THE STABILITY AND CHARACTERIZATION OF METAL-SULFIDE SPECIES UNDER OXIC CONDITIONS

A THESIS

By

KATRINA ROSE SUKOLA

Submitted to

The University of Manitoba

in partial fulfillment of the requirements

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To laugh often and much;
To win the respect of intelligent people and the affection of children;
To earn the appreciation of honest critics and to endure the betrayal of false friends;
To appreciate beauty;
To find the best in others;
To leave the world a bit better whether by a healthy child, a garden patch or a redeemed social condition;
To know even one life has breathed easier because you have lived.

This is to have succeeded.

Ralph Waldo Emerson

Chance is always powerful. Let your hook always cast; in the pool where you least expect it, there will be a fish.

Ovid

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Finally, I am forever indebted to my family for their understanding, endless patience and whose constant encouragement and love I have received.

CHEERS!!

ABSTRACT

Most metal ions of environmental concern (e.g., Cd, Cu, Pb, Zn, Hg) tend to form strong complexes with sulfide. Despite the instability of the free sulfide in oxic waters, some metal-sulfide complexes have recently been reported to resist oxidation and can be present in surface waters. However, the stabilities and identities of these complexes remain poorly known. Here my studies on the stability and characterization of the metal-sulfide species under oxic conditions are reported.

The stability of metal-sulfide species was studied by exposing metal-sulfide solutions to air over periods of time and measuring the sulfide remaining in the solution using the Methylene Blue (MB) and the Chromium(II) Volatile Sulfide (CVS) methods. The results indicated that some metal-sulfide species appear to be stable in oxic waters. The sulfide solutions of Cd, Zn, Pb, Cu can be stable in oxic waters for a prolonged period (a few weeks to a few months), while the sulfide concentration in other solutions (Fe, Mn, Ni) decreased below detection limits within a few hours.

Transmission electron microscopy (TEM) indicated that metal-sulfide species composed of Zn-S and Cu-S appear to vary in size, but are morphologically similar, initially appearing as aggregates of metal-sulfide particles and eventually as typical colloids. Additional structural information was obtained using mass spectrometry (MS).

UV-Visible spectrometry measurements and TEM results both indicate that laboratory solutions of metal-sulfide species are fine particles or colloids and not dissolved

nanoclusters, as previously suggested in the literature. For natural samples, it is difficult to directly identify metal-sulfide species with current analytical techniques. However, the results presented here indicate that metal-sulfide species can exist in surface waters, but are more likely to occur as dissolved complexes and colloids, as opposed to clusters. To understand the dynamics of metals in the aquatic environment, the stoichiometry, structure and stability of metal-sulfur complexes warrant further studies.

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LIST OF ABBREVIATIONS

ACS	American Chemical Society
AVS	Acid volatile sulfide
CLS	Chromium (II) labile sulfide
CVS	Chromium (II) volatile sulfide
DDW	Deoxygenated deionized water
DIC	Dissolved inorganic carbon
DMPD	N, N'-dimethyl-p-phenylene diamine sulfate
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
ELA	Experimental Lakes Area
EM	Electron microscopy
ESI-MS	Electrospray ionization mass spectrometry
EXAFS	Extended x-ray absorption fine-structure spectroscopy
FIAM	Free-ion activity model
FTMS	Fourier transform mass spectrometry
HPLC-ICP-MS	High performance liquid chromatography-inductively coupled plasma-mass spectrometry
HPLC-MS	High performance liquid chromatography - mass spectrometry
ICP-MS	Inductively coupled plasma – mass spectrometry
IDMS	Isotope dilution mass spectrometry
LC-MS	Liquid Chromatography - Mass Spectrometry
LMB	Leuco-derivative of methylene blue
MALDI	Matrix assisted laser desorption ionization
MB	Methylene blue
MS	Mass spectrometry
PTA	Phosphotungstic acid
PTFE	Polytetrafluoroethylene (Teflon)
rpm	Revolutions per minute
SEM	Simultaneously extracted metals
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
TEM-EDS	Transmission electron microscopy energy dispersive spectroscopy
TIMS	Thermal ionization mass spectrometry
UV-VIS	Ultra Violet – Visible
WHAM	Windermere humic acid model

Chapter 1. OBJECTIVES

The intention of this research project was to investigate the presence and characteristics of metal-sulfide species in aqueous solutions under oxidizing conditions, particularly those relevant to surface, freshwater systems. The following questions were addressed:

I) Are metal-sulfide species stable in oxic surface waters? Do metals stabilize S(-II) in aqueous solutions?

II) What are these metal-sulfide species in terms of their chemical composition (e.g., stoichiometry) and morphology (e.g., cluster versus colloid)? and,

III) Are these metal-sulfide species present in natural oxic surface waters? If yes, how important are they in controlling metal speciation in these surface waters?

Chapter 2. INTRODUCTION

2.1 Speciation and Bioavailability of Trace Metals in the Aquatic Environment

Trace metals are important constituents of the environment. Many of these metals (*e.g.,* Cu, Zn) are micronutrients and are essential for the health of some or all biota (Chapman *et al.*, 1998), while others (*e.g.,* Pb, Hg) have no known biological functions. Regardless of whether they are essential or not, at elevated concentrations they can be highly toxic to biota. Although they are naturally occurring in the environment by processes such as chemical weathering and volcanic activities, human activities (*e.g.,* burning of fossil fuels, metal mining and smelting, municipal sewage and industrial effluents) have significantly increased their fluxes from the Earth's crust to the environment and changed their distribution in the environment (Nriagu *et al.*, 1988).

Once they enter the aquatic environment, trace metals undergo a variety of chemical processes (*e.g.,* complexation, reduction, oxidation) that affect their partition between solid and aqueous phases and alter chemical speciation (Kuwabara and Luther, 1993). Chemical speciation refers to the distribution of an element amongst defined chemical species in a system. A chemical species is defined (Templeton *et al.*, 2000) as an isotopic composition (*e.g.,* ^{204}Pb, ^{206}Pb, ^{207}Pb, ^{208}Pb), electronic or oxidation state (*e.g.,* Pb(II), Pb(0)), and/or complex or molecular structure (e.g., $Pb(OH)^{+}$, $Pb(HS)^{+}$).

It has been well documented that toxicity of a metal in natural water is not related to its total metal concentrations but rather related to chemical speciation of the metal

(Campbell, 1995). Many laboratory and field studies have suggested that the toxicity of a metal in natural water is strongly related to the activity or concentration of the "free metal ions" (or more appropriately metal aquo complexes or $Me(H_2O)_x^{n+}$) (Campbell, 1995; Wiklund and Sundelin, 2002), giving rise to the so-called "free-ion activity model" (FIAM) (Morel and Hering, 1993; Campbell, 1995). This, however, does not mean that the free metal ions are the only bioavailable metal species; other labile metal species and non-chemical variables may also determine metal bioavailability (Chapman *et al.*, 1998).

