of sulfide ($H_2S$, $HS^-$ and $S^{2-}$) may not be dominated by the sulfide ion ($S^{2-}$) because of rapid protolytic exchanges of the dibasic acid, $H_2S$. The distribution of the sulfide species is pH-dependent and further details will be discussed in Chapter 2.

At the deposition potential (-0.2 V in this thesis) the mercury electrode is oxidised to produce $Hg^{2+}$, which in the presence of sulfide forms insoluble HgS. The amount of HgS formed on the electrode surface is proportional to deposition time and the concentration of sulfide in the solution. During the cathodic stripping process a negative scan direction is applied and the reduction current of the deposited material is measured

### 1.3 Automated flow-analysis

A high sampling resolution is sometimes needed to study the spatial and temporal processes occurring in the marine environment. Analytical systems with a high sample through-put are therefore required. Automation

of voltammetric techniques using flow-analysis increases the sample processing speed by reducing the amount of manual sample handling, thereby also reducing the risk of sample contamination.

The work described here has led to the development of a flow-cell with which sulfide, and thiols, can be measured by flow-analysis with detection by CSV (Chapter 2). This flow-cell was used to study the thiol distribution in coastal waters of the North Sea and the Channel, at a very high data intensity. The data show a clear relationship with biological parameters suggesting that micro-organisms are the source of the thiols (Chapter 4).

### 1.4 The importance of sulfur compounds to marine systems

Sulfur is the twelfth most abundant element on earth (Wedepohl, 1995). It occurs in a large number of chemical compounds. The sulfur atom ranges in oxidation steps from +6 as in sulfate ($SO_4^{2-}$) to 2- as in hydrogen sulfide ($H_2S$). The ability of sulfur to undergo changes in oxidation state over an eight electron shift bears resemblance to the properties of carbon and nitrogen. It is therefore not surprising that sulfur also has many important functions in the living organisms and marine environment.

Sulfide is the major inorganic form of sulfur compounds under anoxic condition. Anoxic conditions occur when the consumption rate of oxygen exceeds that of supply. In the marine environment, the consumption of oxygen is intimately link to the oxidation of organic matter. It was thought that complete exhaustion of oxygen could occur only in closed basins where the combination of physical barriers and density stratification of the water limit the presence of oxygen in the deep water. However, there are also some open-ocean areas in which the concentrations of dissolved oxygen are reduced almost to zero values. These areas are close to regions of upwelling, and consequently of high primary productivity (Deuser, 1975; Chester, 1990). In the anoxic environment, the formation of hydrogen sulfide has a significant impact on the fate of deposited organic matter and it leads to the production of variety of organic sulfur compounds (e.g. glutathione, 3-mercaptopropionate (3-MPA), methanethiol (MSH)) (Luther et al., 1986; Vairavamurthy and Mopper, 1987; Kiene and Taylor, 1988; Kiene et al., 1990; Taylor and Gilchrist, 1991) .

Sulfide, thiols (R-SH) and organic sulfur compounds are potential complexing ligands for many metals in natural waters. Therefore, they can

affect the speciation of metals in the marine system. The uptake and toxicity of copper, cadmium and zinc to marine organisms are thought to depend on free rather than the total or complexed metal concentrations (e.g. (Jackson and Morgan, 1978; Bernhard and George, 1986)). Thus, the bioavailability of trace metals may be strongly influenced by their speciation. Further, the presence of strong complexing ligand can reduce the toxicity of a trace metal to marine organisms.

Sulfide is hypothesised to be present in oxic water because of the hydrolysis of carbonyl sulfide (COS) (Elliott et al., 1987). Culture studies have shown that marine phytoplankton could act as a source of sulfide (Walsh et al., 1994). Moreover, recent studies have confirmed the existence of sulfide in oxic seawater (Cutter and Krahforst, 1988; Luther and Tsamakis, 1989; Radford-Knoery and Cutter, 1994). Linear free energy relationship techniques have been used to estimate the stability constants of metal sulfide complexes (Dyrssen, 1988; Elliott, 1988) showing that sulfide is strongly bounded to copper. Therefore sulfide could play an important role in the speciation of trace metals in oxic seawater. Also, in anoxic water, metal sulfide species could be important because they may enhance the solubility of trace metals over that predicted on the basis of metal sulfide solubility

products. Moreover, these species are intermediates in the formation of sulfide minerals and are products during minerals dissolution (Rickard, 1989; Luther, 1991).

Thiols and other organic sulfur compounds exist at high concentrations in the living tissue of marine organisms (e.g. glutathione, dimethylsulfoniopropionate (DMSP), cysteine). Therefore, these compounds are expected to be present in the marine environment as dissolved and particulate forms. Thiols are the key components of some coenzymes and of metal lothioneins which, through selective complexation, are involved in the intracellular availability, or detoxification of trace metals, such as copper, zinc and mercury (Sanders et al., 1983).

The chemical and microbial transformations between various forms of organic sulfur compounds, and biodegradation of organic matter are the most important pathway for the production of thiols and organic sulfur compounds (Mopper and Taylor, 1986; Kiene and Taylor, 1988; Kiene et al., 1990; Mopper and Kieber, 1991; Taylor and Gilchrist, 1991; Kiene, 1996). Thiols and organic sulfur compounds are formed, also, through reactions of sulfide and polysulfide with organic compounds such as those containing

activated unsaturated carbon-carbon bonds via Michael addition (Boulegue et al., 1982; Mopper and Taylor, 1986; Vairavamurthy and Mopper, 1987). These mechanisms are thought to affect the bioavailability of the reactive organic matter in marine sediments (Vairavamurthy et al., 1992), and hence may play an important role in the preservation of organic matter in anoxic marine sediments. In addition, thiols and organic sulfur compounds were shown to form strong complexes with metals (Boulegue et al., 1982).

### *1.4.1 Examples of the most common thiols and organic sulfur compounds and their role in the marine environment*

Glutathione is thought to be the most abundant non-protein thiol in animals, plants, and various bacteria (typical concentrations range between 0.1 and 10 mM) (Kosower, 1976; Giovanelli, 1987). It is important for many biochemical reactions such as maintaining the redox and the detoxification status of the cell or being a substrate or a product of a considerable number of enzymatic reactions (Kosower, 1976). (Matrai and Vetter, 1988) measured nanomolar levels of particulate glutathione in coastal waters and showed that chlorophyll and glutathione distributions covaried in some instances. However, little is known about the distribution of dissolved glutathione in

oxic conditions. Low, nanomolar, levels of glutathione have been detected in the water column of the Atlantic (Le Gall and van den Berg, 1998). In contrast, high levels (nanomolar to micromolar) of glutathione in anoxic conditions have been detected in marine porewaters (Luther et al., 1986), marine sediments (Mopper and Taylor, 1986; Kiene et al., 1990), and seawater (Luther et al., 1991; Mopper and Kieber, 1991), where it was produced by anaerobic degradation of organic matter via anaerobic bacteria or by oxidation of sulfide minerals (pyrite). The consumption of glutathione by the larvae *Ciona intestinalis* was shown to be important for its fin formation (Robinson et al., 1991)a. It is therefore clear that glutathione is present in the marine environment and plays a fundamental role in the ecosystem.

Dimethylsulfoniopropionate (DMSP) is present in marine phytoplankton at concentrations ranging between 0.2 to 0.4 mol DMSP $l^{-1}$ cell volume (Dacey and Wakeham, 1986; Keller et al., 1989). It is believed to play a role in the osmotic regulation in marine phytoplankton (Vairavamurthy et al., 1985), and is cleaved to produce dimethylsulfide (DMS). DMS is the major volatile sulfur compound in surface seawater (Andreae and Barnard, 1984; Wakeham et al., 1984; Turner and Liss, 1985; Turner et al., 1988), and is

thought to play a major role in global climate regulation through affecting the radiative properties of marine clouds (Charlson et al., 1987). It is thought that DMS accounts for 50% of biogenic sulfur emission entering the atmosphere. DMS is thought to be correlated with phytoplankton chlorophyll in the oceanic water of Bering Sea (Barnard et al., 1984).

In general conclusion, sulfur compounds are very reactive and exist in oxic and anoxic environments. They are intermediate compounds in many reactions and are produced by marine organisms, react with trace metals and undergo to different chemical and microbial reactions, Therefore, they play a major role in the marine environment.

### 1.5 The objective of this thesis

The studies that were performed here, have been subdivided into separate chapters in order to improve the readability of this thesis. The chapters have been written in such a way that they can be interpreted as individual pieces of research and can be read on their own.

In Chapter 2 the problems of sulfide measurements in the batch mode voltammetry were investigated. One of the first tasks of this study was

to build a flow cell to facilitate sulfide measurements in natural waters. The construction of the flow cell and its components are outlined.

In Chapter 3 a study of sulfide speciation is presented. This study was performed by flow-analysis with detection by CSV (FA-CSV). The conditional stability constants of metal-sulfide complexes were determined using two methods; one method used was titration of the sulfide by metal with detection of the free sulfide by FA-CSV. The second method took advantage of ligand competition between sulfide and 8-hydroxyquinoline (oxine) for free metal ions. Thus the stability constants of sulfide complexes with copper, cobalt, nickel, lead, cadmium, zinc, aluminium, chromium, silver, iron, manganese in seawater were determined. The importance of sulfide on copper speciation and vice versa was investigated and calculated using a computer programme.

In Chapter 4 the distribution of thiols in the coastal waters of the western North Sea is described. This was determined during a cruise with RRS Challenger (July 1995). Samples from the water column of the Black Sea obtained during a cruise with the RV "Professor Vodyanistkiy", were analysed by CSV and the thiol distribution determined. The biogeochemical

factors that could influence the distribution of the thiols are discussed. The possible effect of the thiols on the speciation of copper was investigated. Thereto complexing ligands and conditional stability constants of complexes of copper were determined in the Black Sea samples using ligand competition methods. The importance of thiols as potential ligands for copper is then discussed.

Main results of this work are a novel, simple, flow-cell by which sulfides can be detected, a new set of stability constants describing metal complexation by sulfide in seawater, and the distribution of thiol compounds in the North Sea and the Black Sea. Covariation with chlorophyll suggests that marine phytoplankton produce the thiols. The work shows the importance of sulfide and thiol to the speciation of metals in the marine aquatic system.

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# Chapter 2

## The determination of sulfide

## in seawater

## by flow-analysis with voltammetric detection

### *2.1 Abstract*

Preliminary measurements of sulfide in seawater using cathodic accelerate stripping voltammetry and a hanging mercury drop electrode (HMDE) in batch-mode showed that the sulfide peak decreased rapidly with time. This decrease was not caused by $O_2$, $H_2O_2$ or $IO_3^-$, and the sulfide peak was not stabilised by trace metal additions. A home-made flow-cell was constructed to enable the determination of sulfide in seawater using voltammetry with an HMDE. A stable sulfide peak was obtained by flow-analysis with voltammetric detection, with a precision of 2.8% and detection limit of 0.5 nM at a 60 s adsorption time. Several thiol compounds were found to produce a peak at, or very close to, the peak potential for sulfide. Their interference was evaluated by allowing the sulfide peak in conventional (batch) voltammetry to decay. Comparative experiments showed that waste metallic mercury is responsible for removal of sulfide in batch-mode analysis due to formation of insoluble mercuric sulfide salts causing the rapid decay of the sulfide peak. The problem is circumvented by using flow-analysis to determine sulfide.

## *2.2. Introduction*

Sulfur(-II) is an important element from environmental point of view as it is one of the major elements in living organisms. Sulfide enters the aquatic environment when organisms die or as by-products from bacterial processes. Sulfide (as bisulfide) is known to form stable complexes with metal ions (Dyrssen, 1988; Zhang and Millero, 1994) and form metal sulfide compounds of very low solubility (Martell and Smith, 1976).

### 2.2.1 Methods of sulfide determination

Several techniques have been developed to determine sulfide in natural waters. The usual method is by reaction with methylene blue with spectrophotometric detection (Cline, 1969). Concentrations which can be determined by this technique are in the range 1-1000 μM sulfide which is suitable for anoxic waters. Recently, spectrophotometric determination of sulfide was used to determine sulfide in spring water based on reaction of sulfide with magenta (Safavi and Ramezani, 1997). The detection limit was 15 ng/l. Iodimetry is also used for the determination of sulfide but it is usually used as a method for calibration of sulfide standard solutions.

The determination using voltammetric techniques has been used by a number of workers. Normal pulse polarography (NPP), differential pulse polarography (DPP) and direct current (DC)were used by (Davison, 1977; Davison and Heaney, 1978; Davison and Gabbutt, 1979) to measure sulfide down to low levels (micromolar) in lake waters. (Luther et al., 1991) used various voltammetric techniques, the selection depending on the concentrations of sulfide in Black Sea samples. They used sampled DC for high sulfide concentrations (>50 μM). For intermediate levels (200 nM-50 μM) square wave voltammetry was used whereas for concentrations lower than 200 nM cathodic stripping voltammetry (CSV) was applied. In addition, CSV has been used for the determination of sulfide (Luther and Tsamakis, 1989) at very low concentrations (few nanomolar). Possible advantages are that no reagents are required and that only a small sample volume is used, combined with good sensitivity and minimal contamination problems.

### 2.2.2 Electrode mechanisms of sulfide determination by voltammetry

The polarographic behavior of sulfide has been investigated by a number of workers using different techniques; DC polarography

Potentiometry was also used for sulfide determination in natural marine system. (Boulegue et al., 1982; Dyrssen and Wedborg, 1986) measured sulfide and other sulfur species at pH 13 using Hg(II) as a titrant via a silver/silver sulfide electrode as an indicator electrode. However, potentiometric measurements are tedious and require extensive precautions to prevent contamination during titration. In addition, they are not as sensitive as polarographic techniques (generally, the response slope is 30 mV decade$^{-1}$ and the detection limit $10^{-5}$-$10^{-6}$ mol $l^{-1}$). However, recently, a new potentiometric method was used for sulfide determination, in sodium tetraborate buffer (pH=9), with a detection limit of 50 nM using a carbon paste electrode (Hu and Leng, 1996), but its application was tested only for waste water.

Gas chromatography (Radford-Knoery and Cutter, 1993, 1994) has been used for its direct and indirect (preconcentration of sulfide via precipitation as zinc sulfide) determination in surface seawater. Hydrogen sulfide is generated by acidification and trapped in a liquid-nitrogen-cooled trap followed by gas chromatographic separation and detection by flame photometry. The detection limit is 0.5 pM and 5 pM for the direct and indirect procedures, respectively.

(Canterford, 1973), DPP (Canterford and Buchanan, 1973), and NPP (Turner et al., 1975).

Hydrogen sulfide, $H_2S$, is a dibasic acid which dissolves easily in water and thereafter dissociates to bisulfide ($HS^-$) and sulfide ($S^{2-}$)

$$H_2S \Leftrightarrow HS^- + H^+ \qquad 2.1$$

$$HS^- \Leftrightarrow S^{2-} + H^+ \qquad 2.2$$

The first ($pK_1$) and second ($pK_2$) dissociation constants for the hydrogen sulfide are 6.67 (Hershey et al., 1988) and 18.51 (Schoonen and Barnes, 1988), respectively. Thus below pH 6.67 the dominant species is $H_2S$ whereas above pH 6.67 it is $HS^-$. The equilibria of sulfide species is very quickly so the electroactive species can be considered to be $S^{2-}$.

For low sulfide concentrations (less than $10^{-4}$M), (Turner et al., 1975) have shown that the electrode process is a two electron reversible reaction involving the oxidation of mercury and the formation of the insoluble mercuric sulfide

$$Hg + S^{2-} \Leftrightarrow HgS + 2e^- \qquad 2.3$$

The halfwave potential of this reaction is at -0.6 V. At more positive potentials this reaction goes to the right whereas at more negative potentials it goes to the left, and any precipitated HgS is redissolved. At high sulfide concentration multiple signals were observed as a result of formation of

insoluble films of mercuric sulfide (Canterford, 1973; Canterford and Buchanan, 1973; Renard et al., 1975; Youssefi and Birke, 1977), which are unsuitable for analytical work. These phenomena suggest that these electrode processes are poorly reversible (Renard et al., 1975). It is therefore important to choose the correct experimental conditions and polarographic technique which reduce sulfide concentration at the mercury drop surface to low concentrations. NPP was used to overcome this problem using short pulses to ensure that the multi-film does not occur (Turner et al., 1975; Davison and Gabbutt, 1979). However, DPP is the desired technique when the concentration of sulfide is low (less than micromolar) (Canterford and Buchanan, 1973; Davison and Gabbutt, 1979). Recently, cathodic stripping voltammetry was used to study sulfide speciation in seawater at low concentrations (nanomolar to few micromolar) (Luther and Tsamakis, 1989; Zhang and Millero, 1994). They deposited mercuric sulfide at a potential more positive than the wave potential (more positive than -0.6V) and then scanned in a negative direction to get the signal. However, potential scans in a positive direction ( with holding the initial potential more negative than the potential of the wave) is preferable when the solution contains high levels of sulfide.

### 2.2.3 Minimisation of sulfide loss

It is difficult to preserve the sulfide in samples for an extended period, and immediate analysis is recommended. Sulfide can be oxidised by oxidants naturally present in seawater (i.e. $O_2$, $H_2O_2$ and $IO^-_3$). The oxidation of sulfide in marine water by oxygen (Avrahami and Golding, 1968; Almgren and Hagstrom, 1974; Millero, 1991), hydrogen peroxide (Millero et al., 1989), and iodate (Zhang and Whitfield, 1986) have been studied. Oxygen is a primary oxidant in surface seawater, with the half-life time for sulfide in surface seawater being about 22 h (Millero, 1991). The sulfide in seawater originating from anoxic conditions is oxidised much more rapidly (30 minutes) upon ingress of oxygen due to the presence of high concentrations of the redox couple Fe(II)/Fe(III) (Millero, 1991).

Loss of sulfide during collection and determination in natural waters using polarographic and voltammetric techniques is a major challenge for investigators. When sulfide samples are collected from natural waters, it comes in contact with the atmosphere. Loss of sulfide could be due to oxidation by oxygen or as a result of evaporation in the form of hydrogen sulfide gas. The latter problem is related to the low partial pressure in the open atmosphere (i.e. Henry's law for the solubility of gases).

Methods for sampling, handling procedures and determination of sulfide by voltammetric methods can be obtained from (Boulegue, 1977; Davison, 1977; Davison and Heaney, 1978; Davison and Gabbutt, 1979; Boulegue et al., 1982; Luther et al., 1985, 1986; Davison et al., 1988; De Vitre et al., 1988; Dyrssen and Kremling, 1990; Luther and Ferdelman, 1993). Their recommendations for minimising sulfide loss can be summarised as:-

1- Collection of the sample is recommended to be in trace-metal cleaned bottles.

2- Samples from porewaters should be handled in a nitrogen filled bag

3- Bottles should be kept in a nitrogen-filled container until analysis.

4- In weakly buffered sample (e.g. lake water), change in the pH of the solution is possible because it is controlled by the partial pressure of carbon dioxide. When the sample is exposed to air the equilibria with the atmosphere will shift toward release of carbon dioxide to the atmosphere and consequently the pH of the solution rises. In this case, carbon dioxide gas can be used to keep the partial pressure of carbon dioxide the same as in the in-situ sample.

5- The voltammetric cell should be kept in a glove box which is flushed with inert gas.

6- Continuous running of a stream of inert gas over the surface of the solution, in the cell, is also recommended.

7- Chemical additives such as formaldehyde, glutaraldehyde, hydroxylamine and ascorbic acid were shown to prevent the loss of sulfide.

8- In lake waters, the measurements are recommended to be at higher pH (e.g. 10-11). This is because at the natural pH (6-7), a high proportion of the sulfide in the solution is in the volatile form of hydrogen sulfide which could escape from the solution.

Although the loss of sulfide can be minimise as discussed above, the direct in-situ determination was shown to be the most effective methods to ensure against loss of sulfide. (De Vitre et al., 1988) measured sulfide by pumping the water into a glass bottle followed by transfer in a sealed flow system to the voltammetric cell. Comparison between direct measurement of sulfide in the field with laboratory measurement after 6-8 hours of collection indicated that large losses (up to 80%) sulfide occurred in the case of laboratory measurement although that the sample was collected in airtight completely filled container. This was attributed to the formation of gas microbubbles on the wall of the container due to a pressure change (De Vitre et al., 1988). Loss of sulfide in samples collected was also observed by (Luther et al., 1991). Loss of sulfide was also reported during the

voltammetric and polarographic measurements by (Davison and Gabbutt, 1979; De Vitre et al., 1988), and was attributed to volatilisation. The loss of sulfide during polarographic measurements was shown to obey first order kinetics, therefore the amount of sulfide which was lost over a certain time could be calculated (Davison and Gabbutt, 1979).

Unexpectedly (in view of its expected rapid oxidation by dissolved oxygen), sulfide has been found to occur in surface seawater, but reported levels possibly depend on the analytical technique. Up to 19 pM free sulfide and up to 0.5 nM combined (particulate and total dissolved) sulfide have been detected by gas chromatography (Radford-Knoery and Cutter, 1994), whereas levels up to several nM of sulfide have been detected by voltammetry (Luther and Tsamakis, 1989) albeit in different waters. Systematic differences in sulfide concentrations are important as sulfide could be an important ligand for several metal ions if it occurrs at nanomolar levels (e.g. Dyrssen, 1988).

A stable peak at the potential corresponding with that for sulfide in seawater analysed by CSV has been ascribed to sulfide (Luther and Tsamakis, 1989). In preliminary experiments, we show here that free sulfide

in seawater (consisting of $HS^-$, $H_2S$ and $S^{2-}$ at decreasing concentrations) does not give a stable peak when a standard voltammetric (batch) cell is used with a mercury drop electrode; instead the peak decreases rapidly. We show the mechanism for this effect which makes it very difficult or impossible to determine sulfide by conventional (batch) voltammetry without unpredictably large systematic errors, in contradiction with previous work. It is also shown that this peak is not stabilised by metals contrary to previous suggestions. For these reasons a flow-cell was constructed which was found to be suitable for the determination of sulfide. The flow-cell and its application to the measurement of sulfide in seawater are reported here, along with experiments showing the cause for peak instability in the batch cell.

## *2.3 Methods and materials*

### 2.3.1 Instrumentation

An Autolab voltammeter (Eco Chemie) was interfaced with a Metrohm 663 VA electrode stand with a hanging mercury drop electrode, and controlled by a IBM-compatible personal computer. The reference electrode was double-junction: the Ag/AgCl/ 3 M KCl reference cartridge was separated by a frit from a salt bridge filled with 3 M KCl: the bridge

was freshly filled with KCl solution at the beginning of the experiments, and once during the experiments. The leakage rate of the salt bridge was imperceptibly small, so any releases of $Ag^+$ in the voltammetric cell during the experiments were negligible. The counter electrode was a glassy carbon rod. UV-digestion was carried out using a 1000 W high-pressure mercury vapour lamp. A glass voltammetric cell was used for batch analysis.

### 2.3.2 Voltammetric components for sulfide determination by flow-cell

The complete system used for the sulfide determinations is illustrated in Fig. 2.1. It consists of a personal computer, an Autolab voltammeter, an in-line purging system, a pump and the sample container. The flow-cell and the in-line purging system were constructed in the workshop at the Oceanography Laboratory in Liverpool and are shown in Fig. 2.1B and C. Further details are explained below.

### 2.3.3 The flow-cell

A flow-cell was made from extruded acrylic. The liquid is transported via a jet to the electrode. The jet is located in the bottom of flow-cell, and is

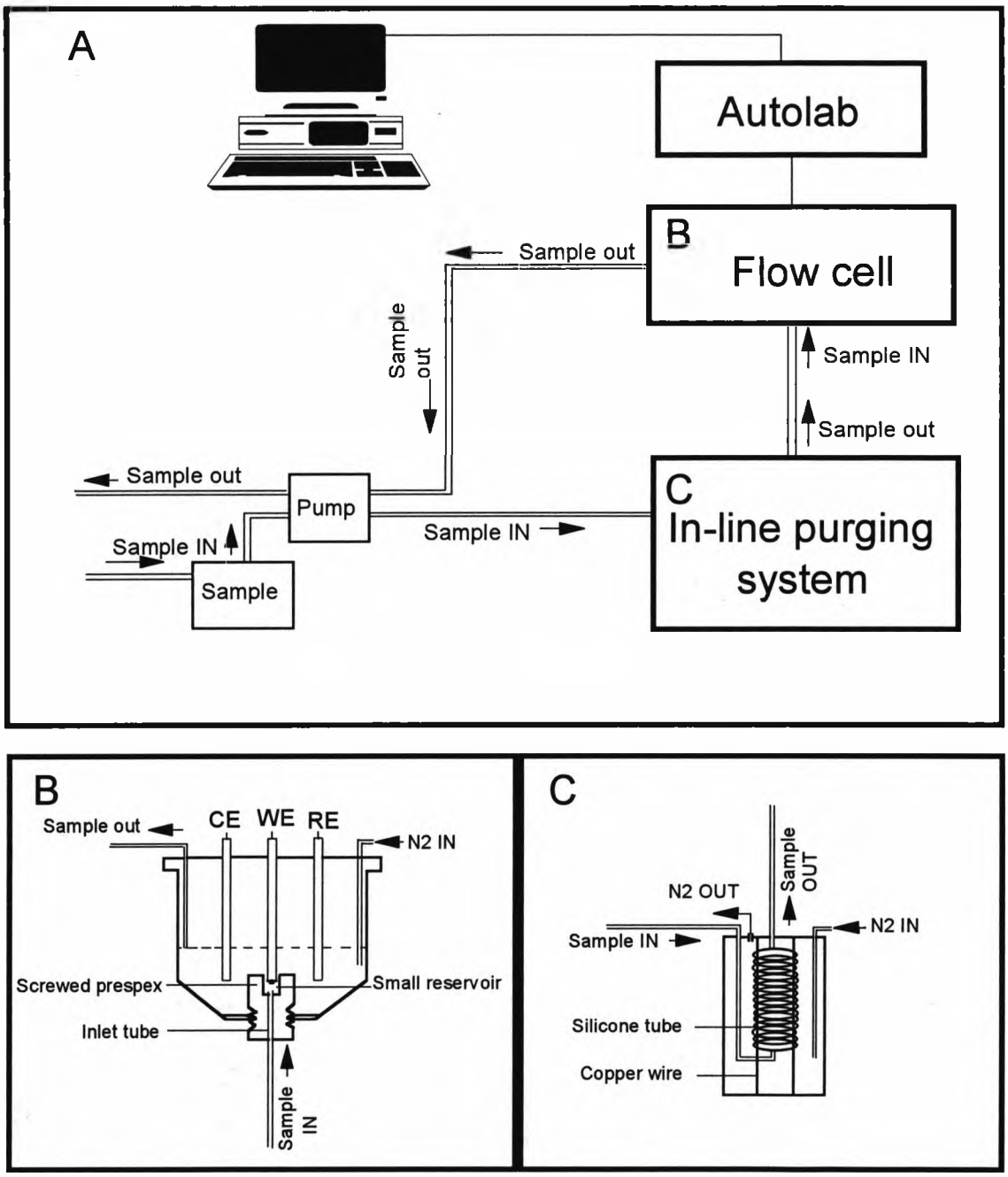


Fig. 2.1. The voltammetric system for sulfide determination by the flow-system consists of a personal computer, Autolab, an in-line purging system (part C), a pump, sample container and the flow-cell (part B).

contained in a rounded acrylic tube with a small conical reservoir (volume=60 µl at the level of the mercury drop) of 0.5 cm diameter and 0.4 cm height. The waste mercury which accumulates in the reservoir is flows away via two gaps in the edge of the reservoir (not shown in the figure). The acrylic tube can be screwed up or down to achieve a close fit to the HMDE. The overall shape and size of the cell is similar to the standard 50 ml voltammetric cell and fits a Metrohm 663 VA stand. The level of the solution inside the cell was kept constant by pumping liquid from the surface (Teflon tube, o.d.=1.6 mm, i.d.=0.8 mm). The solution in the cell was purged with water-saturated nitrogen when water was pumped into the cell; the purging was stopped when the pump was stopped. A Minipuls 3 (Gilson) peristaltic pump was used to pump water in and out of the cell, with 1.14 mm (i.d.) pump tubing for the in-flow and 1.85 mm (i.d.) for the out-flow. Thus, a fixed water level inside the cell was maintained as excess water was immediately pumped out.

### 2.3.4 In-line purging system

The in-line purging system was made according to (Colombo et al., 1997). Briefly, it consisted of a 30 ml polystyrene (Bibby Sterilin Ltd, Cat.No.128A) container and gas-permeable silicone tubing (i.d.=0.5 mm,

wall thickness=0.25 mm) of 1.5 meter length (Fig. 2.1, part C). The silicone tube was coiled around two copper wires fixed inside the container. Nitrogen gas was led to the bottom of the container via a Teflon tube (o.d.=1.6 mm, i.d.=0.8 mm) and allowed to flow out at a rate of ~50 mL $min^{-1}$ via a hole in the cap. The effect of the in-line purging on sulfide removal was tested using a solution of 100 nM sulfide. The peak height before and after purging was similar indicating that the effect of purging on sulfide concentration was negligible.

### 2.3.5 Reagents

Stock solutions of 0.1 M NaHS were prepared daily from $Na_2S.9H_2O$ (Aldrich) in MQ water. Metal standard solutions were prepared by dilution of atomic adsorption spectrometry standard solutions (Merck, Spectrosol grade) in MQ water and then acidified by addition of 10 µl of 50% HCl to 10 ml of solution. Hydrochloric acid was Analar (BDH) grade. A stock solution of 0.1 M hydrogen peroxide was prepared by dilution of 30% w/v stock solution (Analar, BDH) and stored in a refrigerator until use. A pH buffer was prepared by dissolving 1 M tris(hydroxymethyl)methylamine, $NH_2C(CH_2OH)_3$ (Analar,BDH) in 0.5 M HCl; 100 µl of this TRIS buffer in 10 ml seawater yielded a pH of 8.0-8.1. Other reagents used were stock

solutions of 0.1 M potassium iodate ( Analar, BDH) and 0.1 M mercuric chloride (Analar, BDH). A stock of elemental sulfur was prepared in toluene. Thiol compounds were: thioglycollic acid (BDH), 3-mercaptopropionic acid (Aldrich), cystine (BDH), thioacetamide (BDH), thiourea (BDH), 2-mercaptoethanesulfonic acid (Sigma), 3,4 dimercaptotoluene (Aldrich), dimethylsulfoniopropionic acid (Research Plus Inc.), 2-naphthalenethiol (Aldrich), xanthine (BDH), adenine (BDH) and dimethyl sulfide (Fluka). Water for preparation of reagents and rinsing was purified by reverse osmosis (Milli RO, Millipore) followed by deionization (Milli-Q, Millipore). The seawater used for the experiments originated from the Mediterranean (salinity=37.5) and the North Sea (salinity=35).

### 2.3.6 The experimental procedure to determine sulfide by voltammetry

The procedure for the determination of sulfide by CSV using the batch cell was as follows: 10 ml seawater was transferred to the voltammetric cell and purged with $N_2$ gas for 5 min. A deposition potential of -0.2V, and a deposition time of 60 sec were used, and a scan was carried out from -0.2 to -0.9V. The square-wave modulation was used for the voltammetric scans. A square-wave pulse-height of 25 mV and a frequency

of 150 Hz with a 2.5 mV scan increment was used. A flow rate of 1.2 ml $min^{-1}$ was used during measurements with the flow-cell. Purging was continued during the deposition time, and the flow as well as purging were stopped 3 sec prior to the scan.

### *2.4 Results and discussion*

#### 2.4.1 The decrease of the sulfide peak in the batch cell

Preliminary experiments using the standard batch voltammetric cell showed that the peak for 200 nM sulfide decreased rapidly, disappearing altogether within 30 minutes (Fig. 2.2A). Loss of sulfide was reported by %%211(Davison and Gabbutt, 1979); 295(De Vitre et al., 1988)%%. Time-series measurements of sulfide was carried out using a voltammetric flow cell by %%295(De Vitre et al., 1988)%%. Their measurements showed large losses of sulfide with time (e.g 25% after 10 minutes ) and this was attributed to volatilisation.

