ABSTRACT

Title of Thesis: **IN SITU MEASUREMENT OF SULFIDE IN NATURAL WATERS**

Michael Sean Rearick, Master of Science, 2004

Thesis Directed By: **Dr. Robert P. Mason, Marine Estuarine Environmental Science**

Dissimilative sulfate reduction is a major source of sulfide in sediments and the water column of lakes and estuaries. Resulting dissolved sulfide can be incorporated into organic or inorganic sulfur complexes. When present in aquatic systems, sulfide is the dominant ligand for many trace metals and may control metal bioavailability to organisms.

Laboratory tests were performed to validate the coupling of the diffusive gradient in thin films technique to a solid-state ion selective electrode to quantify in situ sulfide concentrations. Diffusive gradient in thin films probes were deployed in three lakes and estuary pore water and compared with parallel sulfide measurements using the Cline method and potentiometry. Differences between the recently validated method and the other methods were found with the former resulting in lower concentrations. Laboratory experiments examined these differences, and the experimental results identified measurement artifacts associated with sulfide antioxidant buffer usage.

IN SITU MEASUREMENT OF SULFIDE IN NATURAL WATERS

By

Michael Sean Rearick

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2004

Advisory Committee:
Professor Robert P. Mason, Chair
Research Associate Professor Jeffery C. Cornwell
Dr. Cynthia Gilmour
Dr. Andrew Heyes

Acknowledgements

I thank my advisor, Dr. Robert P. Mason, for the opportunity to study and work in his laboratory at CBL. I am thankful for his support and guidance throughout my stay. I am also thankful for the many travel opportunities that he has granted. I appreciate the many lively and fruitful discussions between us on our weekly training runs on Solomons Island. I also want to thank my additional committee members, Dr. Jeffery Cornwell, Dr. Cynthia Gilmour, and Dr. Andrew Heyes for their support and suggestions. I thank my lab mates, especially Carrie Miller, for their advice and technical assistance. I thank the CBL Graduate Education Committee, EPA STAR grant (R827653-01-0), and CSTL Exploratory Research grant for financial assistance. I thank my parents, Brent and Marilyn Rearick, for their support. In particular, I thank dad for introducing his sons to chemistry and the outdoors. Finally, I thank Rachael, my wonderful and loving wife, for her steadfast support of all my activities even through demanding times. You have inspired me, and I only hope that I can return the favor.

Table of Contents

Acknowledgements ii
Table of Contents iii
List of Tables v
List of Figures vi
List of Acronyms viii

Chapter 1: Introduction 1
Background 1
Available analytical techniques 5
Basic DGT theory 8
Merits of DGT 14
Statement of research and primary hypothesis 18
Thesis structure 18

Chapter 2: DGT methodology and validation: Laboratory studies 19
Materials and procedures 19
Probe assembly 19
Elution of sulfide from binding gel 21
Analysis of sulfide in traps following elution 22
Method assessment 23
Laboratory testing conditions 23
Sulfide elution efficiency 23
Validity of DGT equations 24
Testing over pH gradient 26
Comments and recommendations 28

Chapter 3: Field measurements of dissolved sulfide and laboratory investigations of potential measurement artifacts 29
Introduction 29
Field study sites 34
Laboratory studies 35
Methods 36
Pavin Lake, France 36
Lake Lariat, Calvert County, Maryland 37
Lake 658, ELA 40
Laboratory study pore water from Mackall Cove, Maryland 42
Zinc sulfide clusters laboratory experiment 44
Thioglycolate laboratory experiment 46
Results and discussion 47
Pavin Lake, France 47

Lake Lariat, Calvert County, Maryland 49
Lake 658, ELA 52
Laboratory study using pore water from Mackall Cove, Maryland 54
ZnS clusters laboratory experiment 57
Thioglycolate laboratory experiment 60
Summary of field and experimental results 63
Conclusion 64

Chapter 4: Conclusions and recommendations 67

Appendix A 72
Bibliography 76

List of Tables

Table 1. Observations on common analytical methods for sulfide analysis. 17

Table 2. Historical $[(S\text{-}II)]_t$ by methylene blue and estimated [(S-II)] by DGT-ISE near chemocline in Pavin Lake, France. 49

Table A 1. Sulfide fluxes and estimated concentrations by DGT-ISE at three littoral sites (G1, G2, and G3) located in L658 ELA, NW Ontario during June 2002… 72

Table A 2. Sulfide fluxes and estimated concentrations by DGT-ISE at a littoral site (G2) located in L658 ELA, NW Ontario during August 2002. 73

Table A 3. Sulfide fluxes and estimated concentrations by DGT-ISE at a wetland site located in L658 ELA, NW Ontario during August 2002. 74

Table A 4. Sulfide fluxes and estimated concentrations by DGT-ISE at a littoral site (G2) located in L658 ELA, NW Ontario during July 2003. 75

List of Figures

Figure 1. Generalization of the sedimentary sulfur cycle and potentially formed intermediate sulfur species. 3

Figure 2. Schematic of the three layered system within a typical DGT probe. 10

Figure 3. Schematic of a concentration gradient through the diffusive layer of a DGT probe in a solution with an ion concentration of C. 11

Figure 4. Elution efficiency of sulfide from AgI binding gel as determined by ISE. *Data are from Teasdale et al., 1999 as determined by methylene blue. 24

Figure 5. Measured sulfide accumulation on DGT probes over 24 hours as determined by DGT-ISE. 25

Figure 6. Total sulfide accumulation over varying diffusive gel thickness as determined by DGT-ISE. 26

Figure 7. Percent of total sulfide measured by DGT-ISE at various pH values. 27

Figure 8. DGT-ISE estimated sulfide concentrations and various filtered sulfide concentrations determined by SAOB/ISE in Lake Lariat bottom waters. 51

Figure 9. Graphical determination of DBL and DGT-ISE estimated [(S-II)] by reciprocal plot. 51

Figure 10. Sulfide profiles estimated by DGT-ISE and SAOB/ISE for the profundal of Lake 658. 53

Figure 11. Comparison of sulfide concentrations as determined by various methods for extracted sediment pore water from Mackall Cove, MD. Container 1 (C1) and container 2 (C2) are duplicate samples of extracted pore water. 56

Figure 12. Sulfide as detected by SAOB/ISE in Mackall Cove extracted sediment pore water over 24 hours. Container 1 (C1) and container 2 (C2) are duplicates samples of extracted pore water. 56

Figure 13. Concentrations of synthesized 10.0 $\mu molL^{-1}$ZnS solution (experiment ZnS I) as detected by SAOB/ISE, methylene blue, and DGT-ISE. 58

Figure 14. Concentrations of synthesized 11.0 $\mu molL^{-1}$ZnS solution (experiment ZnS II) as detected by SAOB/ISE, methylene blue, and DGT-ISE. 58

Figure 15. SAOB/ISE concentrations of sulfide derived from the hydrolysis of thioglycolate over 24 hours. 61

Figure 16. Percent recovery of thioglycolate working solutions over 24 hours by means of an iodimetric titration. 62

Figure 17. Thiol III experimental results demonstrating that thiols are not a fraction of the measurable sulfide as detected by methylene blue. 63

Figure 18. Commonly used techniques for sulfide analysis and potentially detected sulfide species. Various sulfide species are in gray ovals and the potential techniques to detect these species are in adjacent gray boxes. 70

List of Acronyms

AVS	acid-volatile sulfide
AVS/SEM	acid-volatile sulfide and simultaneously extracted metals ratio
CBL	Chesapeake Biological Laboratory
CID	computer imaging densitometry
CIS	closer interval sampling system
CRS	chromium reducible sulfur
DBL	diffusive boundary layer
DGT	diffusive gradients in thin films
DGT-CID	diffusive gradients in thin films-computer-imaging densitometry
DGT-ISE	diffusive gradients in thin films-ion selective electrode
DOM	dissolved organic matter
EDTA	ethylenediaminetetraacetic acid
ELA	Experimental Lakes Area
FIA	flow injection analysis
HPLC	high-performance liquid chromatography
ISE	ion selective electrode
MBRS	methylene blue reactive sulfides
MDR	mixed diamine reagents
METAALICUS	Mercury experiment to assess atmospheric loadings in Canada and the United States
NOM	natural organic matter
SAOB	sulfide antioxidant buffer

SEM	simultaneously extracted metals
TEMED	N,N,N’,N’,-tetramethylethylenediame
UV-VIS	ultraviolet-visible
VOC	volatile organic compound

Chapter 1: Introduction

Background

In aquatic environments a portion of the organic matter reaching the sediments is oxidized by anaerobes utilizing sulfate, the most oxidized form of sulfur, as an electron acceptor. While in freshwater environments sulfate reduction is often limited by sulfate concentration, in marine and estuarine environments, this process can be the dominant carbon mineralization mechanism due to the higher sulfate concentrations (Nealson and Stahl 1997). Dissimilative sulfate reduction produces dissolved hydrogen sulfide (H_2S) as the primary product. As H_2S is a diprotic acid the proportion of the various forms are functions of the pH. AtpH values between 6 and 9, the pH range for most natural waters, sulfide (S^{2-}) is the least abundant species. The concentration of dissolved sulfide species builds up in the pore water and can be incorporated into the sediments as reduced organic and inorganic sulfur complexes. A flux of dissolved sulfide from the sediments to the overlying water column may also occur allowing the sulfide to be oxidized or complexed to metals in solution. The oxidation of dissolved sulfide is believed to be quite rapid (Millero 1986). However when sulfide is complexed with a metal, its resistivity towards oxidation is enhanced. These complexed sulfide species can persist in oxic watersfor nearly 30 days (Rozan et al. 2000).

Previous research has demonstrated that sulfide is a dominant ligand for a number of metals in anoxic environments and can potentially control metal speciation and toxicity under low oxygen environments as well (Boulegue et al. 1982; Morse et al. 1987; Ditoro et al. 1990; Hansen et al. 1996; Chapman et al. 1998). When present

under anoxic conditions, dissolved sulfide readily reacts with reduced iron (Fe^{2+}) to form various iron mineral phases. Exchangeable iron mineral phases and dissolved sulfide species have been operationally defined as the acid-volatile sulfide (AVS) fraction of the total sulfide (Morse et al. 1987). The remaining fraction of sulfide, pyrite, is considered refractory and is referred to as chromium reducible sulfur (CRS) because of the chromium reduction technique used for pyritic sulfide extraction (Fossing and Jorgensen 1989). The AVS fraction is more labile than the pyritic fraction and is defined by its ability to be separated using 6 $molL^{-1}$ cold HCl (Cornwell and Morse 1987; Allen et al. 1993). Recently, this acid-labile fraction has been redefined as methylene blue reactive sulfides (MBRS), and this includes ZnS, CdS, MnS, and terminal polysulfides (Mylon and Benoit 2001). Other researchers have made similar conclusions and have even added PbS to the acid labile or MBRS fraction (Cooper and Morse 1998; Bowles et al. 2002). Furthermore, it has been demonstrated that metal sulfide complexes of Co, Ag, Cu, Ni, and Hg have no recoverable acid-labile fraction (Cooper and Morse 1998; Bowles et al. 2002). Figure 1 is a generalization of the sedimentary sulfur cycle and demonstrates the incorporation of reduced sulfide into labile and refractory minerals (Cornwell and Sampou 1995). The dashed lines represent oxidative processes leading to the formation of other reduced intermediates of sulfur and ultimately to the formation of sulfate.

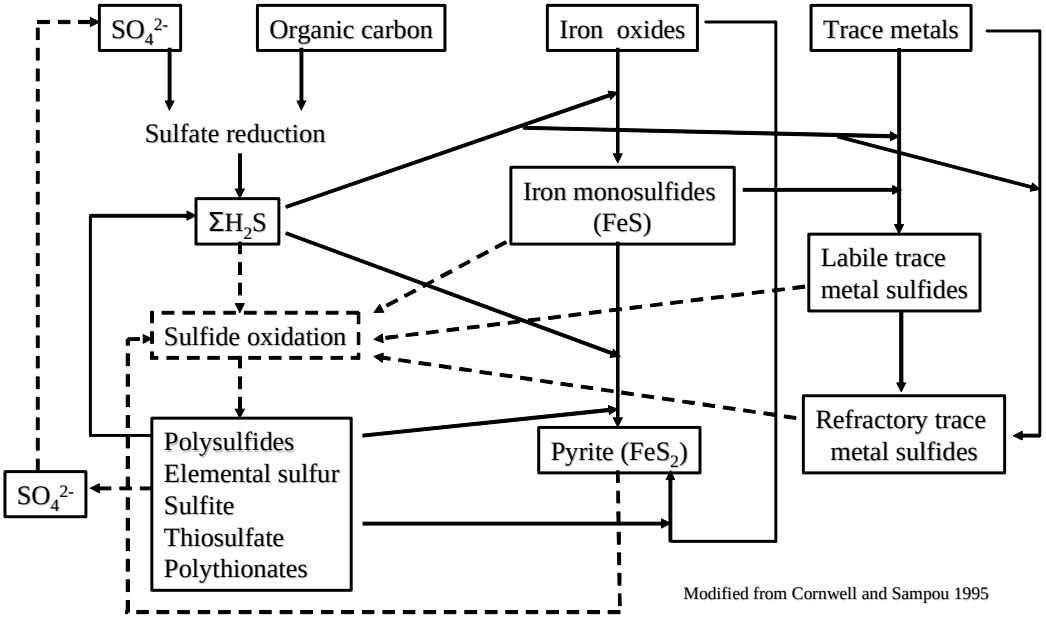

Figure 1. Generalization of the sedimentary sulfur cycle and potentially formed intermediate sulfur species.

Because many MBRS metal complexes have larger solubility products than FeS, other metals may displace Fe^{2+} to form these more insoluble metal sulfide complexes. Equation 1 demonstrates a solubility driven displacement reaction.

$$metal^{2+}_{(aq)} + FeS_{(s)} \rightarrow metal\ sulfide_{(s)} + Fe^{2+}_{(aq)} \quad (1)$$

This displacement process can be predicted by hard and soft acid-base theory. This theory predicts that there is preferential formation of a B-type metal or soft acid with a soft base (Stumm and Morgan 1996). For example, mercury would preferentially bind to a soft base such as sulfide rather than chloride. Because B-type metals form strong bonds with sulfide and are incorporated into the solid phase, the free metal ions become unavailable for uptake by organisms. It should be noted that researchers

have also reported metal sulfide accumulation in invertebrates and bacteria by ingestion of particles and through the passive diffusive of neutral HgS across cell membranes, respectively (Lee et al. 2000; Benoit et al. 2001; Bianchini et al. 2002).

Metal insolubility considerations have led to proposed sediment quality criteria for metal bioavailability to organisms (Ankley et al. 1996). The AVS fraction within the sediments is important in controlling the partitioning of free metal ion concentrations between solid and dissolved phases and potentially lowering metal toxicity in sediment pore waters and anoxic waters. It has been proposed that the ratio of acid-volatile sulfides to simultaneously extracted metals (AVS/SEM) can be indicative of toxic effects. If the SEM is lower than the AVS, then toxicity effects to organisms are not expected (Hansen et al. 1996).

Recent findings have also shown that dissolved sulfide species exist and may be important ligands in fully oxygenated waters (Cutter and Oatts 1987; Luther and Tsamakis 1989; Radfordknoery and Cutter 1993; Adams and Kramer 1999; Rozan et al. 1999; Rozan et al. 2000; Mylon and Benoit 2001; Bowles et al. 2003). Where natural organic matter (NOM) was previously considered to be the primary factor determining the speciation of heavy metals in oxygenated aquatic environments, low levels of MBRS are now thought to be able to out compete the more abundant NOM due to the nature of their solubility constants (Mylon and Benoit 2001). Because dissolved sulfide oxidizes in oxygenated waters, it was previously believed that metal complexation to dissolved sulfide did not appreciably occur. However, the rate at which some MBRS oxidize is much slower than the rate of oxidation for free bisulfide ion (Rozan et al. 2000). Therefore, a viable fraction of labile sulfide can

still exist in the water column for complexation and metal replacement reactions. The bioavailability of these metal sulfide complexes varies, and these complexes can reduce the toxicity of other metals. For example, recent studies have demonstrated that zinc sulfide clusters in fully oxygenated waters appear to suppress the acute toxicity of Ag^{1+} to *Daphnia magna* (Bianchini and Bowles 2002; Bianchini et al. 2002). A correct assessment of dissolved sulfide is essential to allow for more accurate predictions of metal speciation in chemical equilibrium calculations, in order that more reliable assessments of metal bioavailability and toxicity to organisms can be made.

Available analytical techniques

A variety of in situ and ex situ spectrophotometric, electrochemical, and chromatographic techniques for measuring dissolved sulfide and associated complexes exists in thechemical literature. In a recent review of methods available for sulfide analysis, it was noted that spectrophotometric methods of analysis are the most commonly cited methods; followed in popularity by electrochemical and chromatographic methods (Lawrence et al. 2000). Each method has its own set of advantages and disadvantages. The three most cited methods in the literature will be briefly discussed.

Spectrophotometric sulfide analysis usually involves the ex situ treatment of an analytical sample with a set of chemical reagents. The classic methylene blue and Cline method utilize a reaction between sulfide and N,N-dimethyl-p-phenylene-diamine that produces a blue complex (Cline 1969; APHA AWWA-WPCF 1989). These methods have detection limits between 0.3 and 1.0 $\mu molL^{-1}$(Kuhl and Steuckart

2000). Different concentration ranges of N,N-dimethyl-p-phenylene diamine and ferric chloride ($FeCl_3 \cdot 6H_2O$) are required for various sulfide concentrations to ensure proper color development. Therefore, usage of the methylene blue technique can be rather time consuming. Since this is an ex situ method, it is necessary to prepare samples in a manner that minimizes sulfide losses through adsorption, oxidation, and volatilization. The samples are normally fixed in the field and analyzed upon returning to the laboratory. Dissolved organic matter (DOM) present in environmental samples may contribute to absorptive interferences. This issue can be addressed via the coupling of methylene blue to HPLC (high-performance liquid chromatography) which minimizes DOM interferences and significantly lowers detection limits (Tang and Santschi 2000; Mylon and Benoit 2001). Mylon claims a method detection limit of 0.3 $nmolL^{-1}$. An in situ methylene blue method has been developed which utilizes flow injection analysis (FIA) coupled to a submersible vehicle for sulfide concentrations around hydrothermal vents (Johnson et al. 1986; Sakamotoarnold et al. 1986). This technique has a detection limit of 0.1 $\mu molL^{-1}$, but it is not very practical for most environmental sample analyses. Gas chromatographic techniques for low levels of sulfide have also been developed (Cutter and Oatts 1987; Radfordknoery and Cutter 1993). These methods consist of gas stripping of acidified sulfide species from water, pre-concentration via liquid nitrogen cold trapping, and analysis by flame photometric or photoionization detection. Detection limits range between 0.13 $nmolL^{-1}$ and 0.2 $pmolL^{-1}$. These methods are effectively utilized when large volumes of water are available.