In aquatic environments where redox conditions are relatively stable such as in surface waters, the speciation of a metal is predominantly controlled by complexation. A variety of inorganic (*e.g.*, CO_3^{2-}, Cl^-, HS^-) and organic (*e.g.*, fulvic and humic acids) metal-complexing ligands can be found in natural waters. The ligand concentrations are usually greater than those of the metal present (Florence, 1986). As a result, a variety of metal complexes are present in natural waters. These complexes differ greatly in concentration, reactivity, and thus in bioavailability. For example, certain metal complexes are known to be kinetically inert as a result of the electron configuration of the metal. This inertness stems from a stronger-than-expected metal-ligand bond that does not dissociate readily. Examples of kinetically inert metal cations (in octahedral symmetry) are d^3 (Cr^{3+}) and d^6 (low spin) electron configurations (Luther and Tsamakis, 1989). Low spin states are a result of strong ligand fields; the ligand's orbitals interact strongly with the metal orbitals (Miessler and Tarr, 1998). Other metal ions, such as Cu^{2+} (d^9), may also give kinetically stable complexes as a result of the *Jahn-Teller effect* (Luther and Tsamakis, 1989). The *Jahn-Teller* theorem states that there cannot be an unequal occupation of orbitals with

identical energies, resulting in molecular distortion to avoid unequal occupation (Miessler and Tarr, 1998). This effect is responsible for the behavior of Cu(II) complexes: Cu^{2+} tends to form square planar, rather than octahedral complexes and has very low formation constants on the addition of a fifth and sixth ligand (Luther and Tasamakis, 1989; Miessler and Tarr, 1998).

Chemical speciation is thus the key to understanding not only the cycling of trace metals in the aquatic environment, but also their bioavailability and potential toxicity to aquatic biota. It should be noted that the bioavailability of trace metals in natural waters is also determined by the physiology and feeding behavior of the exposed organism. This is however beyond the scope of this research and will not be further discussed in this thesis.

2.2 Sulfide as a Metal-Complexing Ligand

Despite extensive studies concerning the forms of sulfur in the environment, sulfur chemistry in natural waters is far from being well understood. This is due to the large range of oxidation states of sulfur (-2 to +6), its tendency to form numerous compounds with carbon, oxygen and itself, as well as methodological problems in measuring sulfur containing compounds (Howarth et. al, 1992).

Among all the metal-complexing ligands in natural waters, sulfide is of particular importance. From a chemical point of view, most metal ions of environmental concern are Type-B metal cations (or "soft Lewis acids"; *e.g.*, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}) and tend to

from strong complexes with sulfide which is a soft Lewis base, as a result of the electron configuration of the metal and ligand field effects (Stumm and Morgan, 1996). The high affinity of Type B metal ions for sulfide is confirmed by the extremely high stability constants (or formation constants) for metal-sulfide complexes (*e.g.,* NIST, 1997; Bell and Kramer, 1999). As a result, Type B metals usually occur in sulfide ores, and their speciation is dominated by sulfide complexes in both abiotic (*e.g.,* anoxic waters) and biotic media where sulfide is present. The latter gives rise to the term "toxic metals", because they can form complexes with "soft" sulfide-containing functional groups (*e.g.*, -SH) on biological molecules, replacing them and blocking catalytic actions or modifying vital structures.

2.2.1 Metal-Sulfide Interaction

The chemistry of Type B metals can be described by considering changes in behavior of *d*-orbitals (Hinchliffe and Dobson, 1975; Watanabe and Kamimura, 1987). These orbitals are relatively high in energy and are diffused; as the oxidation number increases (*e.g.,* as one goes across a particular series in the Periodic Table), the orbitals are at first effective acceptors, important in bonding, and then become so stable that their electrons behave as "core" electrons. For example, in ZnS (gas-phased), the "valence orbitals" on Zn are 4*s* and 3*d* electrons behave as "core" electrons. A detailed molecular orbital diagram of ZnS is shown in Figure 2.1.

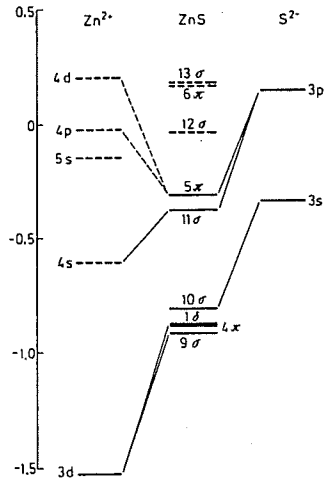


Figure 2.1 *Orbital energy correlation diagram for ZnS (Hinchliffe and Dobson, 1975)*

There is evidence suggesting that complexation of sulfide to Type B metal ions occurs through a covalent chemical bond (*e.g.,* M-S). Observation from voltammetric and other chemical experiments has led researchers to conclude that sulfide is bound in a chemical bond to metals; however metal-ligand bonds are frequently labile (Luther and Tsamakis, 1989):

$$[Cu(H_2O)_x(S)]^0 + H^+ \leftrightarrow \ [Cu(H_2O)_x]^{2+} + HS^-$$

Sulfide complexes with Type B metal ions are important not only because of their thermodynamic stability but also their kinetic inertness. This kinetic effect results from high ligand field stabilization energies associated with the electron configuration of the metal (*e.g.,* d^3 and d^6 low spin octahedral metal complexes.) These metals are likely to have an electron configuration consistent with inert complexes, which do not dissociate

readily to form free sulfide unless in acidic solutions (Luther and Tsamakis, 1989). In natural systems metals that are complexed with sulfide are also likely to be complexed with organic chelates, which serve to further stabilize metal-sulfide bonds.

2.2.2 Metal-Sulfide Complexes, Colloids and Clusters

It is believed that metal-sulfide species may exist in three forms: complexes, colloids, and nanoclusters.

Metal-sulfide complexes are truly dissolved species, they are in rapid equilibrium with metal ions and the ligand, HS^- (Zhang and Millero, 1994), are thermodynamically strong and kinetically inert to dissociation (Luther *et al.*, 1999). As is true for all truly dissolved species, metal-sulfide complexes have definable chemical potentials (Stumm and Morgan, 1996) and thus can be described by chemical thermodynamics.

By definition, colloids and macromolecules are any organic or inorganic entity in the size range of 1nm to 1μm (Buffle and Leppard, 1995). Colloids are dynamic particles, which are continuously generated and undergo compositional changes and removal from water (Stumm and Morgan, 1996). The colloidal phase is presumed to be composed of organic and inorganic materials, and is the most important component for aqueous metals (Bell and Kramer, 1999). In aquatic systems, they form inherently unstable suspensions due to their tendency to undergo conformation changes, aggregate and then settle to form a sediment. The fine physical structure of colloids, macromolecules and their aggregates is very delicate and easily perturbed by chemical reagents such as added electrolytes or pH

changes. Chemical and physical characteristics of colloids cannot be determined without some analytical manipulation such as sampling, storage, or fractionation, which may often introduce drastic perturbations to colloidal structures. Water samples containing colloids are unstable mainly due to continuing coagulation and microbial activity. Collecting samples representative of all particles, including the largest and smallest colloids is not easy. The largest particles are easily excluded during sampling due to their rapid sedimentation. Sub-micrometer particles represent only a very small proportion of the total colloidal mass. Consequently, the concentration is often low (typically $\leq$ 100μg/L) and losses by adsorption on vessel walls may be important (Buffle and Leppard, 1995), as will be discussed.