Various naturally occurring oxidants (oxygen, iodate, and hydrogen peroxide) were tested to evaluate the cause of the rapid decrease. Usually the solution is purged with nitrogen to remove dissolved oxygen from the sample, but this could also strip the sulfide from solution. To eliminate this possibility, sulfide was added to purged seawater which was kept blanketed

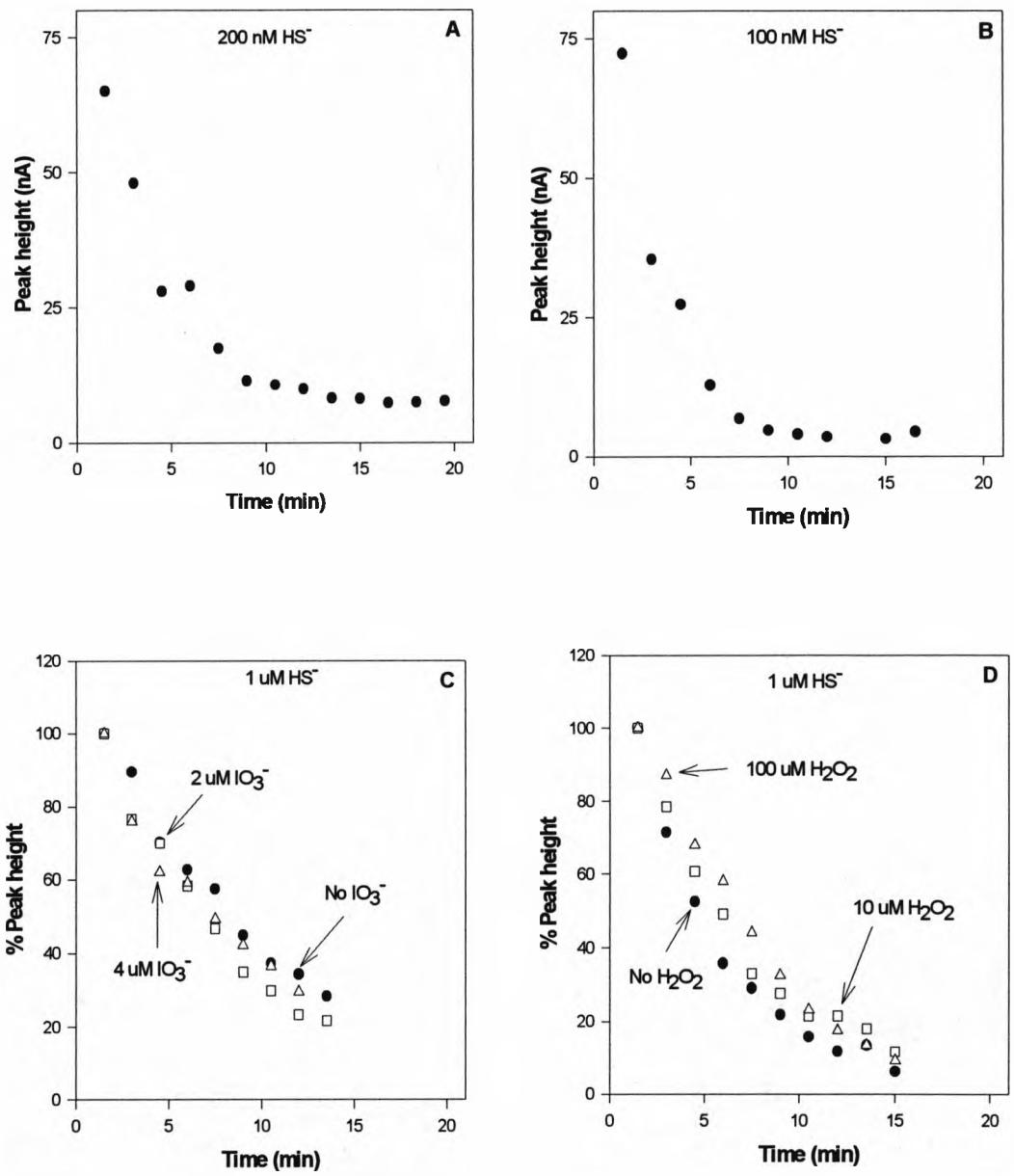


Fig. 2.2. The voltammetric behaviour of sulfide in the batch cell under varying conditions. A) a typical measurement using 200 nM $HS^-$ with 3 sec purging between scans. B) With $N_2$ gas flow over the surface using 100 nM of sulfide and no purging between scans. C) Response for 1 μM $HS^-$ in the presence of 2 and 4 μM of $IO_3^-$. D) Response for 1 μM $HS^-$ in the presence of 10 and 100 μM of $H_2O_2$.

with nitrogen over the surface, but no purging took place over the duration of the experiment. Successive scans showed (Fig. 2.2B) that the peak height decreased as before; neither oxygen nor the purging therefore were the cause of the rapid decrease in the sulfide peak.

Iodate is known to be an effective oxidant of sulfide when present at high concentrations. The half-life of sulfide in seawater containing 9-19 μM iodate (Zhang and Whitfield, 1986) is thought to be less than 1 hour. The concentration of iodate in seawater is typically much less at 200 (in surface waters) to 500 nM (Elderfield and Truesdale, 1980), so a significant effect is not expected. Iodate in oceanic waters has been estimated to cause an oxidation rate of 0.1 nM $h^{-1}$ of free sulfide (Radford-Knoery and Cutter, 1994). We added high levels of iodate (2 and 4 μM) to see whether it could be responsible for the fast decrease in the absence of dissolved oxygen. The decrease in the sulfide peak height was similar to that without the iodate addition (Fig. 2.2C) confirming that iodate did not cause the rapid decrease in the batch cell.

Hydrogen peroxide is known to occur at levels up to 100 nM in surface seawater (Moffett and Zika, 1983). Additions of high concentrations

of hydrogen peroxide (20 and 100 μM) did not increase the rate of sulfide disappearance (Fig. 2.2D). This finding is consistent with the known slow kinetics of its oxidation of sulfide, which is thought to have a half-life of 2,800 hour in the presence of 100 nM hydrogen peroxide (Millero et al., 1989).

### 2.4.2 Effects of metals

Metal additions of 20 and 40 nM were made to seawater to verify whether all or part of 100 nM sulfide would be stabilised against oxidation by dissolved oxygen and produce a stable peak with CSV. Measurements after two days storage (dark, room temperature) showed no evidence for the presence of any stabilised sulfide. A sulfide-like peak equivalent to 2.3 nM sulfide was originally present in the seawater and this peak was largely unchanged during the experiment (Table 2.1). The data showed no systematic stabilisation of sulfide (no free sulfide was detectable in the samples as the residual small stable sulfide-like peak was that originally present in the seawater). Further experiments were carried out in an attempt to identify the nature of the sulfide-like peak in seawater.

It is thought that the half-life of sulfide in seawater is about 22 hour. Therefore, 25 nM of sulfide should have remained in the solutions. This

Table 2.1. Effect of high metal concentrations on the stability of $HS^-$ in seawater. Seawater containing 100 nM $HS^-$ and either 20 or 40 nM added metals was stored for 2 days at room temperature in the dark. The residual $HS^-$ concentrations were determined with the flow cell.

| | Residual $HS^-$ concentration (nM) | |
|---|---|---|
| Metal | 20 nM metal | 40 nM metal |
| Se(IV) | 1.99 | 2.04 |
| V(V) | 2.26 | 2.55 |
| Zn(II) | 2.29 | 1.93 |
| Co(II) | 2.12 | 2.6 |
| Ni(II) | 2.11 | 2.2 |
| Cu(II) | 2.30 | 2.21 |
| Al(III) | 1.90 | 2.08 |
| Cd(II) | ----- | 2.01 |
| Cr(III) | 1.75 | 1.48 |
| Fe(III) | 1.86 | 1.27 |

amount of free sulfide was not detected in the solutions; possible reasons may be the purging of the samples with nitrogen gas (4 minutes purging time), further removal by the mercury drop from the electrode, or oxidation of sulfide which could be accelerated by metals (Vazquez et al., 1989)

### 2.4.3 Sulfide measurements using the flow-cell

The measurement of sulfide using the flow-cell resulted in a peak which did not show the systematic decrease characteristic of the batch measurements. The relative standard deviation of the measurements was 2.8% (n = 16) at a sulfide concentration of 80 nM. The peak heights are shown in Fig. 2.3 along with those obtained in a batch cell experiment illustrating the contrasting behaviours.

### 2.4.4 Comparison of sulfide recovery in the flow and batch cells

The voltammetric response of the flow-cell varied as expected with the sulfide concentration added to seawater. The peak height increased linearly with the sulfide concentration over a tested range of 0-2750 nM (Fig. 2.4). A recovery test was carried out in seawater to which 250 nM sulfide was added to simulate a measurement of sulfide in seawater; using the flow-cell with calibration by standard additions, a concentration of

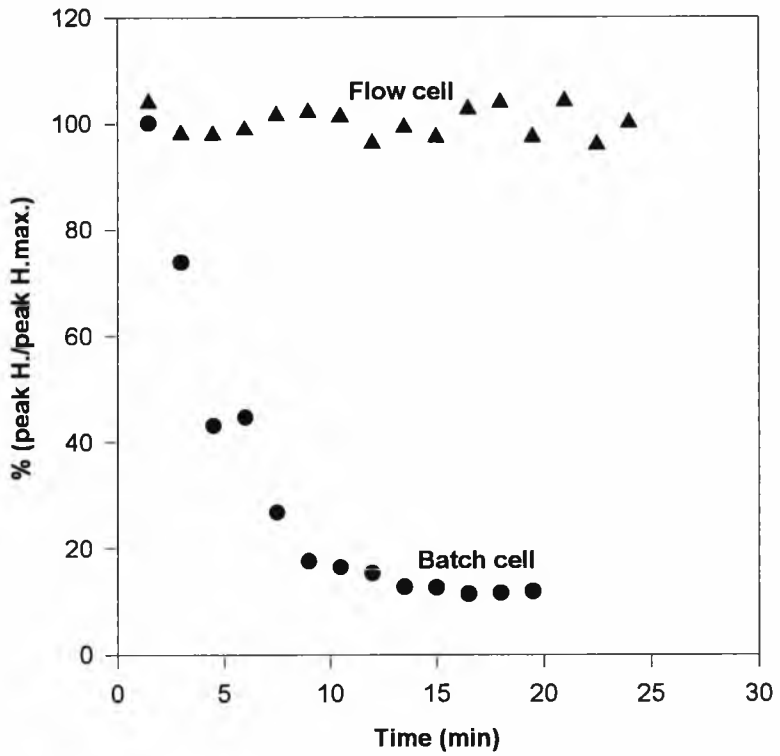


Fig. 2.3. Comparison of sulfide determinations in the flow-cell and the batch cell; flow-cell [$HS^-$] = 80 nM, batch cell [$HS^-$] = 200 nM.

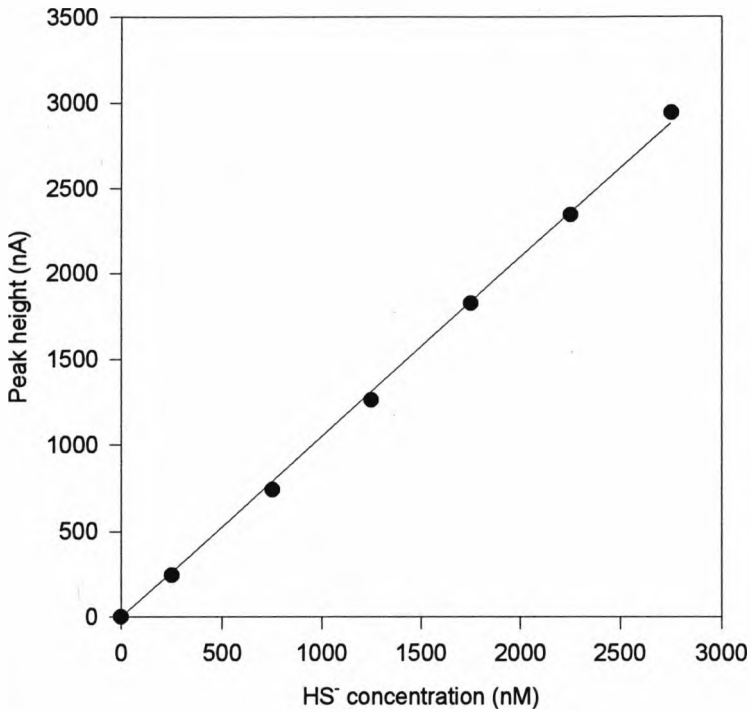


Fig. 2.4. Calibration curve of sulfide using the flow-cell

240 nM sulfide was determined (a recovery of 95%). A comparative measurement using the batch cell gave a recovery of 46% (using the first scan obtained immediately after transferring 10 ml of the sample in the cell and with calibration with thiourea (see below) with cross calibration of the sensitivity against sulfide using the flow-cell).

### 2.4.5 Detection limit

It was found that at low concentrations of thiourea and sulfide that the measurements of thiourea are more reliable than sulfide. It is therefore decided to use thiourea instead of sulfide to calculate the detection limit. The limit of detection was determined in seawater from which organic matter was previously removed by UV irradiation. Thiourea was added to this water to a concentration of 1 nM, and the detection limit was calculated from the standard deviation of repeated scans using a 60adsorption period at 0.05 V. The relative standard deviation of the peak height of repeated scans of thiourea was 6.1% (n=9) from which a limit of detection was calculated ($3\sigma$) of 0.19 nM thiourea, which is equivalent with 0.5 nM sulfide. This detection limit depends on the adsorption time and the flow rate, and can be readily lowered for instance by increasing the adsorption time. The peak height increased linearly with adsorption time up to a tested range of 5

minutes, so the limit of detection can be lowered to 0.1 nM by increasing the adsorption time from 1 to 5 minutes. Also, increasing the flow rate in a range 0.45 to 1.40 ml.min$^{-1}$ increased the peak height linearly, which could be used to further lower the detection limit. Non-linearity may be expected at higher adsorption time and flow rate due to saturation of the drop mercury but these saturation levels were not attained in these experiments.

### 2.4.6 Thiols and other natural organic compounds

Thiols (Luther et al., 1985) and various other natural organic compounds such as glutathione (Le Gall and van den Berg, 1993a), folic acid (Le Gall and van den Berg, 1993b), purines (Househam et al., 1987), and cystine and cysteine (van den Berg et al., 1988) are known to produce voltammetric peaks. Several compounds were tested to verify whether they could interfere with, or be responsible for, the sulfide-like peak in seawater. Scans for several of these thiols can be compared with that for sulfide in Fig. 2.5. Two of the thiols tested, thiourea and thioacetamide, gave a peak at the same potential as that for sulfide in seawater at -0.53 V (Table 2.2).

Although the peak produced by several of the thiols is very similar to that of sulfide, there are differences in the response when electrochemical

parameters are varied. This could used as a diagnostic tool to verify whether a peak in a sample is due to free sulfide or to a thiol. The effect of varying the adsorption potential on the voltammetric response for sulfide, thiourea and thioacetamide is shown in Fig. 2.6. The response of sulfide was measured in the flow-cell, whilst for thiourea and thioacetamide, it was measured in the batch cell. The potential at which maximum response it obtained for thiourea and thioacetamide differs from that for sulfide in that the maximum for thiourea and thioacetamide is obtained at an adsorption potential of 0.05V, whereas for sulfide it is between 0 to -0.2V. Thiols like thiourea or thioacetamide clearly interfere with sulfide measurements by CSV in natural waters, and could be responsible for the unknown stable peak which is apparent by CSV.

### 2.4.7 Sulfide removal by reaction with mercury

The cause for peak instability for sulfide in the batch cell was further investigated with the flow-cell. The solubility of mercuric sulfide is very low (solubility product = $10^{-52.7}$ (Martell and Smith, 1976)) suggesting that mercuric ions from oxidised waste mercury in the batch cell could be the cause for the peak instability. Seawater aliquots of 10 ml were spiked with

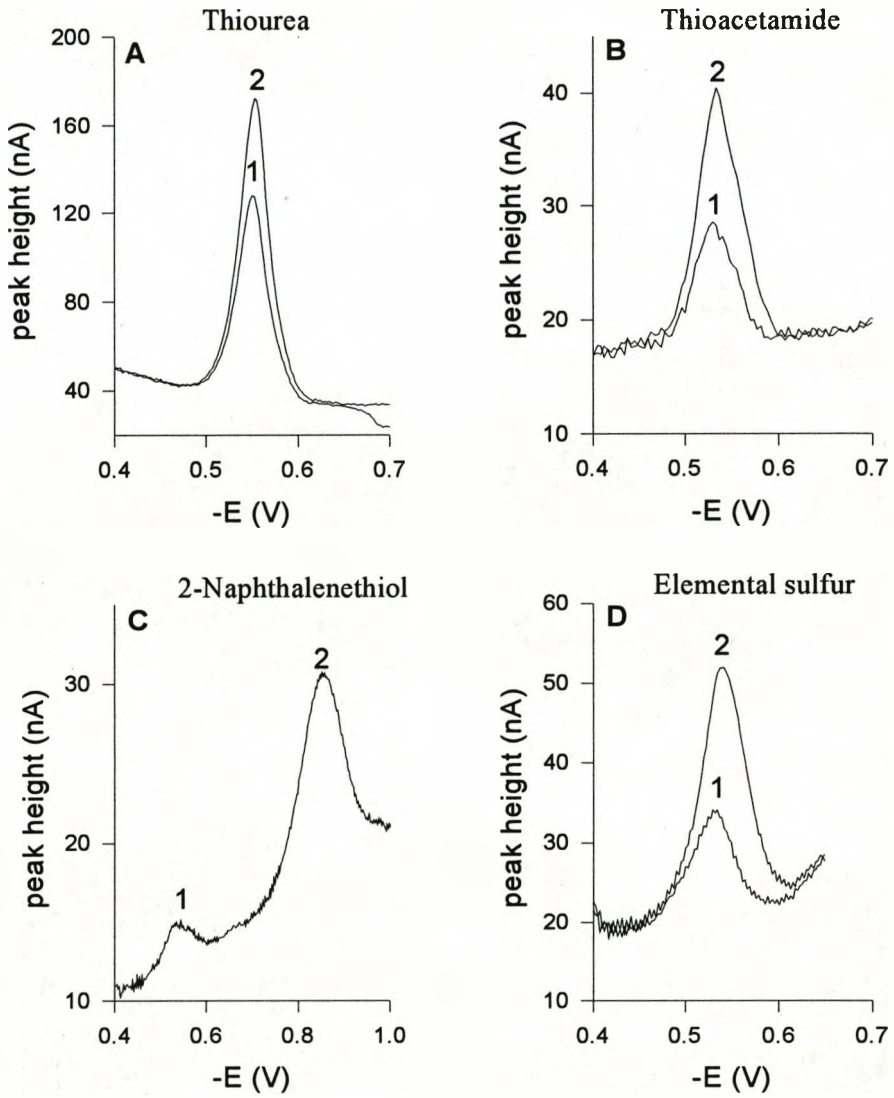


Fig. 2.5. Square-wave voltammograms for sulfide, several thiols, sulfur, and the unknown sulfide-like peak in seawater. A1: 70 nM $HS^-$; A2: 20 nM thiourea. B1: unknown natural sulfide-like peak; B2: after addition of 1 μM thioacetamide. C1: natural sulfide-like peak; C2: after addition of 60 nM elemental sulfur. D1: natural sulfide-like peak; D2: 85 nM 2-naphthalenethiol.

Table 2.2. The peak potential of several thiol and natural compounds in seawater. (Other compounds used but no peak found xanthine, adenine, dimethyl sulfide, dimethylsulfoniopropionic acid, DL-methionine).

| Compound's name | peak potential (volt) |
|---|---|
| Thioglycollic acid | -0.431 |
| 3-mercaptopropionic acid | -0.445 |
| Cystine | -0.471 |
| 2-mercaptoethanesulfonic acid | -0.478 |
| $HS^-$ | -0.533 |
| Thioacetamide | -0.533 |
| Thiourea | -0.533 |
| Elemental sulfur | -0.534 |
| 3,4 Dimercaptotoluene | -0.773 |
| 2-Naphthalenethiol | -0.845 |

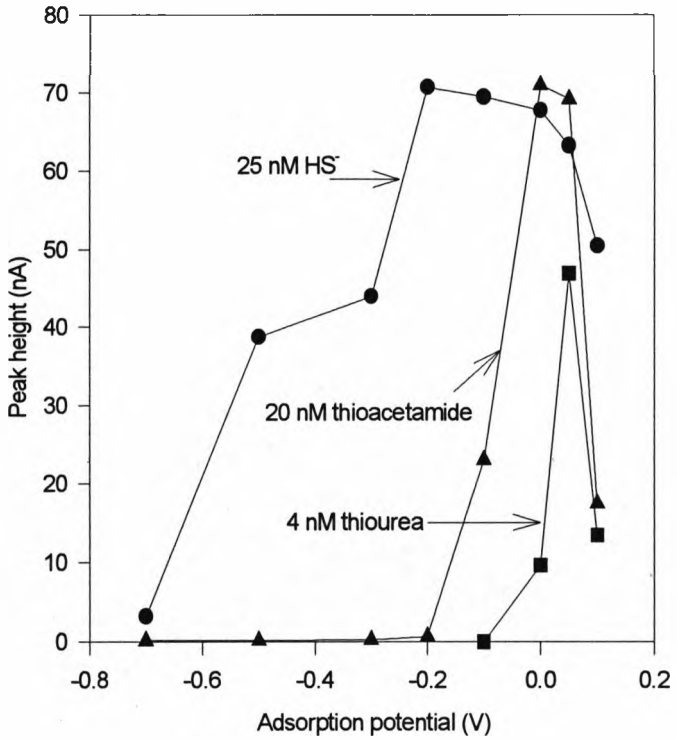


Fig. 2.6. The CSV response for free sulfide, thiourea and thioacetamide after deposition at different adsorption potentials. The response of free sulfide (25 nM) was measured using the flow-cell; thiourea (4 nM) and thioacetamide (20 nM) were measured in the batch cell.

0.1, 1, and 10 μM mercuric ions, and with metallic mercury (0.15g). Sulfide (1 μM final concentration) was then added and the aliquots placed on a shaker and equilibrated for 45 minutes; the residual sulfide concentration was measured using the flow-cell and the sensitivity was calibrated with seawater aliquots to which sulfide was freshly added. The residual sulfide concentrations were suppressed by 10% (0.1 μM $Hg^{2+}$), 97% (1 μM $Hg^{2+}$), 99% (10 μM $Hg^{2+}$), and 99.7% (metallic mercury) showing that dissolved, oxidised mercury(II) from metallic mercury in the batch cell may be the prime cause for the masking of sulfide in seawater in the batch cell. An additional pathway is the continued reaction of sulfide with waste mercury droplets in the cell on which it precipitates, forming a mercuric sulfide (clearly visible as a black coating at high sulfide concentrations). However, the latter process is comparatively slow as it involves diffusion of all bulk sulfide to the mercury drop, so it is more likely that the bulk sulfide removal in the first few minutes when the sample gets in contact with the voltammetric cell and receives traces of oxidised mercury is caused by precipitation with the dissolved mercury(II). This process is eliminated in the flow-cell as the sulfide containing seawater flows along the mercury electrode prior to being mixed with the waste solution containing enhanced mercury levels.

### 2.4.8 Discussion

The preliminary experiments using the batch cell showed that the CSV signal for sulfide added to seawater rapidly decreased to non-detectable levels within 15 minutes. In contrast, use of the flow-cell for sulfide detection showed that stable signals were obtained. Separate experiments in which residual sulfide was detected with the flow-cell after brief equilibration with mercury(II) or metallic mercury showed that the sulfide is removed rapidly from solution. Mercury (metallic as well as ionic) is always present in a voltammetric cell from waste mercury drops, but also from equilibration with the mercury drop on the tip of the electrode when the solution is placed in the cell and deaerated. The presence of metallic mercury rapidly produces dissolved mercury(II) and increasingly masks sulfide in the sample, making accurate measurements impossible. Assuming a practical half-life of 7 minutes of free sulfide in the voltammetric cell (probably less as our first scan would already have decreased significantly from the sulfide present initially), it is shown that around 50% of the sulfide is masked by the time the first scan is carried out (assuming 1 minute sample handling, 5 minutes purging, and 60 second adsorption prior to the scan). The further rapid decrease in the peak height makes it impossible to obtain

reproducible repeat scans to verify the first one and to calibrate the measurement by standard addition. It is likely that the sulfide removal is less at very high (micromolar to millimolar) sulfide concentration, but this is well out of the range for natural water samples except perhaps for pore waters in coastal sediments or the deep waters of anoxic basins such as the Black Sea. It is worthwhile to mention that the mercury(II) is not only produced by dissolved oxygen, but also by the presence of the sulfide as it shifts the oxidation potential of metallic mercury to more negative potentials (Turner et al., 1975), therefore facilitating its oxidation.

The problem of sulfide masking by mercury(II) in voltammetry is eliminated by using the flow-cell analysis. Flow-analysis with CSV eliminates reaction of samples with waste products of the measurements, and enables faster measurement and smaller sample volumes. Its application to metal analysis as well as metal-sulfide speciation in natural waters is currently being evaluated for further work.

### 2.4.9 Speculations on the nature of the sulfide-like peak in seawater

Previous voltammetric (batch) measurements have indicated the presence of nanomolar levels of a sulfide-like compound in seawater (Luther

and Tsamakis, 1989). These measurements can be compared with measurements by gas chromatography indicating up to at most 0.5 nM of total sulfide (generally much less) in the oceanic water column (Radford-Knoery and Cutter, 1994). Our work shows that inorganic sulfide gives a rapidly decreasing response and can not be determined reliably by conventional (batch) voltammetry. However, we also found a stable sulfide-like peak in seawater samples (stored as well as fresh from the North Sea). It is likely that some sulfide is strongly complexed by various metals in seawater, providing stabilisation of this bound sulfide against oxidation for kinetic reasons (the oxidation potential of the complex will have shifted to more negative values, but residual free sulfide will continue to be oxidised). However, it is counter-intuitive that this stabilised sulfide would give a stable peak by conventional (batch) voltammetry as any measured sulfide must react with the electrode's mercury and must therefore be subject to the same removal mechanism as free sulfide. Furthermore, our experiments with metal additions showed no evidence that sulfide is stabilised by such complexation. It is therefore likely that the nanomolar level of sulfide-like compounds detected by us, and previously identified as metal-sulfide species (Luther and Tsamakis, 1989), are in fact something different.

Our preliminary experiments indicate that two (thiourea and thioacetamide) of thirteen tested thiols give a peak indistinguishable from that of inorganic, free, sulfide, differing only in that the peak is stable with time. Our work therefore indicates that the unknown sulfide-like compounds could easily be thiols, which could be readily produced by bacteria as well as algae (Vairavamurthy et al., 1985).

### *2.5 Conclusions*

The experiments indicate that two reactions take place in the batch cell

1) $Hg^0 + S(II-) \Leftrightarrow HgS_{(S)} + 2e^-$

2) $Hg^{2+} + S(II-) \Leftrightarrow HgS_{(S)}$

In reaction 1 the sulfide reacts with the mercury drop electrode (and with waste mercury). This is used to preconcentrate the sulfide on the electrode. The second reaction causes losses of sulfide from solution which cannot be taken into account and interfere with its determination. The reaction mechanism leading to the formation of the sulfide peak is based on reaction 1. This reaction proceeds to the right at potentials more positive than -0.6 V, and is reversed during the potential scan where the reduction of mercury(II) causes a reduction current.

Determination of sulfide by conventional voltammetry (batch cell) is hampered by the low solubility of mercuric sulfide, which leads to inaccurate analysis of sulfide due to the rapid decrease in the free sulfide concentration by reaction with free mercury(II) as well as waste mercury. A flow-cell was used successfully for sulfide determination, introducing fresh solution to the electrode at each scan. The relative standard deviation obtained was 2.8%, and the limit of detection was 0.5 nM using an adsorption time of 60s. Measurements of seawater samples in the batch cell show a stable peak at a potential of -0.53 V, which is similar to that for free sulfide. Comparison of the electrochemical behaviour of this peak and of free sulfide, and of that of several thiol compounds, suggests that this peak could be due to thiols and is not due to free sulfide.

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# Chapter 3

## Metal-sulfide complexation

## in seawater

### *3.1 Abstract*

The conditional stability constants of metal-sulfide complexes were determined in pH 8 seawater at various salinities, by flow-analysis with detection by cathodic stripping voltammetry (FA-CSV). Two methods were used. The first method was titration of the sulfide by metal with detection of the free sulfide by FA-CSV. The titrant metals were $Ag^{+}$, $Fe^{2+}$, $Mn^{2+}$, $Cr^{3+}$ and $Al^{3+}$. This method is suitable for comparatively weak complexes (log K <~8). The second method took advantage of ligand competition between sulfide and 8-hydroxyquinoline (oxine) for free metal ions. The ability of the oxine to form electroactive complexes with $Cu^{2+}$, $Cd^{2+}$, $Pb^{2+}$, $Zn^{2+}$, $Co^{2+}$ and $Ni^{2+}$, was used to detect the concentration of the metal-oxine complexes in equilibrium with sulfide. More stable complexes could be detected by this method. The two independent methods could be compared for some metals showing good agreement.

The stability of the sulfide complexes with copper was found to be much greater than previously determined, in line with theoretical prediction. The high stability, greater than that for $Ag^{+}$ , suggests that copper may be complexed by sulfide as copper(I) rather than copper(II). Complex stability with $Co^{2+}$ and $Cd^{2+}$ is also greater than found before, but the difference is small. The stability constants were used to calculate the speciation of sulfide at realistic sulfide and metal

concentrations similar to those present in the open ocean. At a sulfide concentration of 0.5 nM and a copper concentration of 1 nM in seawater, the major form of sulfide is copper(II)-monobisulfide ($Cu(HS)^+$) representing 99% of the total sulfide. In the presence of natural organic complexing ligands a smaller percentage (28%) of sulfide was bound to copper, the remainder occurring as bisulfide, whereas copper was then 71% complexed by organic matter. The new data and the calculations confirm the potential importance of sulfide as a complexing ligand in seawater, more important than major anions and competing effectively with natural organic ligands.

### *3.2 Introduction*

Sulfide is known to occur in anoxic environments where the rate of oxygen removal is close to or exceeds its rate of supply. In such environments (e.g. the Black Sea and Framvaren Fjord), the residual oxygen concentration is present at low or non-detectable levels. At these levels sulfate is used by anaerobic bacteria as an electron acceptor, leading to the reduction of sulfate to sulfide. Anoxic environments are highly dynamic regions, and remarkable variations in the dissolved concentration of trace metals have been found at the oxic-anoxic boundary in anoxic lakes (Davison and Heaney, 1978; Morfett et al., 1988; Balistrieri et al., 1992, 1994;

Achterberg et al., 1997), and anoxic marine waters (Emerson and Jacobs, 1982; Kremling, 1983; Jacobs et al., 1985; Dyrssen and Kremling, 1990).

The improvement in sensitivity of analytical techniques since the mid-1970s have increased our knowledge of the distribution and speciation of the trace metals in seawater (Bruland, 1983). Voltammetry is a powerful tool to study trace metal speciation (e.g. (Duinker and Kramer, 1977; Hasle and Abdullah, 1981; Nürnberg and Valenta, 1983; Donat and Bruland, 1990; Campos and van den Berg, 1994; Gledhill and van den Berg, 1994; van den Berg et al., 1994), and has been used before to determine stability constants of metal-sulfide complexes in seawater (Zhang, J.-Z. and Millero, 1994; Luther et al., 1996). However, the preconcentration of complex species on an electrode to which a potential is applied can alter the speciation in the diffusion layer if the experimental set-up is not carefully considered. Its effect on the redox speciation of studied metals is not yet fully appreciated and may explain variations in results.

Awareness of the potential importance of sulfide to metal speciation has increased due to evidence suggesting the presence of sulfide in oxic seawater as measured by two techniques: gas chromatography (Cutter and Krahforst, 1988; Radford-Knoery and Cutter, 1993, 1994) and CSV (Luther and Tsamakis, 1989). The

hydrolysis of carbonyl sulfide is thought to be the main source of sulfide, at pico to nanomolar concentration, in seawater (Elliott et al., 1987). Sulfide in seawater is thought to be oxidized by several oxidants including oxygen (Millero, 1991), hydrogen peroxide (Millero et al., 1989), and iodate (Zhang, J.-Z. and Whitfield, 1986), and its presence is therefore unexpected. Sulfide is thought to be stabilised by complexation with trace metals (Luther and Tsamakis, 1989). Several metals have been proposed as responsible for the sulfide stabilisation. Estimation of the stability of metal-sulfide complexes using the linear free energy technique suggests that sulfide speciation in seawater is controlled by copper (Dyrssen, 1988; Elliott, 1988).

Experimental work on the rate of sulfide oxidation by metals demonstrated that copper(II)-monobisulfide has a higher oxidation rate than free sulfide, and that $Zn^{2+}$ is more likely to stabilise sulfide (Vazquez et al., 1989). Voltammetry was used to monitor the distribution of sulfide in the water column of the Black Sea (Luther et al., 1991). A distinction was made between free and complexed sulfide, depending on the location of the peak potential, and it was postulated that the complexed sulfide was in the form of manganese-sulfide (MnS) at depths below 100 m. A polarographic peak (at -1.1 V) of sulfide species in anoxic lake waters in the presence of iron(II) and a high level of sulfide was attributed to iron(II)-sulfide (FeS) (Davison, 1977). Iron(II)-sulfide species are also thought to occur at the sulfidic-

nonsulfidic interface in marine porewaters (Luther and Ferdelman, 1993). The sulfide in lake waters can be loosely attached to algae (Davison and Gabbutt, 1979). The iron(II)-sulfide complexes are kinetically labile when their interaction is determined in standard solutions contain iron(II) and sulfide, whereas these complexes are inert in porewaters (Luther and Ferdelman, 1993).

A recent study (Ciglenecki and Cosovic, 1996) has shown that the voltammetric response to a sulfide-like compound in seawater gave a stable voltammetric peak even after acidification and purging, suggesting that it was not due to free sulfide. More recently, the sulfide-like peak in seawater has been ascribed to thiols (Al-farawati and van den Berg, 1997 (Chapter 2)). It is therefore possible that the free sulfide concentration in the marine system is lower than expected, and that part of the voltammetric response is due to thiols.

On the whole the previous work indicates the presence of free sulfide, various metal-sulfide species, as well as sulfide-like substances in fresh water and seawater. For modeling purposes it is important to establish the thermodynamic stability of the major metal-sulfide species. The stability of the sulfide-metal species has been evaluated theoretically (Dyrssen, 1988), and experimentally (Zhang, J.-Z. and Millero, 1994; Luther et al., 1996) by voltammetry.

In addition to voltammetric and theoretical prediction methods, solubility measurements were used to determine the stability constants of metal sulfide complexes for a number of metals (e.g. Cd (Ste-Marie et al., 1964; Daskalakis and Helz, 1992), Cu (Shea and Helz, 1988; Thompson and Helz, 1994), Pb (Giordano and Barnes, 1979),(Zn (Hayashi et al., 1990; Daskalakis and Helz, 1993) and Ag (Gammons and Barnes, 1989)). In that work, the dissolution of sulfide minerals in the presence of dissolved sulfide was used to calculate the constant using measurements of total sulfide and metal. Various type of metal sulfide complexes were used to interpret the solubility of metals (Table 3.1).