Electrochemical analysis of dissolved sulfide usually is performed by amperometry, potentiometry, or voltammetry (Kuhl and Steuckart 2000), with the latter two techniques being the most commonly employed. A typical potentiometric sulfide measurement is made using a silver and sulfide solid-state ion selective electrode (ISE) and a reference electrode (Berner 1963; Thermo-Electron 2003). Potentiometric measurements can be in situ or ex situ, but most are performed ex situ. The sulfide ISE measures only the sulfide ion (S^{2-}) and has a detection limit of 0.1 $\mu molL^{-1}$, but some metal ions present in environmental samples can significantly interfere with sulfide ISE measurements, especially metals which have solubility products lower than that of Ag_2S (Kuhl and Steuckart 2000; Thermo-Electron 2003). Sulfide antioxidant buffer (SAOB) is added to lessen the effects of metal interferences through the presence of ethylenediaminetetraacetic acid (EDTA). Sulfide antioxidant buffer also buffers the environmental sample at an elevated pH to prevent sulfide losses through volatilization. Sulfide antioxidant buffer usage is now common practice in the ex situ measurement of environmental dissolved sulfide (Baumann 1974; Lawrence et al. 2000; Thermo-Electron 2003). Voltammetric methods have been used to measure dissolved sulfide species in surface and interstitial waters (Luther and Tsamakis 1989; Luther et al. 1999). However under environmental conditions, the fouling of an electrode by colloidal, inorganic, and organic materials changes the surface of the electrode and results in altered oxidation and reduction potentials on the surface of the electrode (Kuhl and Steuckart 2000). Problematic electrode fouling and the ambiguity of sulfide peaks within scans can be

potential drawbacks for the in situ use of these types of probes (Ciglenecki and Cosovic 1996; AlFarawati and vandenBerg 1997).

Recently the technique of diffusive gradients in thin films (DGT) has been developed to measure in situ concentrations of dissolved sulfide (Teasdale et al. 1999). In general, in situ measurements are either produced by taking continuous electrochemical measurements or periodic electrochemical scans. DGT differs from typical in situ electrode measurements by means of performing a continuous in situ fractionation of a chemical species, such as dissolved sulfide, over an extended time period, and the chemical analysis of the DGT probe is performed in the laboratory rather than the field. The result is a time integrated concentration of sulfide or other species of interest. DGT probes specific for sulfide have been analyzed by computer-imaging densitometry (CID) and methylene blue methods (Teasdale et al. 1999; Devries and Wang 2003; Motelica-Heino et al. 2003). Densitometry is performed by image analysis software using a gray scale comparison of standards and samples. Using densitometry, a detection limit of 0.26 $\mu molL^{-1}$ was estimated for a 24 hour DGT deployment using a 0.08 cm diffusive thickness (Teasdale et al. 1999). In theory, the detection limit of an in situ DGT probe can be lowered by using longer deployment times and thinner diffusive layers (Zhang and Davison 1995). Methylene blue has also been coupled to a purge and trap technique, similar to that employed for AVS extractions, for analysis of sulfide in DGT probes (Teasdale et al. 1999).

Basic DGT theory

The diffusive gradients in thin films (DGT) technique was developed in the mid 1990's (Davison and Zhang 1994). DGT probes are in situ analytical sensors for

use in speciation, multi-element, and pre-concentration studies of labile ions (Zhang and Davison 1995). DGT probes have been successfully deployed in fresh and marine waters, sediments, and soils (Harper et al. 2000; Twiss and Moffett 2002; Degryse et al. 2003; Dunn et al. 2003; Gimpel et al. 2003). The probes perform an in situ fractionation of chemical species by means of a semi-permeable membrane, and this separation of chemical species is a kinetically based process rather than an equilibrium process (Davison et al. 2000). During their deployment in solutions or natural waters, DGT probes continually accumulate ions. The mass of ions collected is proportional to the concentration of the ions in the bulk solution. Analysis of the fractionated sample is completed in the laboratory by an appropriate analytical method.

The DGT probe is a relatively simple and inexpensive device, consisting of three well-characterized layers (Figure 2). The filter membrane, hydrated polyacrylamide diffusive gel, and the binding gel are sandwiched into a plastic holder that contains a sampling window with a known exposure area. A thin diffusive boundary layer (DBL), of thickness δ, exists between the bulk solution and the filter membrane. Solutes readily pass through the overlying DBL, filter membrane, and polyacrylamide diffusive layer by way of molecular diffusion (Zhang and Davison 1995). The filter membrane acts as only an extension to the diffusive layer and protects the gel (Davison and Zhang 1994; Davison et al. 1994; Davison et al. 2000). A fully hydrated polyacrylamide diffusive gel is composed of 95% water. Diffusion coefficients of ionic species through a properly conditioned polyacrylamide gel are

therefore similar to those found in water (Davison et al. 1994; Zhang and Davison 1999).

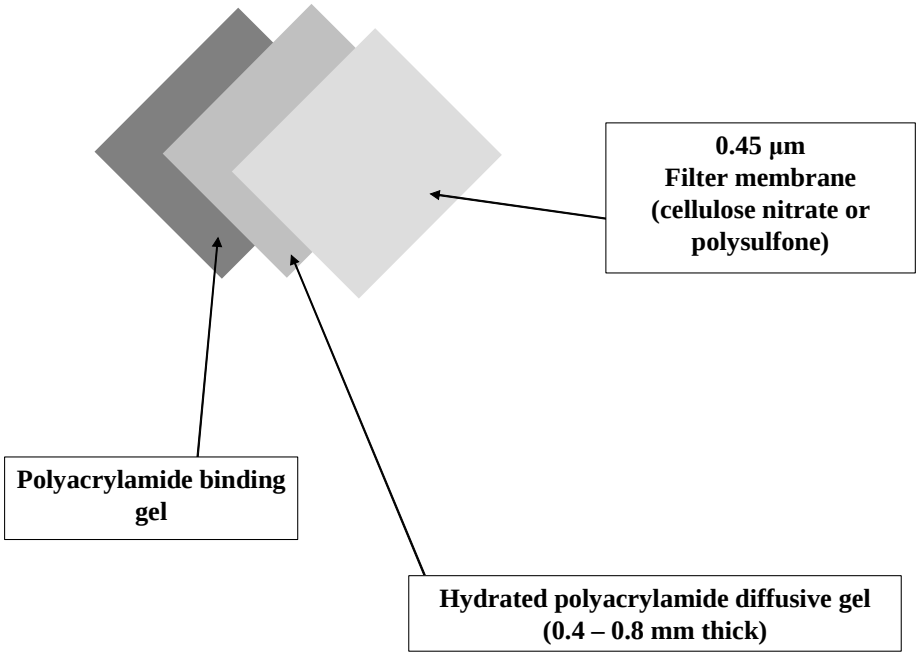

Figure 2. Schematic of the three layered system within a typical DGT probe.

The polyacrylamide binding gel contains a resin or ligand on its surface that selectively immobilizes a target ionic species by means of a large formation constant. Within a few minutes of deployment, a near steady state linear concentration gradient is established between the solution of interest and the binding gel (Figure 3) (Davison et al. 2000). In Figure 3, x represents the thickness of the diffusive layer, δ represents the DBL, C is the concentration of the species of interest within the bulk solution, and $C'$is the concentration of the species of interest at the surface of the binding gel.

The DGT probe's establishment of this steady state condition allows for the measurement of an in situ concentration. Ion fluxes through the diffusive layer, as

determined by Fickian diffusion in one dimension, can be calculated by eq 2 where *D* is the diffusion coefficient (cm^2s^{-1}). It is important that diffusion coefficients be temperature corrected using the Stokes-Einstein equation (Li and Gregory 1974; Zhang and Davison 1995). Because of the existence of a steady state condition, eq 2 simplifies to eq 3.

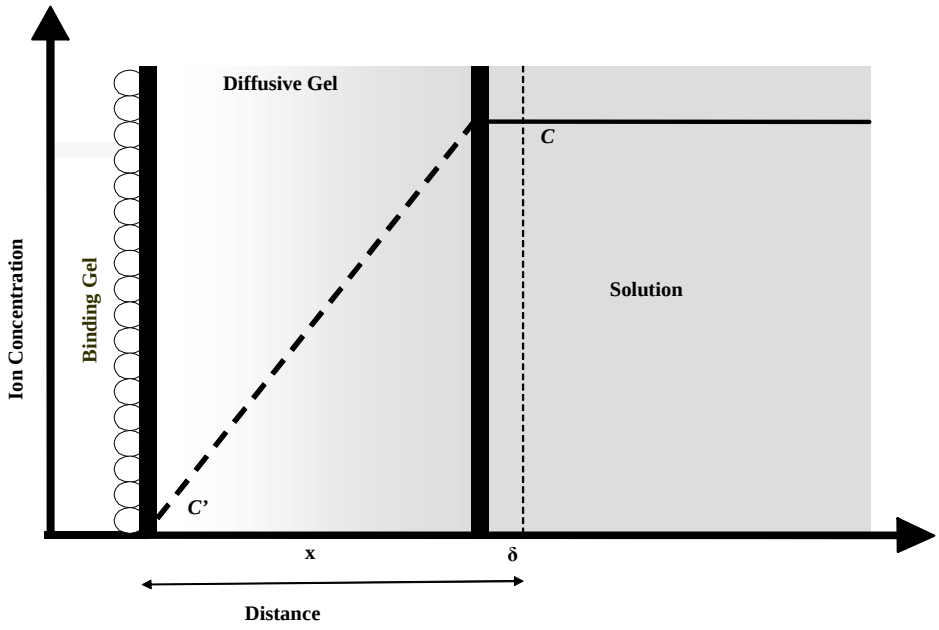

Figure 3. Schematic of a concentration gradient through the diffusive layer of a DGT probe in a solution with an ion concentration of C.

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \qquad (2)$$

$$\frac{\partial^2 C}{\partial x^2} = 0 \qquad (3)$$

Equation 3 implies that the flux doesn't change with time, and that there is no depletion of the ion of interest. In other words, the flux into the diffusive gel is equal to the flux out of the diffusive gel. The solution to eq 3 is simply the equation of a line. This solution is depicted by the dashed line in figure 3, and its slope is the concentration gradient. Using this concentration gradient, the flux (J) can be calculated from eq 4, where C is the concentration ($molL^{-1}$) in the solution, C' is the concentration ($molL^{-1}$) at the interface of the binding and diffusive layers, x is the thickness of diffusive layer (cm), and δ (cm) is the DBL thickness. Alternatively, flux can be written as eq 5, where M is defined as the mass of material (mol), A is the area of the diffusive gel (cm^2), and t is the time (s). By equating and rearranging eqs 4 and 5, the concentration of the ion of interest within the bulk solution can be determined from eq 6 which includes two assumptions. The first assumption is that because of the rapid equilibrium of the species of interest with the binding agent, and a large formation or equilibrium constant between the resin in the binding gel and species of interest, C' is effectively zero. Also the DBL thickness (δ) is usually much smaller than that of the diffusive gel (x) and thus is assumed to be negligible assuming well mixed solutions or waters (Zhang and Davison 1995). The mass of material (M) is found using eq 7 where C_{anal} is the analytical concentration ($molL^{-1}$) as determined by appropriate means, V is the elution volume (L), and f is the elution efficiency. The elution efficiency is a measure of how easily the ion of interest can be retrieved from the binding gel; therefore, it is a measure of recovery. The elution efficiency value may range between 70 and 100% and should be determined for each specific ion and elution method (Davison et al. 2000).

$$J = D\frac{dC}{dx} = D\frac{C - C'}{x + \delta} \quad (4)$$

$$J = \frac{M}{At} \quad (5)$$

$$C = \frac{Mx}{AtD} \quad (6)$$

$$M = {C_{anal}V}\Big/{f} \quad (7)$$

Under field conditions where low solution flow may be an issue (i.e., δ is not negligible), the simultaneous deployment of two or more DGT probes with varying diffusive layer thickness allows for the calculation of *C* and *δ* regardless of mixing and flow. The construction of a plot of 1/*M* versus *x* will result in a line with a slope of 1/*DCtA* and an intercept of δ/*DCtA* (Zhang and Davison 1995; Davison et al. 2000), and then C and δ can be calculated from eq 8. This approach can also be used to correct for biofouling. Biofouling can be viewed as an extension to the DBL of the DGT probe. If assumed that biofouling equally modifies the surface of two DGT probes with differing diffusive thickness, eq 9 can be used to calculate the solution concentration (Zhang et al. 1998).

$$\frac{1}{M} = \frac{x}{CAtD} + \frac{\delta}{CAtD} \quad (8)$$

$$\frac{1}{M_1} - \frac{1}{M_2} = \frac{x_1 - x_2}{CAtD} \quad (9)$$

Merits of DGT

The use of DGT probes presents a number of advantages which are valuable to the capture and analysis of dissolved sulfide. First of all, DGT devices are an in situ technique which captures time-integrated concentrations for chemical or biological processes that are occurring. This is an important consideration for the measurement of a constituent that is produced in situ and is relatively reactive, when regarding the activities of benthic fauna within the sediments or microbial activities around oxyclines. Benthic infaunal activities and sample handling can contribute to variability when using traditional pore water sampling and analysis. For example, many organisms create channels which allow nutrients and oxygen to penetrate potentially anoxic areas resulting in the creation of microenvironments, and improper sample handling (i.e., sulfidic sediments sampled under an oxic environment) can influence changes in chemical speciation. This could result in the tested pore water differing greatly from it original characteristics (Chapman et al. 2002).

Another advantage of the usage of DGT probes is that speciation can be preserved through the design of the probe, using ligands (with large equilibrium constants) that bind very strongly to specific ions. For example, in the case of sulfide as a target analyte, finely powdered high purity AgI is used as the binding agent. Equation 10 shows the reaction of the $AgI_{(s)}$ with migrating sulfide to form $Ag_2S_{(s)}$ ($\log K_{sp} = -49$). This metal sulfide remains insoluble and unreactive towards any oxygen it may encounter upon probe retrieval. Thus the speciation is preserved, and there is no worry of sample loss due to oxidation.

$$2AgI_{(s)} + HS^-_{(aq)} \rightarrow Ag_2S_{(s)} + H^+_{(aq)} + 2I^-_{(aq)} \quad (10)$$

Perhaps the greatest advantage in using DGT is that it is a kinetically based process rather than an equilibrium process. Other in situ methods (i.e., peepers and DET) rely on the reestablishment of equilibrium (Davison et al. 2000). Because the dissolved sulfide ligands are in rapid equilibrium with the binding gel and the rate of water exchange for Ag^{+} is rapid, the overall rate of Ag_2S complexation is fast. The exchange of water is usually the rate limiting step in complex formation. The estimated rate constant of water exchange for Ag^{+} is between log k_{-w} = 8 and 10 (s^{-1}). This value was estimated based on the assumption that the rate of water exchange is a function of the ratio of cation charge to ionic radius, and this value compares favorably to that of Cu^{2+} (log k_{-w} = 9) which has a similar electron configuration (Morel and Hering 1993). Thus the target species is irreversibly removed from the solution resulting in a continual linear concentration gradient.

The use of longer DGT probe deployment times and thinner diffusive layers also enables the user to a pre-concentrate the species of interest and to lower detection limits (DL). In theory, a sample may be collected for weeks as long as the binding capacity of the resin does not become saturated with the ligand or cation of interest (Davison et al. 2000). DGT devices can be used in aqueous environments with a large range of pH values as long as the binding agent effectively performs within that pH range (Zhang and Davison 1995; Zhang and Davison 1999; Gimpel et al. 2001). As previously stated, the use of DGT probes enables correction for biofouling, which is a significant problem with the use of traditional in situ electrodes (Zhang et al. 1998; Davison et al. 2000). Table 1 summarizes several common methods used in

determining dissolved sulfide. Overall, one should carefully choose a technique that is appropriate for their particular application.

Table 1. Observations on common analytical methods for sulfide analysis.

Analytical Method	S(-II) Detection Limit (Reference)	Observations
Methylene blue-spectrophotometric	0.3 – 1.0 $\mu molL^{-1}$ (Kuhl and Steuckart 2000)	ex situ method; reagent concentrations change over sulfide gradient; absorptive interferences from DOM; pre-concentration capabilities
Methylene blue-HPLC	0.3 $nmolL^{-1}$ (Mylon and Benoit 2001)	ex situ method; reagent concentrations change over sulfide gradient; no interferences from DOM
Gas chromatography and cryogenic trapping	<<0.13 nmolL-1 (Cutter and Oatts 1987; Radfordknoery and Cutter 1993)	ex situ method; pre-concentration capabilities; requires large sample sizes
SAOB/ISE	0.1 $\mu molL^{-1}$ (Thermo-Electron 2003)	ex situ method; dynamic linear range; interferences from Hg^{2+} and CN^{-}
Voltammetric	0.01 $\mu molL^{-1}$ (Kuhl and Steuckart 2000)	in situ or ex situ method; biofouling issues; unresolved sulfide peaks
DGT-CID	0.26 $\mu molL^{-1}$ (Teasdale et al. 1999)	in situ method; inexpensive; limited dynamic range; pre-concentration capabilities
DGT-methylene blue	no data	in situ method; reagent concentrations change over sulfide gradient; pre-concentration capabilities
DGT-ISE	0.104 $\mu molL^{-1}$ (this study)	in situ method; dynamic linear range; pre-concentration capabilities

Statement of research and primary hypothesis

Given the problems associated with other measurement techniques and with the current methods of quantifying sulfide by DGT, this study was undertaken to address the feasibility of using the DGT approach and an ISE to measure the sulfide captured by DGT devices. In addition, the study was designed to investigate whether the DGT devices measured true dissolved sulfide levels or whether there were measurement artifacts associated with the technique. Some of the current analytical techniques for sulfide produce concentrations that are understood to be closer to the total sulfide concentration. The primary hypothesis of this research is that DGT probes provide a more accurate assessment of the concentration of dissolved uncomplexed sulfide than other methods currently in use. The use of DGT dissolved sulfide data will therefore allow for more accurate predictions of metal speciation in chemical equilibrium calculations.

Thesis structure

This manuscript consists of four chapters and will discuss the development of an analytical technique and its use under field and laboratory conditions. The appendix will contain field data that will not be discussed in this manuscript. Finally, it is common in the literature for the notation of sulfide and its associated forms (i.e., H_2S, HS^-, S^{2-}, $[S(-II)]_t$, and S(-II)) to vary and perhaps be somewhat misleading. In order to be consistent in this document, I will use $S(-II)_{measured}$ to represent the measured amount or concentration of sulfur with a valence of (-II) and $[S(-II)]_t$ to represent the total amount or concentration of sulfur with a valence of (-II). It should be understood that $S(-II)_{measured}$ does not necessarily have to be equal to $[S(-II)]_t$.

Chapter 2: DGT methodology and validation: Laboratory studies

Materials and procedures

Probe assembly

AgI binding and diffusive gels were prepared in accordance, but with minor changes to methods previously described (Zhang and Davison 1995; Teasdale et al. 1999). AgI binding gels were prepared from a stock solution of polyacrylamide which was composed of 15% by volume acrylamide (Roche Diagnostics) and 0.3% by volume agarose-based cross linker (APA) (DGT Research Ltd., UK). The stock solution was then placed on ice. AgI (Alfa Aesar) was ground by mortar and pestle, and 0.6 g of finely ground AgI was added to a 6 mL aliquot of the polyacrylamide stock solution in a clean 50 mL centrifuge tube. AgI (K_{sp}= 8.51 x 10^{-17}) is insoluble in the binding gel. This solution was vigorously mixed using a Mini Vortexer (VWR) until a dispersed suspension was achieved. The 50 mL centrifuge tube was then placed on ice, and 42 μL of freshly prepared 10% by weight ammonium persulfate (Fisher) and 15 μL of 99% N,N,N',N',-tetramethylethylenediame (TEMED, Sigma) were added to the suspension of AgI and polyacrylamide stock. Ammonium persulfate is the initiator of polymerization and TEMED acts as the catalyst for polymerization. This mixture was inverted once and carefully pipetted into a mold. If the formation of air bubbles was noticed during transfer, the mold was carefully tilted to allow for their release. Polymerization of the solution occurs in less than 2 minutes. Chilling the stock solution was found to slow the polymerization process and allow for complete filling of the mold. The mold consisted of two offset sheets

of glass (15 cm x 7.5 cm) with a 0.37 mm thick plastic spacer inserted between them. The two glass sheets were typically offset by a few millimeters. This mold assembly was held together using binder clips. The mold was completely wrapped with aluminum foil in order to exclude light and was then placed underneath a heat lamp for 60 minutes. The height of the lamp was adjusted in order to produce a temperature of 45 °C. The AgI binding gel was carefully removed and placed into Milli-Q water (18MΩ, Millipore Corp.) for 24 hours in order to hydrate and rinse the gel. The binding gels were then transferred to a freshly prepared 0.01 $molL^{-1}$ $NaNO_3$ (Fisher) solution for storage prior to probe assembly. All hydrated binding gels were between 0.37 and 0.4 mm thick. Binding gel thickness was measured under a microscope.