Metal-sulfide species may also yield quantum-size "particles". These quantum-sized materials are also known as molecular clusters or nanoclusters, consisting of an indefinite number of molecular units with a defined stoichiometry (Luther *et al.*, 1999). The extremely small size of nanoclusters leads to a quantum confinement of the photogenerated electron-hole pair, resulting in a blue shift in the absorption spectrum (Sooklal *et al.*, 1996), corresponding to the exciton energy shifts to shorter wavelengths with decreasing particle size (Korgel and Monbouquette, 1996). Quantum size effects result in unique physical and chemical properties that depend strongly on the size of the cluster (Løver *et al.*, 1997). To mimic clusters and biomineralization processes found in nature, a monodisperse size is required (Korgel and Monbouquette, 1996). Semiconductor research has led to the synthesis and characterization of the photophysical properties of metal sulfide nanoclusters, which are usually prepared at high (mM and

greater) concentrations in the presence of chemical stabilizers or thiols in organic solvents to control the size of the clusters (< 50 nm) and their distribution (Bowles et al., 2002; Luther *et al.*, 1999). These studies cannot easily be extrapolated to understand the properties of naturally occurring metal-sulfides (Bowles et al., 2002).

Persistence of reduced sulfur species in both oxygenated artificial and natural waters has been attributed to the presence of "dissolved" nanoclusters (Rozan *et al.*, 2000; Luther *et al.,* 1999; Bowles *et al.*, 2003). Identification of these particles as nanoclusters is uncertain since it is based on mass spectra of freeze-dried water samples (Rozan *et al.*, 2002) where species analyzed may well have been formed during the drying process. The sulfur-containing particles resistant to oxidation reported in natural or simple artificial waters were more likely dynamic particles or colloids, since they were formed under conditions of oversaturation with respect to the corresponding metal-sulfide solids and without the presence of protective agents.

Knowledge of the identity of the metal-sulfide species in oxic waters (synthesized or natural) is a prerequisite to determining their relative importance for metal speciation in these waters. If the clusters reported in the literature are indeed colloids, they should be distinguished from aqueous metal-sulfide complexes that are truly dissolved species. While the formation and concentrations of metal-sulfide complexes can be described by thermodynamic equilibria, those of metal-sulfide clusters and colloids cannot due to their dynamic nature.

2.2.3 Metal-Sulfide Complexes in Sulfidic Waters

Given the high affinity of Type B metal ions for sulfide, it is not surprising that speciation in sulfidic water is dominated by sulfide complexes (Huerta-Diaz *et al.*, 1998). In fact, a good portion of natural water is anoxic and sulfidic. Micromolar to millimolar concentrations of sulfide have been commonly reported in natural anoxic waters, such as hypolimnetic waters of stratified lakes, seas and estuaries, and most sediment interstitial waters. Sulfide in these waters is mainly produced by bacteria-mediated sulfate reduction under anaerobic conditions. Once formed, the most common mechanism for sulfide removal is via oxidation, which produces a variety of intermediate sulfur-containing species (*e.g.,* elemental sulfur, polysulfides, thiosulfate, sulfite) and ultimately sulfate (Millero, 2001). As shown in Figure 2.2, the sulfur cycle in natural waters is further complicated by its coupling with biogeochemical cycles of many other major and minor element cycles and methane fluxes.

Since in natural waters concentrations of most Type B metals are very low (picomolar to micromolar levels), the predominant metal-sulfide complexes change from simple mononuclear to polynuclear with increasing metal (Me), and sulfide concentrations (Bell and Kramer, 1999; charges are neglected for simplicity):

$$Me \rightarrow Me(HS)_1 \rightarrow Me(HS)_2 \rightarrow MeS_n \rightarrow Me_mS_n$$

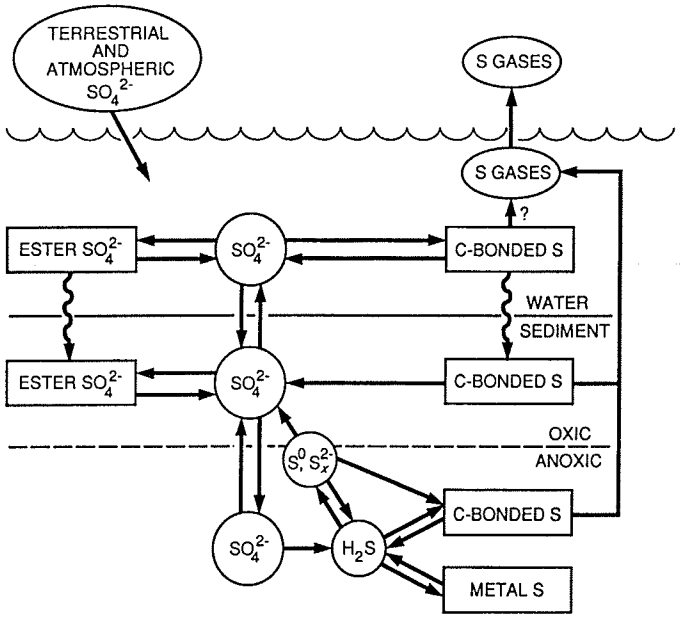


Figure 2.2: Diagram of the sulfur cycle in small lakes (Howarth et al., 1992)

Perry and Pedersen (1993) found that the presence of monosulfides corresponds well with the increase in dissolved sulfide concentrations. Once sulfide levels become sufficiently high, monosulfides can precipitate. As lake depth increases, more time is allowed for monosulfide particles to settle and be converted to pyrite (Perry and Pedersen, 1993). Monosulfide minerals such as FeS or Fe_3S_4 are indicative of weakly sulfidic conditions, insufficient to fully combine with available transition metal, while MnS_2 requires strongly reducing environments to become stable over $MnCO_3$ (Salbu and Steinnes, 1995).

Released metals, initially precipitated as metal sulfides, will be scavenged by or co-precipitate with other substances such as iron, manganese hydroxides and organic matter. Metal-sulfides oxidize as oxygen conditions improve, thus increasing dissolved trace

metal concentrations (Wiklund and Sundelin, 2002). Metal sulfides may also undergo exchange reactions. More strongly S(II$^-$)-bound metals will replace weakly bound metals, even if sulfide is in the solid state. Precipitated metal-sulfides such as Fe(II)-, Zn(II)-, Pb(II)- and Cd(II)-sulfide, having higher solubility, rapidly exchange with macro amounts of Cu(II), Ag(I) and Hg(II) of lower solubility. Hence, if a metal concentration (*e.g.*, Cu(II)) is less than another MeS (*e.g.*, FeS), Cu(II) would be removed from solution (Bell and Kramer, 1999). In addition, since FeS and MnS have higher solubility products (K_{sp}) than all other trace metal-sulfides (MeS), metals will displace Fe and Mn to form more insoluble MeS:

$$Me^{2+} + FeS(s) \leftrightarrow MeS(s) + Fe^{2+}$$

$$Me^{2+} + MnS(s) \leftrightarrow MeS(s) + Mn^{2+}$$

The metal-sulfide formed can be either discrete (pure) metal-sulfide phases or in a solid solution formed by coprecipitation and/or adsorption on FeS (Chapman *et al.*, 1998). However, total dissolved Cu(II) may increase if complexation by other species interferes (Bell and Kramer, 1999). Because solubility products of metal sulfides of environmental interest are far less than that of FeS, the activities of these metals will be very small in the presence of excess acid volatile sulfide (AVS; see below). Consequently if AVS is in excess, the metals are not likely toxic (Chapman *et al.*, 1998). In order to understand how metals will behave in highly dilute systems that they naturally occur, the structure and chemistry of metal-sulfide compounds, both inorganic and organic, must be known (Bell and Kramer, 1999).