Existing knowledge of the composition and stability of sulfide complexes comes mostly from sulfide mineral solubility and voltammetric measurements. (Luther and Ferdelman, 1993; Luther et al., 1996) titrated metal sulfide solutions with acid and base. For Mn, Fe, Ni, and Co, They found that the complexes with sulfide are metal-rich (polynuclear) in the form $MHS^+$, $M_2(HS)^{3+}$, $M_3(HS)^{5+}$ whereas the stoichiometry of Cu and Zn complexes are MS and $M_2S_3^{2-}$. The high positive charge on these species is unexpected in seawater. The solubility measurements indicated that Cd and Zn form mononuclear complexes with respect of metal

(Daskalakis and Helz, 1992, 1993). In other work, silver was shown to form a mononuclear complex (Gammons and Barnes, 1989).

The stoichiometry of Cu was more complicated and it was shown to form various complexes with mononuclear, polynuclear and mixed forms (Shea and Helz, 1988; Helz et al., 1993; Thompson and Helz, 1994). The solubility measurements of PbS in sodium hydroxide solution was investigated in bisulfide solutions at various temperature (30-300 $^{0}$C) (Giordano and Barnes, 1979). The authors reported two mononuclear lead sulfide complexes at 30 $^{0}$C (Table 3.1). At high temperature (300 $^{0}$C) these complexes were unimportant and lead solubility can be explained in the term of another complex.

Recent work has shown that the sulfide peak obtained by conventional CSV in not stable due to precipitation with mercury which is normally present in the voltammetric cell (Al-farawati and van den Berg, 1997 (Chapter 2)). This problem is eliminated by using FA-CSV instead of the conventional, batchwise, voltammetric analysis. FA-CSV is used here to determine complexation of sulfide with several trace metals in seawater. The methods and the results are presented here.

Table 3.1 Examples of stability constants of metal sulfide complexes that were measured by solubility technique.

| Reaction | Medium | $^0$C | pH | log constant |
|---|---|---|---|---|
| Ag (Gammons and Barnes, 1989) | NaOH | 25 | 5.8-7.3 | |
| $1/2Ag_2S + 1/2H_2S + HS^- =$ $Ag(HS)_2^-$ | | | | -3.82±0.10 |
| Cd (Daskalakis and Helz, 1992) | NaCl | 25 | 4-9 | |
| $CdS + H^+ = CdHS^+$ | | | | ≤-6.7 |
| $CdS + H^+ + HS^- = Cd(HS)_2^0$ | | | | ≤-1.0 |
| $CdS + H^+ + 2HS^- = Cd(HS)_3^-$ | | | | 2.08±0.36 |
| $CdS + H^+ + 3HS^- = Cd(HS)_4^{2-}$ | | | | 3.53±0.01 |
| $CdS + H_2O = CdOHS^- + H^+$ | | | | -16.83±0.33 |
| $CdS = CdS^0$ | | | | ≤-9.1 |
| Cu (Shea and Helz, 1988) | NaCl | 25 | 6-11 | |
| $CuS + 3HS^- = CuS(HS)_3^{3-}$ | | | | -4.04±0.01 |
| $CuS + 2HS^- = CuS(HS)_2^{2-}$ | | | | -4.97±0.06 |
| $CuS + HS^- = CuS(HS)^-$ | | | | <-6.4 |
| $CuS = CuS^0$ | | | | <-8.3 |
| $CuS + S_5^{2-} = CuS(S_5)^{2-}$ | | | | -2.631±0.003 |
| $H^+ + 2CuS + 3S_4^{2-} + S_6^{2-} =$ $2CuS_4S_5^{3-} + HS^-$ | | | | 6.467±0.032 |

Continue....

Table 3.1 Continue……

| Reaction | Medium | $^0$C | pH | log constant |
|---|---|---|---|---|
| $H^+ + 2CuS + S_4^{2-} + 3S_6^{2-} =$ | | | | 6.01±0.12 |
| $2Cu(S_5)_2^{3-} + HS^-$ | | | | |
| $Cu^{2+} + 3HS^- = CuS(HS)_2^{2-} + H^+$ | | | | 15.98±0.22 |
| $Cu^{2+} + 4HS^- = CuS(HS)_3^{3-} + H^+$ | | | | 16.91±0.21 |
| $Cu^{2+} + S_5^{2-} + HS^- = CuS(S_5)^{2-} + H^+$ | | | | 18.32±0.15 |
| $Cu^{2+} + 3/2S_4^{2-} + 1/2S_5^{2-} + 1/2HS^- =$ | | | | 24.18±0.15 |
| $CuS_4S_5^{3-} + ½H^+$ | | | | |
| $Cu^{2+} + 1/2S_4^{2-} + 3/2S_5^{2-} + 1/2HS^- =$ | | | | 23.95±0.21 |
| $Cu(S_5)_2^{3-} + ½H^+$ | | | | |
| Pb (Giordano and Barnes, 1979) | NaOH | 30 | ? | |
| $PbS + H_2S_{(g)} + HS^- = Pb(HS)_3^-$ | | | | -6.6±0.20 |
| $PbS + H_2S_{(g} = Pb(HS)_2$ | | | | -7.8±0.20 |
| Zn (Daskalakis and Helz, 1993) | NaCl | 25 | 2.2-9.1 | |
| $ZnS + H_2S = Zn(HS)_2^0$ | | | | -5.65 |
| $ZnS + 2H_2S = Zn(HS)_2^{2-}$ | | | | -5.33 |
| $ZnS + H_2S + 2HS^- = Zn(HS)_4^{2-}$ | | | | -3.83 |
| $ZnS + HS^+ = ZnS(HS)^-$ | | | | -4.64 |

### *3.3 Theory and calculations*

Two methods were used to determine the stability of the metal-sulfide species, the first based on detection of free sulfide in the presence of added metal ions, and the second based on ligand competition with detection of the residual metal concentration in the presence of added sulfide.

Hydrogen sulfide dissociates as follows:-

$$[H_2S] \Leftrightarrow [H^+] + [HS^-] \quad (3.1)$$

$$[HS^-] \Leftrightarrow [H^+] + [S^{2-}] \quad (3.2)$$

where, $[H_2S]$ is the concentration of hydrogen sulfide, $[HS^-]$ is the concentration of the bisulfide ion, and $[S^{2-}]$ is the concentration of the sulfide ion.

The mass balance for sulfide is expressed as:

$$[S]_T = [H_2S] + [HS^-] + [S^{2-}] \quad (3.3)$$

where, $[S]_T$ is the total concentration of sulfide species. Using a value of 6.67 (Hershey et al., 1988) for $pK_1$ (equation 3.1) and 18.51 (Schoonen and Barnes, 1988) for $pK_2$ (equation 3.2), the fractions of sulfide species at pH 8 seawater are 4.46 %, 95.53 % and $2.5\times10^{-4}$ % for $H_2S$, $HS^-$ and $S^{2-}$ respectively. It can therefore be approximated that

$$[S]_T \approx [HS^-] \quad (3.4)$$

and, in the absence of metals,

$$[HS]_T = [HS^-] \tag{3.5}$$

where, $[HS]_T$ is the total bisulfide ion concentration.

### 3.3.1 Method of free sulfide detection

The voltammetric signal for sulfide in the absence of added metal ($I_{max}$) corresponds with the total sulfide concentration. Complexation of $HS^-$ by the major cations in seawater is likely, but its effect is constant. The term [HS'] is used instead of $[HS^-]$ to denote the concentration of bisulfide not complexed by trace metals in seawater, comprising free bisulfide and bisulfide complexed by the major cations. In the absence of added metals:

$$[HS]_T = [HS'] \tag{3.6}$$

After addition of metal to seawater with added sulfide, the signal corresponding to sulfide decreases as a result of metal-sulfide complex formation. The reaction of metal with sulfide is assumed to follow the expression :

$$[M^{n+}] + m[HS'] = [M(HS)_m]^{(n-m)+} \tag{3.7}$$

The conditional stability constant, $\beta_m'$ is

$$\beta_m' = \frac{[M(HS)_m]^{(n-m)+}}{[M^{n+}][HS']^m} \tag{3.8}$$

Where, $[M^{n+}]$ is the free metal concentration, and $[M(HS)_m]^{(n-m)+}$ is the concentration of metal-sulfide species.

Rearrangement of equation (3.8) yields:-

$$[M(HS)_m]^{(n-m)+} = \beta_m'[M^{n+}][HS']^m \quad (3.9)$$

The sulfide signal decreases when metal is added due to complex formation with the added metal ions. In this case, the current is denoted as $I_{P,S}$. The mass balance for sulfide is:

$$[HS]_T = [HS'] + [MHS]_T \quad (3.10)$$

where $[MHS]_T$ is the total concentration of metal-sulfide species:

$$[MHS]_T = \sum m[M(HS)_m]^{n-m+} \quad (3.11)$$

Combining equations (3.8), (3.9), (3.10) and (3.11) yields:-

$$[HS]_T = [HS'] + \sum m\beta_m'[M^{n+}][HS']^m \quad (3.12)$$

The ratio ($R$) of free over total sulfide is accessible by CSV. Its relationship to the sulfide speciation is derived from Eqn. 3.12:

$$R = \frac{[HS']}{[HS]_T} = \frac{1}{1 + \sum m\beta_m'[M^{n+}][HS']^{m-1}} \quad (3.13)$$

Voltammetrically the ratio $R$ = (free sulfide/total sulfide) is obtained from:

$R = \frac{[HS']}{[HS]_T} = \frac{I_{P,S}}{I_{max}}$ which is determined experimentally from the ratio of the voltammetric response for sulfide in the presence ($I_{P,S}$) and absence ($I_{max}$)

of added metals. In the absence of added metals $I_{P,S}=I_{max}$ and the ratio $R$=1. The free metal ion concentration [$M^{n+}$] is computed from the mass balance for the metal:-

$$[M]_T = [M'] + [MHS]_T \tag{3.14}$$

where $[M]_T$ is the total metal concentration, and [M'] is the metal concentration not complexed by sulfide including complexes with the major anion in seawater:

$$[M'] = [M^{n+}]\alpha_M \tag{3.15}$$

where $\alpha_M$ is the side-reaction coefficient of the metal in seawater. $\alpha_M$ equals (total metal/free metal) and is calculated from:

$$\alpha_M = 1 + \Sigma K_i^*[F_j]^i + \Sigma \frac{K_{a,i}^*}{[H^+]^i} \tag{3.16}$$

where $K_i^*$ is the stepwise stability constant of $M^{n+}$ with each major anion $F_j$, and $K_{a,i}^*$ is the stepwise acidity constant. $\alpha_M$ was calculated for each metal using an ion-pairing model to obtain free major anion concentrations, and using stability constants valid for seawater from (Turner et al., 1981). The free metal ion is then obtained from:

$$[M^{n+}] = \frac{[M]_T - [MHS]_T}{\alpha_M} \tag{3.17}$$

The total metal concentration is known as it was added to seawater. $[MHS]_T$ was determined experimentally from the difference of the added

total sulfide concentration and the residual free sulfide concentration as measured by FA-CSV.

Values for conditional stability constants were calculated by two methods: one using non-linear least-squares fitting by fitting $R$ to eqn. (3.13) as a function of $[M^{n+}]$, and the second from the mean of values for $\beta_m'$ from single values of $R$ using the following modified eqn. (3.13):

$$\beta_m' = \frac{\frac{1}{R} - 1}{\sum m[M^{n+}][HS^{m-1}]} \qquad (3.18)$$

A computer programme (Sigma plot) was used for the curve fitting.

### 3.3.2 Ligand competition method

This method depends on competition between sulfide and oxine for metal ions. Oxine forms a complex with the metal which is detected by FA-CSV. After addition of sulfide, the signal of the metal-oxine complex decreases as a result of complexation of the metal by sulfide. The procedure is similar to that used for the CSV methods to determine complexation of zinc, nickel, and copper, by organic complexing ligands in seawater using ligand competition against APDC (ammonium pyrrolidine dithiocarbamate) (van den Berg, 1985), DMG (dimethyl glyoxime) (van den Berg and Nimmo, 1987) and tropolone (Donat and van den Berg, 1992) respectively.

The measured reduction current, $I_p$, is directly related to the dissolved concentration of metal-oxine, [Moxine$_j$] through a proportionality factor $S_1$:

$$I_p = S_1[\text{Moxine}_j] \tag{3.19}$$

where $j$=1, 2, 3,....etc.

In the absence of added sulfide the measured reduction current is maximal so:

$$I_p = I_{max} \tag{3.20}$$

and

$$[\text{Moxine}_j] = [\text{M}^{n+}]\alpha_{M-oxine} \tag{3.21}$$

where $\alpha_{M\text{-}oxine}$ is $\alpha$-coefficient for the complexation of metal by oxine .

$$\alpha_{M-oxine} = \beta'_{M-oxine}[\text{oxine}']^{\gamma} \tag{3.22}$$

where [oxine'] is the oxine concentration not complexed by the metal (the total oxine concentration $[\text{oxine}]_T$ was used instead of [oxine'] as $[\text{oxine}]_T >> [\text{M}]_T$) and $\beta'_{M-oxine}$ is the conditional stability constant of metal-oxine complex. Values for the conditional stability constants $\beta'_{M-oxine}$ were calculated from the thermodynamic stability constant $\beta^*_{M-oxine}$ by correction for side reactions of oxine with the major cations and hydrogen ions in seawater:

$$\beta^{*}_{M\text{-}oxine} = \frac{[\text{Moxine}_j]}{[M^{n+}][\text{oxine}^{-}]^{j}} \tag{3.23}$$

where [oxine$^-$] is the free oxine concentration which is correlated to [oxine'] by :

$$[\text{oxine}^{-}] = \frac{[\text{oxine}']}{\alpha_{oxine}} \tag{3.24}$$

where $\alpha_{oxine}$ is the side reaction coefficient of oxine with hydrogen ion and major cations in seawater. $\alpha_{oxine}$ was calculated from the relation:-

$$\alpha_{oxine} = 1 + \sum K^{*}_{i}[J^{n+}]^{i} + \sum \frac{[H^{+}]^{i}}{K^{*}_{a,i}} \tag{3.25}$$

where $K^{*}_{i}$ is the stepwise stability constant of oxine with J, $K^{*}_{a,i}$ is the stepwise acidity constant, [$J^{n+}$] is the concentration of free cations in seawater, and [$H^+$] is the free hydrogen ion concentration.

Values for $\beta^{*}_{M-oxine}$, $K^{*}_{i}$ and $K^{*}_{a,i}$ were taken from (Martell and Smith, 1989). The values $\beta^{*}_{M-oxine}$, $K^{*}_{i}$ and $K^{*}_{a,i}$ have been subjected to extensive studies by a large number of authors. These values have been evaluated by (Stary et al., 1979) and showed excellent agreement. These values were in agreement with the values which were reported by (Martell and Smith, 1989)

Values for [$J^{n+}$] and [$H^+$] were calculated using an ion-pairing model.

Substitution of (3.24) in (3.23) yields:-

$$\beta^{*}_{M-oxine} = \frac{[\text{Moxine}_j]}{[\text{M}^{n+}]\frac{[\text{oxine}']^{\nu}}{\alpha_{oxine}}} \tag{3.26}$$

which is equivalent to

$$\beta'_{M-oxine} = \frac{\beta^{*}_{M-oxine}}{\alpha_{oxine}} \tag{3.27}$$

$[M]_T$ is related to $[M^{n+}]$ by:

$$[\text{M}]_T = [\text{M}^{n+}][\alpha_M + \alpha_{M-oxine}] \tag{3.28}$$

In the presence of sulfide the current is diminished by the amount of metal complexed by sulfide:

$$I_{P,S} = S_1[\text{Moxine}_j]_S \tag{3.29}$$

where $[\text{Moxine}_j]_S$ indicates the concentration of metal-oxine species in the presence of sulfide:

$$[\text{Moxine}_j]_S = [\text{M}^{n+}]_S \alpha_{M-oxine} \tag{3.30}$$

The mass balance for the metal in the presence of sulfide is written as:

$$[\text{M}]_T = [\text{M}^{n+}]_S[\alpha_M + \alpha_{M-oxine} + \alpha_{MHS}] \tag{3.31}$$

where $\alpha_{MHS}$ is α-coefficient for the complexation of metal by sulfide:

$$\alpha_{MHS} = \beta'_m[\text{HS}']^m \tag{3.32}$$

The ratio, $Q$, of the peak current in the presence ($I_{P,S}$) over that in the absence ($I_{max}$) of sulfide ($Q = I_{P,S} / I_{max}$) is directly related to the concentrations of the metal-oxine species in those conditions:

$$Q = I_{P,S} / I_{max} = S_1[\text{Moxine}_j]_S / S_1[\text{Moxine}_j].$$

After substitution and simplification this ratio is directly related to the free metal concentrations in the presence and absence of sulfide:

$$Q = \frac{[M^{n+}]_S}{[M^{n+}]} \tag{3.33}$$

Substitution for $[M^{n+}]$ and $[M^{n+}]_S$ using eqn. (3.28) and (3.31) with rearrangement gives

$$Q = \frac{\alpha_M + \alpha_{M-oxine}}{\alpha_M + \alpha_{M-oxine} + \alpha_{MHS}} \tag{3.34}$$

This means that the ratio ($Q$) of the peak heights depends on a relatively simple ratio of $\alpha$-coefficients. $Q$ is directly accessible by experiment. $Q$, varies when $\alpha_{MHS}$ (the only unknown) is varied by sulfide addition. The other $\alpha$-coefficients are constant during each experiment.

Substitution for $\alpha_{MHS}$ in eqn. (3.34) yields:

$$Q = \frac{\alpha_M + \alpha_{M-oxine}}{\alpha_M + \alpha_{M-oxine} + \beta_m'[HS']^m} \tag{3.35}$$

[HS'] is calculated from a sulfide mass balance:

$$[HS'] = [HS]_T - [[M]_T - [M]_{labile}] \tag{3.36}$$

where $[M]_{labile}$ is the labile metal concentration which is measured by FA-CSV; and the total sulfide and metal concentrations are known. Values for $\beta_m'$ were obtained by fitting $Q$ to equation (3.35) using non-linear, least square curve-fitting as a function of the sulfide concentration.

Preliminary values for $\beta_m'$ were obtained by calculation of single values from:

$$\beta_m' = \frac{[\frac{\alpha_M + \alpha_{M\text{-oxine}}}{Q}] - [\alpha_M + \alpha_{M\text{-oxine}}]}{[HS']^m} \quad (3.37)$$

### *3.4 Materials and methods*

#### 3.4.1 Equipment

Voltammetric measurements of sulfide and metals were performed by FA-CSV using an Autolab voltammeter (Eco Chemie) that was interfaced with a Metrohm 663 VA electrode stand and a hanging mercury drop electrode (HMDE). The conventional voltammetric cell was replaced by a specially designed flow-cell which was fitted in the bottom of a voltammetric cell made from extruded acrylic (Al-farawati and van den Berg, 1997 (Chapter 2)). For some metals (Ni, Zn and Co) the flow cell was preceded by an in-line deaeration system (Colombo et al., 1997) to remove dissolved oxygen. The in-line deaeration system consisted of a length of silicone tubing (1 m) which was fitted inside a 28 ml container which was flushed with nitrogen at rate of 50 ml $min^{-1}$. The reference electrode was double-junction: an Ag/AgCl/3M KCl reference cartridge (Metrohm, Switzerland) was separated by a frit from a salt bridge filled with 3 M KCl:

the bridge was freshly filled with KCl solution at the beginning of the experiments. The counter electrode was a glassy carbon rod. UV-digestion was carried out using a 1 kW, high-pressure, mercury vapour lamp.

### 3.4.2 Reagents

Water for preparation of reagents and rinsing was purified by reverse osmosis (Milli-RO, Millipore) and ion-exchange (Milli-Q, Millipore). Acid (HCL) and ammonia used for solutions were purified by sub-boiling, quartz distillation. Stock solutions of 0.1 M NaHS were prepared daily from $Na_2S.9H_2O$ (Aldrich) in Milli-Q. Metal stock solutions were prepared by dilution of atomic absorption spectrometry standard solutions (BDH, Spectrosol grade) in Milli-Q and acidified by addition of 10 µl of 50% HCl to 10 ml of solution. Standard of Fe(II) and Mn(II) were prepared from Fe(III) and Mn(II) in an aqueous solution of hydroxylammonium hydrochloride at a concentration 10 times higher than that of the iron and manganese to maintain the iron and manganese in reduced form. A stock solution of 0.1 M hydroxylammonium hydrochloride (Analar, BDH) was prepared prior to use in Milli-Q. The effect of hydroxylammonium hydrochloride on sulfide was tested by double addition and the effect was shown to be negligible. A stock solution of 5 M sodium nitrite (Analar, BDH) was prepared in Milli-Q.

Hydrochloric acid was Analar (BDH) grade. A stock solution of 0.1 M 8-hydroxyquinoline (oxine), (BDH, Analar) was prepared in 0.2 M HCl and diluted with Milli-Q; this solution is stable for several months (van den Berg, 1986). A pH buffer was prepared by containing
1 M tris(hydroxymethyl)methylamine (Analar, BDH) in 0.5 M HCl; addition of 100 µl of this TRIS buffer in 10 ml seawater served to maintain the pH at 8. A solution of 2 M $NH_3/NH_4^+$ was prepared by mixing ammonia with hydrochloric acid, and was used to buffer the pH at 9 during voltammetric detection of cobalt.

Seawater used throughout the experiments originated from the North Sea, and had a salinity of 35. The water was UV-irradiated for 3 h to eliminate organic matter that could interfere with the metal-sulfide complexation measurements. This water was diluted with UV-irradiated Milli-Q water to obtain lower salinities of 21 and 10.5. All reagent solutions were prepared by weighing the solid and Milli-Q into pre-cleaned polystyrene (30 ml) (Bibby Sterilin Ltd, Cat.No.128A) tubes (called Sterilins in the text), or Nalgene high density polyethylene bottles. The final volume of the solutions was adjusted by weight. This procedure for solution

preparation avoids possible contamination with metal ions (such as from glassware or due to excessive manipulation of the solutions).

### 3.4.3 Procedure of the free sulfide detection method

100-150 ml of irradiated seawater was transferred to a 250 ml polyethylene bottle and buffered with Tris to pH 8. A subsample of 10 ml was pipetted into a clean Sterilin, and sulfide was added. This aliquot was used as reference (without added metal) to calculate the ratio, *R*, (equation 3.13). Metal was added to a second, third, fourth and so on (with increasing metal concentrations), aliquot, and the free sulfide concentration was determined by FA-CSV. The analysis was initiated within a minute after sulfide addition. This procedure continued with increasing metal concentrations of metal, until the peak height of sulfide was reduced to approximately a third of the peak height of the reference sample. pH changes by additions of some metal solutions (Al(III) and Cr(III)) caused the sulfide peak to shift to a more positive potential due to acid in these solutions (0.5 N of nitric acid for Al and 0.1 N of perchloric acid for Cr). Ammonia was therefore added along with the Al and Cr additions to neutralize the pH effect.

### 3.4.4 Procedure of the ligand competition method

100-150 ml of the UV-irradiated seawater was transferred to a 250 ml polyethylene bottle. The seawater was buffered to pH 8 by addition of Tris buffer for cadmium, copper, lead, nickel and zinc, and pH 9 by addition of ammonium buffer for cobalt. Oxine (1-100 μM) and metal additions (30-100 nM) were made to the buffered seawater. A subsample of 10 ml was pipetted into a clean Sterilin, spiked with the sulfide standard and mixed. After an equilibration time of 1-2 minutes, the peak height of the metal-oxine was measured by FA-CSV. The pump was run for 2 min prior to initiating the measurements between different concentrations allowing the tubing to become flushed with new sample solution. Between 4 and 6 scans were carried out at each concentration. The procedure continued with increasing concentrations of sulfide until the peak height of the metal was reduced to ~30 % of the initial peak height before the sulfide additions. The voltammetric parameters to determine the stability constants of metal-sulfide complexes are shown in Table 3.2.

### 3.4.5 In-line purging

In-line purging was used for the voltammetric determination of the cobalt, nickel and zinc concentrations, as the oxygen wave was found to interfere. The oxygen wave was a broad peak occurring at ~-1 V, overlapping with the peak potentials for nickel, zinc and cobalt, at -0.93, -1.11 and -1.12 V, respectively. The interference of the oxygen wave with the peaks for copper, cadmium, lead and sulfide was much less due to the much better sensitivity for these elements than for oxygen, and also because their peaks occurred away from that for oxygen; the peak potentials for copper, lead, sulfide and cadmium were at -0.40, -0.51, -0.54 and -0.67 V, respectively, the background current due to oxygen being low between -0.1 and -0.9 V. An advantage of measuring without the in-line purging system was a shorter measuring time, because the travel-time of the solution through the tubing was 20 s less. An titration with sulfide with detection of residual metal took 30-50 minutes, using 4-6 repeat scans for each aliquot.

Table 3.2. The experimental parameters for determination of metal-sulfide complexes using cathodic stripping voltammetry. The methods are indicated by A (free sulfide detection) or B (ligand competition)

| Metal | Deposition time (sec) | deposition potential (V) | Frequency (Hz) | Scan range (V) | Method |
|---|---|---|---|---|---|
| Ag(I) | 25 | -0.2 | 200 | -0.4 to -0.9 | A |
| Cd(II) | 20 | -0.2 | 100 | -0.3 to -0.8 | A/B |
| Co(II) | 15 | -0.05 | 50 | -0.7 to -1.3 | A/B |
| Cu(II) | 30 | -0.2 | 100 | -0.2 to -0.8 | B |
| Ni(II) | 30 | -0.1 | 20 | -0.75 to -1.25 | B |
| Pb(II) | 60 | -0.2 | 100 | -0.3 to -0.8 | A/B |
| Zn(II) | 20 | -0.1 | 200 | -0.85 to -1.3 | A/B |
| Fe(II) | 15 | -0.2 | 200 | -0.3 to -0.8 | A |
| Mn(II) | 15 | -0.2 | 200 | -0.3 to -0.8 | A |
| Al(III) | 15 | -0.2 | 200 | -0.2 to -0.8 | A |
| Cr(III) | 15 | -0.2 | 200 | -0.4 to -0.75 | A |

## *3.5 Results*

### 3.5.1 Titration of sulfide with metal with detection of free sulfide by FA-CSV

Voltammetric scans showing the decrease of the sulfide peak by the addition of metal ions are shown for silver in Fig. 3.1. The sulfide peak is thought to be caused by the precipitation of mercuric sulfide species on the electrode surface (Turner et al., 1975). At low sulfide concentrations it is more likely that the sulfide layer on the electrode consists of adsorbed, rather than precipitated, complex species and that it consists of a single molecular layer. In the flow system these species are formed in the first molecular layer next to the mercury surface where the mercury reacts with sulfide according to reaction 2.3; Chapter 2. These mercury species are generated by oxidation of mercury induced by the presence of the sulfide. It is possible that some of the dissolved metal-sulfide species (other than mercury) dissociate in the diffusion layer of sulfide (if they are labile), but their contribution to the dissolved sulfide concentration is likely to be minor until high metal concentrations when most of the sulfide is complexed and the free concentration of sulfide is much lowered: The total sulfide

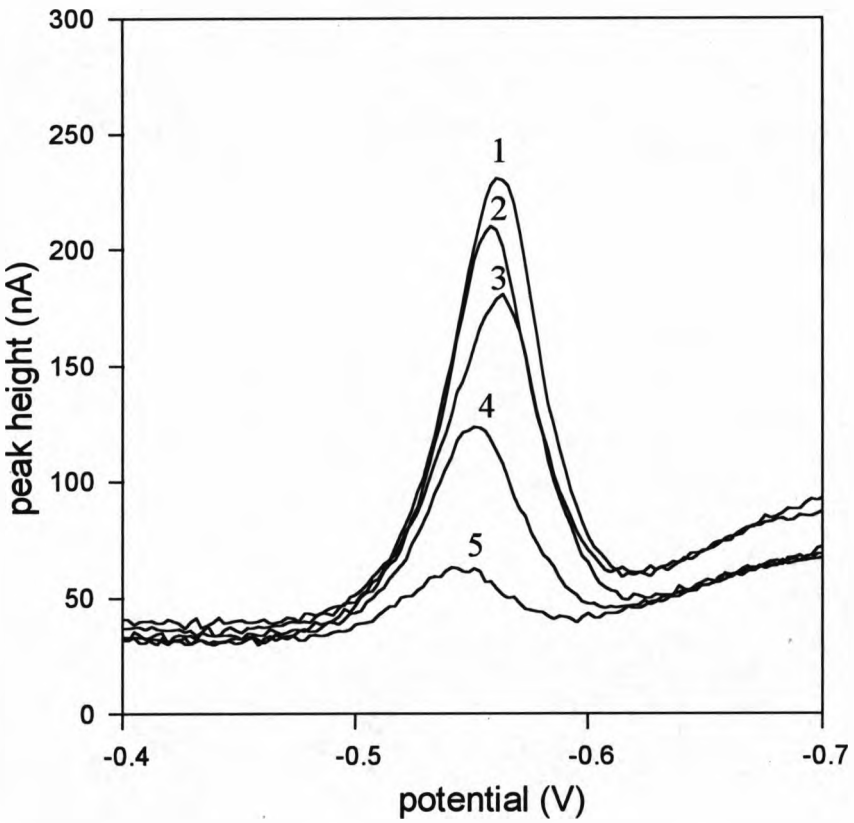


Fig. 3.1. The voltammetric response of sulfide ion: 1) before addition of silver; and after addition of 2) 100, 3) 250, 4) 350, 5) 2000 nM $Ag^{+}$, using 100 nM of sulfide ion and salinity of 35.

consists of free bisulfide and metal sulfide complexes (equation 3.10) and metal sulfide species are formed according to reaction 3.7. At low metal levels the equilibrium lies to the left and most sulfide occurs as free $HS^-$: A lowering of its concentration in the diffusion layer during the measurement is likely to have a relatively small effect on the concentration of MHS. However, if the reaction kinetic of reaction 3.7 are fast then this can be important at higher metal concentrations.

It is worthwhile to mention that a shift in the peak potential in Fig. 3.1 could be attributed to the change in the concentration of sulfide. This was not due to the change of the pH which was monitored at the beginning and end of the experiment.

The titrations of sulfide with metal ions were discontinued when the free sulfide concentration had decreased by 60-70 %, to minimise this possible contribution of the complexed sulfide to the reduction current.

The decrease in the sulfide peak height (the *R*-ratio) with the increase in the metal concentrations is shown in Fig. 3.2. Values for the stability constants were calculated by non-linear least squares curve fitting of *R* to Eqn. 3.13; first assuming the presence of only one metal sulfide species of the type MHS or $M(HS)_2$, and then both simultaneously. Values for the constants

were also calculated for each data point separately (equation 3.18) and averaged. These values were used to corroborate the curve fitting data. The values for the stability constants along with experimental details are presented in Table 3.3.

### 3.5.2 Titration of metals with sulfide with FA-CSV detection of free metal by ligand competition

Ligand competition between sulfide and oxine was used to determine the stability constants for sulfide complexes with copper, lead, cadmium, zinc, cobalt and nickel. The complexes of oxine form electroactive species with these metals which can be determined by FA-CSV (van den Berg, 1986; Colombo and van den Berg, 1997). The decrease of the lead-oxine peak upon addition of sulfide is shown in Fig. 3.3. A peak due to free sulfide appeared at -0.54 V when the lead-oxine peak had completely disappeared and all lead had been complexed by the added sulfide.

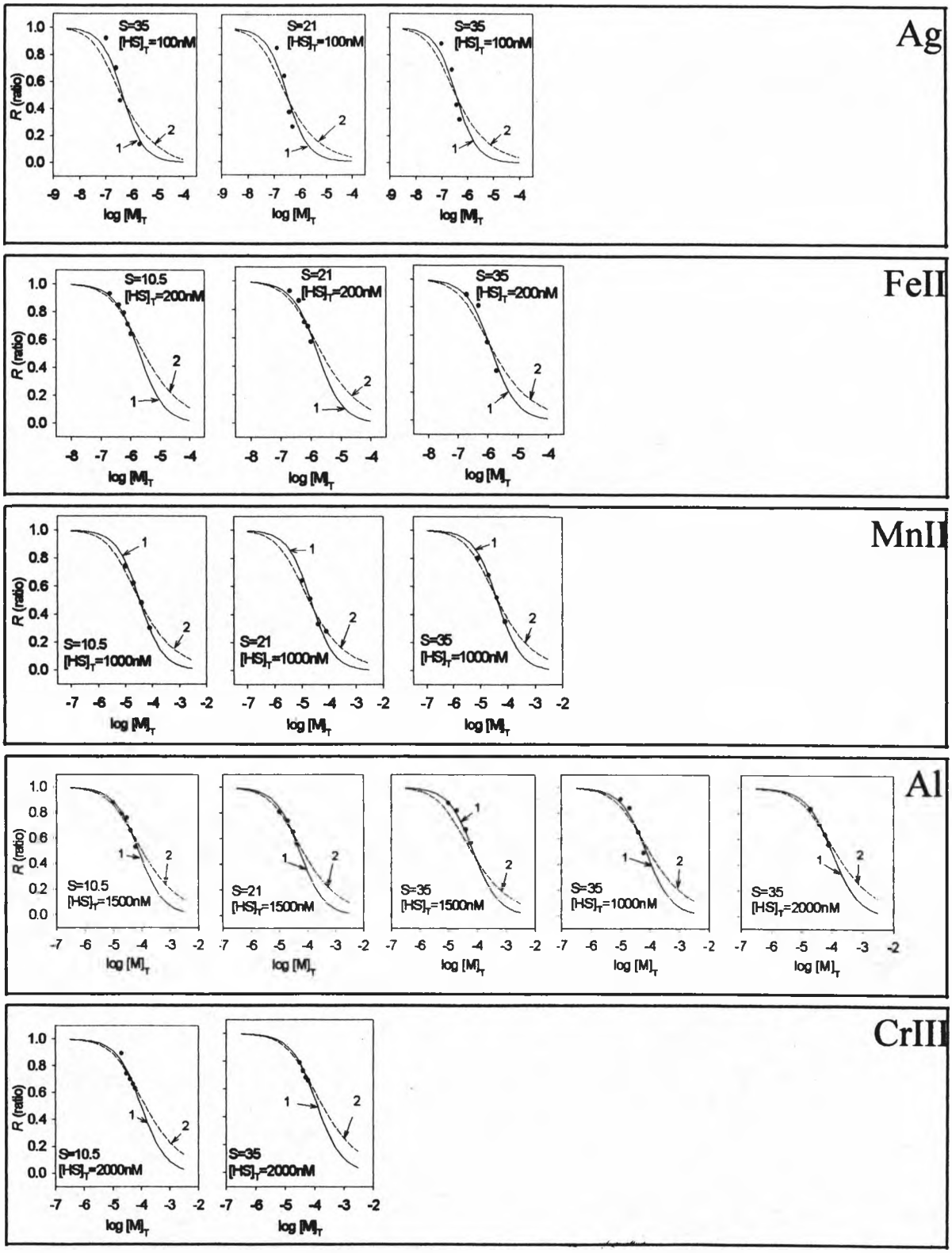


Fig. 3.2. Titration of sulfide with silver, iron(II), manganese(II), aluminum and chromium(III): closed symbols are the data observed; 1) represent the fitting assuming the presence of MHS only or both MHS and $M(HS)_2$ simultaneously; 2) represent the fitting assuming the presence of $M(HS)_2$ only.