Diffusive gels of various thicknesses were produced using 10 mL of the stock solution, 70 μL of 10% by weight ammonium persulfate, and 25 μL of 99% TEMED. The gels were cast and allowed to set in the same manner as described above. Diffusive gels of 0.4 and 0.8 mm thickness were also commercially obtained (DGT Research Ltd., UK). The diffusive gels were hydrated and rinsed with Milli-Q water. They were also stored in a 0.01 $molL^{-1}$ $NaNO_3$ solution prior to probe assembly.

A piston design DGT sampling probe was used for all solution laboratory and field studies. A piston assembly with a 2 cm diameter sample window and appropriate 2.5 cm diameter gel cutter were commercially obtained (DGT Research Ltd., UK). Complete details and schematic of the piston are previously described (Zhang and Davison 1995). Gels were handled and cut on a clean electrophoresis gel handling sheet (Diversified Biotech). DGT sampling probes were carefully assembled

to prevent air bubbles from becoming trapped between the layers of polyacrylamide gel. It is important that the resin or ligand imbedded within the binding gel be facing upward. Care was taken to ensure that the full depth of the piston assembly was filled by polyacrylamide gel. If not, the solution of interest may leak around the diffusive layer and react with the binding gel. The total depth of the piston assembly was approximately 1.34 mm. If a 0.4 mm diffusive layer was used, a similar polyacrylamide gel spacer was placed underneath the binding gel to allow for a secure fit within the piston assembly. No filter membranes were used in this laboratory study. All fully assembled probes were stored in a 0.01 $molL^{-1}$ deoxygenated $NaNO_3$ solution inside an anaerobic (nitrogen filled) vinyl chamber glove box (Coy Laboratory Products). Probes remained within the glove box at least one week before use in laboratory or field studies because it was believed that the plastic pistons may slowly bleed oxygen. Fresh, 0.01 $molL^{-1}$ deoxygenated $NaNO_3$ was added daily.

Elution of sulfide from binding gel

Liberation of the sulfide bound to the AgI binding gel was obtained by a modified version of the acid-volatile sulfide (AVS) purge and trap extraction method (Cornwell and Morse 1987; Brouwer and Murphy 1995). The AgI sample gel was rinsed with Milli-Q water, patted dry with a clean tissue, and placed into a 100 mL three neck round bottom flask (Pyrex). The two side necks were fitted with silicone stoppers (Cole-Parmer) that had Teflon ports through their centers. One port delivered a continual flow (~100 mL/min) of high purity nitrogen gas to the round bottom flask. A disposable oxygen scrubber (Agilent) was placed in line to remove

any residual oxygen that could promote oxidation and therefore result in the loss of sulfide. The other port was fitted with a female Luer lock to 2 way valve (Cole-Parmer) through which 10 mL of 12 $molL^{-1}$ deoxygenated HCl (J.T. Baker) was delivered by a plastic syringe (Henke-Sass, Wolf GMBH). Acid deoxygenation was performed by purging with high purity nitrogen gas for at least an hour. Prior to the addition of acid, nitrogen was allowed to flow over the gel sample for 5 minutes to ensure that oxygen had been purged from the reaction vessel. The round bottom flasks were heated at 65 °C for two hours. The nitrogen and evolved hydrogen sulfide gas (H_2S) departed the distillation apparatus through the top of the condenser by way of a Teflon line and was trapped in 25 mL SAOB (Brouwer and Murphy 1995; Thermo-Electron 2003). The condensers atop each round bottom flask prevented HCl vapor from entering into the traps.

Analysis of sulfide in traps following elution

Analyses of sulfide traps were performed using a solid state ion selective electrode (ISE) and a reference electrode (Thermo Electron Corporation). A six point calibration curve was made daily from a stock solution. The saturated sulfide stock solution was prepared by washing a crystal of $Na_2S \cdot 9H_2O$ (Sigma) with deionized water, drying it with a tissue, and dissolving it in a few milliliters of deoxygenated, deionized water. A new sulfide stock solution was produced every month. This stock solution was stored under nitrogen in a glove box. Production of secondary standards and the standardization of stock solution by means of lead (Pb) titration were performed daily. A 0.1 $molL^{-1}$ $Pb(ClO_4)_2$ standard was used as the titrant (Thermo

Electron Corporation). The titration end point was the point of greatest slope on the titration curve.

Method assessment

Laboratory testing conditions

All testing of dissolved sulfide uptake onto the AgI DGT probes was performed under a nitrogen environment at 25 °C. Once again, the use of the nitrogen glove box minimizes sulfide losses via oxidation. Test solutions were placed into 500 mL amber volatile organic compound (VOC) sampling containers (VWR). The use of these containers helped to minimize the loss of sulfide through volatilization. The pH was kept near 7.0 in order to mimic the pH of most natural waters. An orbital shaker table (Cole-Parmer) set at 85 rpm was used to create proper mixing conditions. At low flows, the formation of a diffusive boundary layer can affect the mass transport of the ion of interest (Gimpel et al. 2001). All test solutions were prepared from aliquots of a standardized Na_2S solution.

Sulfide elution efficiency

Elution tests were performed to ensure that the quantitative recovery of sulfide from the binding gels was adequate. Bare AgI binding gels, without a diffusive layer or piston assembly, were placed into VOC sampling containers with known quantities of dissolved sulfide for 12 hours. The results can be seen in Figure 4. The percent of dissolved sulfide recovered from the gels as detected by ISE compares favorably to data obtained by a similar purge and trap procedure which is followed by the classic

methylene blue method (see data in Figure 4). Even at nanomole levels of dissolved sulfide, an 81 ± 12% recovery was still obtainable**.**

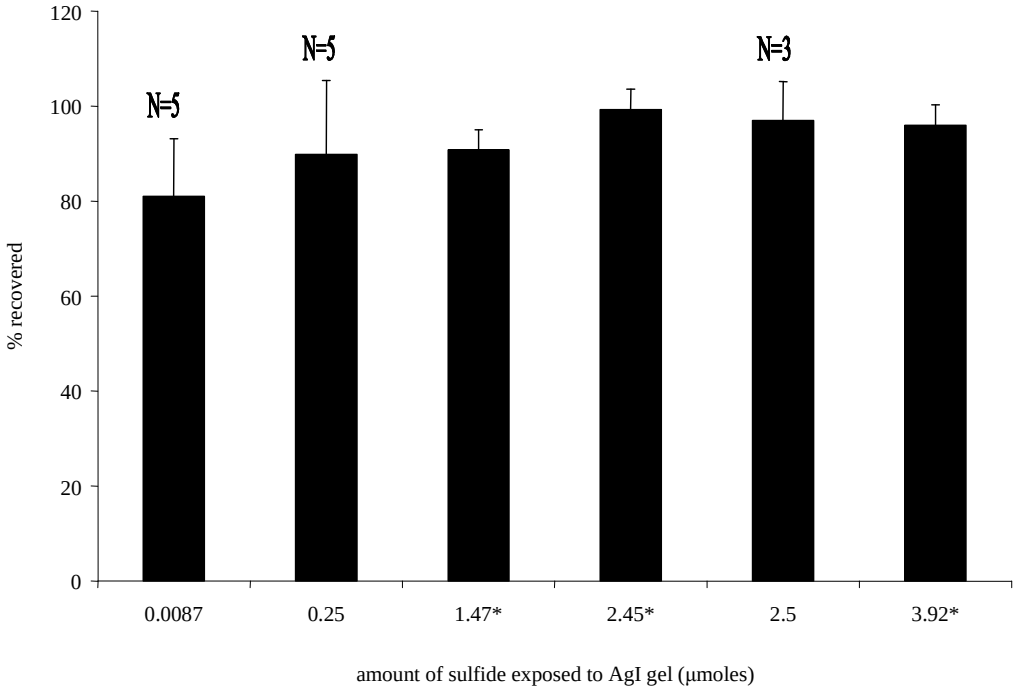

Figure 4. Elution efficiency of sulfide from AgI binding gel as determined by ISE. *Data are from Teasdale et al., 1999 as determined by methylene blue.

Validity of DGT equations

The validity of the standard equation (eq 6) for DGT probes can be tested by measuring the mass or amount of analyte collected over time and with respect to varying diffusive thicknesses (Zhang and Davison 1995). These experiments were conducted using fully loaded DGT probes deployed in an 18 ± 1 $\mu molL^{-1}$ solution of dissolved sulfide under the previously mentioned laboratory conditions. Over a 24 hour time period, sulfide accumulation was linear with an R^2 value of 0.95 (Figure 5).

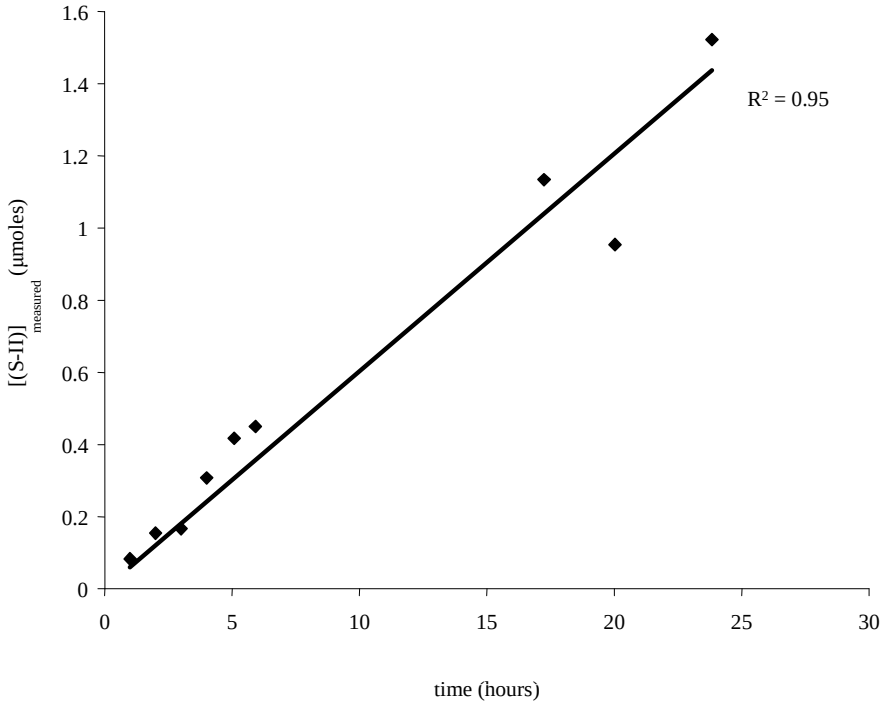

Figure 5. Measured sulfide accumulation on DGT probes over 24 hours as determined by DGT-ISE.

A second validity test was performed by varying the diffusive gel thickness. Duplicate DGT probes with 0.52, 0.67, and 0.83 mm thick diffusive layers were deployed in a 17 ± 1 $\mu molL^{-1}$ solution of dissolved sulfide for 6 hours. The actual mass accumulated is in good agreement with the theoretical mass. The mass as a function of the reciprocal of diffusive thickness has an R^2 value of 0.88 (Figure 6). The dashed line represents the theoretical amount of sulfide which should be detected from the 17 ± 1 $\mu molL^{-1}$ solution of dissolved sulfide over the same deployment time. These results also agree with those of a previous study which utilized the densitometric analysis of dissolved sulfide over varying diffusive gel thickness (Teasdale et al. 1999).

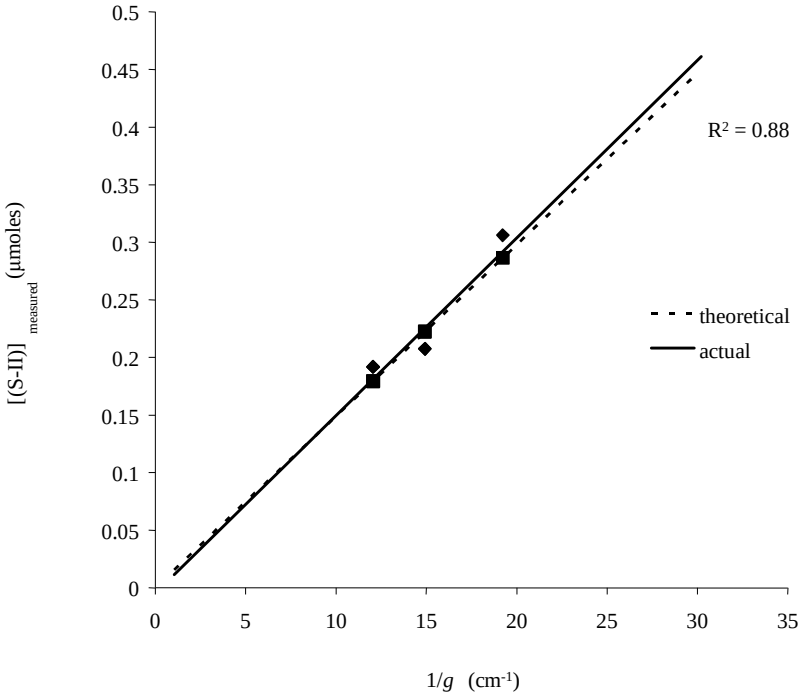

Figure 6. Total sulfide accumulation over varying diffusive gel thickness as determined by DGT-ISE.

Testing over pH gradient

A test of the accumulation of dissolved sulfide by DGT probes over a pH gradient was conducted by adjusting the pH of three solutions containing 25 ± 1 $\mu molL^{-1}$ dissolved sulfide. These solutions were prepared in VOC containers under a nitrogen environment to minimize volatilization and oxidative loses. The pH = 4 and pH = 12 solutions were adjusted by the drop wise addition of concentrated HCl and NaOH solutions respectively. The pH = 7.4 solution was made with a phosphate buffer (Sigma). Complexation of dissolved sulfide to any anion within this buffer solution should not appreciably occur. Duplicate DGT probes with diffusive thickness of 0.83 mm were deployed in the three solutions for 5 hours. The results

can be seen in Figure 7. The pH =4 solution results in the lowest percent of total sulfide detected by a DGT device at 76 ± 8%. At this pH the loss is mostly likely due to the volatilization of dissolved hydrogen sulfide (H_2S) from solution into the container headspace. A calculation of the H_2S concentration in the headspace of the container reveals that approximately 39% of the bulk solution could be lost to volatilization. Regardless of the losses due to volatilization, these results still demonstrate that H_2S easily diffuses into the DGT probe and reacts with the binding agent.

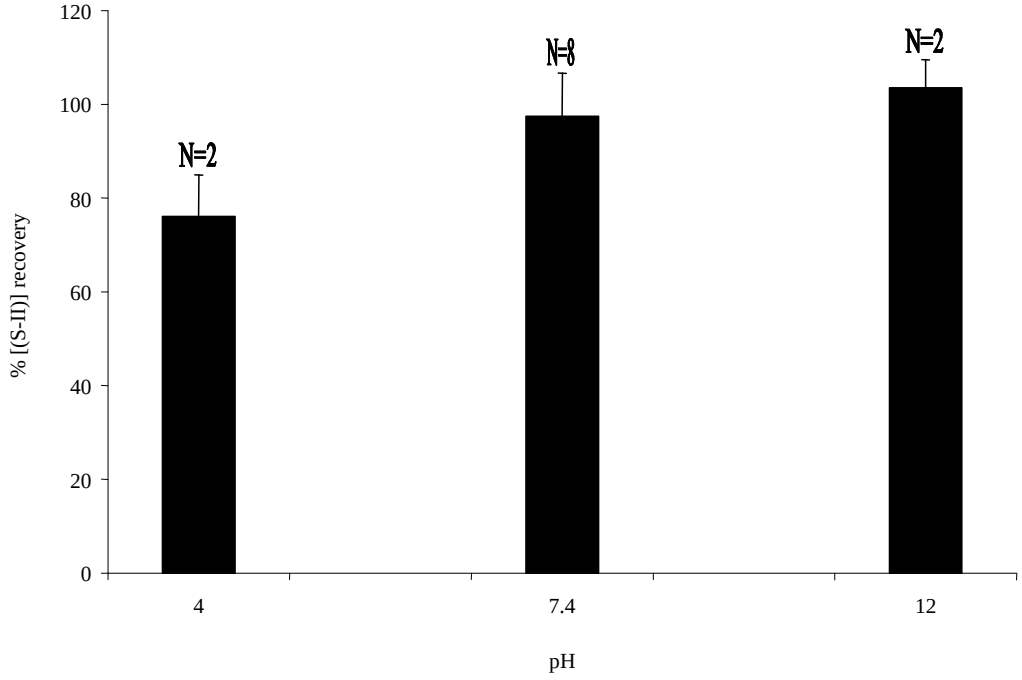

Figure 7. Percent of total sulfide measured by DGT-ISE at various pH values.

Comments and recommendations

The present study demonstrates that sulfide can be quantitatively measured by coupling DGT probes with an ISE. Furthermore, all performance tests compare favorably with previous results which were generated by DGT probes coupled to methylene blue and densitometric measurements. The dynamic linear range of the ISE ($< 10^6$ $\mu molL^{-1}$) enables sample analysis over extreme concentration ranges (Thermo-Electron 2003). This is a substantially larger range than other quantification processes. Image intensity for field samples can exceed the upper limits of the calibration curve for those DGT measurements made by densitometry (Teasdale et al. 1999; Devries and Wang 2003). This is due to black saturation of the gray scale. The relative simplicity of the ISE measurement also has an advantage over the traditional and potentially time consuming methylene blue technique. The classic methylene blue analysis calls for specific reagent concentrations over different dissolved sulfide concentration ranges (Cline 1969). Also methylene blue doesn't offer the dynamic range of an ISE measurement because methylene blue solutions will depart from Beer's Law at higher sulfide concentrations.

Chapter 3: Field measurements of dissolved sulfide and laboratory investigations of potential measurement artifacts

Introduction

The method development data from Chapter 2 suggested that DGT coupled to a silver and sulfide solid-state ISE (DGT-ISE) accurately assess the dissolved sulfide (H_2S, HS^-, and S^{2-}) concentration of simple, well mixed solutions manufactured exclusively from $Na_2S \cdot 9H_2O$. Yet the reality in many natural environments is that sulfide exists not exclusively as the diprotic acid but as many different species due to its affinity for metals and organic material (Ciglenecki and Cosovic 1996; Stumm and Morgan 1996). Because sulfide speciation can influence the bioavailability of trace metals to organisms, there has been a desire to develop methods that allow for the recognition of truly dissolved sulfide and dissolved complexes of sulfide.

A logical step in examining chemical speciation methods is to make comparative measurements between available techniques. If a systematic bias in the concentration appears between two different techniques, then it is likely that the techniques are detecting different pools or species. It is not likely that one speciation method can detect all individual species. In fact, it has been demonstrated in the literature that not all sulfide techniques provide the same quantitative answer. For example, methylene blue detects some labile or reactive metal sulfide complexes but not thiols (Adams and Kramer 1999; Tang and Santschi 2000; Mylon and Benoit 2001; Bowles et al. 2002). The intention of the following comparison of methods (i.e., DGT-ISE versus SAOB/ISE and/or methylene blue) was to determine if differences in measured sulfide concentrations in natural waters exist between

techniques. As differences between the methods were detected, laboratory studies were designed to identify the pools associated by each method and the associated mechanisms.