2.3 Metal-Sulfide Species in Oxic Waters

Despite the well-documented predominant role of sulfide in determining metal speciation in sulfidic waters, its role in oxic waters has been largely neglected. This is because of the instability of free sulfide in oxic waters, due to oxidation. A variety of oxidants including O_2 can readily oxidize sulfide to ultimately sulfate (Millero *et al.*, 1987). As a result, sulfide is not expected in significant concentrations in oxic waters to bind trace metals. Instead, studies on metal speciation in oxic waters have been heavily focused on the complexation by dissolved organic matter (Breault *et al.*, 1996; Xue *et al.*, 1996).

This generally held view, however, has been challenged in recent years. First, new generations of sensitive analytical techniques have detected picomolar to nanomolar levels of sulfide in oxygenated surface waters of open oceans and rivers (Radford-Knoery and Cutter, 1993; Rozan *et al.*, 1999, 2000; Adams and Kramer, 1999; Rozan and Luther, 2002). Since free sulfide is not stable in such oxygenated waters, the measured sulfide must be present in some oxidation-resistant forms such as polysulfides and metal-sulfide species. Rozan *et al.* (1999) conducted various electrochemical studies and found similar (conditional) stability constants for most divalent metal (bi)sulfide complexes (1:1 stoichiometric ratio) as those found in seawater. Theoretically, these results allow metal-sulfide species to be present in oxic waters (Rozan et al., 1999). Rozan *et al.* (2000) was the first to report the presence of metal-sulfide clusters (Me_mS_n) in oxic natural waters based on mass spectrometric analysis. By analyzing vacuum or freeze-dried river water samples, Rozan *et al.* (2000) observed mass spectra that correspond to metal-sulfide clusters of small-molecular mass (<400 Da) that contain no protons. Bowles *et al.* (2002)

assessed that synthetic metal-sulfide solutions containing Zn, Cu, Ag, and Hg are indeed quite resistant to oxidation in oxic waters. These multiple experiments suggested that sulfide species of certain metals are resistant to oxidation and can be present in oxic waters.

These new findings would have profound implications in cycling, speciation and bioavailability of trace metals, if oxidation-resistant metal-sulfide species are ubiquitous in natural surface waters and if they are present at considerable concentrations. Several key questions, however, remain unsolved. The analytical evidence of metal-sulfide species in oxic waters is not convincing. As mentioned above, Rozan *et al.* (2000) has provided the only direct evidence for the presence of metal-sulfide species in oxic waters. Whether the observed clusters were produced artificially during the sample pretreatment process (freeze-drying), however, remains unknown.

Currently, known forms of reduced sulfur species in oxic waters and measurement of their concentrations remain difficult to quantify. Although free sulfide (H_2S and HS^-) is not stable in oxygenated water (Millero *et al.*, 1987), synthetic metal-sulfide solutions (*e.g.,* Zn-S, Cu-S) have shown remarkable resistance to oxidation in oxygenated waters (Bowles *et al.*, 2002). Such resistance to oxidation is contrary with the finding that dissolved and particulate metals increase the rate of sulfide oxidation in oxygenated waters (Vazquez *et al.,* 1989; Zhang and Millero, 1994). As noted earlier, the identity of metal-sulfide species in oxic waters (synthesized or natural) is a prerequisite to determining their relative importance in metal speciation in these waters.

In this study, a weight-of-evidence approach is used to characterize synthesized metal-sulfide species in oxic waters to determine their chemical and physical identities and stability. Synthesized solutions were used for this purpose, since current analytical techniques are not sensitive enough to unambiguously identify metal-sulfide species in oxic natural waters at very low concentrations (if present) and in the presence of complicated matrix (*e.g.*, dissolved organic matter). Field studies were then carried out in two Canadian Shield lakes that have been affected by various degrees of human activities to estimate the ubiquity and relative importance of metal-sulfide species in surface waters.

2.4 Analytical Techniques for Metal-Sulfide Species in Aqueous Solutions

Before moving on to the experimental section, it is worthwhile to have a quick review of analytical techniques available for metal-sulfide species, which are a prerequisite for this study. Unfortunately, there has been no direct analytical technique that can unambiguously identify and quantify individual metal-sulfide species in natural waters, due to their low concentrations and to the presence of interfering species co-existing in water. However, several techniques are available which can provide information on metal-sulfide species in natural waters. These include (i) techniques for measuring sulfide; (ii) mass spectrometry; and (iii) transmission electron microscopy (TEM) and energy dispersive spectroscopy (EDS).

2.4.1 Analytical Techniques for Sulfide

Due to the toxicity of sulfide (particularly H_2S), both environmentally and occupationally, the determination of sulfide is of growing importance to analytical chemists (Lawrence *et al.*, 2000). There is a need for quick, sensitive detection techniques, both in a laboratory and industry (*e.g.,* petroleum industries) and in the field (*e.g.,* evaluation of effluent). Furthermore, sulfide can act as a versatile intermediate, allowing other sulfur-based species to be determined.

Several analytical techniques are currently available for determining sulfide in a variety of sample types, including (Lawrence et al., 2000): spectroscopy, electrochemistry, chromatography and combinations of these (Figure 2.3). UV-visible absorption spectroscopy based on the Methylene Blue (MB) method (Cline, 1969) is by far the most versatile, sensitive, and robust technique; coupled with different sample pretreatment methods, this technique can be used to differentiate different groups of sulfide-containing species in natural waters.

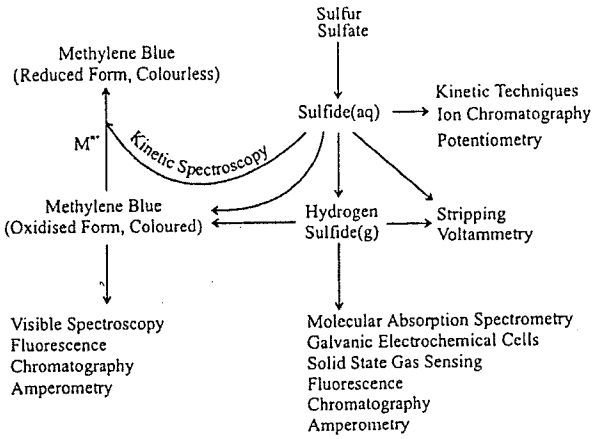
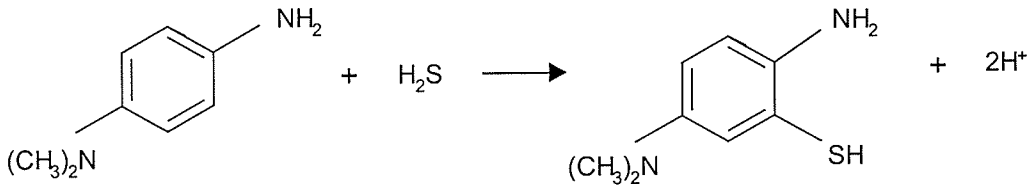


Figure 2.3: Analytical pathways for sulfide detection (Lawrence et al., 2000)