Table 3.3. Values for conditional stability constants of metal-sulfide complexes determined by measurement of free sulfide and calculated by curve fitting

| Metal ions | Salinity (‰) | Total [HS] (nM) | log $K'_1$ | log $K''_1$ | log $\beta'_2$ | log $\beta''_2$ |
|---|---|---|---|---|---|---|
| $Ag^+$ | 35 | 100 | 11.63±0.10 | 6.41 | ---- | ---- |
| | 21 | 100 | 11.16±0.11 | 6.58 | ---- | ---- |
| | 10.5 | 100 | 10.46±0.08 | 6.65 | ---- | ---- |
| $Fe^{2+}$ | 35 | 200 | 6.07±0.06 | 5.91 | ---- | ---- |
| | 21 | 200 | 5.97±0.04 | 5.83 | ---- | ---- |
| | 10.5 | 200 | 5.86±0.02 | 5.73 | ---- | --- |
| $Mn^{2+}$ | 35 | 1000 | 4.51±0.10 | 4.28 | 9.93±0.28 | 9.70 |
| | 21 | 1000 | 4.59±0.20 | 4.42 | 10.57±0.23 | 10.40 |
| | 10.5 | 1000 | 4.50±0.08 | 4.37 | 9.94±0.24 | 9.81 |
| $Al^{3+}$ | 35 | 1500 | 12.97±0.01 | 4.09 | ---- | ---- |
| | 21 | 1500 | 13.09±0.02 | 4.23 | 17.94±0.71 | 9.07 |
| | 10.5 | 1500 | 13.01±0.03 | 4.12 | ---- | ---- |
| | 35 | 1000 | 13.02±0.05 | 4.14 | ---- | ---- |
| | 35 | 2000 | 12.87±0.00 | 3.99 | ---- | ---- |
| $Cr^{3+}$ | 35 | 2000 | 9.54±0.01 | 3.95 | ---- | ---- |
| | 10.5 | 2000 | 9.78±0.03 | 4.01 | ---- | ---- |

$K'_1$ and $\beta'_2$ are conditional stability constants after correction for inorganic side reaction ($\alpha_M$).

$K''_1$ and $\beta''_2$ are conditional stability constants valid for seawater medium, (e.g. $K''_1 = K'_1 / \alpha_M$).

Nioxime and DMG are known to provide high CSV sensitivity for cobalt and nickel in seawater (van den Berg and Nimmo, 1987; Zhang et al., 1990). It was attempted to use nioxime and DMG as competing ligands for sulfide. However, the very high stability of the complexes with nioxime and DMG limited their use in this study, as unrealistically high levels of sulfide were required to compete effectively. Preliminary experiments showed that also cobalt and nickel formed electroactive complexes with oxine, and these had a lower stability than those with nioxime and DMG; the sensitivity was much lower than with DMG or nioxime, but it was sufficiently sensitive to determine the stability of the sulfide complexes as the metal concentrations in the experiments were higher than normally occurring in seawater.

The decrease in the metal peak heights ($Q$-ratio, Eqn. 3.35) with increasing sulfide concentration is shown in Fig. 3.4. The stability constants were calculated by non-linear least squares regression of the $Q$-values to Eqn. 3.35 assuming the presence of one metal-sulfide species of the type MHS and $M(HS)_2$ or both simultaneously. Preliminary values for the

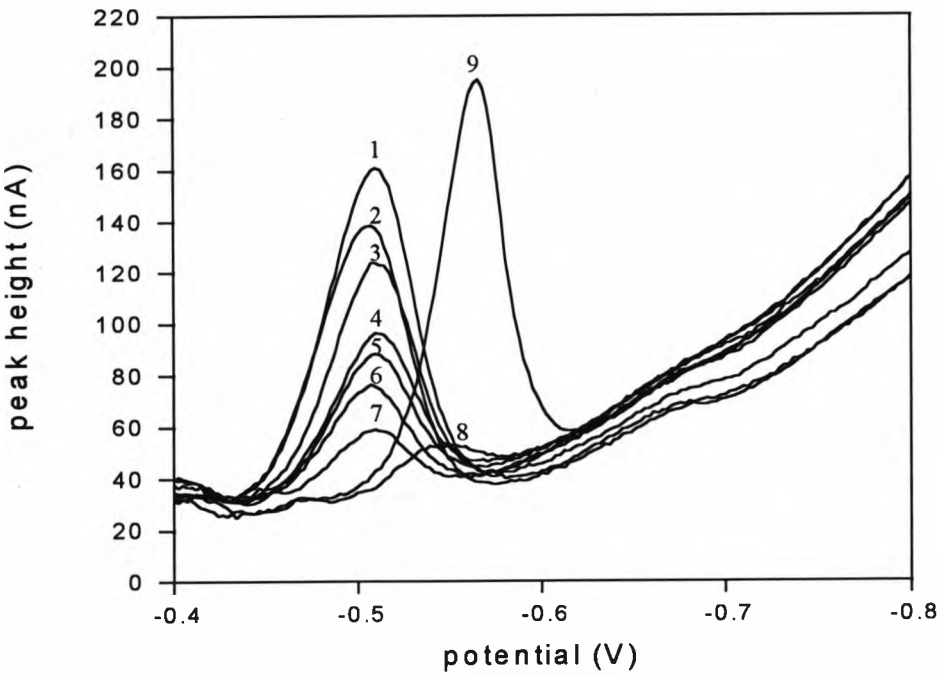


Fig. 3.3. The voltammetric response of lead-oxine complex before and after addition of sulfide standards using 30 nM of Pb, 25 μM of oxine and salinity of 35; 1) solution free of sulfide: after addition of 2) 50, 3) 100, 4) 150, 5) 200, 6) 250, 7) 300, 8) 500, 9) 800 nM sulfide, respectively.

constants were obtained by calculation of individual values using Eqn. 3.37 for each data point followed by averaging. The values of the stability constants are shown in Table 3.4.

### *3.6 Discussion*

The stability constants determined obtained in this work can be compared with those reported in the literature in Table 3.5. The literature data falls into two categories: experimentally determined stability constants, and stability constants estimated by the linear free energy technique. Dyrssen (Dyrssen, 1988) used dithizone as a model ligand to estimate the stability of metal-sulfide complexes using stability constants for cadmium-sulfide (Ste-Marie et al., 1964) and mercuric-sulfide (Schwarzenbach and Widmer, 1963) as reference. The thus estimated values for other metal-sulfide complexes depend therefore on the cadmium and mercury values and on whether dithizone is a good analogue for sulfide. The values of Zhang and Millero (Z&M) (Zhang and Millero, 1994) in Table 3.5 were obtained by CSV using the peak height for free sulfide and by titration of metals in seawater with sulfide. They presented conditional stability constants uncorrected for side-reactions of the metal ions with the major anions in the seawater. We corrected their values for the inorganic inorganic side reactions (Ringbom

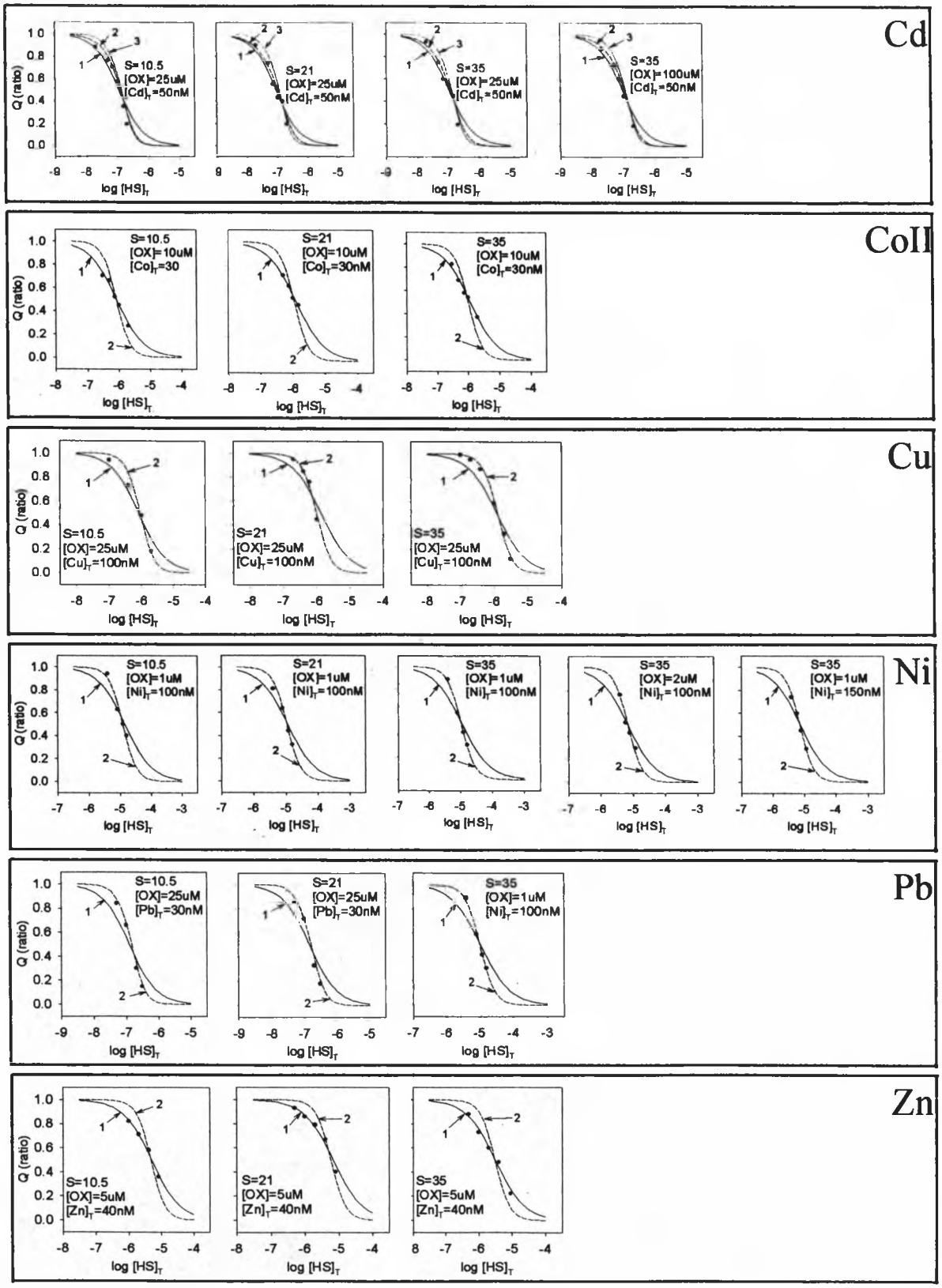


Fig. 3.4. Titration of (cadmium, cobalt(II), copper, nickel, lead and zinc)-oxine complexes with sulfide. The lines represent the calculated variation in *Q* using the fitted values for the constants. The constants were calculated by non-linear curve fitting to either 1) only one species (MHS), 2) two species (MHS and $M(HS)_2$), and 3) only one species ($M(HS)_2$).

Table 3.4. Values for conditional stability constants of metal-sulfide determined by ligand competition technique and calculated by curve fitting

| Metal ions | Salinity (‰) | [Oxine] (μM) | [metal] (nM) | log $K''_{Moxine}$ | log $\beta''_{Moxine}$ | log $K'_1$ | log $K''_1$ | log $\beta'_2$ | log $\beta''_2$ |
|---|---|---|---|---|---|---|---|---|---|
| $Cd^{2+}$ | 35 | 25 | 50 | 4.34 | 7.44 | 8.38±0.11 | 6.83 | 15.36±0.07 | 13.81 |
| | 21 | 25 | 50 | 4.55 | 7.86 | 8.22±0.11 | 7.00 | 15.12±0.14 | 13.90 |
| | 10.5 | 25 | 50 | 4.82 | 8.40 | 7.63±0.16 | 6.72 | 14.99±0.10 | 14.08 |
| | 35 | 100 | 50 | 4.34 | 7.44 | 8.43±0.07 | 6.88 | 15.69±0.07 | 14.14 |
| $Co^{2+}$ | 35 | 10 | 30 | 5.65 | ---- | 6.78±0.04 | 6.43 | 10.44±1.33 | 10.09 |
| | 21 | 10 | 30 | 5.86 | ---- | 6.91±0.05 | 6.53 | neg. | neg. |
| | 10.5 | 10 | 30 | 6.13 | ---- | 7.33±0.05 | 6.88 | 12.05±0.52 | 11.60 |
| | 35 | 20 | 30 | 5.65 | ---- | 6.55±0.24 | 6.20 | 12.25±0.35 | 11.90 |
| $Cu^{2+}$ | 35 | 25 | 100 | 9.00 | 16.90 | 12.88±0.20 | 11.50 | 19.44±0.07 | 18.06 |
| | 21 | 25 | 100 | 9.21 | 17.32 | 12.84±0.75 | 11.53 | 20.18±0.13 | 18.87 |
| | 14 | 25 | 100 | 9.37 | 17.64 | 14.51±0.09 | 13.31 | 20.26±0.19 | 19.06 |
| $Ni^{2+}$ | 35 | 1 | 100 | 6.27 | ---- | ---- | ---- | 10.58±0.07 | 10.25 |
| | 21 | 1 | 100 | 6.48 | ---- | 5.14±0.14 | 4.83 | 10.50±0.07 | 10.19 |
| | 10.5 | 1 | 100 | 6.75 | ---- | 5.42±0.90 | 5.12 | 10.71±0.14 | 10.41 |
| | 35 | 2 | 100 | 6.27 | ---- | 5.22±0.39 | 4.89 | 10.99±0.14 | 10.66 |
| | 35 | 1 | 150 | 6.27 | ---- | 5.02±0.13 | 4.69 | 10.79±0.04 | 10.46 |
| $Pb^{2+}$ | 35 | 25 | 30 | 6.02 | ---- | 7.98±0.19 | 6.44 | 15.41±0.05 | 13.87 |
| | 21 | 25 | 30 | 6.23 | ---- | 7.39±0.60 | 5.88 | 15.61±0.03 | 14.10 |
| | 10.5 | 25 | 30 | 6.50 | ---- | 7.87±0.27 | 6.33 | 15.87±0.01 | 14.33 |
| $Zn^{2+}$ | 35 | 5 | 40 | 5.56 | 9.80 | 6.08±0.06 | 5.76 | 10.24±0.52 | 9.92 |
| | 21 | 5 | 40 | 5.77 | 10.22 | 5.75±0.08 | 5.51 | 10.55±0.21 | 10.31 |
| | 10.5 | 5 | 40 | 6.04 | 10.76 | 6.19±0.05 | 5.98 | 10.28±0.48 | 10.07 |
| | 35 | 2 | 40 | 5.56 | 9.80 | 5.01±0.17 | 4.69 | 11.10±0.04 | 10.78 |

$K'_1$ and $\beta'_2$ are conditional stability constants after correction for inorganic side-reactions ($\alpha_M$).

$K''_1$ and $\beta''_2$ are conditional stability constants valid for seawater uncorrected for side-reactions (e.g. $K''_1 = K'_1 / \alpha_M$).

$K''_{Moxine}$ and $\beta''_{Moxine}$ are conditional stability constants for metals-oxine complexes valid for seawater.

Table 3.5. Comparison of values for conditional stability constants (corrected for side-reactions of the metal ion in seawater) of metal-sulfide in seawater, pH=8 and 25 $^0$C, with literature data

| Metal ions | log $\alpha_{M'}$ | log $K'_1$ | log $\beta'_2$ | references |
|---|---|---|---|---|
| $Ag^+$ | 5.22 | 11.6 | ---- | This study |
| | | ≥9.5 | ≥15.3 | (Zhang, J.-Z. and Millero, 1994) |
| | | 13.6 | 17.7 | (Schwarzenbach and Widmer, 1966) |
| $Cd^{2+}$ | 1.55 | 8.4 | 15.5 | This study |
| | | 7.84 | 14.24 | (Zhang, J.-Z. and Millero, 1994) |
| | | 7.55 | 14.61 | (Ste-Marie et al., 1964) |
| | | ≤7.66 | ≤13.36 | (Daskalakis and Helz, 1992) |
| *$Co^{2+}$ | 0.35 | 6.8* | 10.4* | This study |
| | | 5.53 | ---- | (Zhang, J.-Z. and Millero, 1994) |
| | | 4.7 | 12.2 | (Dyrssen, 1988) |
| | | 4.7 | ---- | (Luther et al., 1996) |
| $Cu^{2+}$ | 1.38 | 12.9 | 19.4 | This study |
| | | 8.38 | 14.38 | (Zhang, J.-Z. and Millero, 1994) |
| | | 14.1 | 21.6 | (Dyrssen, 1988) |
| **$Cu^+$ | 5.15 | 16.67 | 23.17 | This study |
| | | 11.8 | 17.6 | (Zhang, J.-Z. and Millero, 1994) |
| $Ni^{2+}$ | 0.33 | 5.1 | 10.8 | This study |
| | | 5.63 | ---- | (Zhang, J.-Z. and Millero, 1994) |
| | | 3.8 | 11.4 | (Dyrssen, 1988) |
| | | 4.99 | ---- | (Luther et al., 1996) |
| $Pb^{2+}$ | 1.54 | 8.0 | 15.4 | This study |
| | | 8.64 | 15.04 | (Zhang, J.-Z. and Millero, 1994) |
| | | 5.0 | 12.5 | (Dyrssen, 1988) |
| $Zn^{2+}$ | 0.32 | 6.1 | 10.2 | This study |
| | | 6.32 | 14.02 | (Zhang, J.-Z. and Millero, 1994) |
| | | 6.5 | 14.0 | (Dyrssen, 1988) |
| $Fe^{2+}$ | 0.16 | 6.1 | ---- | This study |
| | | 5.46 | ---- | (Zhang, J.-Z. and Millero, 1994) |
| | | 1.4 | 8.9 | (Dyrssen, 1988) |
| | | 5.12 | ---- | (Luther et al., 1996) |
| $Mn^{2+}$ | 0.23 | 4.5 | 9.9 | This study |
| | | 6.93 | ---- | (Zhang, J.-Z. and Millero, 1994) |
| | | -0.5 | 7.0 | (Dyrssen, 1988) |
| | | 4.64 | ---- | (Luther et al., 1996) |
| $Al^{3+}$ | 8.88 | 13.0 | ---- | This study |
| $Cr^{3+}$ | 5.59 | 9.5 | ---- | This study |

* determined at pH=9.0.

** Calculated from the same data as for $Cu^{2+}$.

$K'_1$ and $\beta'_2$ are conditional stability constants corrected for $\alpha_{M'}$ (alpha coefficient for inorganic side reactions) and can be treated as thermodynamic stability constants. (Zhang, J.-Z. and Millero, 1994) data were corrected for $\alpha_M$ except for $Cu^+$. (Daskalakis and Helz, 1992) data were calculated from their experimental results on solubility of CdS (greenockite).

and Still, 1972), using side-reaction coefficients, $\alpha_M$ (Table 3.5) calculated using free major ion concentrations and stability constants for metal complexation valid for seawater (see equation 3.16). The concentrations of the free major ions were calculated from the total ion concentrations and ion-pairing constants using an iterative ion-paring model. The values of Luther et al. (Luther et al., 1996) were also obtained by conventional, batchwise, square wave voltammetry but they used the shift in the peak potential as a measure of complex stability and titrated sulfide solutions with metals. Use of $E_p$ shifts is valid only for reversible reactions. There should be little or no peak suppression for reversible reactions. In the absence of evidence of this reversibility, the use of peak potential shifts is not justified. Their data may therefore be biased.

### *3.6.1 Comparison of the two techniques*

***Comparison of the free sulfide and the ligand competition data:*** The stability constants for several metals could be determined using both methods (ligand competition and detection of free sulfide). The free sulfide data appeared to provide less resolution than the ligand competition data, and only one of the stability constants (either $K'_{MHS}$ or $\beta'_{M(HS)2}$) could be calculated for some of the metals. Comparison of the results (Table 3.6)

shows close agreement once the values for both $K'_{MHS}$ and $\beta'_{M(HS)2}$ are taken into account. For instance, the value for log $K'_{CdHS}$ was 9.1 (with an insignificant value for $\beta'_{Cd(HS)2}$) using the free sulfide method, but 8.4 (with a value of 15.5 for $\beta'_{Cd(HS)2}$) using the ligand competition method. $\beta'_{Cd(HS)2}$ could not be calculated using the free sulfide data so the $K'_{CdHS}$ value takes the contribution of the second species into account. It was attempted to calculate a value for $K'_{CdHS}$ using the value for $\beta'_{Cd(HS)2}$ from the ligand competition method, but this gave a meaningless value for $K'_{CdHS}$. The value for log $K'_{CoHS}$ (6.8) from the ligand competition data was slightly larger than that obtained using the free sulfide data, but the difference was offset by a smaller value for $\beta'_{Co(HS)2}$ (Table 3.6).

The validity of the results obtained by the ligand competition method depends on the accuracy of the stability of the metal-oxine complexes. It was assumed that the interaction of the metal-sulfide complex with oxine was negligible (i. e. no mixed metal-sulfide-oxine complexes were formed). The reaction of a natural organic ligand with iron(II)-sulfide complexes was hypothesized to explain the slow kinetics for iron(II)-sulfide complexes in marine waters (Luther and Ferdelman, 1993). A similar interaction between

the metal-sulfide complex and oxine would lower the apparent value of the stability constants of the metal-sulfide complexes.

Table 3.6. Comparison of conditional stability constants determined by ligand competition and by titration

| | ligand competition | | free sulfide | |
|---|---|---|---|---|
| Metal ions | $\log K_1'$ | $\log \beta_2'$ | $\log K_1'$ | $\log \beta_2'$ |
| $Cd^{2+}$ | 8.4 | 15.5 | 9.1 | --- |
| $Co^{2+}$ | 6.8 | 10.4 | 6.4 | 9.8 |
| $Pb^{2+}$ | 8.0 | 15.4 | --- | 16.4 |
| $Zn^{2+}$ | 6.1 | 10.2 | 6.2 | --- |

A possible advantage of the ligand competition method over the free sulfide method is the elimination of precipitation of metal sulfides. The solubility of the metal-sulfides may be transgressed when either the sulfide or the metal concentration is increased during the titrations, leading to precipitation of the solid phase of the metal-sulfide, although this was not observed in this work or previously(Zhang and Millero, 1994). The free metal ion concentrations were kept low during the experiments due to the presence of the competing ligand, oxine, thus minimizing the potential formation of poorly soluble sulfide species during the experiments.

A potential drawback of the method employing detection of free sulfide to determine the complex stabilities is that dissociation could occur of the complexes in the diffusion layer surrounding the electrode; this would render these complexes labile and they would either not be detected at all or this would lead to underestimation of the stability constant. The oxidation of mercury is enhanced in the presence of free sulfide (the reduction potential for HgS is more negative at -0.58 V) but here the oxidised mercury would be trapped as HgS on the surface of the electrode. Any metal-sulfide complex dissociation would therefore have to occur in the molecular layer(s) immediately surrounding the electrode rather than throughout the diffusion layer. The time scale of such reactions would be much shorter and therefore

the scope for complexes to be labile is small in these experiments. Comparison of the stability constants determined by both methods in Table 3.6 shows no evidence for an underestimation of the complex stabilities using the sulfide detection method; these sulfide complexes were therefore electrochemically non-labile. The situation could be different when a conventional, batch, voltammetric cell is used where free mercury is present which tends to remove free sulfide from solution and which may compete with the added metal ions. On the whole, and in spite of large differences between several individual constants, comparison of the constants found by this work with those reported in the literature and obtained using the batch-voltammetric method (Table 3.5) reveals no systematic underestimation of the complex stabilities by the batch method either.

### *3.6.2 Sulfide complexes with copper and silver*

The stability constant (log $K'_1$) obtained for copper (12.9) is smaller than that (14.1) estimated by Dyrssen, but it is much greater than that (8.4) found by Z&M. The value for silver (11.6) is greater than that (>9.5) found by Z&M but that was below the limit of detection in their procedure; our value is smaller than that (13.6) obtained from sulfide solubility (Schwarzenbach and

Widmer, 1966). The high value for the copper-sulfide constant, is similar to that for silver-sulfide where silver occurs as Ag(I). Recent work (Leal and van den Berg, 1997) has indicated that copper(I) complexes with thiols like glutathione are very stable in seawater with a value for log $K''_{CuGS}$ of 10.6 (equivalent to a value for log $K'_{CuGS}$ of 12.2), similar to that for copper with sulfide. In view of the similarity of the ligands (the sulfide group is involved in both complexes), and the similarity with silver(I), it may be hypothesized that the copper occurs as copper(I) in the sulfide complex. Z&M have attempted to determine a value for the copper(I) complex separately and found a value for $K'_{Cu(I)HS}$ of 11.8. This is equivalent to a value of 6.8 for log $K''_{CuHS}$ (calculated from their constant by removing the correction for side-reactions of $Cu^+$ by division of $K''_{CuHS}$ by $\alpha_{Cu(I)'}$) which is almost the same as the value (7.0) for log $K''_{CuHS}$ which is obtained when the correction for side reactions of $Cu^{2+}$ is removed from their constant for $Cu^{2+}$ complexation (log $K'_{Cu(II)HS}$ = 8.38) (Table 3.5). A possible explanation for the apparent similarity is that the redox reaction of Cu(I)/Cu(II) is electrochemically reversible; it is therefore not possible to retain the original redox state of free copper in the diffusion layer of the electrode when a potential is applied or when the potential is scanned. Z&M used a deposition potential of -0.1 V where free copper would have occurred as copper(II) in the diffusion layer

regardless of the original oxidation state of dissolved copper in the bulk solution. It is therefore likely that they determined the stability of the copper(II)-sulfide complex twice. Luther et al. (1996) scanned without a deliberate preconcentration step, but they would nevertheless have oxidized Cu(I) to Cu(II) during the beginning of the scan and back to Cu(I) during the remainder of the scan, so they have suffered from changes in the speciation during the measurement of which the net effect is not known. The previous work therefore does not conclusively show whether copper in seawater is complexed by sulfide as copper(I) or as copper(II).

Using ligand competition between oxine and sulfide we used a slightly more negative deposition potential than that of Z&M of -0.2 V which would tend to cause some reduction of free copper(II) to copper(I) as the reduction potential for free copper is around -0.17 V in seawater. However, experiments using a specific copper(I) binding ligand (glutathione) have shown that the effect of the deposition potential in the ligand competition method is very slight or insignificant (Leal and van den Berg, 1997) presumably as most copper is stabilised as copper(II)-oxine complexes leaving only a small fraction (<0.1%) as free uncomplexed copper. It is therefore likely that CSV measurements using the ligand competition

method do not significantly affect the redox state of copper. Assuming that the sulfide complex detected by us is indeed with copper(I) (in view of the high complex stability greater than that with silver, and the similarity with glutathione) then this species would be predominant and the presence of free sulfide would tend to cause reduction of dissolved copper(II) to copper(I) in seawater in the absence of applied electrode potentials.

### *3.6.3 Sulfide complexes with divalent metals: Cd, Co, Fe, Mn, Ni, Pb, and Zn*

***Comparison with literature data:*** The values for log $K'_{MHS}$ for cadmium and iron(II) are about 0.5 units greater, and those for nickel, lead, and zinc 0.2-0.5 units smaller, than those obtained by Z&M. The relatively small differences are surprising as the conventional, batch voltammetric method suffers from a continuous and rapid decrease in the CSV response for free sulfide due its reaction with waste mercury (Al-Farawati and van den Berg, 1997 (Chapter 2)). Z&M measured the free sulfide concentration 60 s after each sulfide addition, and continued their sulfide additions to high concentrations (micromolar). Possibly the effect of the sulfide removal was minor in these conditions, or possibly the data treatment was relatively insensitive to the continuous removal of a fraction of the free sulfide.

The value for the constant for cobalt (6.8) is much greater than that found by Z&M (5.5) and Luther et al. (4.7), and also than that (4.7) estimated by Dyrssen. The value for Mn (4.5) is much less than that found by Z&M (6.9), but similar to that of Luther et al. (4.6). It is not clear whether changes in the redox state during deposition have contributed to the differences. The relatively close agreement between our result for cobalt using both methods (free sulfide and ligand competition) is encouraging for the new data obtained using the flow cell.

No stability constants for copper(II) and zinc species of the form $[M(HS)_m]^{(n-m)+}$ were reported by Luther et al., although they did experiments on sulfide complexation by copper(II) and zinc. Their work suggested 1:1 complexes of copper at the beginning the titrations, but the ratio changed at higher sulfide concentrations. Stability constants for complexes of the type $M_2(HS)^{3+}$ and $M_3(HS)^{5+}$ for cobalt, nickel, iron(II) and manganese were reported by Luther et al.. These complexes have a high positive charge so they are unlikely to form in seawater. Instead, complexes (mono or polynuclear) of a lower, near-neutral valency are more likely to exist in seawater (i.e. $[M(HS)_2]^0$, $[M(HS)_3]^-$ and $[M_2(HS)_2]^{2+}$ , for divalent metals).

### *3.6.4 Comparison with solubility measurements*

The determination of the stability constants in this study was obtained assuming the metal sulfide complexes are mononuclear in the form $M(HS)_m^{(n-m)+}$ (equation 3.7). Direct comparison of data with solubility measurements could be only obtained for silver (Schwarzenbach and Widmer, 1966) and cadmium (Ste-Marie et al., 1964). The stability constant ($\log K_1'$) obtained for silver (11.6) in this work is two orders of magnitude smaller than that (13.6) found by (Schwarzenbach and Widmer, 1966). For cadmium, the stability constants ($\log K_1'$ and $\log \beta_2'$) found in this work are one or two order of magnitude higher than those from (Ste-Marie et al., 1964). However, these complexes from solubility measurements present upper limits for solubility of these species.

The stability constants of cadmium sulfide complexes of (Daskalakis and Helz, 1992) (Table 3.5) were calculated from solubility data. Their constants were in agreement with the solubility measurements of (Ste-Marie et al., 1964), but not with our data.

The observations of (Thompson and Helz, 1994) suggested that Cu(II) was reduced under their experimental conditions to Cu(I). Although their data (Table 3.1) did not give evidence for the existence of a copper sulfide complex of the form $M(HS)_m^{(n-m)+}$ (equation 3.7), they were able to estimate a

value of 12.6 for log $K$ for $CuHS^0$ ($Cu^+ + HS^- = CuHS^0$) which is in good agreement with our value for log $K_1'$ (12.9) for $CuHS^+$ (Table 3.5).

For most metals it is clear from Table 3.1 that the direct comparison of their stability constant derived from solubility measurements with data in this work is not readily possible due to a different form of the equations. Instead it was attempted to compare constants with the solubility data by calculating the predicted metal solubility as a function of total sulfide concentrations. The maximum concentration of free metal was assumed to controlled by known pure metal sulfide minerals (see Table 3.8)

$$MS + H^+ \Leftrightarrow M^{2+} + HS^- \quad \text{(for divalent metal)} \qquad 3.38$$

The concentration of bisulfide can be calculated from the total sulfide concentration ant the pH 7(see equations 3.1 and 3.2). The bisulfide concentration is combined with the solubility product of metal sulfide to calculate the free metal concentration. From the predicted soluble free metal ion concentration, the concentration of the major species can be calculated using the thermodynamic constants of metal sulfide either from solubility data or this work, and other inorganic complexes (Turner et al., 1981). The total soluble metal is then obtained from a metal mass balance. The solubility products ($MS \Leftrightarrow M^{2+} + S^{2-}$) for Cu, Pb, Ag and Zn were obtained

from (Stumm and Morgan, 1981) and corrected to the form of equation 3.38 using the second dissociation constant ($pK_2$=18.51) (Schoonen and Barnes, 1988) for $H_2S$. The solubility product for Cd was obtained from (Daskalakis and Helz, 1992).