Recent literature regarding sulfide analysis by DGT suggests that this method may detect a different fraction of dissolved sulfide than what is found with a peeper utilizing a 0.2 μm membrane (Devries and Wang 2003). They used CID to analyze their field samples, and the exact magnitude of the differences between their methods cannot be accurately determined because the sulfide concentration as estimated by DGT-CID had exceeded the upper limit of their gray scale calibration standards (Devries and Wang 2003). However, they concluded that their estimated sulfide concentrations most likely differed due to a localized depletion of sulfide in the sediment pore waters surrounding the DGT probe. Another study, not utilizing simultaneous parallel methods to determine sulfide concentrations, offered similar explanations for differences between DGT estimated sulfide concentrations and colorimetric determined sulfide concentrations estimated from previous years (Teasdale et al. 1999), and the temporal difference makes a direct comparison more difficult. The analyte depletion explanation has been suggested and discussed in detail by other studies which have examined non-steady state DGT measurements in pore waters (Harper et al. 1998; Davison et al. 2000; Harper et al. 2000; Ernstberger et al. 2002; Degryse et al. 2003). Analyte depletion in the area immediately surrounding a DGT sediment probe and the resulting flux changes are real phenomena and make elucidation of any concentration differences between parallel methods much more complicated. Because DGT measurements in sediments are

potentially influenced by localized depletion events and sediment heterogeneity, all sulfide comparisons in this study were performed within the open waters of lakes or in well mixed solutions in an effort to eliminate this effect.

The nature of the entities in the "dissolved fractions" of sulfide is one aspect of current techniques that needs to be addressed. The standard operational cutoff for dissolved substances is conventionally 0.45 μm whereas the pore size for typical polyacrylamide diffusive layers, manufactured with an agarose-based cross linker (APA), within DGT probes are on the order of 2-5 nm (Zhang and Davison 1995). Theoretical calculations of the size of metal sulfide complexes, such as $(ZnS)_x$ clusters, suggest they should readily pass through a 0.45 μm filter (Luther et al. 1999). It has be suggested, however, that the diffusion of these metal sulfide complexes through a DGT probe diffusive layer would be strongly hindered due to the smaller pore sizes of the gels (Zhang and Davison 1995; Teasdale et al. 1999; Devries and Wang 2003). Thus, techniques that measure reactive sulfides and dissolved sulfide following conventional filtration, such as methylene blue, would result in higher concentrations being measured. In another study, different size fractions of dissolved sulfide have been documented using 10 kDa centrifugal filters and standard 0.45 μm polyethersulfone filters (Adams and Kramer 1999). Using methylene blue, Adams and Kramer found $nmolL^{-1}$ concentrations of sulfide in the ≤10 kDa fraction of the wastewater effluent from a water treatment plant and further down stream of the treatment plant suggesting that this technique quantified relatively stable reactive sulfide species (i.e., MBRS).

During this research, size exclusion was used as the method for species separation, and concentrations of sulfide in lake and extracted pore water were determined by filtration through a 0.45 μm filter followed by analysis by SAOB/ISE and/or methylene blue. These methods were compared to estimated dissolved sulfide concentrations by DGT-ISE. The results of the field comparisons show significant differences among the various techniques. In addition to artifacts produced by filtration relative to truly "dissolved fractions", chemical reagents (i.e., SAOB) used in specific techniques may react differently with various forms of sulfide. Therefore, a series of controlled laboratory experiments were performed in order to distinguish potential causes for the systematic differences between DGT-ISE and discrete sampling measurements for dissolved sulfide.

In order to discern some of the field study differences, laboratory experiments focused on the effects of the buffering reagent SAOB on dissolved material containing forms of reduced sulfur as well as on the size fractions of dissolved sulfide complexes and the potential for release of dissolved sulfide from these compounds. It is estimated that sulfur in natural organic matter (NOM) can occur at levels as low as 0.1% to a few percent (Smith et al. 2002). It has been estimated that nearly 50% of the sulfur associated with some aquatic NOM is in a reduced oxidative state, such as sulfhydryl or disulfide groups (Xia et al. 1999). Sulfide antioxidant buffer (SAOB) contains sodium hydroxide (NaOH), ethylenediaminetetraacetic acid (EDTA), and ascorbic acid (Thermo-Electron 2003). The individual reagents that constitute the SAOB reagent have chemical properties which may potentially alter dissolved species within a conventionally filtered sample of natural water over time. For example,

SAOB buffering solutions have high pH's (≥ 12), and dissolved organic molecules that contain reduced sulfur, in the forms of sulfhydryl groups and disulfides, may easily hydrolyze therefore resulting in the release of free sulfide ions. It is known that treating organic molecules with a strong base is a common way to selectively cleave molecules (i.e., hydrolysis of glycerides) (Morrison and Boyd 1987). Equation 11 represents such a proposed alkaline hydrolysis reaction. The leaving group (i.e., sulfhydryl) in the equation is represented by SH^-, and NaOH represents the source for a strong nucleophilic reagent.

$$RCOSH + NaOH \rightarrow RCOOH + SH^- \quad (11)$$

Finally, the presence of excessive EDTA may also facilitate the release sulfide, which can be associated with metal sulfide complexes, through competitive binding with metal cations and thereby allowing the liberated dissolved sulfide to be detected by the ISE. Equation 12 is a proposed mechanism in which sulfide is liberated. A derived stability constant for the proposed reaction is log K = 7.4.

$$ZnS + H^+ + EDTA^{4-} \rightarrow ZnEDTA^{2-} + HS^- \quad (12)$$

It is therefore hypothesized that the release of sulfide generated by SAOB reactions, as represented in eqs 11 and 12, would be detected by the ISE.

Given this discussion, it was hypothesized that DGT-ISE measures only truly dissolved sulfide complexes and that the SAOB/ISE technique measures dissolved sulfide plus some fraction of the sulfide in metal sulfide complexes and thiol containing substances. Finally, the premise put forward concerning the methylene blue method, that it detects $H_2S_{(aq)}$, dissociated $H_2S_{(aq)}$ species, and reactive metal sulfides but not thiols, is hypothesized to be correct. The following sections of this

chapter will give an overview of the field sampling sites and laboratory experiments, discuss the methods for sulfide analysis used at each field location and in each laboratory experiment, and report and discuss the results from each location and the laboratory experiments.

Field study sites

Sulfide data obtained by DGT was compared to data taken by more traditional ex situ techniques, such as methylene blue or ISE, at sites within three lakes (Lariat, Pavin, and 658) and also interstitial water from the Patuxent River estuary. Lake Lariat (38°36'N, 76°44'W) is a man-made recreational reservoir (1.88×10^6 m^3) located in eastern Maryland (Sveinsdottir 2002). It reaches an approximate maximum depth of 7 m. Pavin Lake (45°55'N, 2°54'E), a meromictic crater lake, is located in the Mont-Dore range of central France. It reaches a maximum depth of 92 m and exhibits a permanent chemocline at 60 m. The bottom waters are permanently anoxic. Because of its unique hydrologic and geochemical features, abundant literature exists discussing its water chemistry (Cossa and Mason 1994; Michard et al. 1994; Viollier et al. 1995). Lake 658 is located on the Canadian Shield at the Experiment Lakes Area (ELA) in northwestern Ontario (49°40'N, 93°44'W). It is a dimictic lake with a maximum depth of 14 m, and its anoxic hypolimnion is usually established by late summer (Ogrinc et al. 2003). This lake and its watershed are currently being studied in a whole ecosystem mercury (Hg) loading experiment (METAALICUS Project). Lastly, pore water was collected from Mackall Cove, which is located in St. Leonards Creek on the Patuxent River estuary.

Laboratory studies

For this set of experiments, ZnS clusters and sodium thioglycolate were chosen as representative compounds for a dissolved metal sulfide complex and an organic reduced sulfur compound. Zinc sulfide was chosen because a simple method for its synthesis exists (Bowles et al. 2002) and strong evidence of its existence in a cluster form exists (Luther et al. 1999). Also zinc exists in one relatively stable oxidation state (+2) in the environment thus making it easier to handle. Iron sulfide complexes naturally dominate over zinc or other metal sulfide complexes in the environment because iron typically exists in higher concentrations. Numerous phases of iron sulfide with varying degrees of stoichiometry have made this large pool of reduced iron sulfur complexes difficult to characterize (Morse et al. 1987; Davison et al. 1999). However, while the experiments performed here used ZnS clusters, it is probable that the results are applicable to Fe_xS_y solid phases and other metal sulfides. Thioglycolate, which is easily dissolved and readily available as >95% purity, was chosen to represent a small molecular weight organic compound. Thioglycolate is known to oxidize over time to dithiodiglycolate (Cook and Steel 1959). However, the oxidation to dithiodiglycolate is simply the result of two sulfhydryl groups forming a disulfide bond, and compounds with disulfide linkages also represent a portion of the total reduced sulfur content in organics. Therefore thioglycolate oxidation should not pose a significant problem in the experiments with SAOB. However, as a precaution, the prepared thioglycolate stock solutions were monitored by a back titration with a potassium iodate solution to assess whether appreciable amounts of degradation occurred upon aging in oxygenated environments (Steel 1958). These laboratory

experiments were setup to evaluate whether SAOB has the capability to hydrolyze the carbon to sulfur bond in a thiol like compound and as a result, facilitate the release of sulfide from metal sulfide complexes.

Methods

Pavin Lake, France

Sampling in Pavin Lake occurred in November of 2002. The production of polyacrylamide diffusive and AgI binding gels and their assembly into DGT piston probes are described in detail in Chapter 2. Thirteen DGT piston probes with diffusive layers of 0.56 mm were assembled for deployment in the lake. The probes were conditioned in an anaerobic glove box for 5 days using deoxygenated 0.01 $molL^{-1}$ $NaNO_3$. Probes were transferred to the field station in a vacuum sample saver (VWR). Individual probes were placed inside of clean plastic bags filled with 0.01 $molL^{-1}$ deoxygenated $NaNO_3$. These bags were then packed into the vacuum container, and the headspace of the vacuum container was filled with additional deoxygenated $NaNO_3$. Filling the headspace of these containers with degassed solution allows them to remain oxygen free for several hours. All probe packaging was carried out inside the anaerobic glove box to ensure minimal contact with oxygen. Upon arrival to the field station, fresh deoxygenated degassed $NaNO_3$ was again added. Because in situ sulfide concentrations are being measured, it is essential that the probes remain deoxygenated before deployment.

Twelve DGT probes were deployed in the water column, in close proximity to the chemocline, at nine depths by attaching the probe to a weighted line using monofilament fishing line. Duplicate probes were deployed at three of the nine

depths. One probe was held in the 0.01 $molL^{-1}$ $NaNO_3$ storage solution for a blank measurement. Sample probes were deployed in the water column for 46.5 hours at a temperature of 6°C. Upon retrieval to the lake surface, the probes were removed from the line and were immediately rinsed with deionized water and placed into clean plastic bags. Once back in the laboratory, the probes were disassembled and the binding gels, including the blank binding gel, were placed into clean plastic bags for storage until sulfide elution and analysis. The elution and analysis procedure of sulfide from the binding gels was performed according to the procedures discussed for DGT-ISE in Chapter 2. The calibration curve for sulfide standards was linear (R^2 = 0.99). The blank gel mass was 2.7 nmol $[(S\text{-}II)]_{measured}$ and this was subtracted from the analytical samples eluted from the binding gels. Distillation recoveries, as determined by a known sulfide spike, were 85%.

Lake Lariat, Calvert County, Maryland

Sampling in Lake Lariat occurred in September of 2003. The production of polyacrylamide diffusive and AgI binding gels and their assembly into DGT piston probes is described in detail in Chapter 2. Twelve DGT piston probes were assembled for deployment in the water column of the lake. Four probes were fitted with diffusive layers of 0.8 mm thickness and the remaining eight probes were fitted with 0.4 mm diffusive layers. In addition, four of the twelve probes were covered with cellulose nitrate filter membranes (i.e., two probes outfitted with 0.4 mm and two probes outfitted with 0.8 mm diffusive layers). This was done to evaluate whether the filter membrane truly behaved as an extension to the diffusive layer (Davison and Zhang 1994; Davison et al. 2000). All probes were conditioned inside

an anaerobic glove box for 10 days prior to deployment with a 0.01 $molL^{-1}$ solution of deoxygenated $NaNO_3$. Probes were transferred to the field site in a vacuum sample saver. Individual probes were placed inside of clean plastic bags filled with a 0.01 $molL^{-1}$ deoxygenated $NaNO_3$ solution. These bags were then packed into the vacuum sample saver, and the headspace of the sample saver was filled with additional deoxygenated $NaNO_3$. Filling the headspace of these containers with degassed solution allows them to remain oxygen free for several hours. All probe packaging was carried out inside the anaerobic glove box to ensure minimal contact with oxygen.

The DGT probes were secured within two identically designed frames for deployment in the bottom water. This was done in order to maintain similar sampling orientations within the water column over the length of the deployment, and this framework ensured that all the DGT probes were secured at equal distances from the lake bottom. Each frame was constructed from two (12 cm x 30 x cm x 0.2 cm) sheets of acrylic and could hold six probes. The DGT probes were placed face first into the frame. Holes (3.2 cm diameter) in each frame were cut using a hole saw. The back sheet was placed against the undersides of the probes and secured to the front sheet by nylon screws. Each frame was secured vertically to a platform which was lowered to the lake bottom at an approximate depth of 9 m. On the bottom, the DGT probes rested 20 cm above the sediment and water interface. An appropriate length of 3/8" OD polypropylene tubing was attached to the platform at equal depth to the DGT probes in order for bottom water to be pumped to the surface for total dissolved sulfide analysis by ISE. A 90° elbow was fastened to the end of the tubing

to allow for bottom water in the same plane as the DGT probes to be sampled. A subsurface buoy was used to keep the line and tubing taut, and a small surface marker was attached to mark the location of the platform. The platform was deployed for 48 hours.

On the day of DGT probe retrieval, bottom water samples were pumped to the surface using a battery powered peristaltic pump. Pumped bottom water was discarded for 5 minutes prior to actual sample collection. Samples were filtered through a 0.05 μm Fiberflo cartridge (Minntech Corp.), 0.2 μm polysulfone Acrodisc (Pall Gelman), or a 0.45 μm polysulfone Acrodisc (Pall Gelman) filter membrane. For 0.2 μm and 0.45 μm filtered samples, water was collected in clean, disposable 10 mL syringes (Henke-Sass, Wolf GMBH) and a 5 mL sample aliquot was filtered and placed into 5 mL of freshly prepared SAOB. A new syringe and filter were utilized for each sample. The 0.05 μm filter cartridge was rinsed with bottom water for 5 minutes prior to the collection of bottom water into a new 10 mL syringe. The cartridge was flushed to ensure that any residual oxygen present would be flushed out. A 5 mL aliquot from the syringe was also placed into 5 mL of SAOB. After the discrete bottom water samples were collected, the platform was pulled to the lake surface and the probes were removed and immediately rinsed with deionized water and placed into clean plastic bags. It was noted that the bottom water temperature was 11 °C. Upon return to the laboratory, all DGT probes were disassembled, and the binding layers were placed in clean plastic bags. One DGT probe was damaged during deployment or retrieval process and was disregarded. All 0.45, 0.2, and 0.05 μm filtered water samples were analyzed by SAOB/ISE upon return to CBL. This

was approximately four hours after sampling. Elution and analysis of sulfide from the binding gels were performed according to the DGT-ISE procedures discussed in Chapter 2. The calibration curve constructed from freshly prepared sulfide standards was linear ($R^2 = 0.99$). The blank gel was found to be below detection limit of the ISE. Distillation recoveries as determined by a spike were 110%. The calibration curves for the quantification of the filtered bottom water samples were also linear ($R^2 = 0.99$).

Lake 658, ELA

In situ sampling for sulfide in Lake 658 occurred in September of 2003. Twelve DGT piston probes were assembled for deployment in the hypolimnion of the lake. The production of polyacrylamide diffusive and AgI binding gels and their assembly into DGT piston probes was described in detail in Chapter 2. Six of the assembled probes had diffusive layers of 0.4 mm and the remaining six had 0.8 mm diffusive layers. Probes were transferred from the Chesapeake Biological Lab (CBL) to the ELA field station in a vacuum sample saver. Immediately upon arrival, the probes were placed inside an anaerobic glove bag into a bath of freshly deoxygenated $NaNO_3$. This bath was gently stirred with a Teflon stir bar for 5 days prior to field deployment. The 0.01 $molL^{-1}$ $NaNO_3$ conditioning solution was exchanged with a freshly deoxygenated $NaNO_3$ solution two days prior to deployment. On the day of deployment, probes were once again transferred to the field site by a vacuum sample saver.

One DGT probe of each thickness was deployed at one of five heights above the sediment water interface. The extra probes remained in the $NaNO_3$ conditioning

solution and were used as blanks. The use of two diffusive layers permits the calculation of sulfide concentrations and DBL thickness irrespective of flow. The probes were attached to an acrylic panel at 5, 10, 20, 40 and 80 cm intervals above the sediment surface. This panel was attached by diver to the frame of an existing platform on the lake bottom which housed the Close Interval Sampling (CIS) system. This panel was also designed to be retrieved by a surface line at the end of the deployment period. The CIS was developed by Dr. Chris Babiarz of the University of Wisconsin and will be described in greater detail in a future publication (Babiarez 2004). In brief, the CIS system consists of a network of sampling tubes and pumps used to sample the water column over small distances. It is a useful tool for examining redox chemistry over chemoclines. The acrylic panel housing the DGT probes was designed in order for the DGT probe deployment heights to match the existing heights of the CIS sampling ports. Total DGT probe deployment time was 75 hours. Discrete sulfide samples were taken just prior to DGT probe deployment and retrieval. The discrete sampling was performed using CIS, and 5 mL aliquots were placed into SAOB for analysis by ISE. Analysis by SAOB/ISE was performed approximately five hours after sampling. The bottom water temperature was 4°C. The elution and DGT-ISE analysis procedure of sulfide from the binding gels was performed according to the procedures discussed in Chapter 2. A linear ($R^2 = 0.99$) calibration curve was constructed from sulfide standards. Blank gels were found to be below the detection limit of the ISE. Distillation recoveries, as determined by a sulfide spike, were between 85 and 105%. The calibration curves for discrete sampling of profundal water were linear ($R^2 = 0.99$).

Laboratory study pore water from Mackall Cove, Maryland

Bulk sediments from Mackall Cove, located within the Patuxent River estuary in Maryland, were collected in August of 2003 and brought back to CBL. Fresh 10 μm filtered bay water was added to the existing overlying water, and the sediment was allowed to sit for four weeks. The overlying water was siphoned off and the bulk sediment was placed in an anaerobic glove box where it was homogenized by mixing with a clean plastic spatula. During this study we were not concerned with the actual vertical profiles of dissolved sulfide. Our primary interest was obtaining a homogeneous pore water sample that could be sampled by various techniques. Approximately 350 g of homogenized sediment was weighed into 250 mL Teflon centrifuge bottles. The pore water was extracted by centrifugation at 3000 rpm for 30 minutes. The centrifuge bottles were then returned to the glove bag where the pore water was collected and filtered through 0.45 μm cellulose nitrate Nalgene disposable filter units (Nalge Nunc International). All filtered pore water was collected in a 1 L acid cleaned borosilicate glass container and mixed by shaking for five minutes. At this point, it was assumed that the filtered pore water was homogenous in nature. A volume of 300 mL of pore water was then placed into two 500 mL VOC containers.