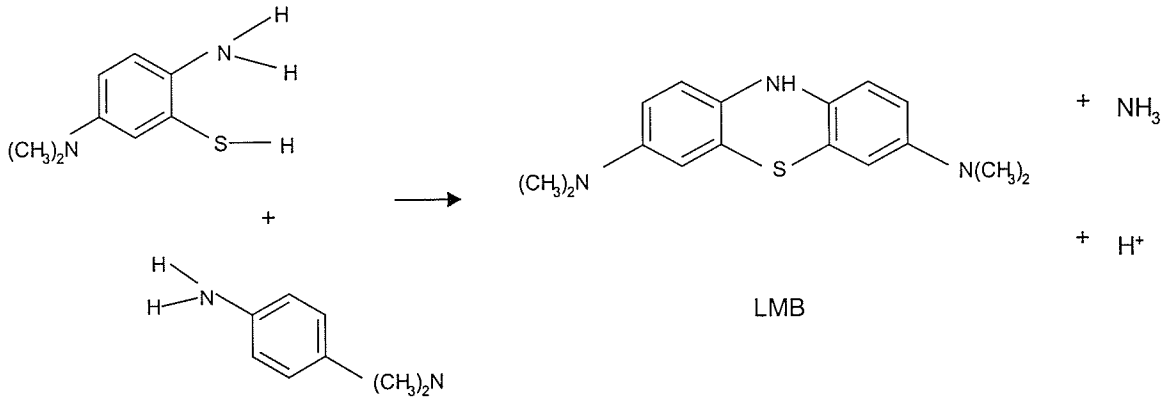
2.4.1.1 Methylene Blue Method (MB)

The Methylene Blue method for determining sulfide is based on the oxidative coupling of N, N'-dimethyl-p-phenylenediamine (DMPD; also known as 4-amino-N,N'-dimethylaniline) and sulfide. The method can be traced back to the 1880s (Fischer, 1883) and the major revision was done by Cline (1969) for the determination of sulfide in natural waters. It involves two chemical reagents, referred to as Cline's Reagents: DMPD and ferric chloride; both are prepared in 6M HCl. Sulfide reacts with DMPD to produce mercapto-3-dimethyl-p-phenylenemiamine:



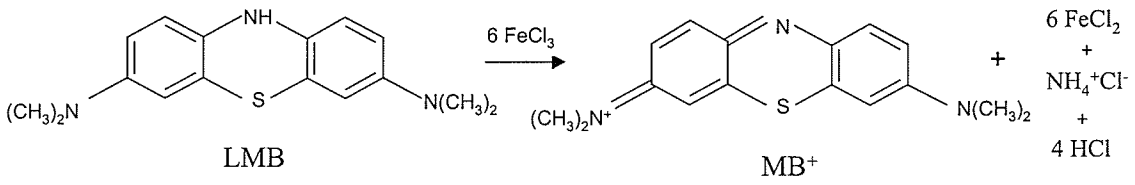
DMPD

The mercapto-3-dimethyl-p-phenylenemiamine reacts with another DMPD molecule, producing the leuco-derivative of methylene blue (LMB):



LMB

Finally, in the presence of Fe(III), LMB is oxidized to produce methylene blue (MB; Cline, 1969; Lawrence *et al.*, 2000):



Sulfide concentrations in water samples can therefore be determined spectroscopically by the intensity of the blue color produced; that is, by measuring the absorbance of the methylene blue complex at a wavelength of 667 nm. By using different concentrations of the Cline Reagents, the MB method is capable of analyzing sub-micromolar to millimolar levels of sulfide in natural waters (Cline, 1969; F. Wang, unpublished results). The detection limit can be further lowered down to sub-nanomolars with flow injection technique (F. Wang, unpublished results).

It is important to note that the final pH of the measuring system (sample plus the Cline Reagents) is extremely acidic (pH ~ 1). This low pH is required not only to promote the methylene blue reactions which occur only when sulfide is in the protonated form of H_2S (pK_{a1} for H_2S is 7.02; NIST, 1997), but also to keep the methylene blue in the MB^+ form (pK_{a1} for MBH^{2+} is around –0.8). Since many metal-sulfide complexes dissociate completely or partially at this low pH (Kuban *et al.*, 1992; Luther *et al.*, 1996), the MB method not only measures the free sulfide ($H_2S + HS^-$), but also these acid-dissociable

metal-sulfide complexes. The later is usually negligible in highly sulfidic waters when compared with the free sulfide, but is expected (as discussed later in this study) to dominate the sulfide speciation in oxic waters where free sulfide is not stable.

2.4.1.2 Acid Volatile Sulfide

The direct MB method is prone to interference in samples with high dissolved organic matter or suspended particles, which may affect the absorption at 667 nm. It is thus desirable to remove the matrix interference. One of the most popular methods is the acid volatile sulfide (AVS) method (Allen *et al.*, 1993). It involves acidifying the sample with HCl to convert the free sulfide and acid-dissociable sulfide to H_2S, which is purged and trapped in a NaOH solution followed by analysis of the trapping solution for sulfide by the MB method.

The method was originally developed for the analysis of "reactive" sulfide in sediments (Allen et al., 1993). It was found that AVS measured in sediments is mainly metastable iron sulfides (*e.g.,* amorphous iron monosulfide, FeS; mackinawite, FeS_{1-x}; pyrrhotite, $Fe_{1-x}S$), manganese monosulfides, and less abundant trace metal sulfides such as ZnS, PbS, CdS (Allen *et al.*, 1993; Cooper and Morse, 1999). The less active mineral pyrite ($FeS_2(s)$) is not measured by the AVS method. This finding is subsequently incorporated into the AVS-SEM (simultaneously extracted metals) model which predicts that no

toxicity of a Class B metal ions is expected in anoxic sediments when the molar concentrations of AVS is in excess of that of SEM (Di Toro *et al.*, 1996).

Bowles *et al.* (2003) recently adapted the AVS method for the determination of sulfide in oxygenated waters. By adding a 0.8 M HCl (final concentration in the water sample), they reported a very good recovery and precision for the free sulfide (e.g., Na_2S) and synthesized Zn-sulfide clusters.

2.4.1.3 Chromium Volatile Sulfide

Both the direct MB and the AVS methods use acidification to strip sulfide from metal-sulfide species. However, not all the metal sulfides are acid dissociable. For example, HgS is not soluble in HCl, and CuS and NiS are poorly soluble in HCl (Allen *et al.*, 1993; Copper and Morse, 1999; Luther *et al.*, 1996). More aggressive extraction techniques are thus needed for these sulfides. In geochemistry, this is traditionally done by the chromium(II)-volatile sulfide (CVS) (also known as chromium(II) labile sulfide or CLS) method (Canfield *et al.*, 1986; Mylon *et al.*, 2002).

In the CVS method, reduced sulfur species (pyrite + acid volatile sulfur + elemental sulfur) are dissociated to H_2S by their reductive dissolution by a strong reductant, Cr(II) in an acidic solution:

$$MeS + 2Cr^{2+} + 2H^{+} \leftrightarrow Me + 2Cr^{3+} + H_2S$$

The H_2S is purged from the sample and trapped in a NaOH solution and subsequently analyzed by the MB method.

Since Cr(II) is extremely unstable and readily oxidizes upon exposure to air (Kolthoff *et al.*, 1963), it has to be prepared freshly from the reduction of $CrCl_3$. The most commonly used reducing agent is amalgamated metal zinc in an acidic solution:

$$Cr^{3+} + Zn \leftrightarrow Cr^{2+} + Zn^{2+} + e^-$$

Amalgamation of Zn is needed to prevent the dissolution of zinc in the acid (Kolthoff *et al.*, 1963) while allowing the (amalgamated) zinc to react readily (Vogel, 1961).