The results of the calculated total metal solubilities as a function of total sulfide using constants of metal sulfide from the literature are compared in Fig 3.5 with those from this work. Large differences were found for most metals (Cd, Pb and Zn) . The solubility of copper is very similar using both data set. For the other metals the solubilities show more differences: for silver, the difference is only one order of magnitude, but for cadmium, lead and zinc the differences are greater. In all cases (except for lead) the data show at least good agreement within a relatively narrow band of sulfide concentrations, deviating at greater or lower levels. Clearly there is no general agreement yet on the speciation of the sulfide system in natural waters

### *3.6.5 Modeling of the sulfide speciation in seawater*

Oxygenated seawater contains sulfide at levels of 0.1 to 2 nM (Radford-Knoery and Cutter, 1994) (Cutter and Krahforst, 1988; Luther and Tsamakis, 1989). This sulfide concentration is of the same magnitude as that of copper,

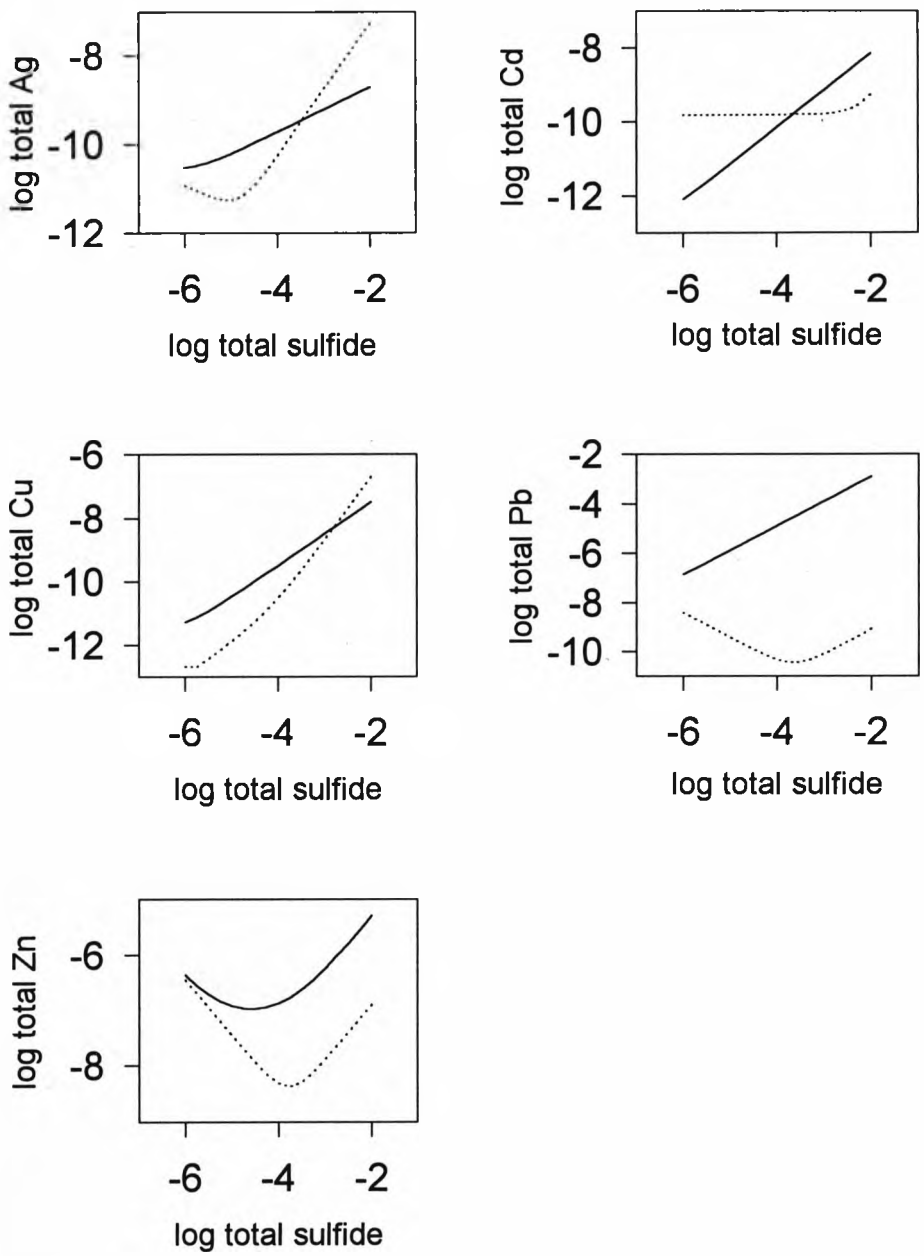


Fig 3.5 Comparison of calculated total metal solubilities for Ag, Cd, Cu, Pb and Zn as a function of total sulfide using constants of metal sulfide from the literature (Table 3.1) and from this work; Solid line: this work, dot line: the literature.

and similar to, or greater than, that of other metals for which stability constants were determined in this study. Because of the very great stability of the copper-sulfide complexes it is likely that copper controls the speciation of sulfide and vice versa sulfide controls the speciation of copper. The sulfide speciation in the presence of several trace metals is calculated here in a manner similar to that done before (Dyrssen, 1988; Elliott, 1988; Dyrssen and Wedborg, 1989; Zhang, J.-Z. and Millero, 1994). The calculation was carried out using realistic total metal and sulfide concentrations, and mass balance equations for metals and bisulfide

The speciation of sulfide with copper was calculated in the presence and absence of organic complexing ligands, and without making assumptions regarding complexation of copper as copper(I) or (II) by using the conditional stability constant ($K''_{CuHS}$). A stability constant and organic ligand concentration was selected from copper complexing ligands occurring in seawater (van den Berg, 1984a). Concentrations of organic ligands and their conditional stability constants for copper complexation in waters originating from the Atlantic Ocean have been determined by CSV using catechol as competitive ligand (van den Berg, 1984a). Two ligands

were observed at concentrations of 11 and 33 nM with conditional stability constants (log values) of 12.2 and 10.2 respectively.

The concentrations of cadmium, lead, nickel, cobalt and zinc were taken from ( (Bruland, 1983), of aluminum and silver from (Broecker and Peng, 1982) and for chromium(III) from (van den Berg et al., 1994). Sulfide concentrations of 0.5 and 2 nM, and copper concentrations of 1, 5 and 10 nM, were used in the presence and absence of organic ligands.

*At a total sulfide concentration of 0.5 nM*: Table 3.7 shows the speciation of sulfide at several copper concentrations and in the absence and presence of organic ligands. In the absence of the organic copper binding ligand and at a concentration of 0.5 nM sulfide the sulfide occurs predominantly complexed with copper, amounting to more than 99% of all sulfide at all copper concentrations. This finding is in general agreement with the previous work although it was attributed to a different copper-sulfide

Table 3.7. Percentage speciation of sulfide species in the presence and absence of organic ligand at various concentrations of copper. The sulfide concentration is 0.5 or 2 nM, and the trace metal concentrations are from (Broecker and Peng, 1982; Bruland, 1983); conditional stability constants and organic ligand concentrations are from (van den Berg, 1984a)

| **$[HS]_T$=0.5 nM** | *In the absence of organic ligand* | | | *In the of presence organic ligand* | | |
|---|---|---|---|---|---|---|
| $[Cu]_T$ | 1 nM | 5 nM | 10 nM | 1 nM | 5 nM | 10 nM |
| [HS'] | 0.62 | 0.07 | 0.03 | 71.77 | 23.90 | 5.98 |
| $CuHS^+$ | 99.38 | 99.93 | 99.97 | 28.08 | 76.01 | 93.99 |
| $Cu(HS)_2^0$ | 0 | 0 | 0 | 0.07 | 0.06 | 0.02 |
| $PbHS^+$ | 0 | 0 | 0 | 0.03 | 0.01 | 0 |
| $NiHS^+$ | 0 | 0 | 0 | 0.01 | 0 | 0 |
| $AlHS^{2+}$ | 0 | 0 | 0 | 0.03 | 0 | 0 |
| AgHS | 0 | 0 | 0 | 0.01 | 0 | 0 |
| Others | 0 | 0 | 0 | 0 | 0.02 | 0.01 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 |

| **$[HS]_T$=2 nM** | *In the absence of organic ligand* | | | *In the of presence organic ligand* | | |
|---|---|---|---|---|---|---|
| $[Cu]_T$ | 1 nM | 5 nM | 10 nM | 1 nM | 5 nM | 10 nM |
| [HS'] | 49.9 | 0.1 | 0.04 | 79.1 | 31.1 | 8.99 |
| $CuHS^+$ | 49.7 | 99.9 | 99.96 | 20.6 | 68.6 | 90.9 |
| $Cu(HS)_2^0$ | 0.3 | 0 | 0 | 0.2 | 0.3 | 0.1 |
| $PbHS^+$ | 0.02 | 0 | 0 | 0.04 | 0.01 | 0 |
| $NiHS^+$ | 0.01 | 0 | 0 | 0.01 | 0 | 0 |
| $AlHS^{2+}$ | 0.02 | 0 | 0 | 0.03 | 0.01 | 0 |
| AgHS | 0 | 0 | 0 | 0.01 | 0 | 0 |
| Others | 0.01 | 0.01 | 0 | 0 | 0.03 | 0.01 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 |

species (Dyrssen, 1988; Elliott, 1988). In the presence of organic ligands some of the copper is organic-bound; this releases a large fraction of the sulfide as free bisulfide (72% at a copper concentration of 1 nM), and copper-monobisulfide amounts to 28% of the sulfide. This is interesting because free sulfide is known to be oxidized by iodate (Zhang, J.-Z. and Whitfield, 1986) and oxygen (Millero, 1991) in seawater whilst one could expect the copper-sulfide species to be stabilised. Free sulfide is thought to amount to ~20% of total sulfide in seawater originating from the western North Atlantic and is readily purged with inert gas at natural pH (Radford-Knoery and Cutter, 1994). This may confirm the importance of the indirect influence of organic ligands on the speciation of sulfide. At copper concentrations of 5 and 10 nM, the copper-monobisulfide dominates the sulfide speciation.

*At a total sulfide concentration of 2 nM*: At a copper concentration of 1 nM and in the absence of organic ligand, half of the sulfide occurs as free bisulfide whereas the rest is copper-monobisulfide. At higher copper concentrations the sulfide speciation is dominated by copper-monobisulfide. In the presence of organic complexing ligands the calculated free sulfide concentration is increased to 79, 31 and 9 % at copper concentrations of 1, 5

and 10 nM, respectively, and the copper-monobisulfide concentration is decreased to 21, 69 and 91 % respectively.

These calculations show that the ratio of total copper and total bisulfide determines sulfide speciation in the absence of organic complexation of copper. At a S:Cu ratio of 2 sulfide occurs as copper-monobisulfide, and half of the sulfide occurs as free bisulfide. In the presence of copper complexing organic ligands some of the sulfide is released and occurs mainly as free bisulfide due to competitive complexation of the copper by the organic ligands.

### *3.6.6 Copper speciation*

Due to the great stability of the sulfide copper complexes it is likely that the sulfide is a major ligand for copper even at low sulfide concentrations. Disregarding sulfide the inorganic speciation of copper in seawater is dominated by carbonate complexation (Turner et al., 1981; van den Berg, 1984b). Organic complexing ligands are known to bind most copper in seawater, and are present in excess (Hirose et al., 1982; Mills et al., 1982; van den Berg, 1984a; Coale and Bruland, 1988; Zhou and Wangersky, 1989). The speciation of copper over organic and sulfide complexes is

recalculated here as function of the sulfide concentration at a total copper concentration of 1.2 nM using the new constants for the copper-sulfide species, and is shown in Fig. 3.6. At total sulfide concentrations of 0.5-1000 nM the copper-monobisulfide is the major species. This species can be expected to play a major role in determining the geochemistry of copper in oxic-anoxic boundaries in the marine environment. At total sulfide concentrations >1000 nM copper-dibisulfide is the main species.

In the presence of organic complexation the copper-organic species are dominant at sulfide <1 nM, but at higher levels of sulfide, first copper-monobisulfide, and then copper-dibisulfide complexes constitute the major species.

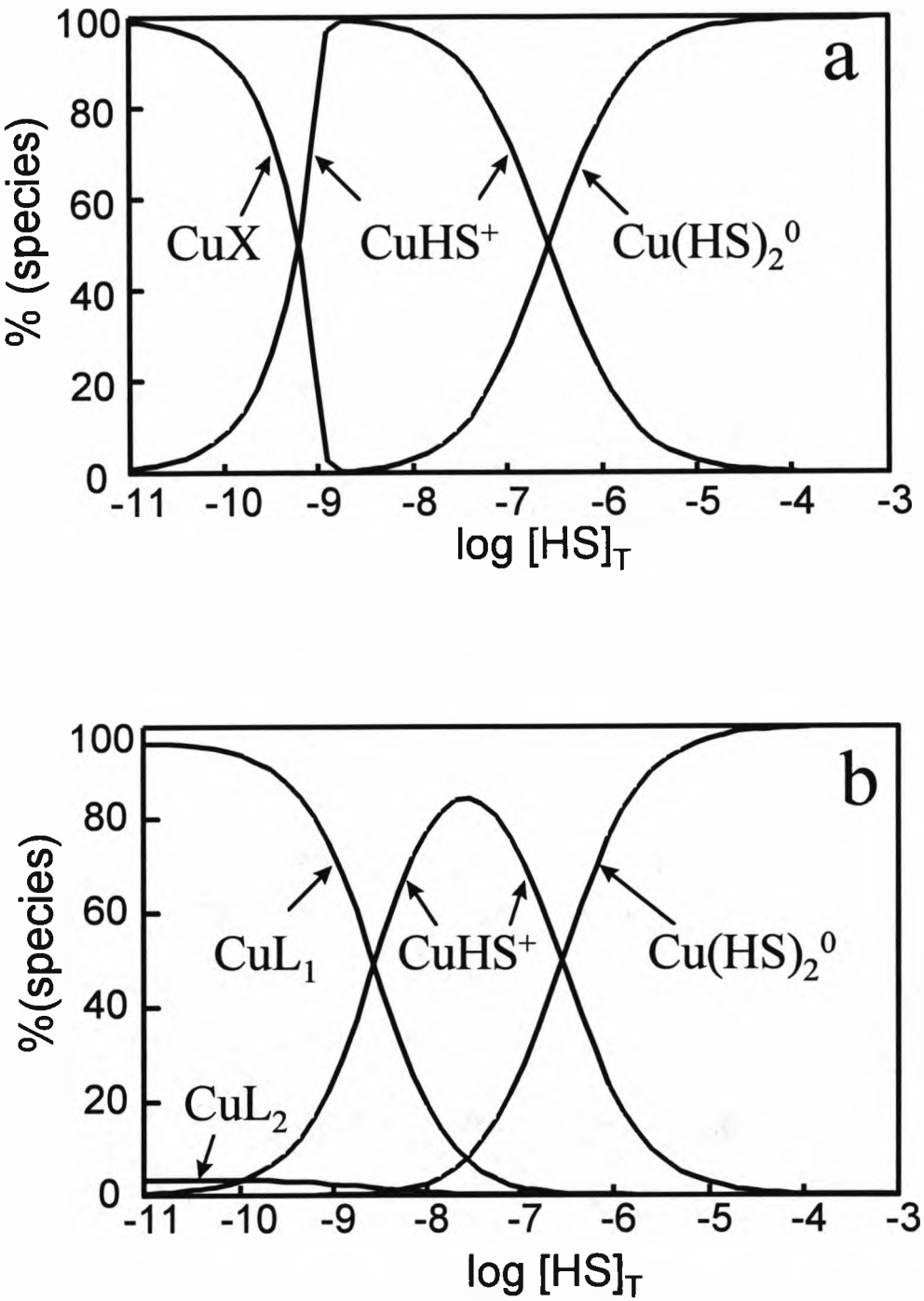


Fig. 3.6. Copper speciation as a function of log $[HS]_T$: a) in the absence of organic ligand; b) in the presence of organic ligand. $[Cu]_T$=1.2 nM; total organic ligand ($L_1$) =11 nM and log $K'_{CuL1}$=12.2; total organic ligand ($L_2$) = 33 nM and log $K'_{CuL2}$=10.2. (X) inorganic ligands.

### *3.6.7 Application of the constants to anoxic marine waters*

Most studies show that the metal sulfide complexes may enhance the solubility of metals in anoxic waters (Emerson et al., 1983; Kremling, 1983). The stability constants in this study were used to predict the solubility of metal sulfide (ion activity product(IAP)) and then compare it with values from literature. (Jacobs et al., 1985) determined the total metal and sulfide, and calculated the IAPs of various metals from Framvaren Fjord.

For a divalent metal

$$IAP = ([M^{2+}].[HS^-]) / [H^+] \qquad 3.39$$

Using the total metal and sulfide concentrations from (Jacobs et al., 1985) at selected depths, The IAPs for various metal can be calculated for a given pH, here chosen as equal to 7. The results of the calculated IAPs in this study are presented in Table 3.8 along with the observed values from (Jacobs et al., 1985).

The calculated IAPs for Cd, Co and Fe are in a good agreement with the observed IAPs and usually the difference is less than one order of magnitude. However, the calculated IAPs for Cd and Fe are higher than the observed values whereas the calculated IAP for Co is less than that observed. Large differences are found for Zn and Ni. The calculated IAP for Mn is less than that observed but in relatively good agreement. The Cu value is less by two orders of magnitude.

Comparison with solubility products ($K_{sp}$) (Table 3.8) suggests that Cd, Cu, Fe, Mn and Zn are undersaturated with respect to pure metal sulfide minerals. Various process could affect the solubility of metals: either adsorption of metals on surface of the iron sulfide which could be an important process (Dyrssen and Kremling, 1990) or irreversibility of metal metal sulfide precipitation under anoxic conditions (Jacobs et al., 1985). It is also possible that the solubility of metals are controlled by other solid phases of sulfide minerals which lower their solubilities.

### *3.7 Conclusions*

Good agreement for stability constants for metal-sulfide complexation are obtained when determined using a flow cell combined with either ligand competition or detection of the free sulfide concentration. Comparative measurements shown that the conditional stability constants (log $K'_1$) of cobalt(II) and zinc sulfide species determined by both methods show a close agreement. The stability constants for sulfide complexes with copper in seawater determined with the flow cell are much greater than those determined previously using a conventional cell. The differences are less for other metals. The data suggest that copper in seawater is complexed strongly by sulfide, but it is not clear whether it is complexed as Cu(I) or as Cu(II). Using non-linear, least-squares, curve fitting it was demonstrated that the predominant sulfide species with silver, iron(II), aluminum

and chromium(III) in seawater are mono-bisulfide species. Comparison of calculated total metal solubilities using metal sulfide constants from this work and with stability constants derived from solubility measurements show large differences for most metals. Clearly there is no agreement wet on the speciation of the sulfide in natural waters.

Calculation of the sulfide speciation in seawater typical for oceanic waters but free of organic ligands indicates that the speciation of sulfide is dominated by complexation of copper and occurs mainly as copper monobisulfide. In the presence of organic ligands, about two third of the total sulfide is present as free sulfide and the rest as copper monobisulfide. However, the sulfide speciation depends greatly on the ratio of copper to sulfide, and on the stability and concentration of organic copper complexing ligands. Conversely, the inorganic speciation of copper is strongly affected by sulfide ions when the sulfide concentration is ≥ the copper concentration.

Table 3.8 Comparison between calculated ion activity products (-log IAPs) and observed -log IAPs at selected depth from Framvaren Fjord. Data of total sulfide, metal and observed -log IAPs are obtained from (Jacobs et. al., 1985). The observed -log IAP of (Jacobs et. al., 1985) were estimated from their figures. Solubility products of known metal sulfide minerals are also presented.

| Depth(m) | 80 | | 160 | |
|---|---|---|---|---|
| Metal | calculated -log IAPs | Observed -log IAPs | calculated -log IAPs | Observed -log IAPs |
| Cd | 16.8 | 17.4 | 16.5 | 17.3 |
| Co | 9.2 | 8.5 | 9.2 | 8.8 |
| Cu | 19.9 | 17.5 | 19.5 | 18.1 |
| Fe | 5.9 | 6.6 | 5.9 | 6.9 |
| Mn | 3.5 | 2.6 | 3.9 | 2.3 |
| Ni | 8.9 | 4.2 | 9.0 | 5.0 |
| Zn | 8.7 | 13 | 8.4 | 12.5 |

$-\log K_{sp}(Cd) = 14.36$*

$-\log K_{sp}(Cu) = 17.59$

$-\log K_{sp}(Fe) = 1.94$**

$-\log K_{sp}(Mn) = -5.01$

$-\log K_{sp}(Zn) = 6.19$

The solubility products ($-\log K_{sp}$) ($MS \Leftrightarrow M^{2+} + S^{2-}$) for Cu, Mn and Zn were obtained from (Stumm and Morgan, 1981) and corrected to the form of $MS + H^+ \Leftrightarrow M^{2+} + HS^-$ using second dissociation constant ($pK_2 = 18.51$) (Schoonen and Barnes, 1988) for $H_2S$.

* (Daskalakis and Helz, 1992).

** (Davison and Heaney, 1980).

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# Chapter 4

## Thiols in the coastal waters of the western North Sea, English Channel and the water column of the Black Sea

### *4.1 Abstract*

Thiols were determined in the coastal waters of the western North Sea, English Channel and the water column of the Black Sea. The measurements were performed on-line using flow analysis for the coastal waters of the western North Sea and the English Channel, whereas batch analysis was used for the water column of the Black Sea. Detection was by cathodic stripping voltammetry calibrated with thiourea. Diel variation and water column profiles were also obtained in the North Sea and the English Channel. Correlation between thiols and various parameters were calculated for the surface seawater. The concentrations of thiols in the surface water of the western North Sea and the English Channel ranged from 0.70 to 3.60 nM. Thiol concentrations were found to vary over relatively short distance. Low concentrations were evident at the Humber-Wash area whereas highest concentrations were observed in the Tees-Tyne area. Contradicting co-variation was found with salinity indicating that the thiols did not originate in low-salinity waters. However, variations in the surface thiol concentrations were closely related to variations in the concentrations of chlorophyll. The depth profiles in the North Sea and The Black Sea showed maxima in thiol concentrations coincident with maxima in the chlorophyll concentrations. The data reveals the importance of marine phytoplankton in

the production of thiols. In addition, the data shows that other processes could act as source for thiols in the surface water such interaction between sulfide and organic matter in the water column in bottom water.

The thiol concentrations in the anoxic deep water of the Black Sea were much higher than the oxic surface layer which could be attributed to the interaction of sulfide with organic compounds leading to the production of thiol coupled with the absence of an oxidative breakdown process.

Titration experiments using samples from the Black Sea indicated that the thiols are powerful complexing ligands forming very stable complexes with copper. The complex stability is similar to that observed previously for complexes in oxygenated seawater from oceanic origin. The widespread occurrence of thiol compounds in surface seawater as well as in high concentrations in the deep waters of the Black Sea suggests that these compounds may be important ligands to control the speciation of copper and possibly other metals in the marine system.

### *4.2 Introduction*

Transformations between the various forms of inorganic and organic sulfur compounds are key factors in the global sulfur cycle (Vairavamurthy

and Mopper, 1987; Andreae, 1990; Taylor and Gilchrist, 1991; Flock and Andreae, 1996)

The inorganic sulfur is found in valence states ranging from +6 in $SO_4^{2-}$ to -2 in sulfides. In the presence of oxygen $SO_4^{2-}$ is the major form of inorganic sulfur, which can be assimilated by marine organisms and subsequently reduced into organic sulfur form. Sulfide is the predominant form in reduced conditions, but its presence has also been found in aerobic conditions at low concentrations (i.e. pico to nanomolar) (Cutter and Krahforst, 1988; Luther and Tsamakis, 1989). These authors found similarities in the depth profiles of sulfide and chlorophyll. The depth maximum of sulfide coincided with that of chlorophyll. Hydrolysis of carbonyl sulfide (COS) has been hypothized to be the major route for the production of sulfide (Elliott et al., 1987).

The sulfide concentration has been shown to be subject to daily variations (Cutter and Krahforst, 1988). Highest concentrations of sulfide occur in the early morning which was attributed to photochemical processes. The spatial distribution of sulfide indicates that sulfide could be affected by a variety of coastal sources (e.g. anoxic estuarine, marsh, and subtidal

sediments) leading to concentrations in the coastal waters higher than those in the open seawater (Cutter and Krahforst, 1988). Cultures studies have shown that marine phytoplankton could act as a source of sulfide (Walsh et al., 1994).

COS is present in seawater at pico to nanomolar concentrations (Ferek and Andreae, 1984; Turner, S.M. and Liss, 1985).Pronounced diel variations in the COS concentration in surface seawater are thought to be due photochemical reactions (Ferek and Andreae, 1984). A photosensitised reaction of organic sulfur compounds is a source for COS (Zepp and Andreae, 1994). Furthermore, studies with model organic sulfur compounds in synthetic and coastal seawater revealed that COS could be produced efficiently from compounds with divalent sulfur atoms (e.g. thiols) (Zepp and Andreae, 1994). The spatial variability (i.e. offshore and nearshore) in COS distribution is associated with the distribution of organic compounds which are photolysed to COS, and this could be the reason why the concentrations of COS in the coastal waters is higher than those in open seawater (Andreae, 1990).

Perhaps the most important organic sulfur compound is dimethylsulfoniopropionate (DMSP), which is believed to play a role in the osmotic regulation in marine phytoplankton (Vairavamurthy et al., 1985). It is present in high concentrations in the algal and phytoplankton tissue (Tocher et al., 1966; Craigie et al., 1967; White, 1982). The volatile sulfur compound, dimethyl sulfide (DMS) is produced via cleavage processes from DMSP (Dacey and Wakeham, 1986; Kiene and Taylor, 1988; Ledyard and Dacey, 1994). In anoxic marine sediment, DMSP is shown to produce other natural sulfur compounds such as 3-methiolpropionate (MMPA) and 3-mercaptopropionate (MPA) via demethylation reactions (Taylor and Gilchrist, 1991).

Due to its potential impact on the atmospheric sulfur cycle, DMS (also other sulfur compounds such as COS, and $H_2S$) has received considerable attention. DMS is the most abundant volatile sulfur compound in surface ocean waters (Andreae and Barnard, 1984; Wakeham et al., 1984; Turner, S.M. and Liss, 1985; Turner, S.M. et al., 1988), and is thought to enter the atmosphere via sea-air exchange. It was suggested that biological production and consumption are the most important factors in controlling DMS concentrations in the ocean (Kiene and Bates, 1990). The relationship

between DMS and (culture and marine) phytoplankton was subjected to extensive studies by several authors (Barnard et al., 1984; Turner, S.M. et al., 1988, 1996; Malin et al., 1993). Their results demonstrated that the distribution of DMS is taxon-dependent and that groups of phytoplankton like dinoflagellates and coccolithophores are the most significant DMS producers.

(Dyrssen et al., 1985) measured thiol concentrations in the water column of the Black Sea and concluded that organic analysis of the Black Sea should include the search for sulfur-containing compounds. The authors found high concentrations of thiols ranging from 10-30 μM with highest concentrations in the deep water. They suggested that sulfur-containing proteins could be the source for thiols in the Black Sea water. Thiols and the unknown reduced sulfur compound concentrations were determined by (Mopper and Kieber, 1991) and showed an increase in their concentrations with depth.

The spatial and temporal distribution of DMS and DMSP in the North Sea has been studied before (Turner, S.M. et al., 1988; Nedwell et al., 1994). The concentrations of DMS in winter were found to be lower than in

summer. This was attributed to the biological activity of marine phytoplankton during the summer which enhanced the production of DMS.

Chromatography was used for the determination of volatile sulfur compounds like COS, hydrogen sulfide ($H_2S$) and DMS in seawater (Turner, S.M. et al., 1988; Malin et al., 1993; Radford-Knoery and Cutter, 1993, 1994).

Voltammetry has been used before to measure sulfide-like compounds in various marine waters and the voltammetric response was ascribed to either a metal-sulfide complex (Luther and Tsamakis, 1989), sulfur associated with organic matter (Ciglenecki and Cosovic, 1996), or thiols (Al-farawati and van den Berg, 1997a (Chapter 2)). The concentrations of these compounds ranged between <0.1 and 2 nM (after calibration with sulfide standard) in the western North Atlantic Ocean and Mediterranean Sea (Luther and Tsamakis, 1989). In the Adriatic Sea, the concentrations ranged between 10 and 50 nM (equivalent to sulfide concentrations) with high concentrations during phytoplankton bloom when mucous aggregates cover the surface water (e.g. 500 nM) (Ciglenecki and Cosovic, 1996). Incubation experiments of phytoplankton with DMSP solution have shown that the voltammetric response for sulfide-like compounds increased

implying the production of sulfide-like compounds by DMSP degradation (Ciglenecki and Cosovic, 1996).

In coastal waters, the processes which control the distribution of minor constituents of seawater tend to be varied: inputs can be from rivers, sediments, atmosphere and biodegradation; removal can be by biological uptake, sorption in or on to sedimentary particles or by flushing with ocean water. Collection of sufficient data should facilitate our understanding of the dominant processes that control the distribution of these compounds which occur at low levels. Automated voltammetric methods, on board ship, were used before for in-line monitoring of trace metals in surface and coastal waters of the western North Sea (Achterberg and van den Berg, 1994; Colombo et al., 1997).

Here, thiol concentrations are presented for coastal water of the western North Sea and English Channel. Flow-analysis was used with detection by CSV (FA-CSV), and the measurements were on-line, in water which pumped on-board the ship. A very large data set was obtained of some 7000 thiol measurements in these waters. These data were used to determine relationships of thiols to various other parameters (e.g. salinity,

temperature, chlorophyll---etc). Thiols were measured in discrete samples from the water column of the Black Sea. These data also are presented here along with the surface water data.

### *4.3 Materials and methods*

#### 4.3.1 Equipment

Water was pumped at a rate of 4 L $min^{-1}$ from a depth of ~4 m on-board ship using a peristaltic pump via a 20 meter polyvinyl chloride (PVC) hose of 12 millimetre internal diameter. To hold the hose under water, the hose was attached to a heavy, metallic "fish". The "fish" was placed to the left of the bow of the ship. The distance between the "fish" and the hull was approximately 3-4 meters. A high density polyethylene funnel was attached to the front of the "fish". The funnel was connected to the inlet of the hose. In-line filtration of the seawater was achieved using a home-made filtration unit which was flushed continuously in a cross-flow fashion. The seawater was flowing tangentially to a 0.45 μM membrane filter through a hole in a Swinnex 47 filter holder (Millipore part No. SX00 047 00).

Samples from the water column of the North Sea and the English Channel were collected using CTD-rosette sampler with modified pre-cleaned 10 litre FEP-coated Go-Flo bottles, filtered and measured by FA-CSV. For reasons of comparison, filtered samples were collected using the in-line pumping system and were measured on-board ship by flow and batch-mode voltammetry.

Samples (20-40 ml) of the Black Sea were collected using CTD hydrocast MARK-III and Go-Flo rosette bottles, filtered, purged with nitrogen gas to remove dissolved oxygen, and frozen.

All discrete samples were analysed in duplicate. Polystyrene (Bibby Sterilin Ltd, cat.No. 128A) vials (30 ml) were used for copper-complexing ligand titrations; those vials are called Sterilins. UV-digestion was carried out using a 1 kW, high-pressure, mercury vapour lamp.

### 4.3.2 Reagents

A stock solution of 0.1 M thiourea (BDH) was prepared in Milli-Q water. Acid (HCL) and ammonia used for solutions were purified by

sub-boiling, quartz distillation. A pH buffer was prepared containing 1 M tris(hydroxymethyl)methylamine (Analar, BDH) in 0.5 M HCl; addition of 100 µl of this TRIS buffer in 10 ml sea water gave a pH of 8. An aqueous stock solution containing 0.01 M salicylaldoxime (SA) was prepared in 0.1 M HCl. Water used to prepare reagents was purified by reverse osmosis (Milli-RO, Millipore) followed by ion-exchange (Milli-Q).

### 4.3.3 Analytical procedure to detect thiol compounds

Voltammograms were recorded using a Autolab voltammeter (Eco Chemie) with a Metrohm 663 VA electrode stand and hanging mercury drop electrode (HMDE). The voltammetric cell was replaced by a home-made flow-cell and in-line purging system to remove oxygen (Al-farawati and van den Berg, 1997a (Chapter 2)). The system was controlled by an IBM-compatible computer. The software (EAS from Eco Chemie) had been altered to enable continuous on-line analysis. A square-wave modulation (150 Hz) was applied to the CSV scans at a pulse-height of 25 mV and step height of 2.4 mV. Potentials were set relative to an Ag/AgCl, saturated AgCl in 3 M KCl reference electrode. Purified nitrogen gas was used to flush the container holding the semi-permeable tubing for oxygen removal. The

residence time of the seawater in the degassing unit was 20 s.

4.3.4 Voltammetric detection of thiol concentrations

Thiols dissolved in seawater were determined by CSV. A flow-cell was used for in-line analysis using the following voltammetric parameters: the deposition potential was set to 0.05 V; four mercury drops were discarded before a new mercury drop was extruded. A deposition time of 30 sec was used during which the solution was purged. A quiescent period of 3 sec was allowed before the potential scan was initiated at -0.2 V and was terminated at -0.7. The system was intermittently calibrated at 6-8 hour intervals. The calibration was achieved by collecting a sample in 250 ml clean polyethylene bottle using the in-line pumping system. The sample was divided into two subsamples. A total 2.5 nM of thiourea was added to one of them. The sensitivity was calculated from the difference of the average peak heights of the two samples (roughly 20 scan for each sample)

The same voltammetric procedure was used for the analysis of the Black Sea samples but here a conventional, batch voltammetric cell was used, and the deposition time was 60 sec with a quiescent period of 8 sec.