After allowing the pore water to equilibrate in the VOC containers for 30 minutes, 5 mL grab samples for sulfide by methylene blue and SAOB/ISE analysis were collected from each container prior to DGT probe deployment. Analysis by SAOB/ISE was performed one hour later. It was assumed that most of the adsorptive losses of sulfide to the container walls would occur during the 30 minutes prior to DGT deployment. The methylene blue samples were fixed by the addition of the

sample aliquot to the mixed diamine reagents (MDR). Stock solutions for the MDR were prepared according to the Cline method for reactive sulfides in the 3-40 $\mu molL^{-1}$ range (Cline 1969). In brief, 0.5 g of N,N-dimethyl-p-phenylene-diamine (Sigma) and 0.75 g of $FeCl_3 \cdot H_2O$ (J.T. Baker) were dissolved in 125 mL of 50% by volume HCl (J.T. Baker). Methylene blue stock solutions were stored under darkness in a refrigerator for up to a month. In addition, several 5 mL samples were collected and added to 5 mL of a 5% by weight zinc acetate. These samples were centrifuged for 15 minutes at 1000 rpm. The supernatant was decanted off, and 5 mL of deionized water and the MDR were added to the remaining precipitate. Standards for methylene blue analysis were prepared from aliquots of the primary solution and were diluted to 60 mL with deoxygenated, deionized water. Standards and samples were allowed to set for 30 minutes to allow for proper color development. The absorbance of the methylene blue samples and standards was measured at 670 nm using a 96 well plate cuvette with a 1 cm path length on a SPECTRAmax PLUS^{384} microplate spectrophotometer (Molecular Devices).

Two DGT probes utilizing 0.4 mm and 0.8 mm diffusive layers were added to each VOC container. No filter membranes were used with these probes. The solutions were mixed inside the glove box on an orbital shaker table at 85 rpm. The temperature inside the glove box was 25 °C. After 4 hours the DGT probes were removed from the pore water solution, rinsed with deionized water, and disassembled. Binding layers were placed in clean plastic bags until elution and analysis. Elution and analysis of sulfide were performed according to the DGT-ISE procedures discussed in Chapter 2. The calibration curve for sulfide standards was linear (R^2 =

0.99). The blank gel mass was 0.2 nmol $[(S\text{-}II)]_{measured}$ and this was subtracted from the sample gels. Distillation recoveries as determined by a spike were 83%. The calibration curves for discrete sampling of pore water were linear ($R^2 = 0.99$). The calibration curve for sulfide standards using methylene blue was linear ($R^2 = 0.97$). All methylene blue sulfide samples were blank corrected.

Zinc sulfide clusters laboratory experiment

Evidence for the existence of soluble ZnS clusters as intermediates during zinc sulfide mineral formation has been recently demonstrated (Luther et al. 1999). In this study, using zinc and sulfide reactants not exceeding 15 $\mu molL^{-1}$, the authors proposed ZnS, Zn_3S_3, and $Zn_4S_6^{4-}$ as potential soluble intermediate species and provided electrochemical and UV-VIS spectroscopic data for their existence. The synthesis of zinc sulfide clusters for use in laboratory toxicological studies has also been described (Bowles et al. 2002). Briefly, on two separate occasions, approximately 2.7 μmol of zinc nitrate ($Zn(NO_3)_2 \cdot 6H_2O$) and 2.5 μmol $Na_2S \cdot 9H_2O$ were added to deionized, deoxygenated water under a nitrogen environment to give a final ZnS cluster concentration of approximately 10 $\mu molL^{-1}$. Acid cleaned borosilicate glassware was used in all ZnS cluster preparations. The prepared solutions of ZnS clusters were allowed to age in the presence of air for 2 days. For clarification, the individual experiments will be referred to as ZnS I and ZnS II. In their study, Bowles et al. (2002) allowed their ZnS clusters to age for a minimum of 3 days because their toxicological studies mandated that no unreacted sulfide ligands remained in solution. Free bisulfide and sulfide may serve as potential ligands for free metal ions and lead to incorrect conclusions for their toxicological studies. Final

synthesized ZnS cluster concentrations for ZnS I and ZnS II were approximately 10 and 11 $\mu molL^{-1}$ZnS. During ZnS I, 300 mL of the aged ZnS solution was placed into a VOC container inside the nitrogen filled glove box for the duration of each experiment. During ZnS II, 300 mL aliquots of the aged ZnS cluster solution were dispensed into three VOC containers. In each experiment, the containers and solutions were allowed to equilibrate for 15 minutes. It was assumed that equilibration between the ZnS solution and container occurred within this time span.

Sample aliquots (5mL) from the 10 and 11 $\mu molL^{-1}$ solutions were removed for analysis by conventional methylene blue (Cline 1969) and SAOB/ISE prior to the addition of DGT probes. Samples were fixed for sulfide analysis by the addition of the mixed diamine reagent (MDR) or SAOB respectively. Sample analysis by SAOB/ISE was performed two hours later. Samples for analysis by spectrophotometry sat for approximately 30 minutes to allow for proper color development, and the methylene blue measurements were once again taken at 670 nm using a 96 well plate cuvette with a 1 cm path length on a SPECTRAmax PLUS^{384} microplate spectrophotometer. DGT probes were deployed in duplicate with each probe having a different thickness of diffusive layer. A total of eight DGT probes were deployed into the ZnS cluster solutions, two DGT probes into the 10 $\mu molL^{-1}$ solution for ZnS I and six DGT probes (two per VOC container) into the 11 $\mu molL^{-1}$ solution for ZnS II. The probes were deployed for 4 hours at 85 rpm on an orbital shaker table. Probes were then removed, rinsed with deionized water, and disassembled. Binding gels were stored in clean plastics bags until elution by purge and trap. Sulfide elution and subsequent DGT-ISE analysis are described in detail in

Chapter 2. The temperature within the glove box was 25°C. The ISE calibration curves generated for ZnS I and ZnS II were linear ($R^2 = 0.99$). The methylene blue calibration curves for ZnS I and ZnS II were also linear ($R^2 = 0.99$). All methylene blue samples absorbance values were blank corrected.

Thioglycolate laboratory experiment

As previously stated, the primary reasoning behind this experiment was to determine the effects that a highly alkaline solution, such as SAOB, has on an organic compound containing a thiol group, and whether free sulfide was released as a result. A secondary objective was to monitor the effects of the MDR on thioglycolate. The literature states that thiols should not react with the MDR (Adams and Kramer 1999; Tang and Santschi 2000; Mylon and Benoit 2001). On two separate occasions an experiment testing the effects of SAOB on thioglycolate was performed using several thioglycolate solution concentrations. For clarification, the individual experiments will be referred to as Thiol I and Thiol II. Thioglycolate stock solutions were made from 99% sodium thioglycolate salt (Sigma), and stock solutions of 13 (Thiol I) and 19 (Thiol II) $mmolL^{-1}$ were prepared. Working solutions were made from each stock as needed. Thiol I experiment consisted of the addition of numerous 5 mL aliquots of a 382 $\mu molL^{-1}$ thioglycolate working solution to 5 mL of SAOB. These solutions were monitored at 0, 3, and 24 hour time points. Thiol II experiment was similar except a 1000 $\mu molL^{-1}$ thioglycolate working solution was prepared and the sample aliquots were monitored at 0, 3, 18, and 24 hour time points. Dissolved sulfide at each time point was measured by SAOB/ISE. Because of the potential for thioglycolate degradation by reactions other than those catalyzed by added chemical

agents (i.e., oxidation by O_2), a diluted sample made from the 13 $mmolL^{-1}$ stock solution was monitored by iodimetry at the completion of the 24 hour experiment. The recovery was determined to be 99%. Furthermore, all thioglycolate stock solutions and analytical solutions were stored inside a nitrogen filled glove box as precautionary measures to reduce losses due to oxidation.

An additional experiment involving individual solutions of thioglycolate and Na_2S and their subsequent mixture was also performed. For clarification, this experiment will be referred to as Thiol III. In Thiol III, three working solutions were prepared from concentrated stock solutions, 84 $\mu molL^{-1}$ thioglycolate, 176 $\mu molL^{-1}$ Na_2S, and a mixture of 84 $\mu molL^{-1}$ thioglycolate and 176 $\mu molL^{-1}$ Na_2S, respectively. The individual solutions and the mixture of thioglycolate and Na_2S were analyzed by methylene blue. For experiments Thiol I-III, the SAOB/ISE calibration curve for known sulfide standards was linear ($R^2 = 0.99$). A new calibration curve was created at every time point in order to avoid problems associated with drift. The calibration standards were stored inside the nitrogen filled glove box between time points in order to reduce losses due to oxidation. All methylene blue samples absorbance values were blank corrected. The methylene blue calibration curve for sulfide standards was linear ($R^2 = 0.99$).

Results and discussion

Pavin Lake, France

Estimated in situ sulfide concentrations by DGT-ISE probe ranged from 8.3 $nmolL^{-1}$ to 6.7 $\mu molL^{-1}$over a 7 m span near the lake's chemocline. Estimated in situ sulfide concentrations from all depths are shown in Table 2. The largest sulfide peak

as estimated by DGT-ISE occurred at a depth of 64 m. Historically, filtered sulfide concentrations within the lake peak between 62.5 and 67.5 m depth with maximum concentrations around 20 $\mu molL^{-1}$(Michard et al. 1994; Viollier et al. 1995). Because the sulfide profile by DGT-ISE did not span the entire lake, a complete method comparison of the profile is not possible. Only a few of the DGT depths were in close proximity to the depths of the historical data. However, it appears that the historical ex situ concentrations of sulfide by methylene blue are roughly 2-4 times larger than the in situ concentrations by DGT-ISE at corresponding depths. Dissolved iron data suggests that amorphous iron sulfides may be present and dominate sulfide speciation. It has been suggested that the restrictive nature of DGT diffusive gel does not permit dissolved solids (i.e., colloidal material) from reaching the binding gel (Zhang and Davison 1995; Zhang and Davison 2001). It has also been suggested that metal sulfides may not cross the diffusive layer (Devries and Wang 2003), and the diffusion coefficients of colloidal metal sulfides (i.e., FeS) may be sufficiently small and will not contribute a significant fraction to the overall total sulfide measurement (Teasdale et al. 1999). But because of significant temporal differences in the measurements by the two differing methods, reasons for the inconsistencies in concentration can only be speculative.

Table 2. Historical $[(S\text{-}II)]_t$ by methylene blue and estimated [(S-II)] by DGT-ISE near chemocline in Pavin Lake, France.

Sample depth (m)	Concentration of DGT-ISE $[(S\text{-}II)]_{measured}$ This study ($\mu molL^{-1}$)	Sample depth (m)	Concentration of filtered $[(S\text{-}II)]_t$ Michard et al. ($\mu molL^{-1}$)	Concentration of filtered $[(S\text{-}II)]_t$ Viollier et al. ($\mu molL^{-1}$)
57.0	0.02	55.0	-	-
57.5	0.02 ± 0.01	57.5	-	-
58.0	0.01	60.0	0.6	5.0
58.5	0.04	62.5	13.6	27.3
59.0	0.44	65.0	14.2	22.4
59.5	0.74	70.0	14.5	21.6
60.0	1.57 ± 0.35	75.0	15.2	20.2
61.0	4.01	80.0	14.2	-
64.0	6.70 ± 0.37	85.0	12.7	20.8

Lake Lariat, Calvert County, Maryland

The average in situ sulfide concentration by DGT-ISE in the bottom water was 1.46 ± 0.46 $\mu molL^{-1}$ (n=11). The average ex situ sulfide concentration for filtered bottom water as determined by SAOB/ISE was 3.91 ± 0.37 $\mu molL^{-1}$ (n=12). A single factor ANOVA reveals that the measured concentrations of sulfide by DGT and ISE are significantly different ($p<0.05$). The ex situ SAOB/ISE measurements are approximately 2-4 times larger than those concentrations obtained by DGT-ISE. The average sulfide concentration in unfiltered bottom water as determined by SAOB/ISE was 12.43 ± 0.45 (n=4). Unfiltered lake water was analyzed for measurable sulfide concentrations in order to examine the extent to which sulfide may be released from solid phases. The large difference between filtered and unfiltered water suggests that significant amounts of sulfide associated with solids and dissolved solids is extractable into SAOB. Therefore colloidal material derived from the disaggregation of larger solids may significantly contribute to the total filterable

sulfide concentrations measured by methylene blue or SAOB/ISE. Measured sulfide concentrations for all filtered bottom water samples and those estimated by DGT-ISE can be found in Figure 8. The three size classes of filter show no apparent differences in the concentration of measured sulfide. However, the lower estimated sulfide concentrations as determined by DGT suggest that colloidal sulfides, in sufficient amounts, may be present and easily pass through conventional 0.45 μm filters. The small pore sizes characteristic of a diffusive gel may restrict materials containing significant amounts of sulfide in the form of metal colloids or perhaps sulfide incorporated into large organic molecules as thiol groups.

Finally, this lake is a bottom release lake, and the associated bottom water flows most likely negate any sulfide concentration differences originating from DBL effects. Nonetheless, utilizing eq 8 and plotting the reciprocal of the measured mass of sulfide versus the diffusive layer thickness for eleven DGT probes, results in an estimated sulfide concentration of 1.76 $\mu molL^{-1}$and an estimated DBL of 154 μm (Figure 9). A DBL of this thickness may cause approximately a 16 to 39% bias in the measured flux of sulfide to the binding gel and a similar uncertainty in the estimated bulk solution concentration. However, this graphically extrapolated sulfide concentration as determined by considering any DBL effects is also in good agreement with the mean sulfide concentration ($1.46 \pm 0.46\ \mu molL^{-1}$) of the eleven individual DGT-ISE measurements shown in Figure 8.

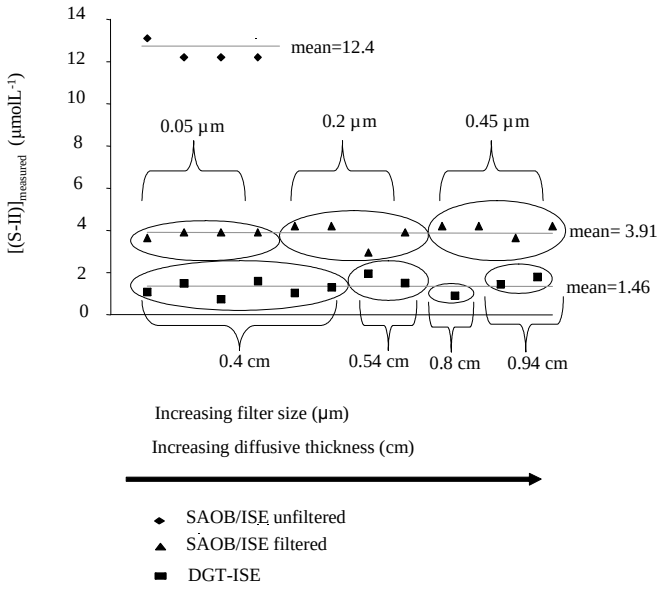

Figure 8. DGT-ISE estimated sulfide concentrations and various filtered sulfide concentrations determined by SAOB/ISE in Lake Lariat bottom waters.

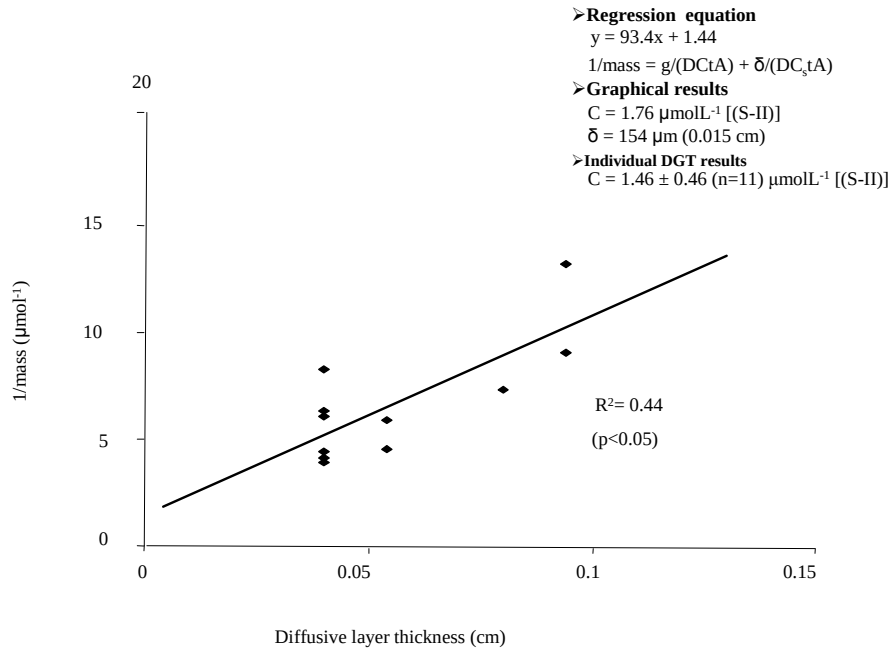

Figure 9. Graphical determination of DBL and DGT-ISE estimated [(S-II)] by reciprocal plot.

Lake 658, ELA

Profiles of the estimated sulfide concentration by DGT-ISE and filtered samples by SAOB/ISE are in Figure 10. Filtered sulfide concentrations are an average of those measured by SAOB/ISE on the days of DGT probe deployment and retrieval. The error bars represent one standard deviation from the mean for each measurement. The DGT-ISE sulfide concentrations at all heights from the sediment interface, excluding the 20 cm height, were calculated using 0.4 and 0.8 mm diffusive gels. The sulfide concentration at the 20 cm height was only calculated from one DGT probe containing a 0.8 mm diffusive gel. The duplicate probe from the 20 cm height was lost during the distillation process, perhaps the result of either a bad trap or too high of N_2 flow. The sulfide concentrations represented by the dashed line in Figure 10 were calculated incorporating a DBL thickness of 270 μm. It was believed that the 40 and 80 cm heights were not influenced by a DBL effect. The DBL thickness of 270 μm was produced by utilizing eq 9 and replicate probe measurements taken with probes containing two differing diffusive layer thicknesses near the sediment and water interface (at the 5 and 10 cm heights). Not incorporating a DBL thickness of 270 μm into the individual sulfide estimates using eq 6 would produce a 33 to 60% error in the estimated sulfide concentration depending on the existing diffusive layer thickness. The thinner diffusive layer is affected to a greater degree. However in this situation, the extrapolated DBL was based upon only two data points (i.e., a DGT probe containing 0.4 and 0.8 mm diffusive gel at each sampling height), and the actual numerical values and final DBL influences are questionable. Two DGT probes containing different diffusive thicknesses may be

used to calculate a DBL, but the use of three or more DGT measurements with differing diffusive layers is more appropriate for DBL calculations (Zhang et al. 1998). The large error bars at 40 and 80 cm heights may reflect an additional DGT probe problem. One possible explanation for the variability in DGT-ISE sulfide concentrations at the 40 and 80 cm heights may be insufficient deoxygenation of the probes or the presence of a gas bubble between the diffusive and binding layers. However, all of DGT probes were deoxygenated for 5 days. The formation and trapping of a gas bubble between layers during deoxygenation is a more realistic scenario. A gas bubble would effectively interfere with the diffusion of a dissolved species through the DGT probe. Theoretically, the reduced ability of a dissolved species to pass through a diffusive layer would result in a lower estimated concentration.