Reduction with amalgamated zinc is usually carried out in a Jones reactor (Canfield *et al.*, 1986). This consists of a glass tube containing a column of amalgamated granulated zinc. A stopcock is used to draw the solution that is to be reduced. A zinc column is more efficient due to the large surface exposed when compared to pieces of zinc placed in the solution. The glass tube should be of an appropriate length to hold a column of zinc 25 to 35 cm in height and have an internal diameter of 2 cm. A pad of glass wool that is supported by a perforated porcelain plate supports the zinc column. Finally, the system is connected to a 500 mL suction flask (Kolthoff *et al.*, 1963).

If hydrochloric (or sulfuric) acid solutions or reducible elements, such as Cr(III), are passed through the tube, quantitative reduction to Cr(II) occurs (Vogel, 1961). Upon reduction, the chromium solution changes from green to blue, indicative of the valence

change from chromic (Cr(III)) to chromous (Cr(II)) ion (Canfield *et al.*, 1986). Due to atmospheric oxidation the $CrCl_2$ solution must be prepared every 2-3 days and is stored in a sealed bottle in a glove box.

The sulfide measured by the CVS method includes not only free sulfide and metal-sulfide complexes, but also other sulfur-containing species such as elemental sulfur, sulfite and thiosulfate (Canfield *et al.*, 1986; Fossing and Jørgensen, 1989), although the concentrations of the latter species are not likely high enough to interfere with the measurement (Mylon *et al.*, 2002). Whereas most studies (Canfield *et al.*, 1986; Bowles *et al.*, 2003; Fossing and Jørgensen, 1989) reported that the CVS method does not measure sulfate and organic sulfur, Mylon *et al.* (2002) suggested that a portion of the sulfur in sulfate and organic sulfur compounds could also be measured by the CVS method.

Originally developed for measuring sulfide (particularly pyrite) in sediments, the CVS method has recently been used to measure acidification-resistant sulfide in aqueous samples (Rozan *et al.*, 2000; Bowles *et al.*, 2003). However, such application may be questionable for natural oxic waters, as a recent paper by Mylon *et al.* (2002) indicated that at high $Cr:SO_4^{2-}$ ratios, SO_4^{2-} can be quantitatively converted to H_2S. They found that the quantity of measured CLS occurred proportionally to the amount of Cr(II) added to the solution. This may be insignificant for anoxic sediments were sulfide levels are high and sulfate low, but in oxic waters the amount of H_2S liberated from sulfate and reduced organic sulfur compounds is large enough to interfere with the ambient concentration of

CVS. Mylon *et al.* (2003) suggested that decreasing the Cr(II) concentration may avoid sulfate reduction, but noticed that quantitative liberation of all CVS in solution at low concentrations may not be possible at low Cr(II) concentration.

2.4.2 Characterization of Metal-Sulfide Species

2.4.2.1 Mass Spectrometry

Mass spectrometric techniques for aqueous metal-sulfide species characterization are poorly studied. When appropriately used, this technique can provide insight on the type of species that are present in natural aqueous samples while also revealing the stoichiometries of the metal sulfide species. Using laser ablation - Fourier transform mass spectrometry (FTMS), Rozan et al. (2000) were the first to report that these metal-sulfide species are not simple $M(HS)^+$ complexes, but are higher order unprotonated clusters, such as M_3S_3, M_4S_6, M_2S_4. As mentioned earlier, it remains unknown whether the clusters were artificially created during the vacuum drying and laser ablation processes. To avoid artifacts, a direct liquid sample introduction technique is needed. As shown later in this thesis, several attempts were made during this study to directly introduce liquid samples to mass spectrometer; unfortunately, the results were not satisfactory.

2.4.2.2 Transmission Electron Microscopy

As discussed later in the thesis, one of the key questions regarding metal-sulfide species in oxic water is whether they are truly dissolved species (complexes) or particles. Electron microscopy (EM) is a powerful technique that can be potentially used to answer this question. Typically, submicron particles found in natural waters may be studied using a scanning electron microscope (SEM). However, to determine high-resolution images as well as elemental compositions of individual submicron particles, a transmission electron microscope (TEM) must be used. TEM can not only provide highly magnified images (1 to 200 000 times and greater), but also provide information on the crystal structure, morphology and elemental composition of a submicron part, based on observation of electron diffraction, characteristic X-ray emission and energy losses of the incident electrons (Nomizu *et al.*, 1987). When coupled with energy dispersive spectroscopy (EDS), TEM also allows for the determination of chemical composition (stoichiometric ratios) of single particles (Buffle and Leppard, 1995).

Although TEM has been used to identify the structure of metal-sulfide nanoclusters in semiconductor materials, to my knowledge this thesis is the first study using TEM to study the morphology and chemical composition of metal-sulfide species in oxic waters, as detailed in the later sections of the thesis. However, there have been studies examining metal-organic species and submicron particles (*i.e.* clay minerals, microorganisms) in oxic waters using TEM.

Chapter 3. MATERIALS AND METHODS

All water used for sample and reagent preparation was deionized water prepared from a Milli-Q (Millipore) or Nanopure (Barnstead) system. Deoxygenated deionized water (DDW) was prepared by boiling deionized water and cooling to room temperature while degassing with N_2. All reagents used were of ACS reagent grade or higher unless otherwise specified.

3.1 Preparation of the Solutions

3.1.1 Metal Stock Solution

Metal stock solutions (50mM) were prepared in DDW with the following salts: $Zn(NO_3)_2 \cdot 6H_2O$, $Pb(NO_3)_2$, $MnSO_4 \cdot H_2O$, $Cu(NO_3)_2 \cdot 21/2H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$ and $Cd(NO_3)_2 \cdot 4H_2O$ (Fisher Scientific) and $FeSO_4$ (Sigma-Aldrich).

3.1.2 Sulfide Stock Solutions and Standards

Crystals of sodium sulfide ($Na_2S \cdot 9H_2O$, Fisher Scientific) were placed in a weighing dish and washed with a few drops of DDW and sponged with a "Kimwipe" to wash the oxide form of sulfide from the surface. The sulfide stock solution (approximately 50mM) was prepared by dissolving 1.2 g of the crystals in 100 mL of DDW and 30 μL of 1M NaOH

(the NaOH minimizes volatile sulfide species, *e.g.*, H_2S). The stock solution was kept in a refrigerator.

Due to the oxidation and volatility of sulfides, the concentration of the stock solution was standardized before every use using iodometry (APHA, 2000). In brief, an excess amount of 0.0125 M I_2 solution (prepared by dissolving 2.0-2.5 g of KI and 0.32 g of I_2 and diluting to 100 mL with DDW) was added into the sulfide stock solution. The remaining I_2 is then back titrated with a 0.025 M $Na_2S_2O_3$ standard solution (Fisher Scientific).

$$HS^- + I_2 \leftrightarrow S^o + 2I^- + H^+$$

$$2\ S_2O_3^{2-} + I_2 \leftrightarrow S_4O_6^{2-} + 2I^-$$

The sulfide concentration of the stock solution was then calculated by comparison with a blank that does not contain sulfide.

Calibration standards of sulfide were prepared by diluting the sulfide stock solution into 5 working standards ranging from 0.25 to 25 μmol/L. Standards were prepared prior to each calibration and analyzed for sulfide using the MB method.