### 4.3.5 Determination of copper complexation by thiols in seawater

Black Sea samples (station 3, depths of 1000 and 1500 m) were diluted (dilution factor ranged between 7-100) to 100 ml using UV-irradiated clean seawater with a salinity of 35. The seawater was buffered to pH 8 by addition of Tris buffer. A concentration of 1 μM SA was used for the copper complexation measurements. Subsamples of 10 ml were pipetted into clean Sterilins. Cu was added to the Sterilins in 5-10 increments increasing from 0 to 160 nM and the solutions were allowed to equilibrate for a period of 3 hours. The voltammetric measurements were carried out in batch-mode. The labile copper was determined using an adsorption potential of -0.2 V and an adsorption period of 60 s, followed by a quiescence period of 8 s. The voltammetric scan using square-wave mode was from -0.1 to -0.75 V at a scan rate of 25 $mVs^{-1}$. The sensitivity (see equation 4.6) was obtained by calibration in UV-irradiated seawater (free of thiols) and by addition of high concentrations of copper to the titrated thiols samples. The concentration of dissolved copper initially present in the clean seawater was determined by UV-digestion at pH~2.

4.3.6 Theory: determination of complexing ligands and conditional stability constants of copper-thiols complexes

Ligand concentrations, $C_L$, and conditional stability constants, $K'_{CuL}$, for complexes of copper with thiols were calculated using the following relationship (van den Berg and Kramer, 1979; Ruzic, 1982; van den Berg, 1982)

$$\frac{[Cu_{labile}]}{[CuL]} = \frac{[Cu_{labile}]}{C_L} + \frac{\alpha'}{K'_{CuL}\, C_L} \qquad (4.1)$$

where [CuL] is the concentration of copper complexes with thiols L, and $[Cu_{labile}]$ is the labile copper concentration.

The labile copper concentration is defined by

$$[Cu_{labile}] = \Sigma[Cu(SA)_X] + [Cu'] \qquad (4.2)$$

where $[Cu(SA)_X]$ is the concentration of copper complexes by SA and [Cu'] is the concentration of inorganic copper. $\alpha'$ is the overall $\alpha$-coefficient (Ringbom and Still, 1972) of the copper in seawater (excluding complexation by L),

$$\alpha' = \alpha_{Cu'} + \alpha_{CuSA} \qquad (4.3)$$

where $\alpha_{Cu'}$ is the $\alpha$-coefficient for inorganic complexation of $Cu^{2+}$; and $\alpha_{CuSA}$ is the $\alpha$-coefficient for complexation of copper by SA.

$$\alpha_{Cu'} = 1 + \Sigma K_i^*[F_j]^i + \Sigma \frac{K_{ai}^*}{[H^+]^i} \qquad (4.4)$$

where $K_i^*$ is the stepwise stability constant of $Cu^{2+}$ with each major anion $F_j$, and $K_{a,i}^*$ is the stepwise acidity constant. A value was calculated for $\alpha'$ using an ion-pairing model to obtain free major anion concentrations, using stability constants valid for seawater from (Turner, D.R. et al., 1981).

$$\alpha_{CuSA} = K'_{CuSA}[SA'] + \beta'_{Cu(SA)2}[SA']^2 \quad (4.5)$$

where $K'_{CuSA}$ and $\beta'_{Cu(SA)2}$ are the conditional stability constants for the formation of CuSA and $Cu(SA)_2$ respectively, and [SA'] is the concentration of SA not complexed by copper ( invariably [SA'] equalled the total SA concentration as this was much greater than the copper concentration). Values of $\alpha_{CuSA}$ were calculated using constants from (Campos and van den Berg, 1994). Values for $[Cu_{labile}]$ were obtained from the measured peak height, $i_p$:

$$[Cu_{labile}] = i_p/s \quad (4.6)$$

where $s$ is the sensitivity (peak current/copper concentration)

Concentrations of CuL were calculated from the total and labile copper concentrations:

$$[CuL] = C_{cu} - [Cu_{labile}] \quad (4.7)$$

where $C_{cu}$ is the total dissolved copper concentration at each point of the titration. A value for $C_L$ was obtained by least square linear regression from the slope$^{-1}$ of a plot of $[Cu_{labile}]/[CuL]$ as a function of $Cu_{labile}]$ whereas

$K'_{CuL}$ was obtained from

$K'_{CuL}=\alpha' \times$ slope/Y-axis intercept. Concentrations of $Cu^{2+}$ were calculated from $[Cu^{2+}]=[Cu_{labile}]/\alpha'$.

### *4.4 Data source for salinity, chlorophyll, transmissometry, temperature, phosphate, nitrate, nitrite and silicate*

Salinity, transmissometry, temperature, SPM, and chlorophyll for the North Sea study were obtained from the British Oceanographic Data Centre (BODC) held with Proudman Oceanography Laboratory (POL). Phosphate, nitrate, nitrite and silicate were measured by members of Plymouth Marine Laboratories (PML), and were limited to parts of the cruise.

### *4.5 Study areas*

Fig. 4.1 shows the study area and cruise track ( RRS Challenger, 1-13 July 1995) for the North Sea and the English Channel. The area investigated was divided into three major regions:

1) Humber-Wash grid starting from the north of the Humber Estuary

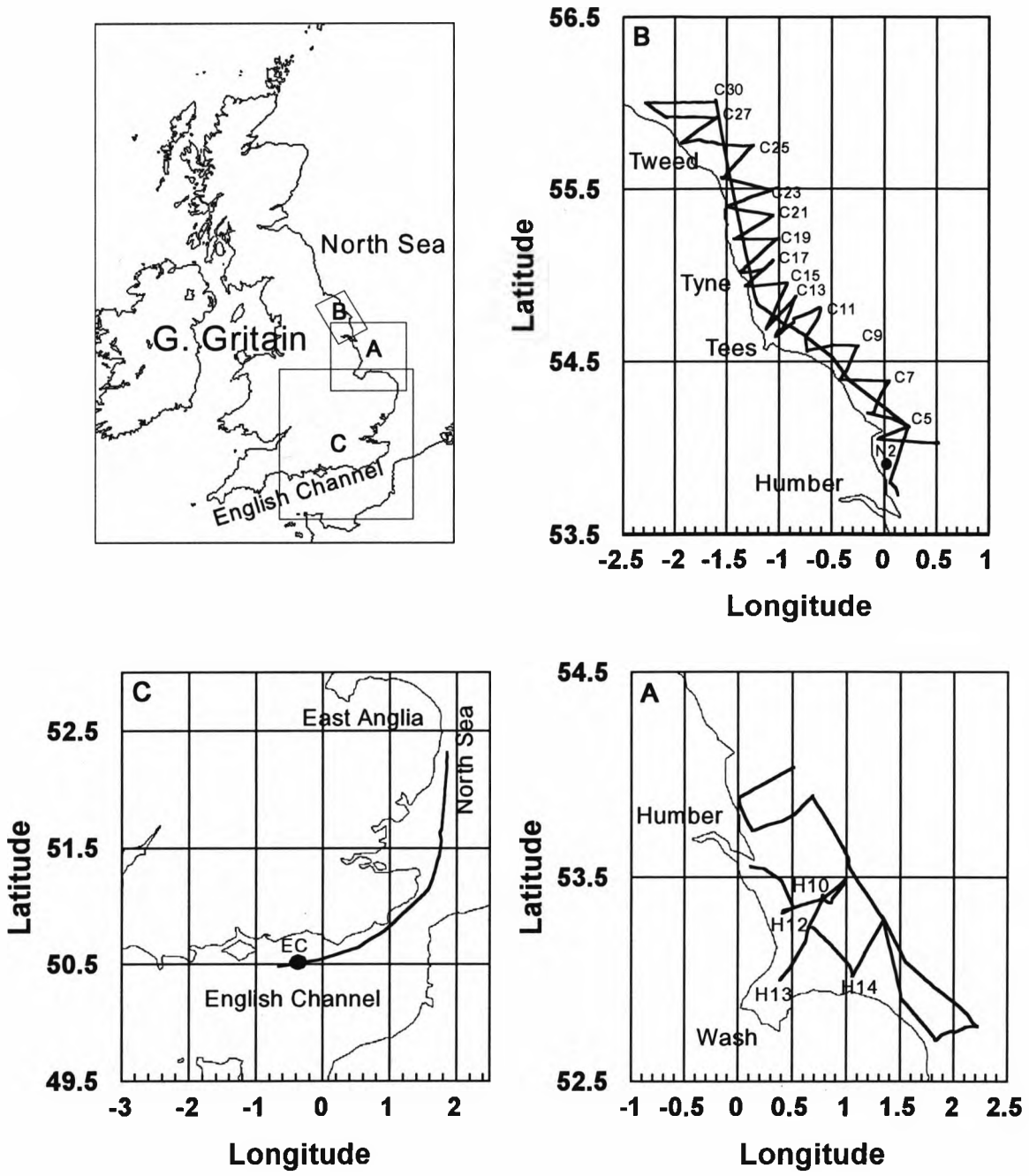


Fig. 4.1. Study area of the North Sea Cruise: A) Humber-Wash grid including the Humber, Wash and part of the East Anglia; B) north east coastal grid between the Humber and the Tweed rivers; C) East Anglia-English Channel grid.

going south to 52.8° N; this followed a box-like pattern (Fig. 4.1A).

2) North east coastal grid where the ship followed a zigzag pattern starting from the north of the Humber Estuary and going north to Tweed (Fig. 4.1B) and then back parallel to the coast.

3) East Anglia-English Channel grid where the ship steamed parallel to the coast from the south of East Anglia along the southwest of the North Sea to the northern side of the English Channel (Fig. 4.1C).

This cruise was part of the Land Ocean Interaction Study (LOIS) project of the National Environmental Research Council (NERC).

Samples of the Black Sea were collected during a cruise with research vessel "Professor Vodyanistkiy" (17 July-1 August 1995), as part of the EROS 2000 programme of the EU. The location of the stations are shown in Fig. 4.2. Samples were collected in shallow coasts in the northwestern part of the Black Sea. A deep cast was taken in water of a depth of 1425 m.

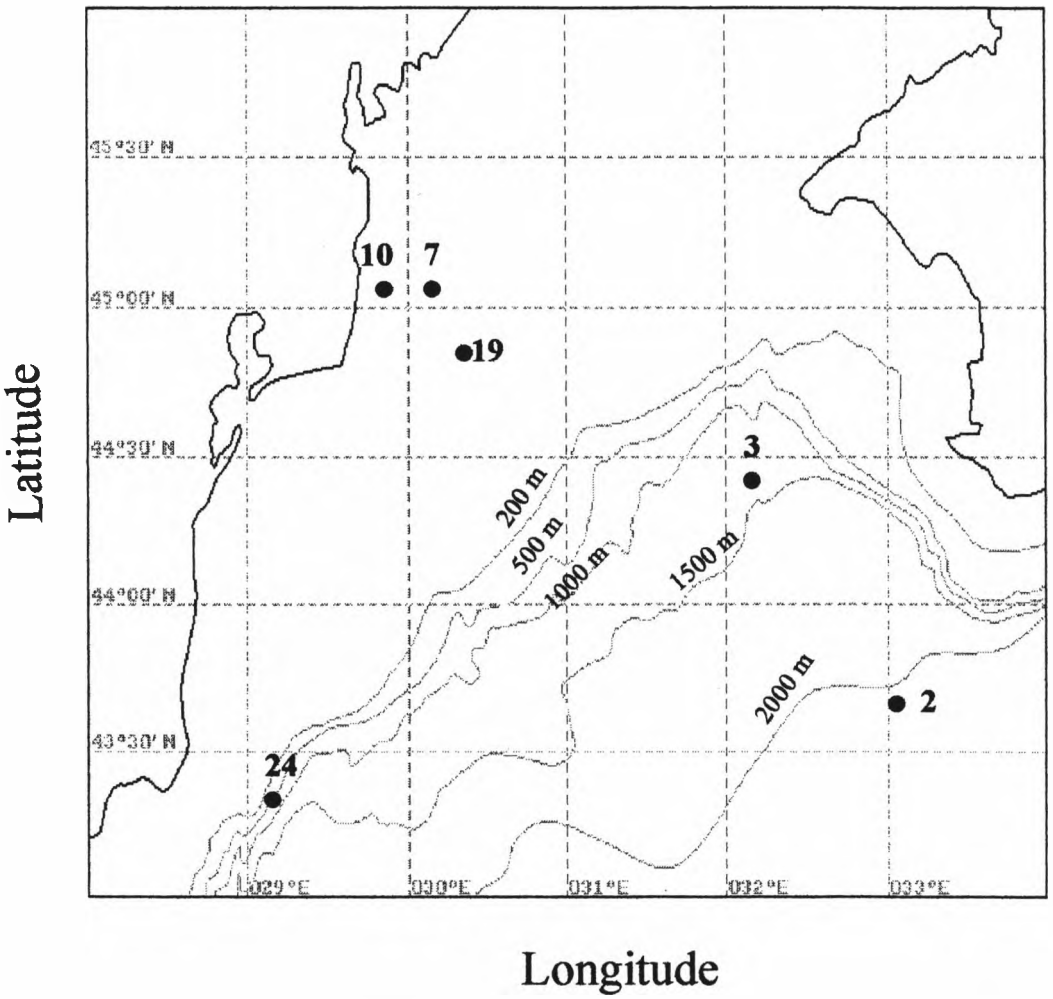


Fig. 4.2. Map of the northwestern Black Sea including the geographical locations of the stations occupied during the Black Sea cruise.

## *4.6 Results and discussions*

### 4.6.1 North Sea data

#### 4.6.1.1 Results

##### 4.6.1.1.1 Verification for the negligible presence of sulfide compared to thiol compounds

Voltammetry of seawater collected during the RRS Challenger cruise revealed the presence of a sulfide or thiol-type peak at -0.54 V (Fig 4.3).

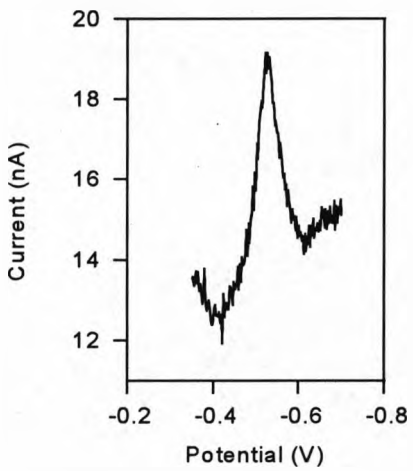


Fig. 4.3. The voltammetric response of sulfide/thiol compounds from the North Sea water

Batch-mode voltammetry of several (10) filtered samples was used on-board ship to verify whether the response was due to sulfide or to thiol compounds. The voltammetric response was stable for a period of 10 minutes, therefore it was concluded that the response was not due to sulfide but to thiol compounds (Al-farawati and van den Berg, 1997a (Chaprer 2)). Additional filtered samples were analysed on-board ship of surface water of the North Sea at selected CTD-stations. Thiol concentrations were measured by flow and batch-mode voltammetry. The results are presented in Table 4.1. Stable peak heights of the batch-mode measurements showed again that the sulfide concentrations was negligible compared to that of the thiols. The data shows a good agreement confirming that the samples contained insignificant levels of sulfide, and thus the response was due to thiols compounds. In the view of average standard deviation of 5% of successive scan in the batch cell, the sulfide concentration must amount to less than 5% of thiols. This would mean that sulfide levels would amount to less than 0.3 nM, in agreement with oceanic concentration found by (Radford-Knoery and Cutter, 1994).

Table 4.1. Comparison of thiol measurements by flow and batch-mode voltammetry the measurements were carried out on-board ship during Challenger cruise (CH119C) on the North Sea in samples taken at CTD-stations.

| Station | batch analysis | Flow analysis |
|---|---|---|
| H12 | 1.51 | 1.64 |
| H13 | 1.70 | 1.72 |
| H18 | 1.77 | 1.80 |
| C5 | 1.52 | 1.61 |
| C10 | 1.80 | 1.96 |
| C12 | 3.12 | 2.99 |
| C18 | 2.37 | 2.36 |
| C21 | 1.65 | 1.71 |
| C24 | 1.90 | 1.73 |
| C29 | 1.86 | 1.91 |

#### 4.6.1.1.2 Thiol in the western North Sea and the English Channel

Thiol concentrations in North Sea and Channel waters in the cruise track of the ship are shown in Figs. 4.4, 4.5 and 4.6. Concentrations are indicated using colour bands. A total of 7030 data were collected throughout the cruise at a rate of 50 measurements/hour. The thiol concentrations were found to range from 0.77 to 3.54 nM with a mean of 1.81±0.54 nM (n=7030) (calibration with thiourea). Highest concentrations were found in areas adjacent to the Tees-Tyne, the Tweed (North east coastal region) and a narrow part of the Strait of Dover (East Anglia-English Channel region). The Humber-Wash region was characterised by lower thiol concentrations.

Previous voltammetric studies utilizing the same voltammetric peaks reported concentrations of 10-50 nM sulfide-like substances in the northern Adriatic ( (Ciglenecki and Cosovic, 1996)) which is known for its large algal blooms, and 0.1-2.0 nM in the northwestern Atlantic ( (Luther and Tsamakis, 1989)). Those studies used calibration against sulfide.

Cross calibration of thiourea with sulfide showed that each nanomole of thiourea yields a voltammetric response equivalent to 2.76 nanomole of sulfide.

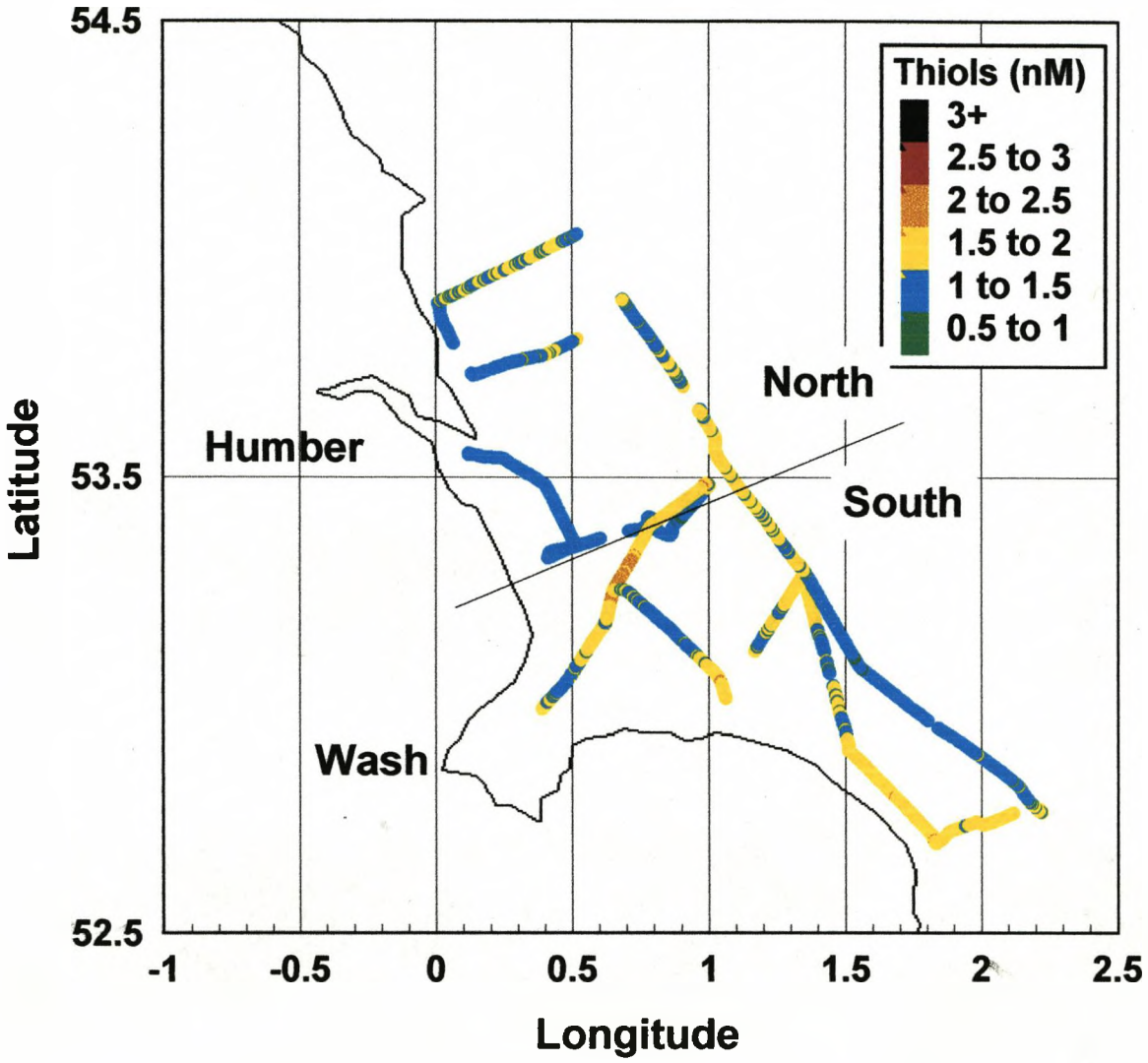


Fig. 4.4. Thiols distribution in the Humber-Wash region. Colour on the map track indicate detected thiols concentrations.

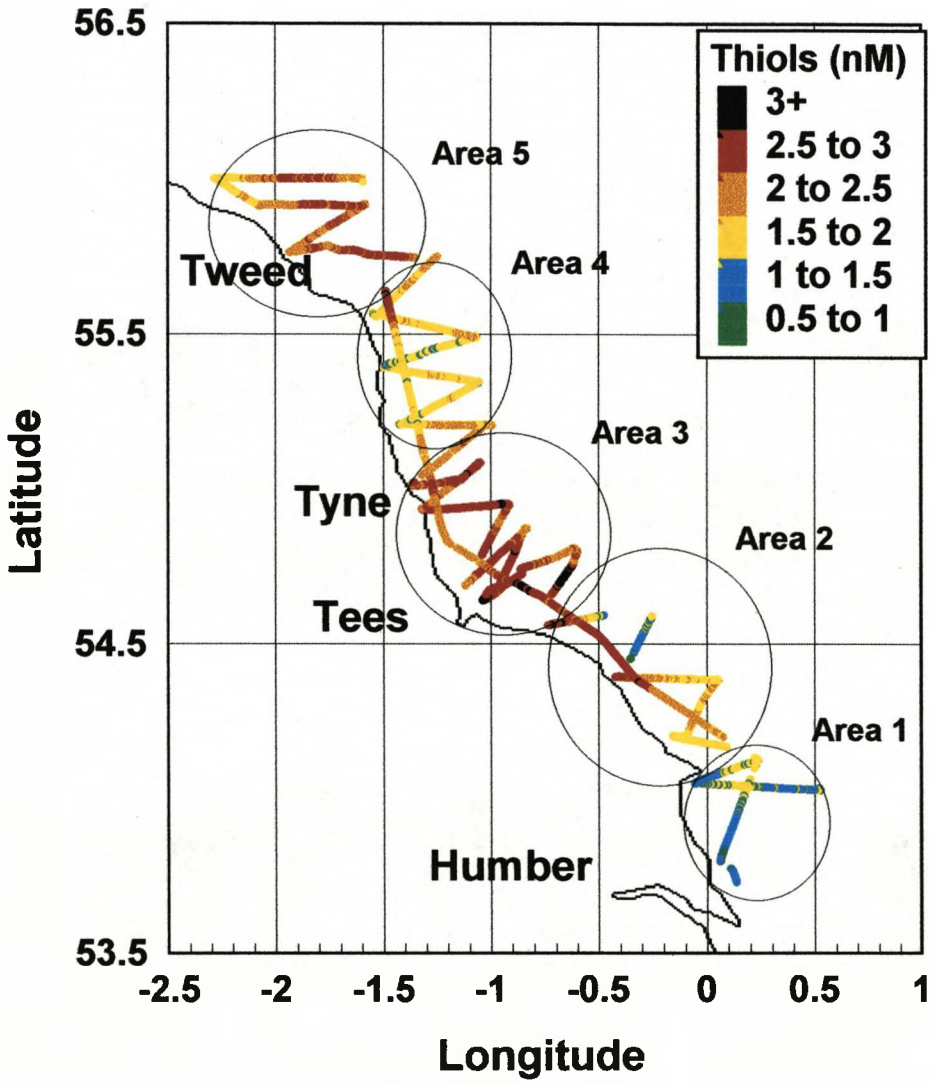


Fig. 4.5. Thiols distribution in the five areas of the north east coastal region. Colour on the map track indicate detected thiols concentrations.

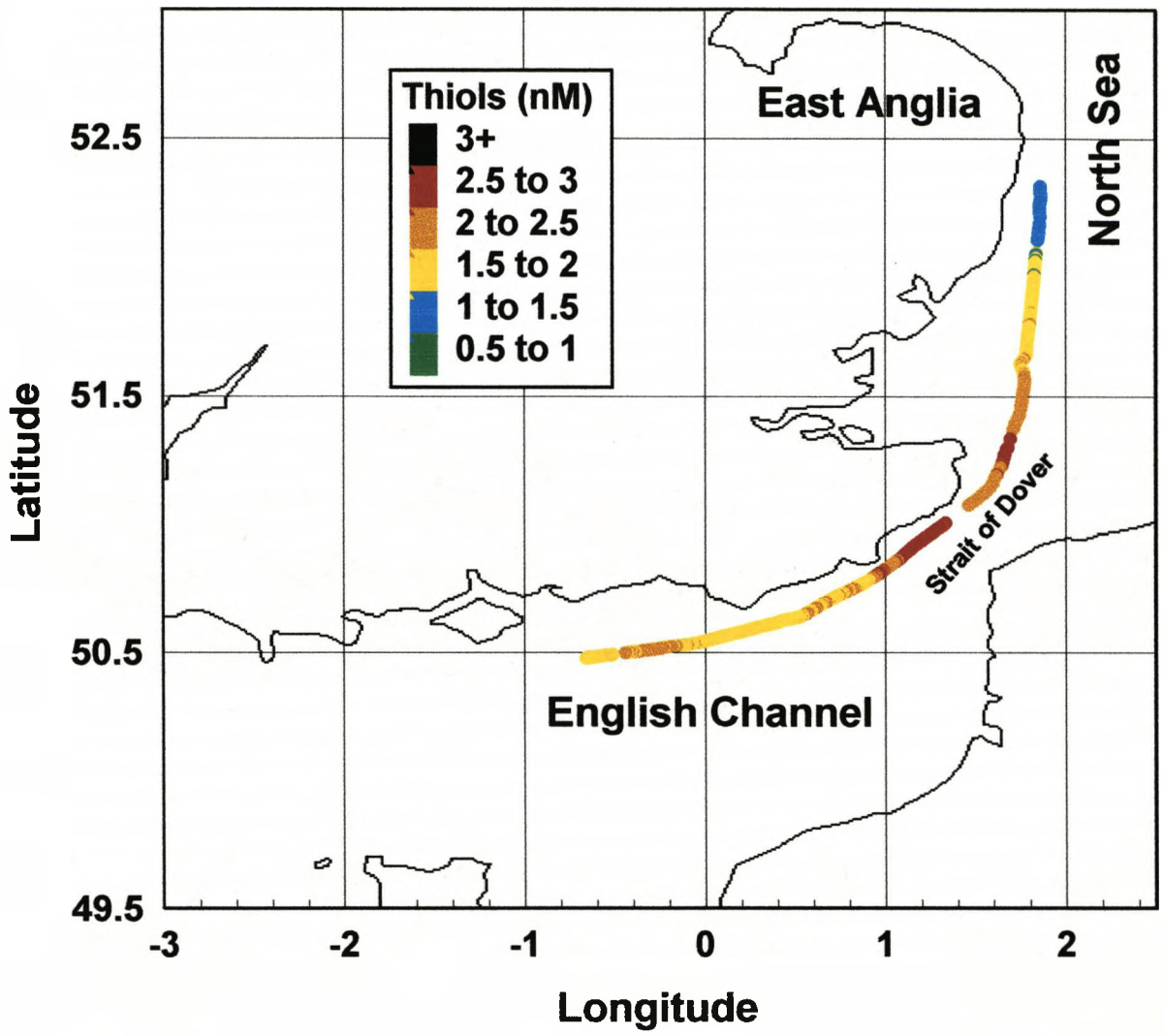


Fig. 4.6. Thiols distribution in the East Anglia-English Channel region. Colour on the map track indicate detected thiols concentrations.

Comparison therefore shows that the Ciglenecki and Cosovic data are equivalent to 3.6-18.1 nM thiourea, whereas the Luther and Tsamakis data are equivalent to 0.04-0.72 nM thiourea. The North sea and Channel waters concentrations lie between these two levels. The thiol concentrations are greater than those of total sulfide in coastal and open waters (0.002-1.1 nM) ( (Cutter and Krahforst, 1988; Radford-Knoery and Cutter, 1994)) as determined by gas chromatography.

The distribution of the thiols, and its relation to other parameters (temperature, salinity, chlorophyll, nitrate, nitrite, phosphate, silicate and suspended particulate material (SPM)) in the three regions of the cruise will be discussed in detail below.

*4.6.1.1.2.1 Humber-Wash grid*

The distribution of thiols in the Humber-Wash region is presented in Fig. 4.4. The data have been plotted on a map showing the cruise track, using colours to indicate concentrations. The thiol concentrations ranged between 0.92 nM and 2.41 nM, with a mean of 1.51±0.26 (n=1867). There is no systematic, clear areal trend in the distribution of the thiols. The south of Humber-Wash area has a mean concentrations of 1.62±0.26 (n=969), which is slightly higher than that in the north (1.39±0.21, n=898), but it is an arbitrary

definition and the difference is small. Highest concentrations were found in the Wash area near station H12 between stations H10 and H13 and near the coast of East Anglia.

*4.6.1.1.2.2 North east coastal grid*

In this part of the study the track followed a zigzag pattern 20 miles to and from the coast. The distribution of the thiols along the north east coast is shown in Fig. 4.5. Here the thiol concentrations of are variable, ranging over several nM over relatively short distances. Thiols ranged from 0.88 nM to 3.54 nM, with a mean of 2.14±0.48 (n=5179). Geographically the data from this region could be subdivided in five areas to see whether there are differences in the thiol distribution (Table 4.2 and Fig. 4.5). The thiol concentrations are distinct for these areas, the highest levels occurring in the Tees-Tyne area, and the lowest in the most southerly part (Humber to station 5). Possible reasons for the variations apparent when all data were compared with other parameters. This will be discussed below.

*4.6.1.1.2.3 East Anglia-English Channel grid*

The thiol distribution along East Anglia-English Channel region are presented in Fig. 4.6. The thiol concentrations ranged from 1.12 nM to

Table 4.2. Areas of the North East coastal region according to the distribution of thiols. Minimum, maximum and average thiol concentrations (nM) are shown. Measurements were calibrated with thiourea.

| Area | Min. | Max. | Ave. | n |
|---|---|---|---|---|
| Area 1 (between North Humber and station C5) | 0.90 | 1.82 | 1.40±0.19 | 349 |
| Area 2 (between station C5 and station C10) | 0.88 | 3.22 | 2.06±0.43 | 527 |
| Area 3 or Tees-Tyne zone (between station C10 and station C19) | 1.36 | 3.54 | 2.54±0.28 | 1066 |
| Area 4 (between station C19 and station C25) | 1.19 | 3.02 | 1.85±0.27 | 1470 |
| Area 5 or Tweed zone (between station C25 and station C30) | 1.70 | 2.96 | 2.32±0.27 | 572 |

2.95 nM, with a mean of 2.04±0.39 (n=1867). Highest concentrations were evident in the Strait of Dover.

#### 4.6.1.2 Discussion

##### 4.6.1.2.1 Relationships between thiols and other parameters

Thiol concentrations can be compared with data for chlorophyll, nitrite, nitrate, phosphate, silicate, temperature, salinity, and SPM as a function of the cruising time in the three main regions (Humber-Wash, north east coast and east Anglia-English Channel) in Figs. 4.7, 4.8 and 4.9. Variability in the Humber-Wash and north east coast was particularly pronounced because of the zigzag or box-like pattern of the cruise track, whereas the variability in the east Anglia-English Channel area was less pronounced because the cruise track was here parallel to the coast. In the case the ship followed a track which zigzagged to and from the coast, the salinity can be seen to decrease and increase with this pattern. The origin of species dissolved in coastal waters can usually be evaluated by comparison with salinity: a decrease with increasing salinity would then suggest a coastal origin. However, the thiol data do not show a systematic variation with salinity indicating that their distribution is not related to a specific fresh

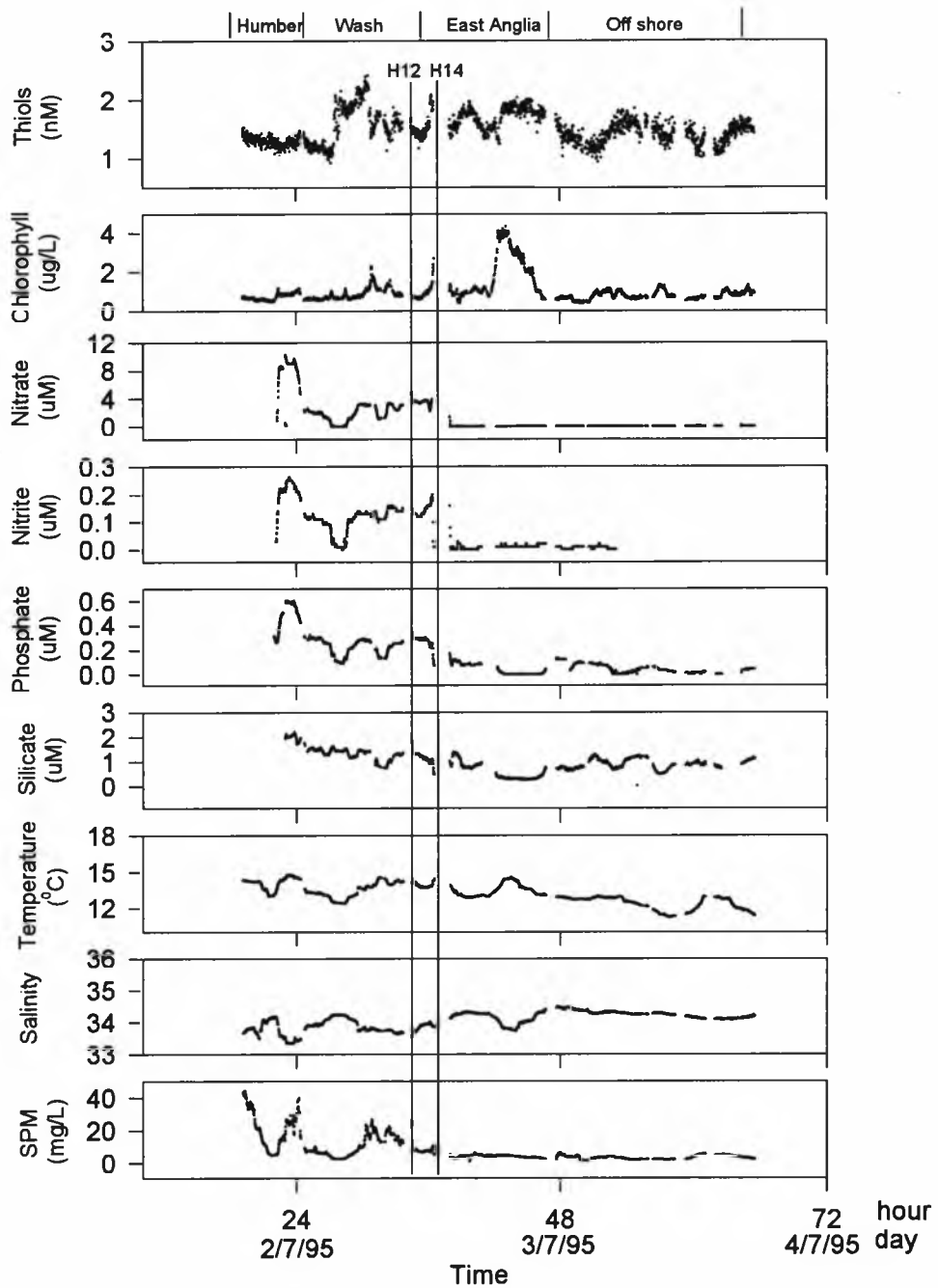


Fig. 4.7. Concentrations of thiols and other parameters as a function of cruising time in the Humber-Wash region during RRS Challenger cruise in the North Sea. Vertical lines illustrate the areas where a good correlation were found between thiols and chlorophyll.