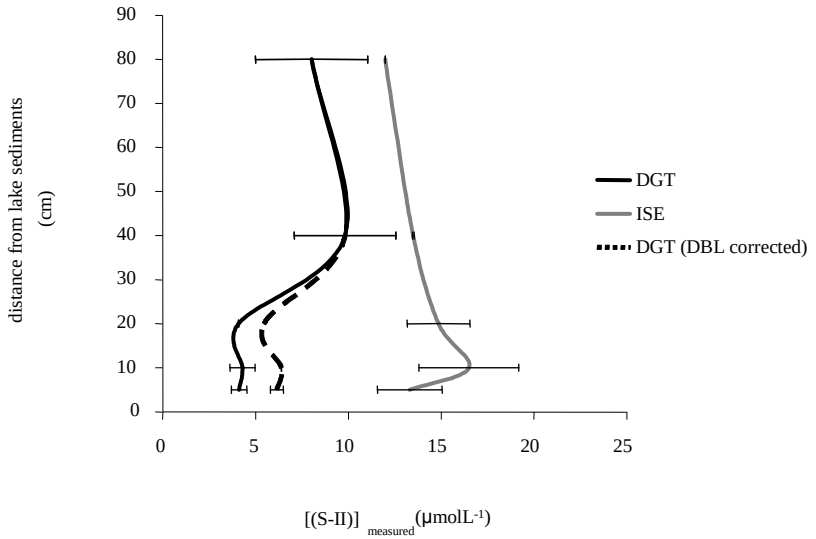

Figure 10. Sulfide profiles estimated by DGT-ISE and SAOB/ISE for the profundal of Lake 658.

Laboratory study using pore water from Mackall Cove, Maryland

The average sulfide concentrations as measured by DGT-ISE for containers 1 and 2 were 1.21 ± 0.32 (n=2) and 1.16 ± 0.27 (n=2) $\mu molL^{-1}$ respectively. Because the results from the validation studies in Chapter 2 suggest that solutions stirred at 85 rpm on an orbital shaker table were well mixed (i.e., negligible DBL), a DBL thickness was not calculated for this experiment. Filtered sulfide concentrations by methylene blue and SAOB/ISE analysis can be seen in Figure 11. The error bars represent one standard deviation from the mean. Filtered sulfide concentrations determined by methylene blue both with and without pre-concentrating by zinc acetate are nearly six times as large as those determined by DGT-ISE. This could be interpreted as metal sulfides being the dominant pool of sulfide in the extracted pore water. However, filtered sulfide concentrations as determined by SAOB/ISE are not different than those determined by DGT-ISE as found in the previous studies. A plausible explanation is that very little unbound free sulfide existed within the pore water (values were lower than found in previous studies), and the majority of the sulfide was present as dissolved solids, such as colloidal metal sulfides, which could not diffusive through the polyacrylamide layer. Kinetically, the metal sulfide and MDR complexation is relatively quick (i.e., minutes), and perhaps the rate at which SAOB reacts with the metal sulfide complexes is much slower (i.e., hours). In this instance, analysis of the SAOB preserved samples occurred about one hour after collection. In the other studies, the time between collection and analysis was somewhat longer (i.e., four to five hours later). The metal sulfide complexes within the extracted pore water may be coated with a layer of NOM which may slow the

release of sulfide by SAOB. Re-analysis of the pore waters by SAOB/ISE at various times up to 24 hours after sampling indicated that sulfide had been released from colloids or dissolved metal sulfides which passed through the filter (Figure 12). This substantiates the claim of slower kinetics for release of sulfide from complexes in the presence of SAOB. Error bars represent one standard deviation from the mean. Statistical analysis by ANOVA shows that differences in sulfide concentration between the initial sampling and 24 hour sampling is significant ($p<0.5$). This suggests that the chemical composition of SAOB may have the ability to extract sulfide from dissolved metal sulfide complexes or perhaps organic sulfides found in natural waters, as hypothesized in eqs 11 and 12, thus allowing it to be detected by the ISE. A chemical speciation model for dissolved ZnS clusters supports the claim of released dissolved sulfide from dissolved metal sulfide complexes. The speciation model will be discussed in the following section.

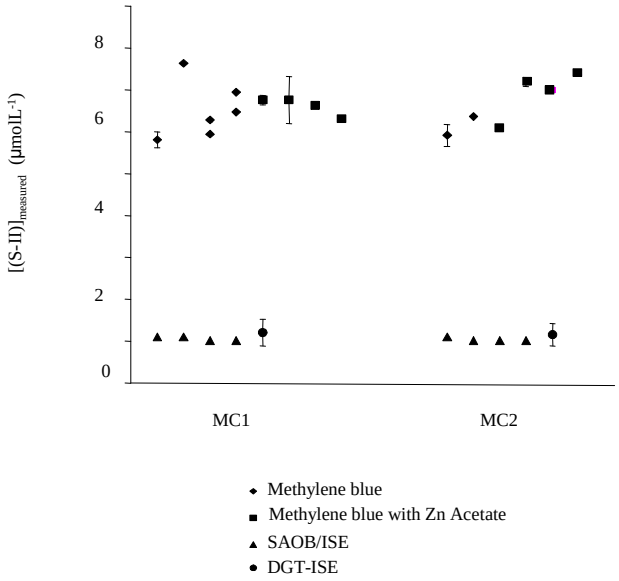

Figure 11. Comparison of sulfide concentrations as determined by various methods for extracted sediment pore water from Mackall Cove, MD. Container 1 (C1) and container 2 (C2) are duplicate samples of extracted pore water.

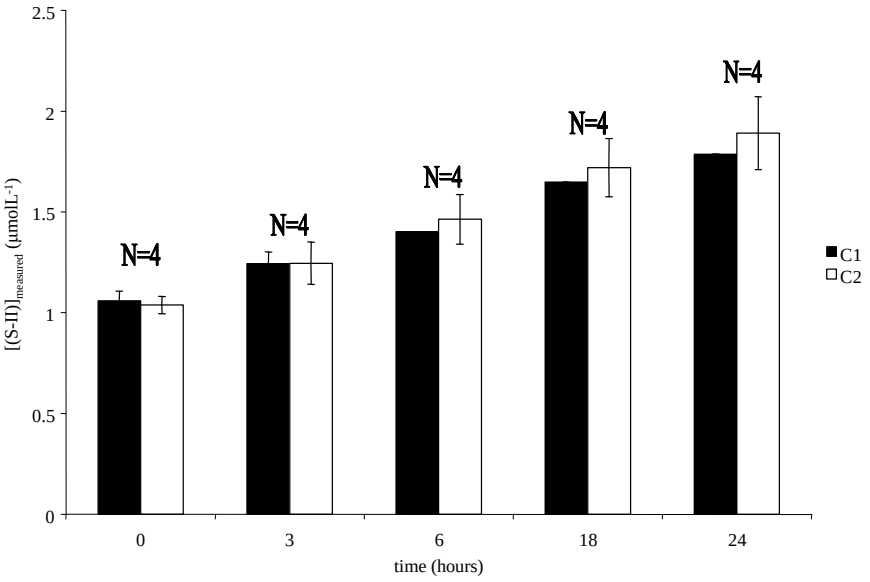

Figure 12. Sulfide as detected by SAOB/ISE in Mackall Cove extracted sediment pore water over 24 hours. Container 1 (C1) and container 2 (C2) are duplicates samples of extracted pore water.

ZnS clusters laboratory experiment

The results from ZnS I experiment (10 $\mu molL^{-1}$ synthesized ZnS clusters) confirmed that methylene blue analysis accurately estimates the concentration of the synthesized ZnS as 9.86 ± 1.10 $\mu molL^{-1}$ (n = 8), as expected. Analysis by SAOB/ISE, which is not supposed to detect metal sulfides, yielded 3.61 ± 0.37 $\mu molL^{-1}$ (n = 8). This is approximately 30% of the synthesized cluster concentration (Figure 13). Measured synthesized ZnS concentrations as estimated by DGT-ISE were 0.041 ± 0.058 $\mu molL^{-1}$ (n = 2). Results from the ZnS II experiment (11 $\mu molL^{-1}$ synthesized ZnS clusters) also demonstrate the ability of the SAOB/ISE to detect a fraction of the synthesized metal sulfide clusters. Methylene blue analysis reveals a synthesized metal sulfide concentration of 10.63 ± 1.10 $\mu molL^{-1}$ (n = 7). Analysis by SAOB/ISE yields a dissolved sulfide concentration of approximately 4.82 ± 0.41 $\mu molL^{-1}$ (n = 6). The concentration of synthesized ZnS as measured by SAOB/ISE is nearly half of that determined by methylene blue analysis (Figure 14). Synthesized ZnS concentrations as estimated for DGT-ISE for ZnS II are 0.11 ± 0.14 $\mu molL^{-1}$ (n = 6). The results from ZnS I and ZnS II suggest that dissolved ZnS species and potentially other dissolved metal sulfide complexes are not stable in SAOB.

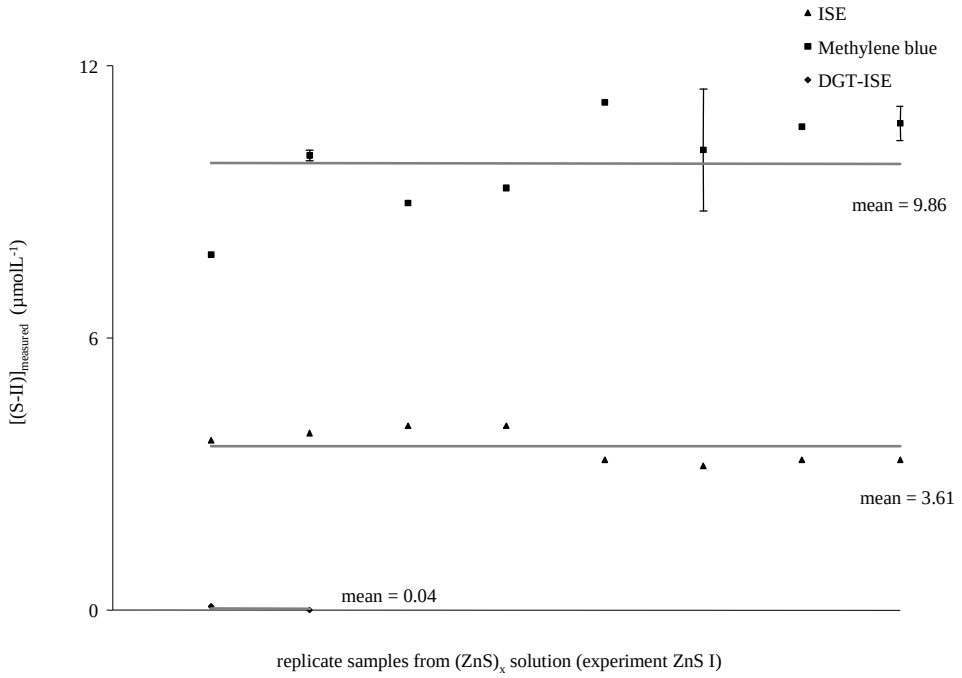

Figure 13. Concentrations of synthesized 10.0 µmolL^{-1}ZnS solution (experiment ZnS I) as detected by SAOB/ISE, methylene blue, and DGT-ISE.

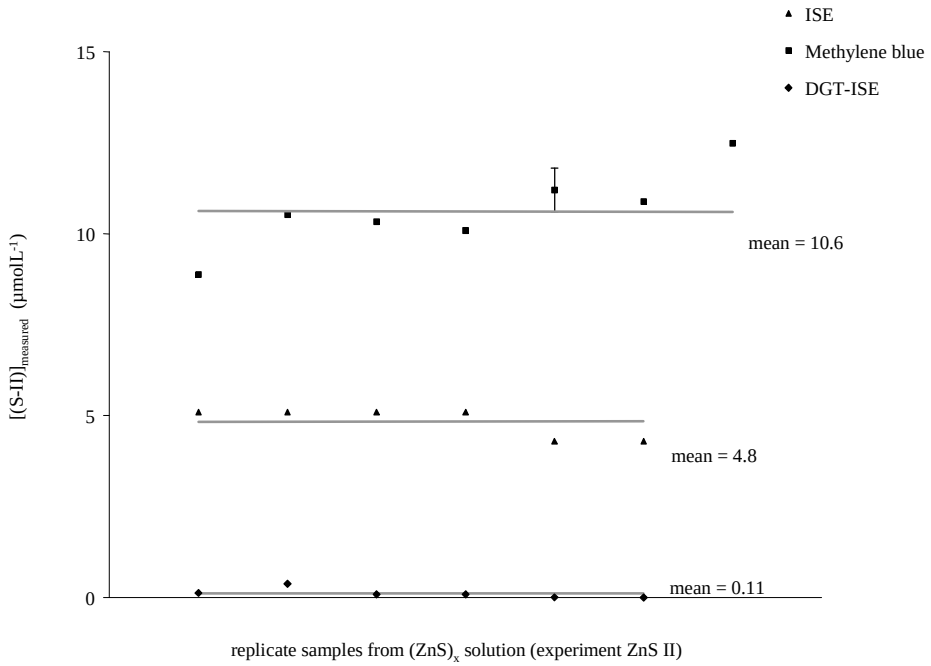

Figure 14. Concentrations of synthesized 11.0 µmolL^{-1}ZnS solution (experiment ZnS II) as detected by SAOB/ISE, methylene blue, and DGT-ISE.

Chemical speciation calculations using MINEQL+ (Version 4.5, Environmental Research Software), performed under conditions similar to those found in SAOB (i.e., pH $\geq$ 12 and 0.2 molL^{-1} EDTA), support the idea that the excessive EDTA is able to competitively bind the zinc from the clusters. The resulting break up of the dissolved ZnS clusters and the formation of various zinc and EDTA complexes increases the dissolved sulfide concentration. The calculated dissolved sulfide concentrations from the model are nearly equal to those of the initial ZnS clusters. This means that nearly 100% of the dissolved ZnS clusters dissociated. The results of this chemical equilibrium model support the hypothesis that the chemical properties of SAOB can liberate sulfide from metal sulfide complexes which in turn can be detected by an ISE.

Our working hypothesis states that only simple dissolved inorganic sulfide complexes are measured by DGT-ISE. It is possible that labile metal sulfide complexes or clusters could react with the AgI binding layer if they are able to pass through the diffuse layer. Most metal sulfide complexes classified as MBRS should react with the binding layer. The DGT-ISE estimations from experiments ZnS I and ZnS II, using the synthesized clusters, were 0.04 and 0.11 μmolL^{-1} respectively. This demonstrates that negligible amounts of the synthesized ZnS clusters, which are classified as MBRS, reacted with the AgI binding gel and suggests that size exclusion of these compounds due to the small gel pore size is the most feasible explanation for their lack of detection. However, this experiment should be revisited. Bowles et al. (2002) found that filtering their synthesized ZnS cluster resulted in some losses due to adsorption and not size exclusion. In future experiments, they were able to overcome

the adsorptive losses by manufacturing their metal sulfide clusters in solutions of NOM (Bowles et al. 2003). Others have also found that coating metal sulfides with an organic agent is an effective way of terminating the polymerization or passivating the reactive surface of a metal sulfide cluster (Herron et al. 1990; Kortan et al. 1990). Therefore, it cannot be completely concluded that size exclusion is the only mechanism behind the lower DGT-ISE estimates for ZnS I and ZnS II, and that adsorption may also play an important role. Nonetheless, these results are similar to a recent DGT and dissolved metal sulfide uptake study. Using DGT sediment probes outfitted with a combination of AgI and chelex binding layers, Motelica-Heino et al. (2003) demonstrated that dissolved metal sulfides were negligible. They proposed that if a dissolved metal sulfide species reacts with the AgI binding gel, it must be considered labile, and it should react with the chelex binding gel and be included as a fraction of the total metals estimated by DGT. Their DGT estimated metal concentrations in their chelex binding gel were much lower than their DGT estimated sulfide concentrations therefore suggesting metal sulfide complexes were negligible.

Thioglycolate laboratory experiment

Measurable sulfide concentrations in sample aliquots (5 mL) taken from each thioglycolate working solution in experiments Thiol I and II increased by factors of 5 and 50 in detected sulfide concentration after 24 hours as compared to the initial solution concentration (Figure 15). Over the 24 hour time period, nearly 10% of the original thioglycolate solution is converted into a detectable product. A titration of diluted samples from the original thioglycolate stock solution reveals that no significant amount of non-SAOB related degradation had occurred (Figure 16). This

demonstrates that the SAOB reagent must be assisting or catalyzing the degradation of thioglycolate into products detectable by the ISE.

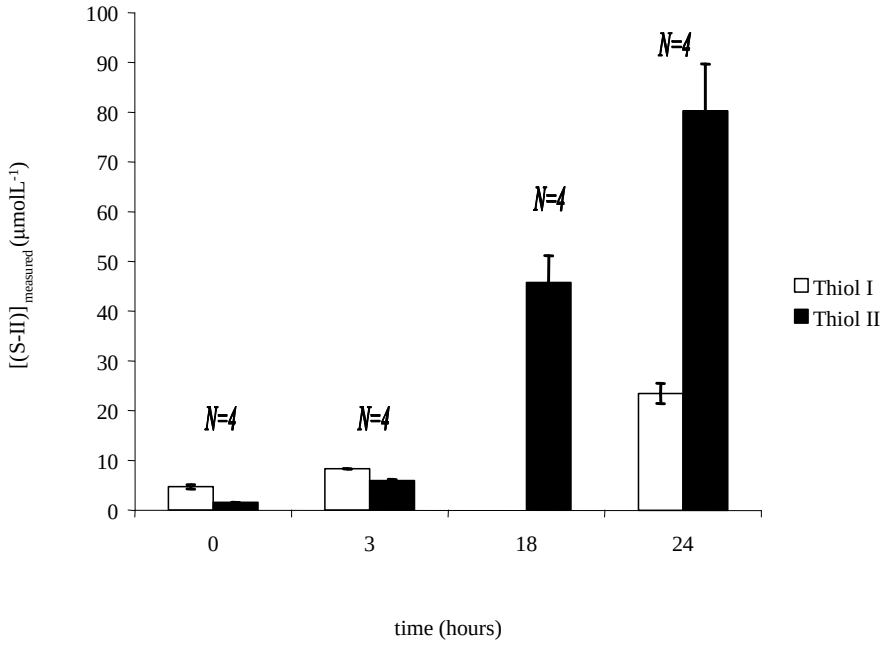

Figure 15. SAOB/ISE concentrations of sulfide derived from the hydrolysis of thioglycolate over 24 hours.

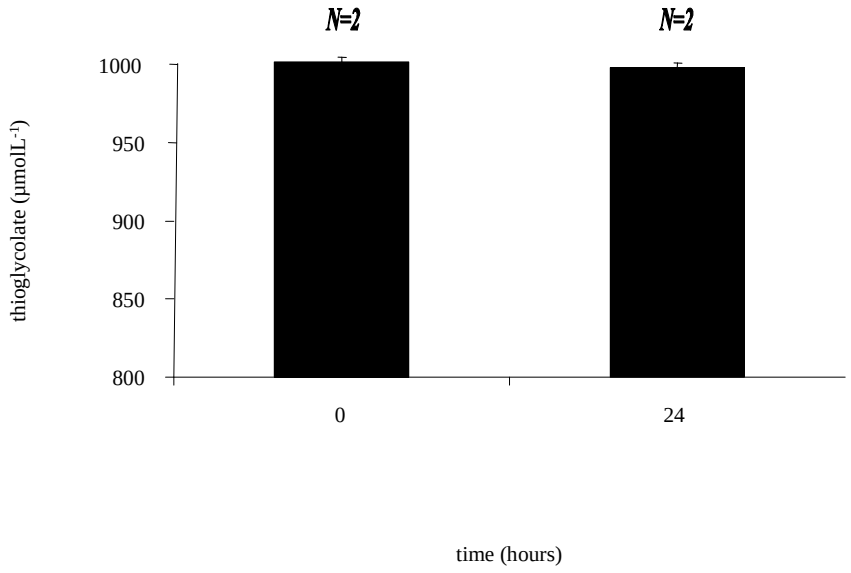

Figure 16. Percent recovery of thioglycolate working solutions over 24 hours by means of an iodimetric titration.