3.1.3 Metal Sulfide Solutions

The technique for the preparation of metal-sulfide species affects the number of sulfur vacancies hence emission intensity, and effects how the complex was formed. The addition of S^{2-} solution to a solution of stirred metal ions, as was done here, results in a

colloidal dispersion that grows in a local environment rich in metal ion. Addition of less than a stoichiometric amount of S^{2-} in the presence of metal ions, yields a colloid that is deficient in S^{2-} and results in more sulfur vacancies (Hao *et al.*, 1998).

Nominal concentrations of 5 μM, 10 μM or 50 μM metal-sulfide solutions were synthesized in the laboratory by mixing equimolar concentrations of the metal stock solution and sulfide stock solution under an argon blanket. An appropriate amount of a metal solution was added to 50 mL of DDW in a 60-mL amber glass container, covered with parafilm, and purged continuously with Ar. A PTFE (Teflon) coated magnetic stirrer bar was placed inside and used to stir the solution, as shown in *Figure 3.1*. The solution was stirred and titrated with an equimolar amount of a Na_2S solution under the condition that no metal-sulfide was precipitated from the solution. The solution was stirred continuously under the Ar blanket for 2 hours. The Ar blanket acts as an anoxic stabilization period during solution preparation (Bowles *et al.*, 2002). Finally, the parafilm was punctured or perforated to allow the solution to equilibrate with the laboratory atmosphere.

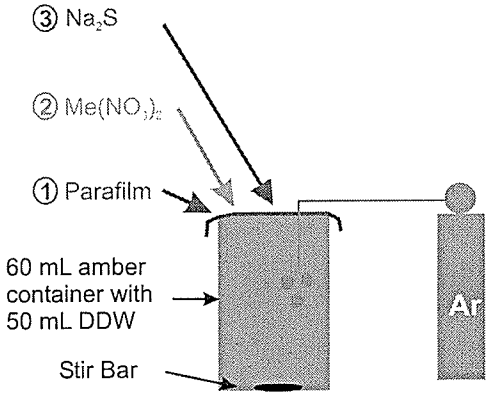


Figure 3.1: Experimental setup of metal-sulfide solution preparation

3.1.4 Cline's Reagents

Cline Reagent A was prepared by dissolving 0.063 g of DMPD (Fluka) in 50 mL 6 M HCl. Cline Reagent B was prepared by dissolving 0.075 g of $FeCl_3 \cdot 6H_2O$ (Sigma-Aldrich) in 50 mL 6 M HCl. Both solutions were stored in amber polyethylene bottles and kept refrigerated.

3.1.5 $CrCl_2$ Solution

Preparation of amalgamated Zn: 300mL of 2% $Hg(NO_3)_2$ solution and 1-2 mL of concentrated HNO_3 was added to 300g of pure 20 mesh zinc (Alfa Aesar) in a beaker. The mixture was stirred thoroughly for 5-10 minutes. The solution was decanted from the zinc metal, and was washed 2 or 3 times by decantation. The amalgamated Zn had a bright silvery luster.

Preparation of the reduction column: The zinc column rested on a pad of glass wool and the top of the column extended to within a short distance of the bottom of the upper reservoir. The reduction column was filled with water, and the zinc added slowly until the column was completely packed. The column was then washed with 500mL of deionized water, using gentle suction. The reduction column remained filled with water after the washing to prevent the formation of basic salts, which would clog the column.

Procedure for Jones reduction: The solution to be reduced had a volume of 100-150mL. Within a nitrogen glove bag, a 1.0 M $CrCl_3$ (Fisher Scientific) solution (in 0.5 M HCl) was drawn under vacuum through the packed column, which was rinsed first with a 1:5 (v:v) HCl:H_2O solution. A clean flask was attached to the reduction column, and using gentle suction, the solution was passed through the column at a rate not exceeding 75mL/min. The emerald green $CrCl_3$ solution became bright blue as it became reduced to $CrCl_2$ through the column. The level of liquid in the reductor should not fall below the top of the zinc column during the reduction. When all of the solution was drawn, the column was washed with 3 portions of 1:5 (v:v) HCl:H_2O solution, each portion drawn through before adding the next. The reductor was washed out with water to remove acid and prevent excessive consumption of the zinc. The solution was stable for 2-3 days, and was stored in an airtight vessel in a nitrogen-purged box.

3.2 Stability Study

The stability of the metal-sulfide complexes was studied by measuring the remaining sulfide in the synthesized metal-sulfide solution as a function of time. The solution was stirred vigorously for approximately 1 minute with a PTFE stir bar before the sulfide analysis. Otherwise, the solution was left quiescent.

The sulfide was analyzed by two different techniques depending on the metal-sulfide affinity: direct MB method and CVS method. Since the synthesized solutions were absent

of particles and organic matter, the direct MB method was used instead of the AVS method.

3.2.1 Direct MB Procedure

For the direct MB method, Cline's reagent A and Cline's reagent B were added directly to the sample vessel in a 1:1:25 (v:v:v) Cline Reagent A: Cline Reagent B: sample ratio. The solution was left to react in the dark for 30 minutes. Samples were then analyzed for absorbance at 667 nm on a Vary 50 Bio UV-Visible spectrophotometer or a Shimadzu UV-VIS spectrophotometer with a 1cm quartz cell. A blank was prepared using Cline's Reagent A and B and DI water in the appropriate volume ratio. Sulfide concentrations were determined from a standard calibration curve of absorbance versus concentration. The detection limit (as determined by measuring replicates of the lowest sulfide standard and calculating the standard deviation) was 5nM.

3.2.2 CVS Procedure

For the CVS measurement, 30 mL metal sulfide samples, prepared as described above, were purged with N_2. Concentrated HCl was added (to achieve a final molarity of 6M) in addition to 5mL of the 1 M $CrCl_2$ solution (As described above). The system was heated for 45 minutes to reflux to improve yields of recovery. The H_2S produced was purged by a low flow of N_2 and trapped in a 20 mL solution of 0.05M NaOH. The sulfide in the trapping solution was analyzed using the MB method as described above.

3.3 Characterization of Metal-Sulfide Species

3.3.1 UV-Vis Spectroscopic Analysis

A Cary 50 Bio UV-Visible spectrophotometer was used to perform the full spectrum analysis of the synthesized Zn-S, Cu-S and Cd-S solutions. Solutions were prepared as described above in 10μM and 200μM concentrations. The 200μM was also diluted to 10μM for further analysis. The analysis was done with the following parameters: medium scan rate and scan length of 190nm to 700 nm. Scans were performed periodically over 60 days. Samples were stirred vigorously for 1 minute and aliquots were taken directly from the prepared sample, with no additional sample preparation.

3.3.2 Mass Spectrometric Analysis

Mass spectrometric analysis was performed on Zn-S, Pb-S and Cd-S solutions at two different concentrations (1 μM and 10 μM), which were prepared following procedures described above and stored in a refrigerator for no more than 1 month. Samples were prepared at two concentrations to measure instrumental response and to determine isotopic response. Standards of metal nitrate or sodium sulfide were also prepared (10μM).

Both electrospray ionization (ESI)-MS and liquid chromatography (LC)-MS analyses were performed. The ESI-MS analysis was performed on a Quattro LC ESI-MS

(Micromass; in Dr. Hélène Perreault's laboratory in the Department of Chemistry) and an API 2000 triple quadrupole LC-MS/MS (MDS Sciex; in Dr. Gregg Tomy's laboratory at the Freshwater Institute). The LC-MS analysis was also performed on the API 2000 triple quadrupole LC-MS/MS.