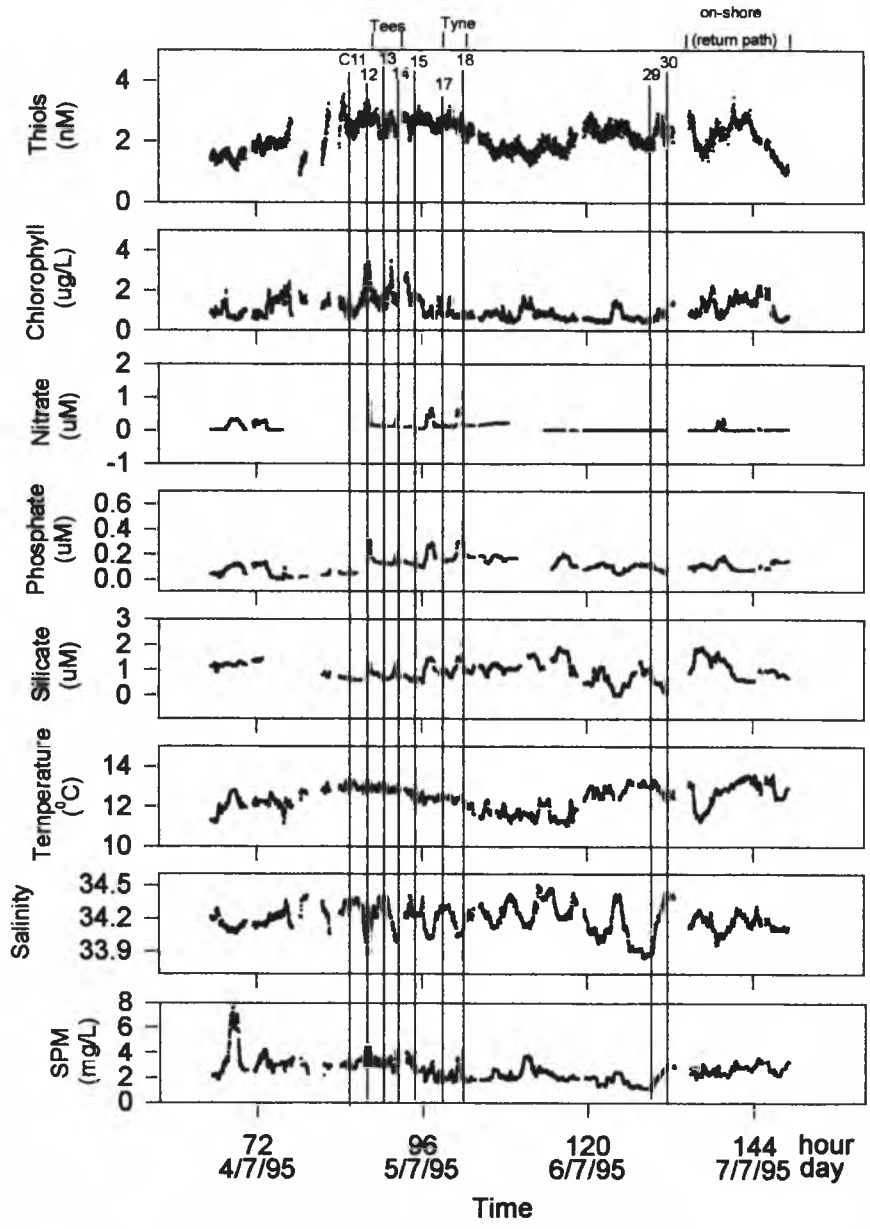


Fig. 4.8. Concentrations of thiols and other parameters as a function of cruising time in the north east coastal region during RRS Challenger cruise in the North Sea. Vertical lines illustrate the areas where a good correlation was found between thiols and chlorophyll.

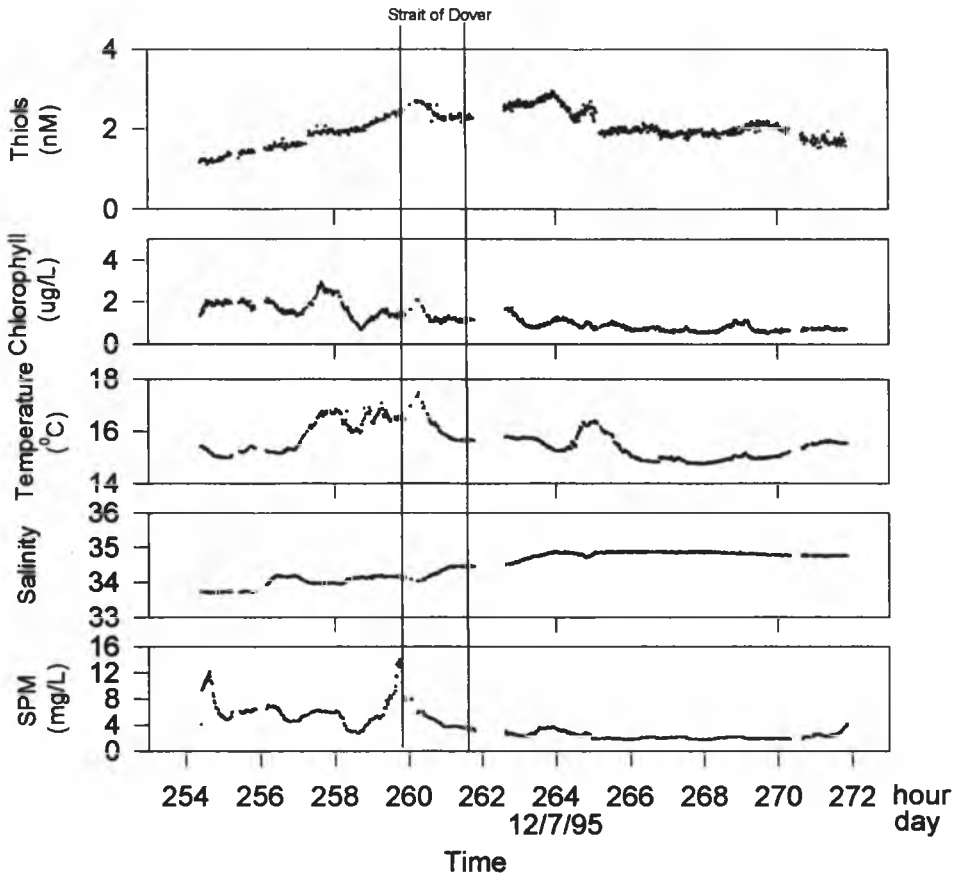


Fig. 4.9. Concentrations of thiols and other parameters as a function of cruising time in the East Anglia-English Channel region during RRS Challenger cruise in the North Sea. Vertical lines illustrate the areas where a good correlation was found between thiols and chlorophyll.

water or oceanic source. Occasionally an increase in the thiol concentration is associated with decreased salinity and increased nutrient concentrations (C12). However, sometimes the opposite is the case and the decreased thiol levels are associated with decreased salinities and increased nutrient concentrations (C14 and C18). Overall the data suggests that the thiols are non-conservative. Apparently the thiol concentrations are subject to short-term variations. Comparison with chlorophyll shows that in most cases the thiol concentration co-varies with chlorophyll. Examples of the covariation of thiol and chlorophyll are shown expanded in Fig. 4.10. The covariation between chlorophyll and the thiols suggest that thiols are produced by phytoplankton.

#### 4.6.1.2.2 Correlation between thiols and other parameters

The correlation coefficient (r) between thiols and chlorophyll was calculated to verify whether the thiol concentration is related to chlorophyll. Furthermore, the correlation coefficient was calculated for thiols and other parameters (Table 4.3). The correlation coefficient with chlorophyll was small (0.09) when all data were used. However, significant correlations were obtained for individual sites (Table 4.3). The distribution of thiols did not show clear differences between the concentration of thiols near-shore and

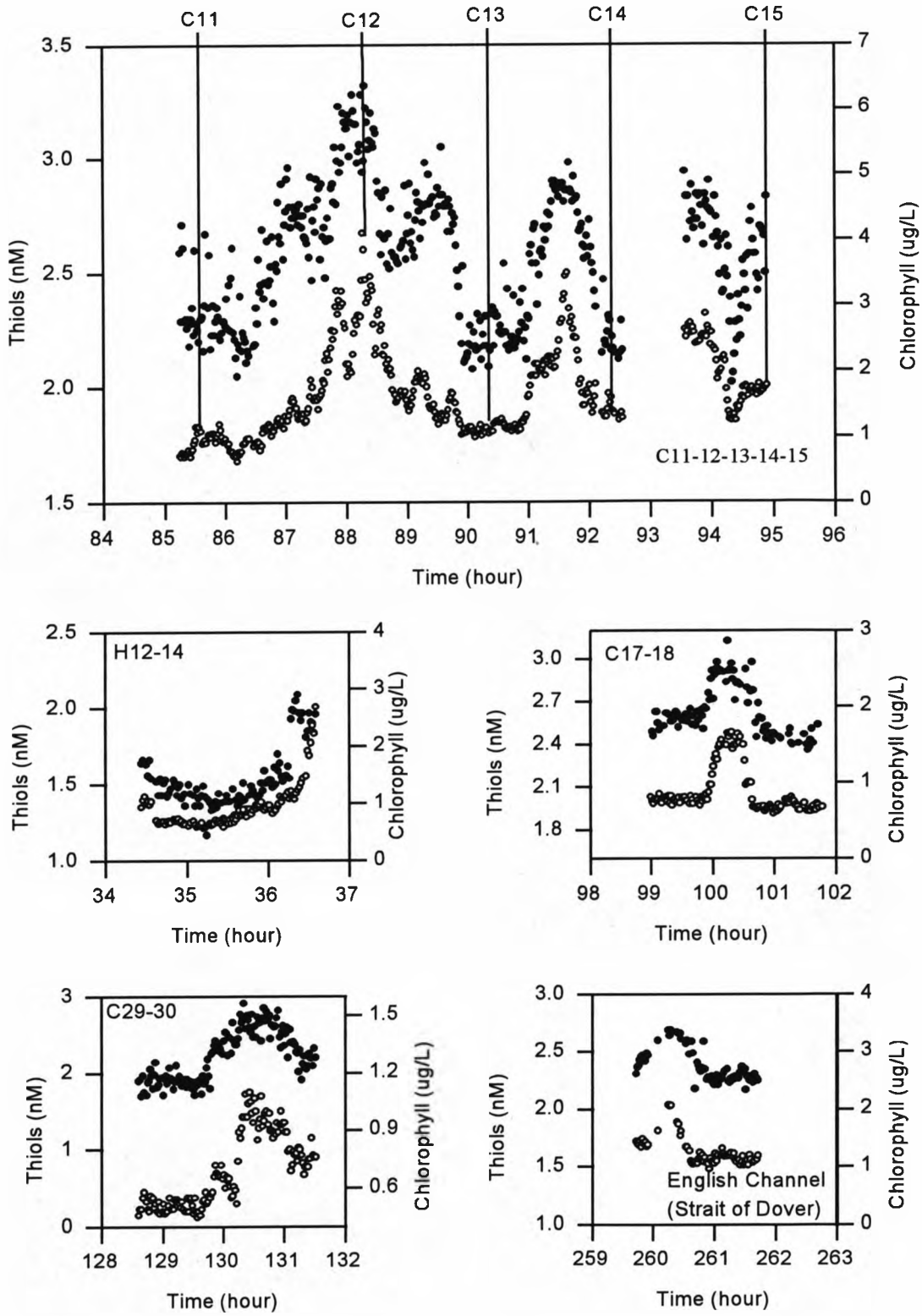


Fig. 4.10. Comparison of thiols and chlorophyll concentrations of selected areas during RRS Challenger cruise to the North Sea.

Table 4.3. Correlation coefficients (r) of linear least-square regressions between thiols and other parameters in data obtained during RRS challenger (CH119c) cruise. Shaded numbers show significant correlations

| Location | chlor-ophyll | temp. | salinity | Nitrate | Nitrite | Phos-phate | Silicate | SPM |
|---|---|---|---|---|---|---|---|---|
| **Total** | 0.09 | 0.00 | 0.07 | 0.03 | 0.00 | 0.01 | 0.04 | 0.04 |
| **NE Coast (total)** | 0.17 | 0.00 | 0.48 | 0.00 | ---- | 0.00 | 0.04 | 0.01 |
| **NE Coast (area 1)** | 0.30 | 0.07 | 0.05 | 0.01 | ---- | 0.15 | 0.36 | 0.02 |
| **NE Coast (area 2)** | 0.03 | 0.20 | 0.19 | 0.25 | ---- | 0.00 | 0.53 | 0.05 |
| **NE Coast (area 3)** | 0.15 | 0.04 | 0.01 | 0.15 | ---- | 0.00 | 0.08 | 0.08 |
| **NE Coast (area 4)** | 0.07 | 0.48 | 0.00 | 0.06 | ---- | 0.27 | 0.18 | 0.09 |
| **NE Coast (area 5)** | 0.09 | 0.08 | 0.30 | ---- | ---- | 0.26 | 0.28 | 0.28 |
| **Near NE Coast (return path)** | 0.25 | 0.25 | 0.03 | 0.00 | ---- | 0.34 | 0.04 | 0.02 |
| **CTD (off-shore)** | 0.18 | 0.59 | 0.00 | 0.08 | ---- | 0.21 | 0.62 | 0.29 |
| **CTD (near-shore)** | 0.85 | 0.40 | 0.13 | 0.78 | ---- | 0.60 | 0.12 | 0.68 |
| **Humber-Wash** | 0.13 | 0.01 | 0.00 | 0.03 | 0.03 | 0.04 | 0.07 | 0.01 |
| **Notrh Humber-Wash** | 0.01 | 0.14 | 0.15 | 0.11 | 0.22 | 0.12 | 0.02 | 0.06 |
| **South Humber-Wash** | 0.10 | 0.14 | 0.17 | 0.26 | 0.04 | 0.00 | 0.02 | 0.05 |
| **English Channel** | 0.05 | 0.11 | 0.16 | ---- | ---- | ---- | ---- | 0.03 |
| **English Channel (Strait of Dover)** | 0.51 | 0.73 | 0.67 | ---- | ---- | ---- | ---- | 0.29 |
| **Diel variation** | 0.02 | 0.06 | 0.18 | ---- | 0.08 | 0.01 | 0.35 | 0.02 |
| **H12-14** | 0.59 | 0.17 | 0.00 | ---- | 0.00 | 0.33 | 0.14 | 0.02 |
| **C11-12** | 0.49 | 0.19 | 0.38 | ---- | ---- | 0.32 | 0.05 | 0.42 |
| **C12-13** | 0.47 | 0.10 | 0.62 | 0.35 | ---- | 0.27 | 0.44 | 0.44 |
| **C13-14** | 0.74 | 0.44 | 0.11 | 0.13 | ---- | 0.08 | 0.01 | 0.09 |
| **C14-15** | 0.64 | 0.05 | 0.02 | 0.29 | ---- | 0.23 | 0.12 | 0.00 |
| **C11-12-13-14-15** | 0.52 | 0.01 | 0.33 | ---- | ---- | 0.18 | 0.06 | 0.14 |
| **C17-18** | 0.68 | 0.14 | 0.34 | 0.36 | ---- | ---- | 0.55 | 0.02 |
| **C29-30** | 0.67 | 0.04 | 0.46 | ---- | ---- | 0.48 | 0.35 | 0.50 |

off-shore. However, calculation of the correlation coefficients of thiols and other parameters from the data obtained at the CTD stations where the ship was stationary for periods of 45-90 minutes gave different results. These data were used to provide two subsets, one of near-shore, and the other of off-shore, at the extremes of the zigzag cruise pattern. The correlation coefficient of thiols and chlorophyll for the near-shore data was 0.85 whereas it was 0.18 for the off-shore data. It is likely that a stronger trend in chlorophyll and thiol concentrations in the near-shore is the cause for the better relationship of the near-shore data.

Significant correlations between thiols and chlorophyll were obtained for the area of the Tees (C11-12-13-14-15)(r=52), the Tyne (C17-18)(r=0.68), the Tweed (C29-30)(r=0.67) and the Strait of Dover (r=0.51). The Tees area was further divided into sub-areas (C11-12, C12-13, C13-14, C14-15) and showed that C13-14 have the highest correlation between thiols and chlorophyll (r=0.74). The correlation of thiols and other parameters was in general, not significant in the Tees, Tyne and Tweed areas. Fig. 4.11 shows examples of areas where good correlation was found between thiols and chlorophyll.

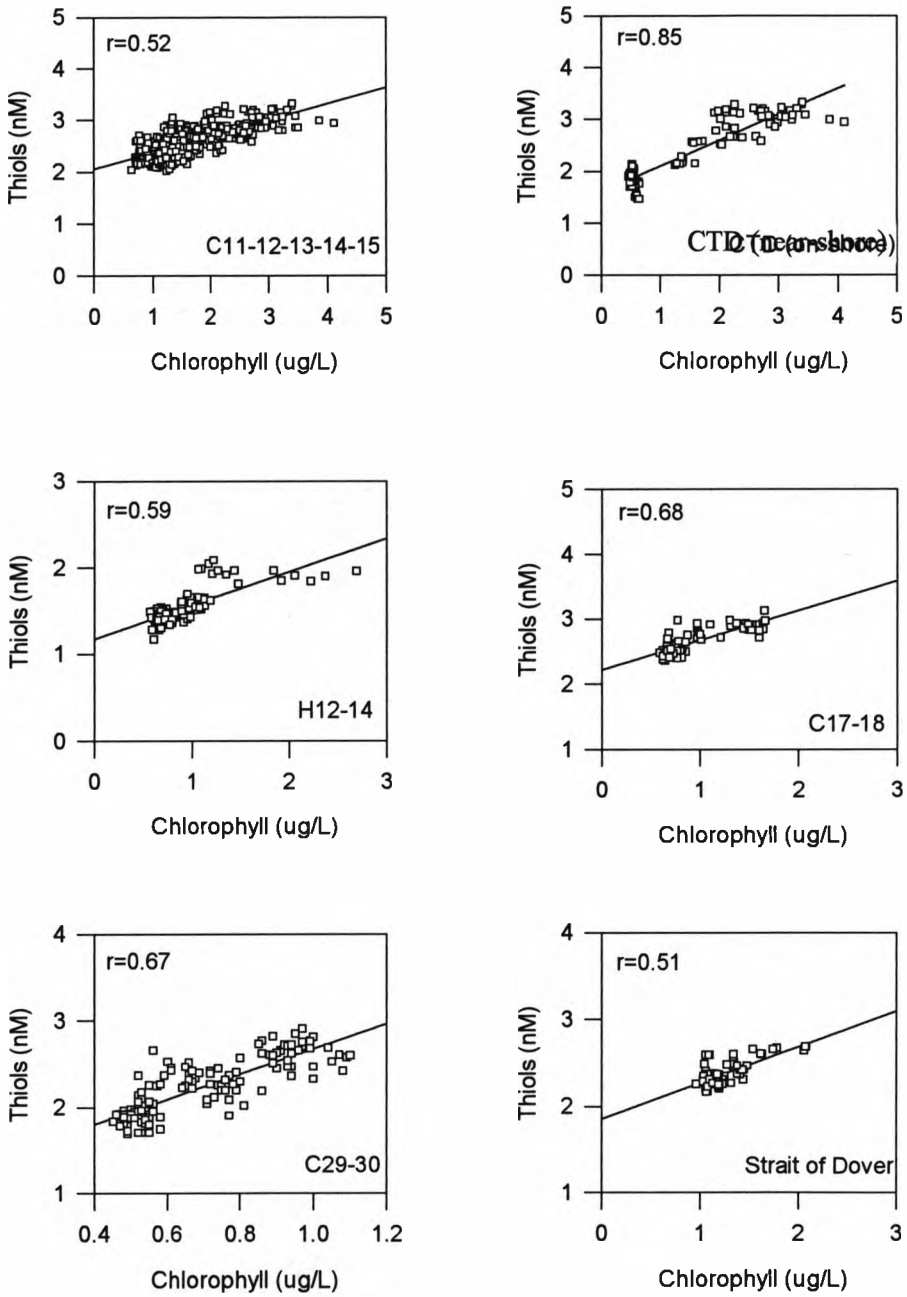


Fig. 4.11. Thiols concentrations as a function of chlorophyll concentrations of selected areas during RRS Challenger cruise to the North Sea.

4.6.1.2.3 Diel variation

Thiol concentrations were followed for a period of 24 hour whilst the ship was anchored at station N2 (Fig. 4.1B). The data shows (Fig. 4.12) that the salinity showed little variation suggesting that the water suffered little from tidal motion at this site. The chlorophyll and SPM concentrations varied during the day, differing from the thiol concentrations which increased more gradually until about 3 PM whereafter it decreased gradually (varying between 0.8 and 1.6 nM thiol). The absence of a strong increase in thiol concentrations suggests that photochemistry did not play a major role in the thiol production. Photochemical processes are thought to cause large increases in sulfide concentrations in the early morning (Cutter and Krahforst, 1988). These data suggest that there is little diel variation in thiol levels. This is supported by the good agreement between the data obtained when the ship was steaming parallel to the coast with the data obtained, at the same locations when the ship returned on the zigzagged track although the measurements were carried out at different times of day (Fig. 4.5).

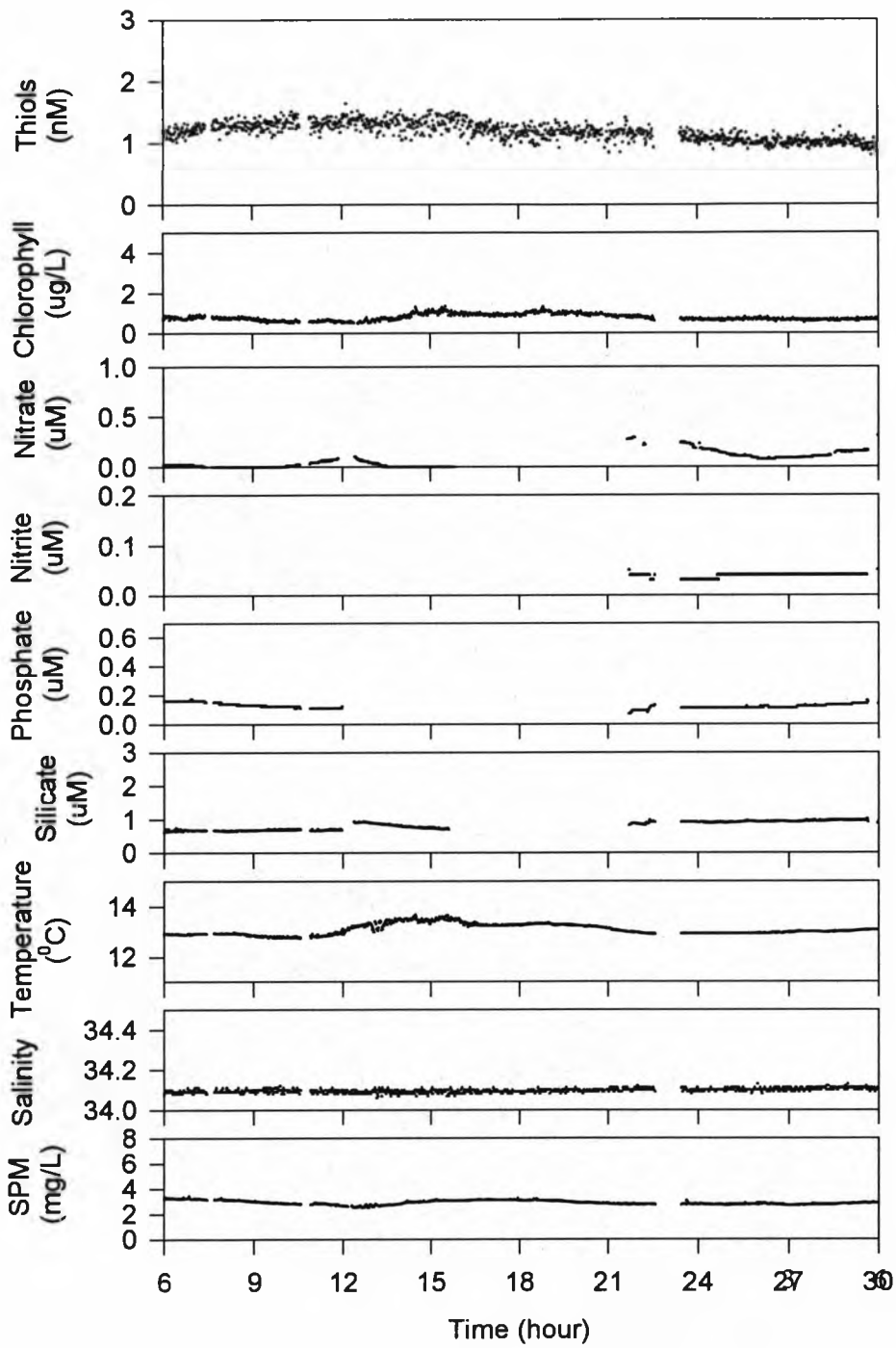


Fig. 4.12. Diel variation of thiols concentrations and other parameters at anchor station N2 during the RRS Challenger cruise to the North Sea.

4.6.1.2.4 Depth profiles

The previous discussion revealed that marine phytoplankton plays a major role in the distribution of thiols. It is not ruled out that other processes (e.g. atmospheric input, in situ degradation of settled phytoplankton biomass and efflux of thiols across the sediment-water interface) could also affect the vertical distribution of thiols. Therefore, the vertical distribution of thiols in the North Sea was determined to verify the importance of such processes.

The depth distribution of thiols, temperature, salinity, oxygen and chlorophyll at three stations (C30, C5 and EC) is shown in Fig. 4.13. These stations reflect different locations and hydrographic conditions (see Fig. 4.1). Stratification was clearly evident at station C30 and less at C5 whereas the thermocline was absent at station EC. In general, highest concentrations of thiols (around 2.1 nM) were found in the surface waters whilst the concentrations decreased with depth to around 1.5 nM at all stations. The decrease in the thiol concentrations was sharp at 50 m depth at station C30. This station shows a well mixed surface layer because of the stratification that separated between the surface and deeper waters. Nevertheless the highest thiol concentrations was found at the surface. At stations EC, and C5

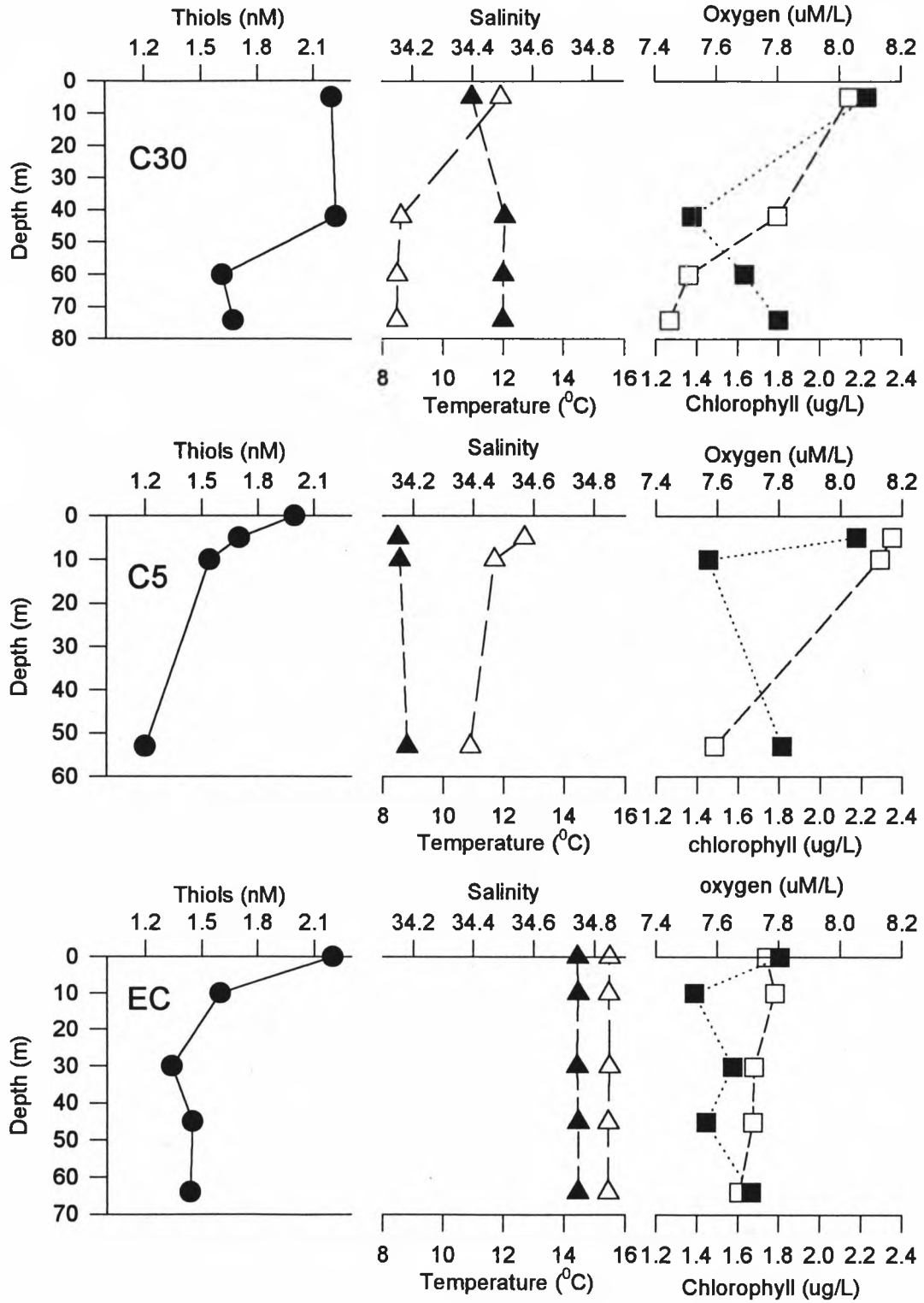


Fig. 4.13. Depth profiles of thiols (●), temperature (△), salinity (▲), chlorophyll (□) and oxygen (■) for CTD stations during the RRS Challenger cruise to the North Sea.

the decreases in the thiol concentrations were more gradual. The vertical profiles of chlorophyll followed those of thiols although some variability is apparent. The increase in thiols concentrations in the surface layer is not yet understood. It is possible that known organic or inorganic sulfur compounds originating from the atmosphere are responsible for this phenomenon or photochemical stress of phytoplankton. The depth profile of thiols at station C30 shows a very small increase near the sediments which could be attributed to releases from porewaters. Marine porewaters and sediments are known to produce a variety of organic sulfur compounds (Luther et al., 1985, 1986; Kiene and Taylor, 1988; Henneke et al., 1991; Vairavamurthy et al., 1994; Maccrehan and Shea, 1995; Vandermaarel et al., 1995). The interactions between hydrogen sulfide and organic compounds are thought to play a major role in the production of thiols in marine sediments (Vairavamurthy and Mopper, 1987). However, The North Sea water column data show little evidence for releases from porewater.

### 4.6.2 The Black Sea

#### 4.6.2.1 General considerations

The Black Sea samples were filtered and then purged with nitrogen

gas on-board immediately after collection ship to remove dissolved oxygen which might have entered the samples to minimise degradation of the thiols. The purging with nitrogen gas would have removed a large proportion of free sulfide from the solutions whilst the thiol compounds should remain. The samples were stored frozen.

Thiols were measured by batch-mode voltammetry at pH 8 using TRIS buffer. Some of the samples were diluted with UV-digested seawater due to the high concentrations of thiols.