Results of the Thiol III experiment show that thiols, or thioglycolate in our case, are not detected by methylene blue. This is in agreement with previous studies (Adams and Kramer 1999; Tang and Santschi 2000; Mylon and Benoit 2001). Figure 17 shows no significant difference between the sulfide concentration as detected by methylene blue for the Na_2S solution and the solution mixture of Na_2S and thioglycolate. Measured sulfide concentrations for Na_2S, 161 ±1 $\mu molL^{-1}$ (n = 5), and the Na_2S and thioglycolate mixture, 159 ± 3 $\mu molL^{-1}$ (n = 4), are lower than the initial working solution concentration of 176 $\mu molL^{-1}$, but the small losses are most likely attributed to oxidation and adsorption.

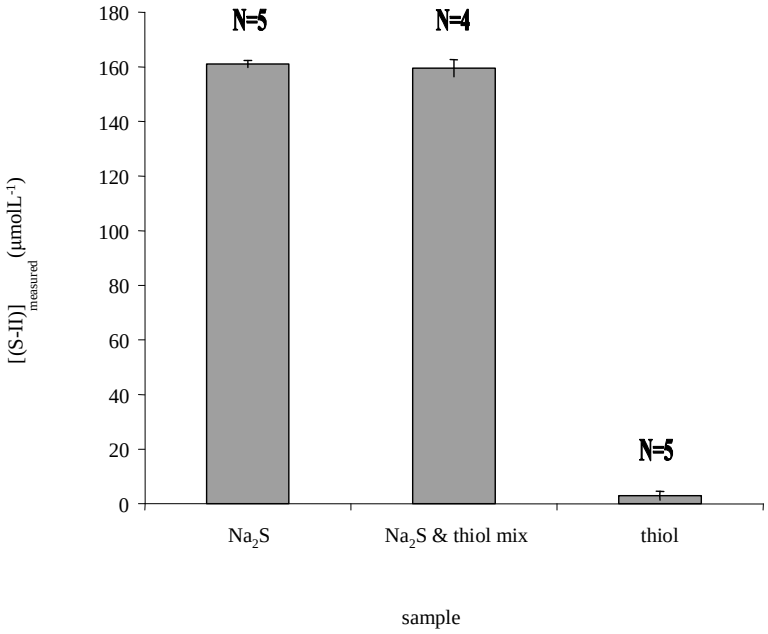

Figure 17. Thiol III experimental results demonstrating that thiols are not a fraction of the measurable sulfide as detected by methylene blue.

Summary of field and experimental results

The methylene blue method measured larger sulfide concentrations than DGT-ISE for Pavin Lake water and Mackall Cove pore water. Methylene blue analysis also detected synthesized ZnS clusters but did not detect a thiol containing compound, supporting the results of other researchers. Sulfide concentrations measured by SAOB/ISE were greater than those measured by DGT-ISE in Lake Lariat bottom water, L658 bottom water, and synthesized ZnS clusters. Using SAOB/ISE, it was determined that sulfide concentrations in Mackall Cove pore water samples and a thiol containing solution increased with time. The thiol hydrolysis reaction was kinetically slower than the competitive binding reaction involving EDTA and the ZnS clusters.

Conclusion

Estimated sulfide concentrations as determined by DGT-ISE for all study lakes were consistently lower than 0.45 μm filtered samples determined by either SAOB/ISE or methylene blue. This strongly suggests that filtered samples analyzed by SAOB/ISE and ethylene blue may overestimate dissolved sulfide concentration and should be in some situations referred to as total sulfide. Only the extracted pore water experiment results showed agreement between SAOB/ISE and DGT-ISE. It was suspected that the sulfide speciation in this sediment from Mackall Cove was dominated by metal sulfide complexes due to the lack of new sources of nutrients and sulfate. The sediment was stored in buckets for a few weeks with no significant flow (i.e., no driving force for pumping or exchange of DOC and sulfate). Yet after 24 hours of storage in SAOB, the sulfide concentrations of these samples increased, by nearly 90%, demonstrating that the highly alkaline nature of SAOB and the presence of excess EDTA have an effect on metal and organic sulfides. The initial agreement between the measured sulfide concentrations by SAOB/ISE and DGT-ISE in Mackall Cove pore water, followed by a significant difference in measured sulfide concentrations after 24 hours, suggests that NOM coatings may play a role in slowing down the rate of reaction between metal sulfides and organic sulfides present and the SAOB reagent.

The laboratory results generated from the interactions of a synthesized metal sulfide complex, a thiol, and the SAOB buffering agent also imply there is a release of sulfide which is detected by ISE from both inorganic and organic molecules containing reduced sulfur. Again, this apparent liberation of dissolved sulfide

becomes greater with time. The reaction rate between the synthesized ZnS clusters and the SAOB is faster than that of the metal sulfide complexes in the Mackall Cove pore water and SAOB. These reaction rates may differ due to the lack of a NOM coating on the synthesized ZnS clusters. The metal sulfides within the extracted pore water were most likely associated with NOM present within the pore water. The field and laboratory findings suggest that ethylenediaminetetraacetic acid (0.2 $molL^{-1}$) in SAOB is able to outcompete and displace the sulfide ligands from metal sulfide complexes due to its high concentration, and SAOB is able to break sulfur and carbon bonds present in thiols resulting in free sulfide which is then detected by the ISE. The use of highly alkaline solutions is also a common method of initiating hydrolysis reactions (Morrison and Boyd 1987). Chemical speciation calculations performed under conditions similar to those found in SAOB support the proposed idea of metal sulfide complexes and EDTA interacting via a competitive ligand exchange reaction. This suggests that analysis of environmental samples for concentrations of uncomplexed or free sulfide by means of SAOB/ISE should be conducted promptly after collection. In many instances, the immediate analysis of environmental samples may not occur because of transport time and results will be interpreted as being higher in concentration. There were also significant differences between measured sulfide concentrations by methylene blue and SAOB/ISE for samples collected from the synthesized ZnS cluster experiments and the Mackall Cove pore water. Measured sulfide concentrations by methylene blue were nearly 3-6 times greater. This may be due to the rate of reaction between the MDR and metal sulfide complexes being greater than that of SAOB and metal sulfide complexes. It should be noted that the

components of the MDR are also dissolved in 6 molL^{-1}HCL, and this acidic medium promotes the release of sulfide from certain complexes (i.e., similar to defined AVS fraction). Overall, the laboratory and field results support the idea that the DGT-ISE estimation of dissolved sulfide may be a closer reflection of the uncomplexed or free sulfide concentration.

Chapter 4: Conclusions and recommendations

The first objective of this thesis was to determine the feasibility of using an ISE to measure the sulfide captured by a DGT device. The successful coupling of ISE to DGT has been demonstrated through a series of validation tests which were addressed in detail in Chapter 2. This successful demonstration provides an alternative means, by which sulfide captured by DGT probes, can be analyzed. This coupling of an ISE to DGT has advantages over DGT-CID and DGT-methylene blue analysis. Drawbacks to these other methods include: (1) the potential to exceed CID gray scale standards and (2) the time consuming nature of methylene blue analysis. The dynamic linear range of the potentiometric method is useful in environments and situations where sulfide production is high, yet the ability of DGT to pre-concentrate sulfide gives the analyst the necessary sensitivity to work in environments of low sulfide concentration ($nmolL^{-1}$). The detection limit (DL = 3 x SD) from this study is 0.104 $\mu molL^{-1}$ (n = 18) for a 24 hour deployment period and can be significantly lowered by extending the deployment time or utilizing a thinner diffusive layer.

The second objective of this thesis was to distinguish between different pools of sulfide species in the environment. The abovementioned validation studies were conducted under controlled conditions which included well mixed solutions derived solely from Na_2S. However, in natural environments, dissolved sulfide is present and may be incorporated into many dissolved organic and inorganic complexes. The filtration of natural waters using 0.45 μm filters can result in the passage of DOC and colloids into analytical samples. Thus if the analytical method used is capable of measuring the resulting sulfide complexes, the dissolved sulfide concentration will be

overestimated. Because DGT probes utilize diffusive layers containing small pore sizes (2-5 nm), it was hypothesized that DGT probes provide a measure of uncomplexed sulfide and perhaps a more accurate assessment of the concentration of truly dissolved inorganic sulfide. Differences in sulfide concentrations measured by DGT-ISE and other methods, where conventional 0.45 μm filtration precedes the analytical measurement, existed in samples from field sites located in Pavin Lake, Lake 658, Lake Lariat, and the Patuxent River estuary. Sulfide concentrations as determined by SAOB/ISE and methylene blue were 2-4 times larger than those determined by DGT-ISE. This suggests that DGT probes are able to effectively exclude many larger dissolved compounds (i.e., metal sulfides, organic sulfides, and metal sulfide associated with DOM) whereas conventional 0.45 μm filtration may contribute to overestimates in the concentration of dissolved sulfide measured by other means. Laboratory experiments using synthesized ZnS clusters (10 and 11 $\mu molL^{-1}$) demonstrated that the clusters did not significantly diffuse through the diffusive layer. However, this experiment should be revisited in order to decipher whether the apparent lack of diffusion was due to size or to significant adsorption effects. This experiment should be repeated with the synthesis of the ZnS cluster taking place in a solution of DOM or some other terminating group. Some literature suggests that the addition of organic groups assists in terminating the polymerization process of metal sulfide cluster growth and lessens the reactive or sticky nature of the clusters. However, the bulkiness of the resulting organic and metal sulfide complex may hinder its passage through a diffusive layer.

During the investigation of size fractions of measurable sulfide in extracted pore water, it was discovered that measurement artifacts for the SAOB/ISE method exist. In Chapter 3, two proposed mechanisms were discussed, and tests were conducted in laboratory experiments using the synthesized ZnS clusters and thioglycolate as model sulfide complexes. Sulfide antioxidant buffer (SAOB), due to its highly alkaline nature, is able to catalyze hydrolysis reactions which results in the release of sulfide which was bound to other materials (i.e., thiol groups) and is then detectable by the ISE. The presence of excessive EDTA in SAOB also "catalyzes" a competitive ligand exchange reaction between EDTA and metal sulfides present. This was demonstrated for field and laboratory samples in Chapter 3 and predicted by the chemical equilibrium modeling program MINEQL+. Depending on the time between preservation by SAOB and analysis by ISE, the measured sulfide concentrations may significantly increase. The laboratory data in Chapter 3 suggests rapid rates of reaction for each proposed mechanism, and the increases in measured sulfide from initial preservation to 24 hours are ($p<0.5$) significant. These artifacts are believed to occur when conventionally filtered water samples are placed into SAOB. There is no reason to believe these artifacts exist when distilled sulfide is trapped into SAOB as is the case of AVS and DGT extractions. When sulfide is liberated from the AgI binding gel, it is completely present as H_2S gas due to the acidic nature of the distillation. Figure 18 summarizes some commonly used methods for sulfide analysis and potential forms of sulfide which these methods may detect.

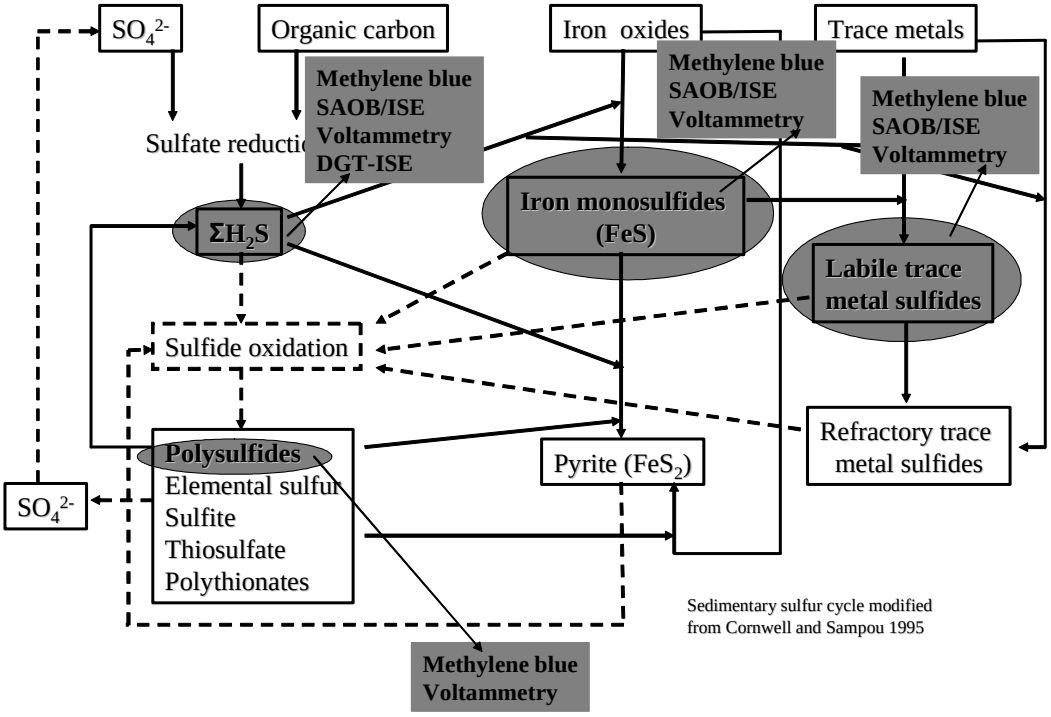

Figure 18. Commonly used techniques for sulfide analysis and potentially detected sulfide species. Various sulfide species are in gray ovals and the potential techniques to detect these species are in adjacent gray boxes.

Over the course of the method development and field deployment stages of DGT-ISE, a number of suggestions have been identified and are recommended for future DGT usage. When dealing with redox sensitive species, proper deoxygenation is a necessary step in to obtain an accurate DGT concentration. It is recommended that the fully assembled DGT probes be deoxygenated for a minimum of one week. It is believed that oxygen slowly bleeds out of the plastic pistons used to house the polyacrylamide gels. Gas bubbles within the DGT probe effectively interfere with the diffusion of a dissolved species through the DGT probe. It is necessary to inspect and release any gas bubbles that may have developed during the deoxygenation period. The use of multiple or "duplicate" DGT probes in the field is necessary. First

of all, the use of two or more DGT devices allows one to determine if and to what extent (magnitude) a DBL occurs. However from a practical point, things can and will go wrong (i.e., tearing a diffusive layer during deployment) with the DGT probes, and thus relying on only two or three probes for duplicate measures or DBL characterization can be problematic. Therefore, it is suggested that three to five differing diffusive layer thicknesses be used in order to calculate a more reliable DBL thickness and the bulk solution concentration. Finally, it is recommended that diffusive layers of less than 0.4 mm thickness not be used in field deployments. Calculated DBL thicknesses on the order of 100 μm and larger begin to have significant effects on the thinner diffusive layers.

Lastly, it is recommended when collecting sulfide samples by DGT that a secondary sulfide measurement by another method is taken in parallel. The comparative measurements may yield valuable information concerning sulfide speciation (i.e., inorganic versus organic complexes). The results from the field deployments in Pavin Lake, Lake Lariat, and L658, where differences existed between DGT and conventional filtration, suggest a large fraction (nearly 50%) of the dissolved sulfide ligands may be associated or incorporated with other materials such as colloids or DOC. The association of sulfide to other materials may have a profound influence on the bioavailability of metals and metal sulfide complexes to organisms. Future studies might examine whether potential relationships exist in aqueous systems between DGT measured sulfide concentration and toxicological measurements.

Appendix A

Table A 1. Sulfide fluxes and estimated concentrations by DGT-ISE at three littoral sites (G1, G2, and G3) located in L658 ELA, NW Ontario during June 2002.

Sediment Probe ID and location	Depth (cm)	Flux ($\mu mol \cdot cm^{-2} s^{-1}$)	$[(S\text{-}II)]_{measured}$ ($\mu molL^{-1}$)
D(G1)	1	4.0×10^{-9}	0.013
D(G1)	-1	7.5×10^{-9}	0.025
D(G1)	-3	2.1×10^{-8}	0.071
D(G1)	-5	3.6×10^{-9}	0.012
D(G1)	-7	4.8×10^{-9}	0.016
D(G1)	-10	1.5×10^{-9}	0.005
F(G1)	1	3.6×10^{-9}	0.012
F(G1)	-1	4.8×10^{-9}	0.016
F(G1)	-3	1.4×10^{-9}	0.005
F(G1)	-5	1.4×10^{-9}	0.005
F(G1)	-7	7.4×10^{-10}	0.002
F(G1)	-10	1.9×10^{-10}	0.001
C(G2)	1	5.1×10^{-8}	0.22
C(G2)	-1	9.9×10^{-8}	0.43
C(G2)	-3	9.9×10^{-8}	0.43
C(G2)	-5	7.1×10^{-8}	0.31
C(G2)	-7	1.2×10^{-8}	0.054
C(G2)	-10	8.3×10^{-9}	0.036
E(G2)	1	1.1×10^{-8}	0.049
E(G2)	-1	6.1×10^{-8}	0.26
E(G2)	-3	4.7×10^{-8}	0.20
E(G2)	-5	2.2×10^{-8}	0.096
E(G2)	-7	7.3×10^{-9}	0.032
E(G2)	-10	2.9×10^{-9}	0.013
B(G3)	1	3.6×10^{-8}	0.12
B(G3)	-1	4.9×10^{-8}	0.17
B(G3)	-3	2.8×10^{-8}	0.096
B(G3)	-5	9.9×10^{-9}	0.033
B(G3)	-7	5.8×10^{-9}	0.020
B(G3)	-10	3.3×10^{-9}	0.011
G(G3)	1	1.6×10^{-8}	0.054
G(G3)	-1	9.0×10^{-9}	0.030
G(G3)	-3	9.0×10^{-9}	0.030
G(G3)	-5	5.2×10^{-9}	0.018
G(G3)	-7	4.6×10^{-9}	0.015
G(G3)	-10	2.3×10^{-9}	0.008

Notes:
G1 site is located in 4 m of water.
G2 site is located in 1 m or less of water.
G3 site is located in 2 m of water.

Table A 2. Sulfide fluxes and estimated concentrations by DGT-ISE at a littoral site (G2) located in L658 ELA, NW Ontario during August 2002.

Sediment Probe ID	Depth (cm)	Flux ($\mu mol \cdot cm^{-2} s^{-1}$)	$[(S\text{-}II)]_{measured}$ ($\mu molL^{-1}$)
G	1	2.4×10^{-9}	0.011
G	-1	4.2×10^{-9}	0.018
G	-3	5.4×10^{-9}	0.023
G	-5	3.9×10^{-9}	0.017
G	-7	2.7×10^{-9}	0.011
G	-9	4.8×10^{-9}	0.021
G	-11	5.3×10^{-9}	0.023
F	1	5.5×10^{-10}	0.002
F	-1	9.4×10^{-9}	0.041
F	-3	1.1×10^{-7}	0.461
F	-5	3.8×10^{-8}	0.165
F	-7	9.9×10^{-9}	0.043
F	-9	6.6×10^{-9}	0.029
F	-11	5.5×10^{-9}	0.024
D	1	8.8×10^{-9}	0.038
D	-1	7.3×10^{-9}	0.032
D	-3	7.3×10^{-9}	0.032
D	-5	7.3×10^{-9}	0.032
D	-7	4.2×10^{-9}	0.018
D	-9	4.9×10^{-9}	0.021
D	-11	9.2×10^{-10}	0.004
E	1	4.3×10^{-9}	0.018
E	-1	1.3×10^{-10}	0.001
E	-3	7.1×10^{-10}	0.003
E	-5	9.2×10^{-10}	0.004
E	-7	3.7×10^{-9}	0.016
E	-9	3.4×10^{-9}	0.015
E	-11	3.2×10^{-9}	0.014
B	1	6.4×10^{-9}	0.028
B	-1	9.0×10^{-9}	0.039
B	-3	4.2×10^{-9}	0.018
B	-5	6.4×10^{-9}	0.028
B	-7	5.9×10^{-9}	0.025
B	-9	9.0×10^{-9}	0.039
B	-11	8.6×10^{-9}	0.037
C	1	7.3×10^{-9}	0.032
C	-1	8.6×10^{-9}	0.037
C	-3	8.6×10^{-9}	0.037
C	-5	1.7×10^{-8}	0.073
C	-7	6.1×10^{-9}	0.027
C	-9	2.5×10^{-9}	0.011
C	-11	6.7×10^{-9}	0.029

Table A 3. Sulfide fluxes and estimated concentrations by DGT-ISE at a wetland site located in L658 ELA, NW Ontario during August 2002.