To confirm that the samples were free of sulfide, successive scans were carried out of diluted samples containing ~50 nM of thiols over a period of 10 minutes. The results showed that the peak height of the thiol/sulfide peak was stable, indicating that the sample contained insignificant amount of sulfide (Al-farawati and van den Berg, 1997a (Chapter 2)), as any sulfide remaining after purging had become oxidised by oxygen during the prolonged storage period. A further experiment was carried out to confirm the absence of any sulfide possibly stabilised by metal complexation. A sample was acidified to low pH (~1.2) and then was purged with nitrogen gas for 10 minutes followed by pH neutralisation with

ammonia solution, and buffered to pH 8 by TRIS solution. The peak heights of the CSV scans before and after this treatment are shown in Fig. 4.14. The peak heights were similar in both experiments confirming that the sample was free of sulfide, including free sulfide as well as sulfide complexed by metals as such sulfide would also have been converted to $H_2S$. There is a minor "bulge" apparent in the scan before acidification, which is lost by the acidification. This bulge did not affect the peak height and may have been caused by other unknown alterations to the seawater by acidification/neutralisation.

In separate experiments the deposition potential was varied in a series of experiments with diluted Black Sea water similar to experiments done using thiourea, thioacetamide and sulfide (section 2.4.6; Chapter 2). The response was similar to thiourea and thioacetamide and the maximum response was obtained at a deposition potential of o.o5 V. This again indicate the absence of sulfide.

#### 4.6.2.2 Results and discussions

The thiol concentrations in the surface water of the Black Sea (up to

100 meters) were similar to that in surface water of the western North Sea. This is surprising in view of the very high thiol concentrations found in the deeper waters of the Black Sea. Depth profiles of thiols and other parameters are presented in Fig. 4.15. The depth profiles for deep water station 3 are shown separately and its upper water column was extended. Stations 2 and 3 are open sea stations. Stations 10, 7, and 19 are coastal and are located in front of the Danube Delta. Therefore, latter stations are influenced by the river discharge. Station 24 is located near the Bulgarian coast.

The depth profiles show interesting systematic variations in the behaviour of the thiols. Stations 2, 3 and 24 showed a maximum in the thiol concentrations at a depth of 20 m coincident with a maximum of oxygen and either fluorescence or chlorophyll. It is likely that this maximum is associated with a maximum in the activity of phytoplankton, in agreement with the relationship with chlorophyll seen in the North Sea waters.

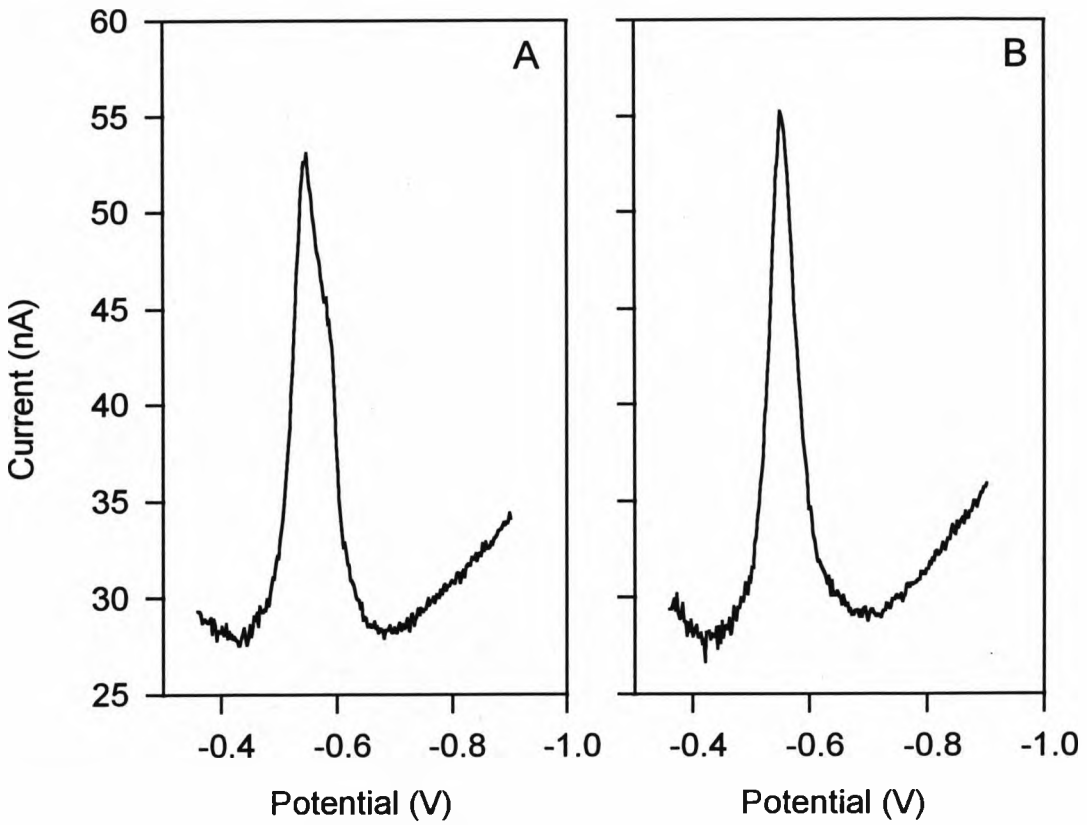


Fig. 4.14. The effect of acidification and purging with nitrogen gas on the peak height of thiols in the Black Sea sample diluted in UV-clean seawater: A) before treatment (pH=8); B) after treatment (acidification and purging with nitrogen gas and then neutralisation to pH 8).

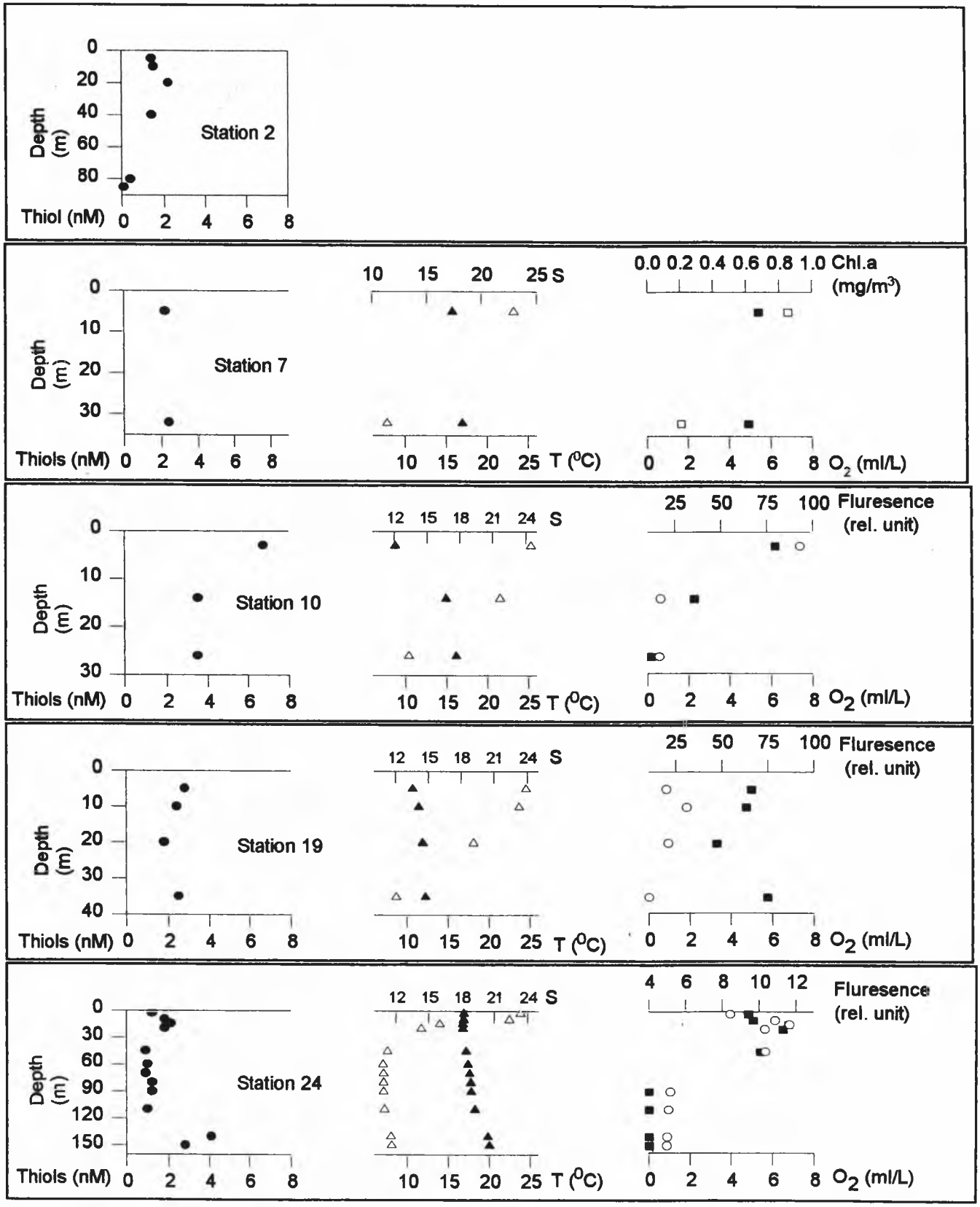


Fig. 4.15. Depth profiles of thiols (●), temperature (△), salinity (▲), Chlorophyll.a (□), fluorescence (○), oxygen (■), nitrate (▽), ammonia (▼), phosphate (◆) and silicate (◇) for the Black Sea stations.

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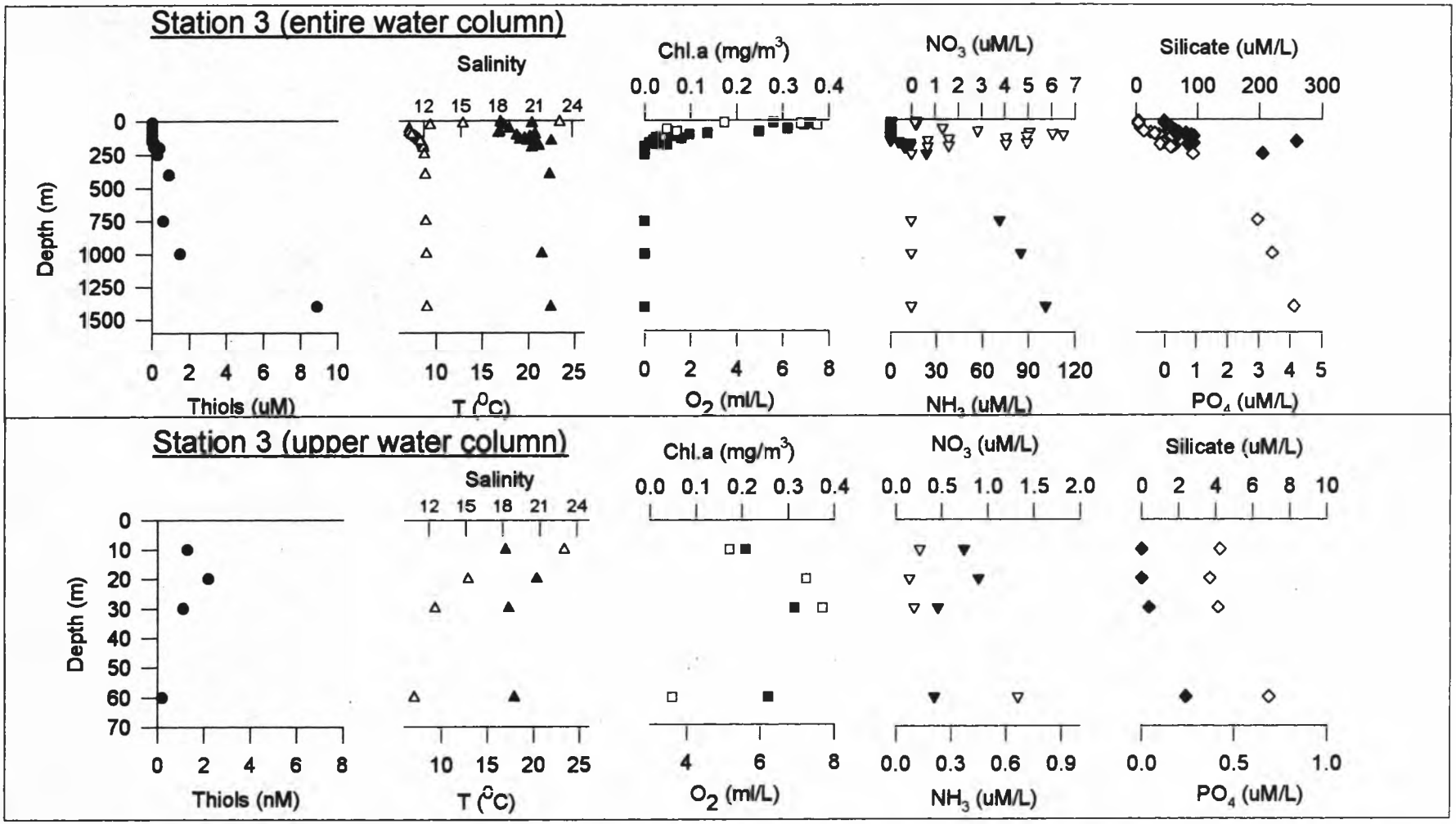


Fig. 4.15. Depth profiles of thiols (●), temperature (△), salinity (▲), Chlorophyll.a (□), fluorescence (○), oxygen (■), nitrate (▽), ammonia (▼), phosphate (◆) and silicate (◇) for the Black Sea stations.

Near the oxic/anoxic interface (at 100 m (Luther et al., 1991)), the levels of thiols can be seen to increase steeply and continuously with depth. The beginning of this increase is apparent at a depth of around 150 m (station 3 and 24), increasing to a concentration of 8.9 μM at 1400 m depth at station 3. Sulfide is shown to be increasingly present at depths below 100 m, reaching levels of 300-400 μM in the deep water (Dyrssen et al., 1985; Luther et al., 1991). The interaction between sulfide and organic matter in anoxic marine sediment is thought to be the most important factor in thiol production (Vairavamurthy and Mopper, 1987). It is probably that similar interactions occur between sulfide and organic matter within the anoxic water column of the Black Sea causing the enhanced levels of thiols in the deep waters.

High concentrations of thiols in the water column of the Black Sea have been found before (Dyrssen et al., 1985; Mopper and Kieber, 1991). The thiol concentrations reported by Dyrssen et al. ranged from 10 (surface water) to 30 μM (deep water) by potentiometric titrations using mercuric chloride and a $Ag/Ag_2S$ electrode as a sulfide sensor. Mopper and Kieber reported levels ranged between 0.1 (surface water) and 0.9 μM (deep water) using HPLC. This compares to concentrations up to 9 μM found in this work

at station 3. It is possible that this difference is due to the use of different calibrant.

4.6.2.3. Copper complexation by thiol ligands

Thiol compounds are characterised by an SH group which forms stable complexes with a number of metals. Especially strong complexes are formed with copper as Cu(I). Complexes are known to be very stable ( (Leal and van den Berg, 1997)). The stability constant for copper (I) complexation in seawater with cysteine has been estimated as $10^{18.5}$ ( (van den Berg et al., 1988)) and that with glutathione as $10^{13.8}$ ( (Le Gall and van den Berg, 1993)). It was investigated whether thiols in the deep waters of the Black Sea act as complexing ligands for copper by titration with copper and detection of labile copper by CSV (van den Berg, 1984; Donat and van den Berg, 1992; Campos and van den Berg, 1994). Recent work ( (Leal and van den Berg, 1997)) has shown that Cu(I) binding thiols can be titrated with copper(II) and can be detected by the usual CSV methods.

The concentration of copper complexing thiol ligands was determined by titrations of Black Sea samples after dilution with UV-irradiated seawater

with copper. Ligand competition was used to determine the reactive copper concentration. Salicylaldoxime (SA) was used as competitive ligand ( Campos and van den Berg, 1994). The samples were collected at station 3 at a depth of 1000 m (one titration, diluted 7-fold) and 1400 m (two titrations, diluted 33- and 100-fold). The increase of the copper-SA peak (at -0.32 V) upon addition of copper is shown in Fig. 4.16 for the 33-fold diluted sample of 1400 m depth. The second peak at -0.54 V is due to the thiols and can be seen to decrease with each addition of copper. This decrease confirms that this thiol is complexed by the copper additions causing the free thiol peak to become masked similar to the response for free sulfide ( Al-farawati and van den Berg, 1997b (Chapter 3)). The complex detected by evaluation of the Cu-SA data is therefore formed with the thiols detected by the peak at -0.54 V. The plot for the titrations and the linearisations are shown in Fig. 4.17A and B. The titration plot (Fig. 4.17A) shows curvature confirming the complexation of copper by thiol ligands. No curvature was present for the UV-irradiated seawater which was used a control as it was free of the original organic matter. The linearisation of the data (Fig. 4.17B) clearly shows a steep slope for the control sample (free of thiols). The slope of the other titrations similarly reflects the magnitude of the concentrations of the thiol ligands in the other diluted seawater samples. The concentrations of

thiol ligands and the conditional stability constant for the thiol-copper complexes, $K'_{CuL}$, are presented in Table 4.4. The value of the conditional stability constant is similar to those found before in seawater of shelf (van den Berg, 1984) or oceanic (Hirose et al., 1982; Coale and Bruland, 1990; Donat and van den Berg, 1992) origin for complexes of copper with natural organic ligands. The composition of the natural organic ligands is not known, but the similar stability of thiol complexes, and the ubiquitous presence of these thiols, suggest that thiols may be an important constituent of the natural organic complexing ligands.

The concentrations of the copper complexing thiol ligands are nominally less than the thiol concentrations (see above) at these depths. The molar ratio of copper complexing thiol ligands/thiol concentrations at 1000 and 1500 m depth was 0.22 and 0.21, respectively. The thiol concentrations were calibrated against thiourea, but the sensitivity for different thiols varies greatly ( Al-farawati and van den Berg, 1997a (Chapter 2)). It is therefore possible that the actual thiol concentrations was more or less than the indicated levels. Furthermore, it is possible that not all thiols form these stable complexes with copper, thus explaining the lower concentration of thiol ligands than that of thiols in these samples.

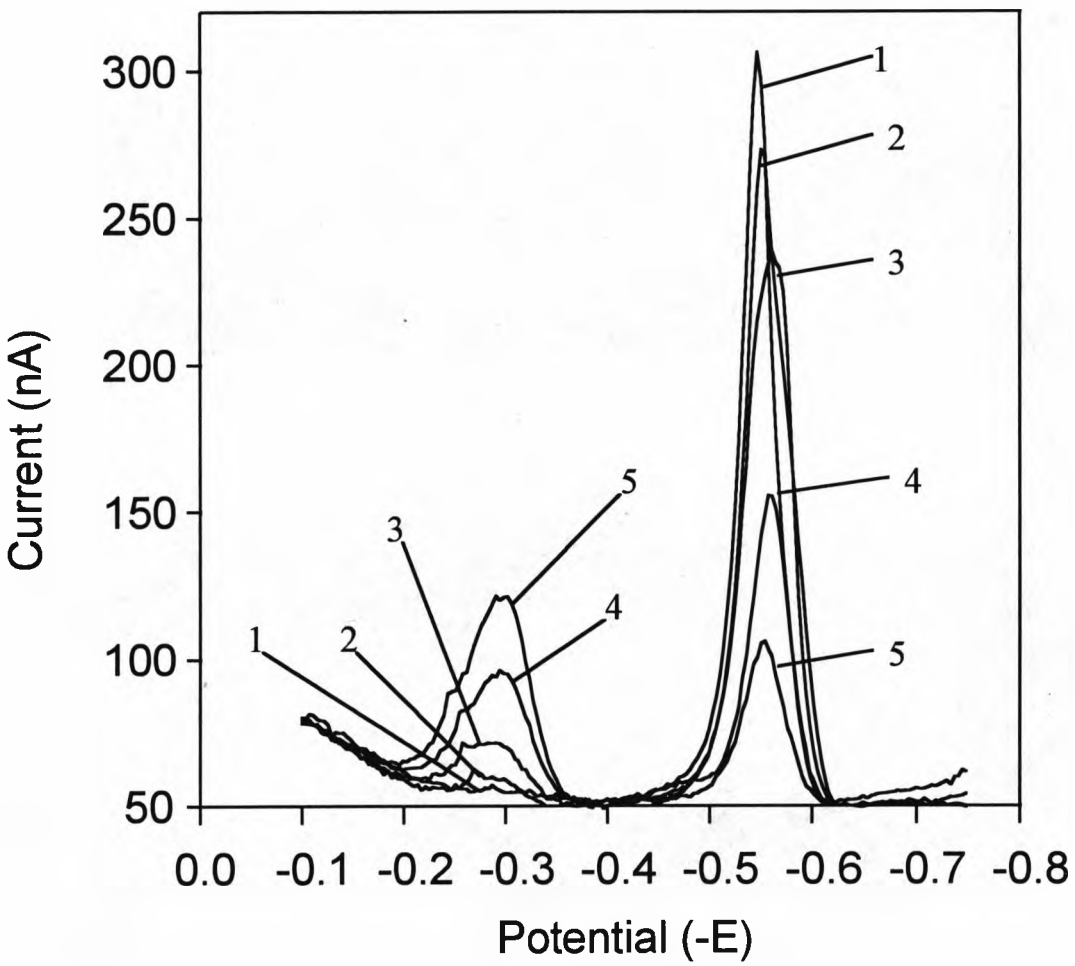


Fig. 4.16. The voltammetric response of copper-SA (-0.32 V) and thiols (-0.54 V) for the Black Sea sample diluted by a factor of 33 in UV-clean seawater: 1) before addition of copper (the initial copper concentration was 1.14 nM); and after addition of 2) 10, 3) 25, 4) 40, 5) 60 nM of copper.

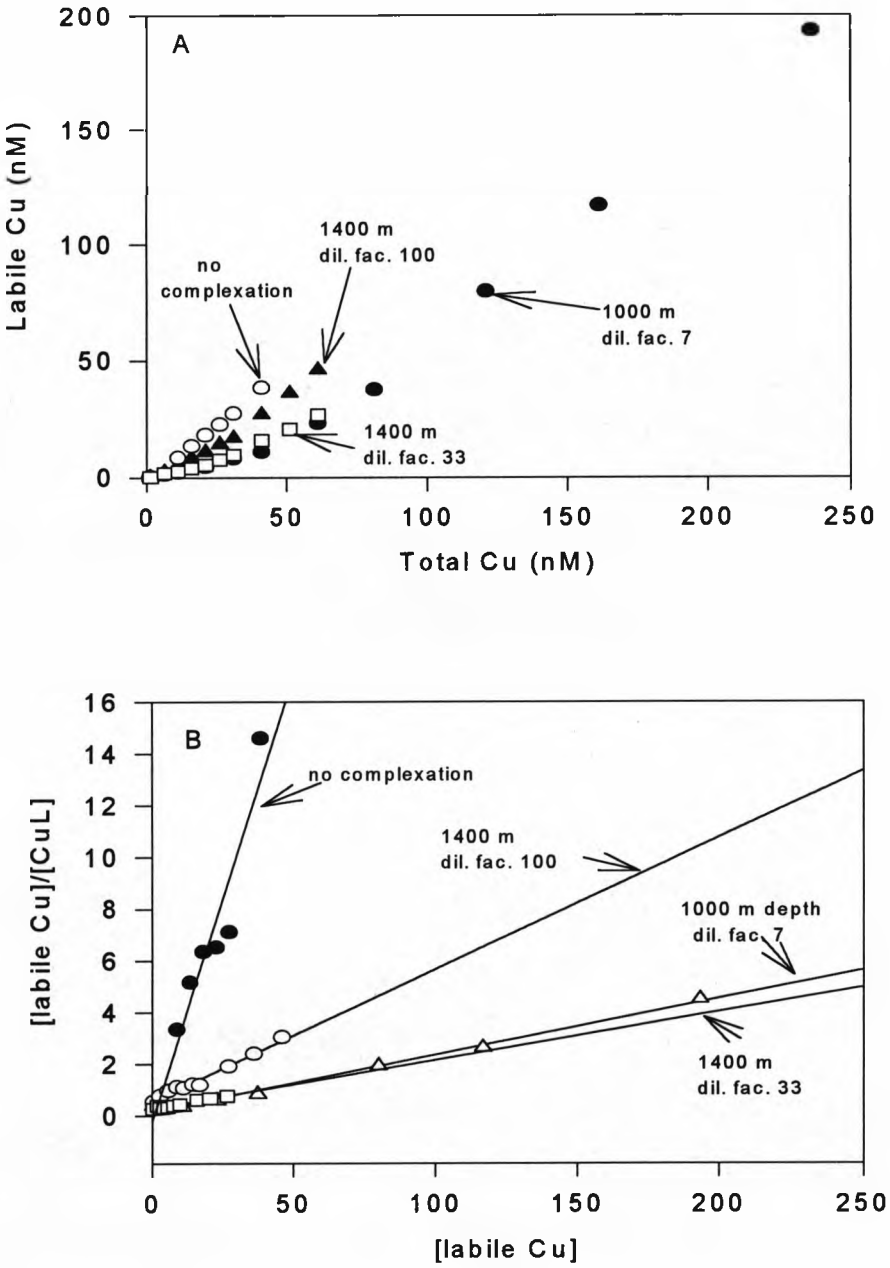


Fig. 4.17. Complexing thiol-ligand titrations of seawater from the Black Sea (station 3). (A) labile copper as a function of total dissolved copper; (B) linearisations of the data.

Table 4.4 Thiol ligand concentrations ($C_L$] and conditional stability constants ($K'_{CuL}$) for diluted Black Sea samples (station 3)

| Depth (m) | $C_L$ (nM) | $K'_{CuL}$ | dilution factor |
|---|---|---|---|
| 1400 | 1778±129 | 11.84±0.02 | 33 |
| 1400 | 1952±98 | 11.98±0.06 | 100 |
| average | 1862 | 11.91 | |
| 1000 | 334±8 | 12.04±0.19 | 7 |

4.6.2.4 Calculation of copper speciation in Black Sea waters

To evaluate the importance of thiol ligands on copper speciation in the upper water column and deep waters of the Black Sea, values were caculated for the alpha coefficient ($\alpha$) of copper-thiols ($\alpha_{Cu\text{-}L}$) and copper-sulfide ($\alpha_{CU\text{-}HS}$) using realistic concentrations of thiols and sulfide. A total sulfide concentration of 0.2 nM was used for surface water (Radford-Knoery and Cutter, 1994) and of 300 μM for deep water (Dyrssen et al., 1985; Luther et al., 1991). Thiol ligand concentrations for the deep water (1400 m) were taken from Table 4.4. The concentration of thiol ligands in the surface water was calculated using the ratio of 0.2 (copper complexing thiols ligands/total thiols) from the average total thiols in the surface water at station 3 (1.5 nM) giving a thiol-ligand concentration of 0.3 nM. Values of conditional stability constants of copper-sulfide were taken from (Al-farawati and van den Berg, 1997b (Chapter 3)). The conditional stability constant of the copper complexes with thiols in surface water was assumed to be similar to that from the deep water at 1400 m depth.

The alpha coefficient of copper-thiols can be defined as:

$$\alpha_{Cu\text{-}L} = K'_{CuL}\,[L'] \qquad (4.8)$$

where [L'] is the concentrations of thiols not complexed by copper.

The alpha coefficient of copper sulfide is:

$$\alpha_{Cu\text{-}HS} = K'_{CuHS}\,[HS'] + \beta'_{Cu(HS)2}\,[HS']^2 \qquad (4.9)$$

where $K'_{CuHS}$ and $\beta'_{Cu(HS)2}$ are the conditional stability constants of Cu-HS complexes (see Chapter 3) and [HS'] is the concentration of bisulfide not complexed by copper.

Using the above equations for $\alpha_{Cu\text{-}HS}$ and $\alpha_{Cu\text{-}L}$ were calculated and are presented in Table 4.5 . The $\alpha_{Cu\text{-}HS}$ and $\alpha_{Cu\text{-}L}$ in surface water are 1518 and 2.44 whereas they are $2.48\times10^{12}$ and $1.51\times10^{6}$ in deep water, respectively.

From these data it is clear that $\alpha_{Cu\text{-}HS} >> \alpha_{Cu\text{-}L}$ in the deep water. For this reason copper complexation by the thiols is negligible compared with that by sulfide in the deep Black Sea. Also $\alpha_{Cu\text{-}HS}$ is bigger than $\alpha_{Cu\text{-}L}$ in surface water so most of copper should be complexed by sulfide. However, the sulfide and thiol-ligand concentrations in surface water are probably less than the copper concentration, so it is likely that copper complexes with sulfide coexist with thiol complexes and other organic species.

Table 4.5. Values of $\alpha_{Cu\text{-}L}$ and $\alpha_{CU\text{-}HS}$ for surface water and deep water of the Black Sea. The value of log $K'_{CuL}$ for the surface water was assumed to be similar to that in the deep water at 1400 m depth (11.91).

| | Thiols data | | | Sulfide data | | | |
|---|---|---|---|---|---|---|---|
| | [L'] | log $K'_{CuL}$ | $\alpha_{Cu\text{-}L}$ | [HS'] | log $K'_{CuHS}$ | log $K'_{Cu(HS)2}$ | $\alpha_{CU\text{-}HS}$ |
| Surface | 0.3 nM | 11.91 | 244 | 0.2 nM | 12.88 | 19.44 | 1518 |
| Deep | 1862 nM | 11.91 | $1.5\times10^{6}$ | 300 μM | 12.88 | 19.44 | $2.48\times10^{12}$ |

### *4.7 Conclusions*

The thiol concentrations in the surface of the North Sea and the English Channel ranged between 0.7 to 3.6 nM.

Comparison shows that automated FA-CSV cam be used successfully to determine thiol compounds at a rate of ~50 measurements per hour. The concentrations in the upper water column of the Black Sea are similar to those in the North Sea. Variations in the thiol concentrations were closely related to variations in the concentrations of chlorophyll which indicate the production of thiols by marine phytoplankton. Photochemical effects and interaction of sulfide with organic matter could be additional sources of thiols.

Anoxic conditions in the deep waters of the Black Sea apparently lead to the presence of very high thiol concentrations, up to 9 μM. Titrations indicated that the thiols are powerful complexing ligands forming very stable complexes with copper. Extrapolation to other, oxygenated, waters suggests that the thiols could be important ligands for copper even at the much lower thiol concentrations predominant there. The complexation of other metals by thiols could be an important subject for further study.

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# Chapter 5

## General conclusions

This study illustrates the importance of analytical developments for the investigation of metals, sulfide and thiols, speciation and distribution, in seawater. The principal analytical development of this research was the automation of a voltammetric analyzer in association with construction of a home-made flow cell. The application of flow-analysis and computer control not only resulted in a higher amount of samples that could be processed per time unit compared with fully manual analysis, it is also reduced the risk of sample contamination by minimisation of sample handling.

The prime aim of this research was to study the interaction between sulfide and metals in seawater. Preliminary measurements of sulfide in seawater using cathodic stripping voltammetry and a HMDE in batch-mode showed that the sulfide peak decreased rapidly with time. The possible effect of oxygen, hydrogen peroxide, iodate ion on the decreases of sulfide peak was negligible. Stabilisation of the sulfide peak by trace metals was considered in seawater free of organic matter but the effect of such stabilisation was negative.

It was therefore decided to build a flow-system to get accurate analysis of sulfide. A home-made flow-cell was used successfully for sulfide determination, introducing fresh seawater containing sulfide to the HMDE at each scan. The relative standard deviation of sulfide measurements by the flow-cell was 2.8%. Using the flow-cell, it was found that the low solubility of mercuric sulfide was the major factor responsible for the decreases of sulfide peak in the batch-cell.

The flow-cell was used for the determination of the conditional stability constants of metal-sulfide complexes in pH 8 seawater at various salinities by two methods: ligand competition and detection of the free sulfide concentration. The stability constants for sulfide complexes with copper in sea water determined by the flow-cell are much greater than literature values determined previously using a conventional cell. The differences are less for other metals. The determination of the stability constants (log $K'_1$) of cobalt(II) and zinc sulfide species were carried out using both methods and show a close agreement. Comparison of the stability constants for sulfide complexes with copper and silver suggests that the copper could be complexed by sulfide as Cu(I) rather than Cu(II).

Calculation of the sulfide speciation in seawater with a "normal" composition indicates that the speciation depends greatly on the ratio of copper to sulfide, and on the concentration of organic copper complexing ligands, as the stability of the sulfide complex with copper is much greater than that with other metals, and as is similar to the stability of copper complexes with organic matter in seawater.

Measurements of surface water samples from the North Sea using conventional CSV show that the sulfide-like peak (at -0.53 V) was stable. This is in contradiction to the behaviour of free sulfide suggesting that this peak was not due to free sulfide. Comparison of the electrochemical behaviour of this peak and of free sulfide, and of thiol compounds, suggests that this peak is most probably due to thiols.

Thiol concentrations were determined successfully in the coastal waters of the western North Sea, English Channel and the water column of the Black Sea using the automated FA-CSV. The automated flow-analysis resulted in ~50 thiol measurements per hour, and more than 7000 thiol measurements were obtained. The thiol concentrations in the surface of the North Sea and the English Channel ranged between 0.7 to 3.6 nM. Comparison of the thiol concentrations with other parameters showed a close co-variation with chlorophyll, suggesting that the thiols are produced by the phytoplankton. The thiol concentrations in the upper water column of the Black Sea were similar to those in the North Sea. However, the thiol concentrations in the deep water of the Black Sea were much higher than those in the surface water indicating that possible interaction between sulfide and organic matter could be the source for thiols in anoxic conditions, or that thiols are stable in the anoxic conditions.

Titrations of the Black Sea samples with copper and detection of the free copper by CSV with ligand competition indicated that thiols are powerful complexing ligands forming very stable complexes with copper. The complexation of other trace metals by thiols could be an important subject for future study.