Probe location	Depth in peat (cm)	Flux ($\mu mol \cdot cm^{-2} s^{-1}$)	$[(S\text{-}II)]_{measured}$ ($\mu molL^{-1}$)
WF-2 *	-10	6.8×10^{-8}	0.29
WF-2 duplicate*	-10	1.6×10^{-8}	0.069
WF-2 *	-20	7.3×10^{-8}	0.32
WF-2 duplicate*	-20	1.7×10^{-7}	0.74
Mid #	-10	2.2×10^{-8}	0.092
Mid duplicate#	-10	9.2×10^{-8}	0.40
Mid #	-20	4.8×10^{-9}	0.021
Mid duplicate#	-20	5.3×10^{-9}	0.023
A-2 !	-10	1.3×10^{-8}	0.055
A-2 duplicate !	-10	2.7×10^{-8}	0.12
A-2 !	-20	6.0×10^{-8}	0.26
A-2 duplicate !	-20	9.8×10^{-8}	0.42

*front of wetland
#2 m behind wetland front
!along front of board walk

Table A 4. Sulfide fluxes and estimated concentrations by DGT-ISE at a littoral site (G2) located in L658 ELA, NW Ontario during July 2003.

Sediment Probe ID	Depth (cm)	Flux ($\mu mol \cdot cm^{-2} s^{-1}$)	$[(S\text{-}II)]_{measured}$ ($\mu molL^{-1}$)
1	1	4.7×10^{-9}	0.030
1	-1	1.3×10^{-8}	0.080
1	-3	2.9×10^{-8}	0.18
1	-5	1.4×10^{-9}	0.009
1	-7	6.6×10^{-10}	0.004
1	-10	6.5×10^{-10}	0.004
2	1	5.6×10^{-9}	0.021
2	-1	4.4×10^{-9}	0.016
2	-3	4.8×10^{-9}	0.017
2	-5	6.6×10^{-9}	0.024
2	-7	4.1×10^{-9}	0.015
2	-10	2.4×10^{-9}	0.009
3	1	5.6×10^{-9}	0.036
3	-1	1.4×10^{-8}	0.087
3	-3	3.6×10^{-9}	0.023
3	-5	1.8×10^{-9}	0.011
3	-7	3.1×10^{-9}	0.020
3	-10	7.1×10^{-10}	0.005
4	1	9.7×10^{-9}	0.035
4	-1	8.3×10^{-9}	0.030
4	-3	8.9×10^{-9}	0.033
4	-5	7.6×10^{-9}	0.028
4	-7	6.0×10^{-9}	0.022
4	-10	5.4×10^{-9}	0.020
5	1	1.9×10^{-9}	0.007
5	-1	3.5×10^{-9}	0.013
5	-3	6.4×10^{-9}	0.023
5	-5	2.0×10^{-9}	0.007
5	-7	1.9×10^{-9}	0.007
5	-10	No detect	No detect
6	1	5.8×10^{-9}	0.037
6	-1	6.3×10^{-9}	0.040
6	-3	5.8×10^{-9}	0.037
6	-5	2.0×10^{-9}	0.012
6	-7	6.1×10^{-10}	0.004
6	-10	1.3×10^{-10}	0.001

Bibliography

Adams, N. W. H. and J. R. Kramer (1999). "Silver speciation in wastewater effluent, surface waters, and pore waters." Environmental Toxicology and Chemistry **18**(12): 2667-2673.

AlFarawati, R. and C. M. G. vandenBerg (1997). "The determination of sulfide in seawater by flow-analysis with voltammetric detection." Marine Chemistry **57**(3-4): 277-286.

Allen, H. E., G. M. Fu, et al. (1993). "Analysis of Acid-Volatile Sulfide (Avs) and Simultaneously Extracted Metals (Sem) for the Estimation of Potential Toxicity in Aquatic Sediments." Environmental Toxicology and Chemistry **12**(8): 1441-1453.

Ankley, G. T., D. M. DiToro, et al. (1996). "Technical basis and proposal for deriving sediment quality criteria for metals." Environmental Toxicology and Chemistry **15**(12): 2056-2066.

APHA-AWWA-WPCF (1989). Standard Methods for the Examination of Water and Wastewater. Washington, DC.

Babiarez, C. (2004). Personal communication. Madison, University of Wisconsin.

Baumann, E. W. (1974). "Determination of Parts Per Billion Sulfide in Water with Sulfide-Selective Electrode." Analytical Chemistry **46**(9): 1345-1347.

Benoit, J. M., C. C. Gilmour, et al. (2001). "The influence of sulfide on solid phase mercury bioavailability for methylation by pure cultures of Desulfobulbus propionicus (1pr3)." Environmental Science & Technology **35**(1): 127-132.

Berner, R. A. (1963). "Electrode Studies of Hydrogen Sulfide in Marine Sediments." Geochimica Et Cosmochimica Acta **27**(JUN): 563-575.

Bianchini, A. and K. C. Bowles (2002). "Metal sulfides in oxygenated aquatic systems: implications for the biotic ligand model." Comparative Biochemistry and Physiology C-Toxicology & Pharmacology **133**(1-2): 51-64.

Bianchini, A., K. C. Bowles, et al. (2002). "Evaluation of the effect of reactive sulfide on the acute toxicity of silver (I) to Daphnia magna. part 2: Toxicity results." Environmental Toxicology and Chemistry **21**(6): 1294-1300.

Boulegue, J., C. J. Lord, et al. (1982). "Sulfur Speciation and Associated Trace-Metals (Fe, Cu) in the Pore Waters of Great Marsh, Delaware." Geochimica Et Cosmochimica Acta **46**(3): 453-464.

Bowles, K. C., R. A. Bell, et al. (2002). "Synthesis and characterization of metal sulfide clusters for toxicological studies." Environmental Toxicology and Chemistry **21**(4): 693-699.

Bowles, K. C., M. J. Ernste, et al. (2003). "Trace sulfide determination in oxic freshwaters." Analytica Chimica Acta **477**(1): 113-124.

Brouwer, H. and T. Murphy (1995). "Volatile Sulfides and Their Toxicity in Fresh-Water Sediments." Environmental Toxicology and Chemistry **14**(2): 203-208.

Chapman, P. M., F. Y. Wang, et al. (2002). "Pore water testing and analysis: the good, the bad, and the ugly." Marine Pollution Bulletin **44**(5): 359-366.

Chapman, P. M., F. Y. Wang, et al. (1998). "Ecotoxicology of metals in aquatic sediments: binding and release, bioavailability, risk assessment, and remediation." Canadian Journal of Fisheries and Aquatic Sciences **55**(10): 2221-2243.

Ciglenecki, I. and B. Cosovic (1996). "Electrochemical study of sulfur species in seawater and marine phytoplankton cultures." Marine Chemistry **52**(1): 87-97.

Cline, J. D. (1969). "Spectrophotometric Determination of Hydrogen Sulfide in Natural Waters." Limnology and Oceanography **14**(3): 454-458.

Cook, A. M. and K. J. Steel (1959). "The Stability of Thioglycollate Solutions .2. Effects of Method of Preparation of Solutions, Ph and Temperature Upon the Oxidation of Thioglycollate." Journal of Pharmacy and Pharmacology **11**(4): 216-223.

Cooper, D. C. and J. W. Morse (1998). "Extractability of metal sulfide minerals in acidic solutions: Application to environmental studies of trace metal contamination within anoxic sediments." Environmental Science & Technology **32**(8): 1076-1078.

Cornwell, J. C. and J. W. Morse (1987). "The Characterization of Iron Sulfide Minerals in Anoxic Marine-Sediments." Marine Chemistry **22**(2-4): 193-206.

Cornwell, J. C. and P. A. Sampou (1995). Environmental controls on iron sulfide mineral formation in a coastal plain estuary. Geochemical transformations of sedimentary sulfur, ACS Symposium Series. M. A. Vairavamurthy and M. A. A. Schoonen. Washington, DC. **612:** 224-242.

Cossa, D. and R. Mason (1994). Chemical Speciation of Mercury in a Meromictic Lake. Mercury Pollution: Integration and Synthesis. C. Watras and J. Huckabee. Boca Raton, CRC Press, Inc.**:** 57-67.

Cutter, G. A. and T. J. Oatts (1987). "Determination of Dissolved Sulfide and Sedimentary Sulfur Speciation Using Gas-Chromatography Photoionization Detection." Analytical Chemistry **59**(5): 717-721.

Davison, W., G. Fones, et al. (2000). Dialysis, DET, and DGT: In situ diffusional Techniques for Studying Water, Sediments, and Soils. In situ monitoring of Aquatic Systems. Chemical Analysis and Speciation. J. Buffle and G. Horvai. Chichester, John Wiley & Sons. **6:** 495-569.

Davison, W., N. Phillips, et al. (1999). "Soluble iron sulfide species in natural waters: Reappraisal of their stoichiometry and stability constants." Aquatic Sciences **61**(1): 23-43.

Davison, W. and H. Zhang (1994). "In-Situ Speciation Measurements of Trace Components in Natural-Waters Using Thin-Film Gels." Nature **367**(6463): 546-548.

Davison, W., H. Zhang, et al. (1994). "Performance-Characteristics of Gel Probes Used for Measuring the Chemistry of Pore Waters." Environmental Science & Technology **28**(9): 1623-1632.

Degryse, F., E. Smolders, et al. (2003). "Relating soil solution Zn concentration to diffusive gradients in thin films measurements in contaminated soils." Environmental Science & Technology **37**(17): 3958-3965.

Devries, C. R. and F. Y. Wang (2003). "In situ two-dimensional high-resolution profiling of sulfide in sediment interstitial waters." Environmental Science & Technology **37**(4): 792-797.

Ditoro, D. M., J. D. Mahony, et al. (1990). "Toxicity of Cadmium in Sediments - the Role of Acid Volatile Sulfide." Environmental Toxicology and Chemistry **9**(12): 1487-1502.

Dunn, R. J. K., P. R. Teasdale, et al. (2003). "Evaluation of the diffusive gradient in a thin film technique for monitoring trace metal concentrations in estuarine waters." Environmental Science & Technology **37**(12): 2794-2800.

Ernstberger, H., W. Davison, et al. (2002). "Measurement and dynamic modeling of trace metal mobilization in soils using DGT and DIFS." Environmental Science & Technology **36**(3): 349-354.

Fossing, H. and B. B. Jorgensen (1989). "Measurement of Bacterial Sulfate Reduction in Sediments - Evaluation of a Single-Step Chromium Reduction Method." Biogeochemistry **8**(3): 205-222.

Gimpel, J., H. Zhang, et al. (2003). "In situ trace metal speciation in lake surface waters using DGT, dialysis, and filtration." Environmental Science & Technology **37**(1): 138-146.

Gimpel, J., H. Zhang, et al. (2001). "Effect of solution composition, flow and deployment time on the measurement of trace metals by the diffusive gradient in thin films technique." Analytica Chimica Acta **448**(1-2): 93-103.

Hansen, D. J., W. J. Berry, et al. (1996). "Predicting the toxicity of metal-contaminated field sediments using interstitial concentration of metals and acid-volatile sulfide normalizations." Environmental Toxicology and Chemistry **15**(12): 2080-2094.

Harper, M. P., W. Davison, et al. (2000). "DIFS - a modelling and simulation tool for DGT induced trace metal remobilisation in sediments and soils." Environmental Modelling & Software **15**(1): 55-66.

Harper, M. P., W. Davison, et al. (1998). "Kinetics of metal exchange between solids and solutions in sediments and soils interpreted from DGT measured fluxes." Geochimica Et Cosmochimica Acta **62**(16): 2757-2770.

Herron, N., Y. Wang, et al. (1990). "Synthesis and Characterization of Surface-Capped, Size-Quantized CdS Clusters - Chemical Control of Cluster Size." Journal of the American Chemical Society **112**(4): 1322-1326.

Johnson, K. S., C. L. Beehler, et al. (1986). "A Submersible Flow-Analysis System." Analytica Chimica Acta **179**: 245-257.

Kortan, A. R., R. Hull, et al. (1990). "Nucleation and Growth of CdSe on ZnS Quantum Crystallite Seeds, and Vice Versa, in Inverse Micelle Media." Journal of the American Chemical Society **112**(4): 1327-1332.

Kuhl, M. and C. Steuckart (2000). Sensors for In situ Analysis of Sulfide in Aquatic Systems. In situ Monitoring of Aquatic Systems. Chemical Analysis and Speciation. Chichester, John Wiley & Sons. **6:** 121-149.

Lawrence, N. S., J. Davis, et al. (2000). "Analytical strategies for the detection of sulfide: a review." Talanta **52**(5): 771-784.

Lee, B. G., S. B. Griscom, et al. (2000). "Influences of dietary uptake and reactive sulfides on metal bioavailability from aquatic sediments." Science **287**(5451): 282-284.

Li, Y. H. and S. Gregory (1974). "Diffusion of Ions in Sea-Water and in Deep-Sea Sediments." Geochimica Et Cosmochimica Acta **38**(5): 703-714.

Luther, G. W., C. E. Reimers, et al. (1999). "In situ deployment of voltammetric, potentiometric. and amperometric microelectrodes from a ROV to determine dissolved O-2, Mn, Fe, S(-2), and pH in porewaters." Environmental Science & Technology **33**(23): 4352-4356.

Luther, G. W., S. M. Theberge, et al. (1999). "Evidence for aqueous clusters as intermediates during zinc sulfide formation." Geochimica Et Cosmochimica Acta **63**(19-20): 3159-3169.

Luther, G. W. and E. Tsamakis (1989). "Concentration and Form of Dissolved Sulfide in the Oxic Water Column of the Ocean." Marine Chemistry **27**(3-4): 165-177.

Michard, G., E. Viollier, et al. (1994). "Geochemical Study of a Crater Lake - Pavin Lake, France - Identification, Location and Quantification of the Chemical-Reactions in the Lake." Chemical Geology **115**(1-2): 103-115.

Millero, F. J. (1986). "The Thermodynamics and Kinetics of the Hydrogen-Sulfide System in Natural-Waters." Marine Chemistry **18**(2-4): 121-147.

Morel, F. and J. Hering (1993). Principles and Applications of Aquatic Chemistry. New York, John Wiley & Sons, Inc.

Morrison, R. and R. Boyd (1987). Organic Chemistry. Boston, Allyn and Bacon, Inc.

Morse, J. W., F. J. Millero, et al. (1987). "The Chemistry of the Hydrogen-Sulfide and Iron Sulfide Systems in Natural-Waters." Earth-Science Reviews **24**(1): 1-42.

Motelica-Heino, M., C. Naylor, et al. (2003). "Simultaneous release of metals and sulfide in lacustrine sediment." Environmental Science & Technology **37**(19): 4374-4381.

Mylon, S. E. and G. Benoit (2001). "Subnanomolar detection of acid-labile sulfides by the classical methylene blue method coupled to HPLC." Environmental Science & Technology **35**(22): 4544-4548.

Nealson, K. H. and D. A. Stahl (1997). Microorganisms and biogeochemical cycles: What can we learn from layered microbial communities? Geomicrobiology: Interactions between Microbes and Minerals. **35:** 5-34.

Ogrinc, N., H. Hintelmann, et al. (2003). "Biogeochemical influence on carbon isotope signature in boreal lake sediments." Hydrobiologia **494**(1-3): 207-213.

Radfordknoery, J. and G. A. Cutter (1993). "Determination of Carbonyl Sulfide and Hydrogen-Sulfide Species in Natural-Waters Using Specialized Collection Procedures and Gas-Chromatography with Flame Photometric Detection." Analytical Chemistry **65**(8): 976-982.

Rozan, T. F., G. Benoit, et al. (1999). "Measuring metal sulfide complexes in oxic river waters with square wave voltammetry." Environmental Science & Technology **33**(17): 3021-3026.

Rozan, T. F., M. E. Lassman, et al. (2000). "Evidence for iron, copper and zinc complexation as multinuclear sulphide clusters in oxic rivers." Nature **406**(6798): 879-882.

Sakamotoarnold, C. M., K. S. Johnson, et al. (1986). "Determination of Hydrogen-Sulfide in Seawater Using Flow-Injection Analysis and Flow-Analysis." Limnology and Oceanography **31**(4): 894-900.

Smith, D. S., R. A. Bell, et al. (2002). "Metal speciation in natural waters with emphasis on reduced sulfur groups as strong metal binding sites." Comparative Biochemistry and Physiology C-Toxicology & Pharmacology **133**(1-2): 65-74.

Steel, K. J. (1958). "A Note on the Assay of Some Sulphydryl Compounds." Journal of Pharmacy and Pharmacology **10**(9): 574-576.

Stumm, W. and J. Morgan (1996). Aquatic Chemistry. New York, Wiley Interscience.

Sveinsdottir, A. (2002). Methylmercury in Largemouth Bass (*Micropterus salmoides*) and Forage Fish from Maryland Reservoirs and Factors Influencing Uptake, University of Maryland.

Tang, D. G. and P. H. Santschi (2000). "Sensitive determination of dissolved sulfide in estuarine water by solid-phase extraction and high-performance liquid chromatography of methylene blue." Journal of Chromatography A **883**(1-2): 305-309.

Teasdale, P. R., S. Hayward, et al. (1999). "In situ, high-resolution measurement of dissolved sulfide using diffusive gradients in thin films with computer-imaging densitometry." Analytical Chemistry **71**(11): 2186-2191.

Thermo-Electron (2003). Orion Silver/Sulfide Electrode Instruction Manual, Model 94-16. Beverly, MA.

Twiss, M. R. and J. W. Moffett (2002). "Comparison of copper speciation in coastal marine waters measured using analytical voltammetry and diffusion gradient in thin-film techniques." Environmental Science & Technology **36**(5): 1061-1068.

Viollier, E., D. Jezequel, et al. (1995). "Geochemical Study of a Crater Lake (Pavin Lake, France) - Trace-Element Behavior in the Monimolimnion." Chemical Geology **125**(1-2): 61-72.

Xia, K., U. L. Skyllberg, et al. (1999). "X-ray absorption spectroscopic evidence for the complexation of Hg(II) by reduced sulfur in soil humic substances." Environmental Science & Technology **33**(2): 257-261.

Zhang, H. and W. Davison (1995). "Performance-Characteristics of Diffusion Gradients in Thin-Films for the in-Situ Measurement of Trace-Metals in Aqueous-Solution." Analytical Chemistry **67**(19): 3391-3400.

Zhang, H. and W. Davison (1999). "Diffusional characteristics of hydrogels used in DGT and DET techniques." Analytica Chimica Acta **398**(2-3): 329-340.

Zhang, H. and W. Davison (2001). "In situ speciation measurements. Using diffusive gradients in thin films (DGT) to determine inorganically and organically complexed metals." Pure and Applied Chemistry **73**(1): 9-15.

Zhang, H., W. Davison, et al. (1998). "In situ measurement of dissolved phosphorus in natural waters using DGT." Analytica Chimica Acta **370**(1): 29-38.