For the ESI-MS analysis, aqueous samples were infused directly into an ESI capillary. Both positive and negative scans were obtained. The analysis was done under the following settings:

Focusing potential: 330.0 V

Entrance potential: 6.00 V

Ion spray voltage: 4800.0 V

MS/MS gas: N_2 at 15 psi

For the LC-MS analysis, a PRP-X200 cation exchange column (Hamilton) was used for the LC separation. Sample flow rate was 0.3 mL/min and the solvents used were methanol:water (60:40 v:v).

3.3.3 Transmission Electron Microscopic Analysis

Metal-sulfide samples (10 μM ZnS and 10 μM CuS) were prepared for TEM analysis. This was done over a period of 65 days to study the changes in the morphology of metal-sulfide species over time. Samples were prepared in two ways. The first technique involved centrifugation at 10,000 rpm for 30 minutes in a TEM grid holder. Carbon-coated nickel grids were used, and 5 mL of sample were added to the centrifuge tube. The second technique used Formvar-coated nickel grids (thin non-reactive plastic film on the surface of the grid); 5μL of sample was pipetted and remained on the grid for 1 minute followed by negative staining with 5μL of 5% phosphotungstic acid (PTA). Both solutions were bloated off using filter paper. The TEM analysis was performed on a Hitachi H-7000 (in the TEM laboratory in the Department of Microbiology) at an accelerating voltage of 75 kV. The images were recorded on a 3.25" x 4" Kodak film (#4489). The TEM-EDS analyses were done on a Hitachi JEOL 2000 FX (in the TEM laboratory in the Department of Mechanical Engineering) at an accelerating voltage of 80 kV.

Luther *et al.* (1999) suggested that nanoclusters of Zn-S can be present in seawater and hypothesized that high ionic solutions act as a protective agent, preventing the nanoclusters from growing. To test this hypothesis, metal sulfide solutions (10 μM Zn-S and Cu-S solutions) were also synthesized at high ionic strength (I = 0.7 M, typical value for seawater) and analyzed by TEM.

3.5 Field Studies

Field studies were performed in two Canadian Shield Lakes: Lake 658 in the Experimental Lakes Area (ELA; 49° 44' N, 93° 45' W) and Lake Wabigoon (49° 44' N, 92° 44' W) in northwestern Ontario. Lake 658 is located in a forested remote area and is relatively uncontaminated, whereas Lake Wabigoon is located near a small town (Dryden; population 6,700) and a large paper and pulp mill. Samples were collected during the summer 2002, and reflect concentrations only at the time of sampling and fail to detect episodic contamination events (Lu *et al.*, 2002).

Samples where collected in 120 mL *in situ* dialysis samplers ("peepers"). The double-sided samplers were made of plexiglass with two separated cells (~ 60 mL each) and built-in nylon bolts for water drainage. Once filled with deionized water, the cells were covered with a 0.2-μm hydrophilic polysulfone membrane (HT-200; Gelman). The assembled samplers were then suspended at different depths in the water column of the lakes; two in the hypoliminion, two in the metalimnion and two in the epilimnion. After an equilibration period of 2 days (A. Tessier, pers. Comm.), the samplers were retrieved from the lake and samples were drained directly (through the nylon bolts) to 300 mL borosilicate AVS/CVS reaction tube, and transferred in coolers to the lab and stored at 4^0C.

Upon return the laboratory, samples underwent the AVS/CVS procedure within 48 hours using a purge-and-trap apparatus (*Figure 3.2*) as described by Bowles *et al.* (2003). In

brief, samples (100mL) were purged first for 10 minutes with N_2 and then acidified with 7.14 mL of 6M HCl, resulting in a final solution of 0.4 M. Samples were purged for 30 minutes, with a trapping solution consisting of 25mL of 0.5M NaOH. After the allotted time has passed, a new trap was connected and the sample underwent the CVS procedure. An acidified 1 M $CrCl_2$ solution (16.67 mL) was added to the sample vessel and purged for 45 minutes, while heated simultaneously to reflux. The sulfide in both traps were analyzed using the methylene blue method as described earlier, except that a 10 cm path length quartz cell was used to measure the absorbance, due to the relatively low concentrations of sulfide in the samples.

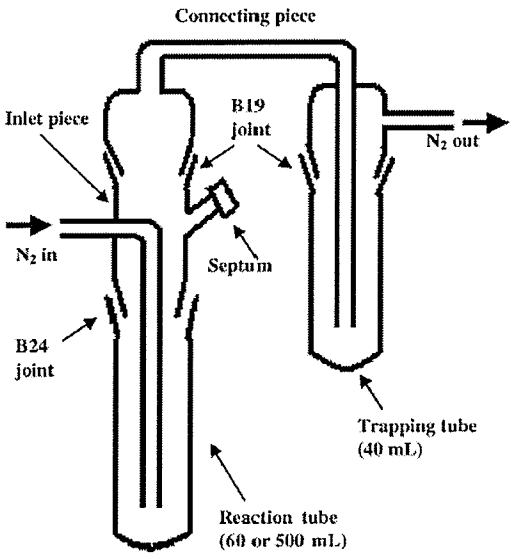


Figure 3.2*: Purge-and-trap apparatus for AVS/CRS determination (Bowles et al., 2003). The borosilicate reaction tube holds the sample, to which acid and/or Cr(II) reagent are added via the septum in the inlet piece. The trapping tube holds a 0.05 M NaOH solution for trapping evolved H_2S.*

3.6 Speciation Modeling

Chemical speciation modeling was done by thermodynamic calculations using the Windermere Humic Aqueous Model (WHAM, Version 6.0; Natural Environmental Research Council, UK), which was based on the Humic Ion Binding Model VI (Tipping, 1998). The thermodynamic constants used in the calculation are listed in Table 3.1.

Table 3.1. Some thermodynamic constants used in the metal speciation calculation by WHAM 6.0 (other constants were taken from NIST, 19997 and Tipping, 1998) (I = 0; t=25 °C)

Reaction	**log K**	Reference
$Pb^{2+} + 2HS^- = Pb(HS)_2$	12.55	Uhler and Helz, 1984
$Pb^{2+} + 3HS^- = Pb(HS)_3^-$	13.8	Uhler and Helz, 1984
$Cd^{2+} + HS^- = Cd(HS)^+$	7.38	Wang and Tessier, 1999
$Cd^{2+} + 2HS^- = Cd(HS)_2$	14.43	Wang and Tessier, 1999
$Cd^{2+} + 3HS^- = Cd(HS)_3^-$	16.26	Wang and Tessier, 1999
$Cd^{2+} + 4HS^- = Cd(HS)_4^{2-}$	18.43	Wang and Tessier, 1999
$Cu^{2+} + 4HS^- = CuS(HS)_3^{3-} + H^+$	18.23	Shea and Helz, 1989
$Cu^{2+} + 3HS^- = CuS(HS)_2^{2-} + H^+$	17.3	Shea and Helz, 1989
$Hg^{2+} + HS^- = HgS^0 + H^+$	28.1	Dyrssen, 1989
$Hg^{2+} + HS^- = Hg(HS)^+$	20.55	Dyrssen, 1989
$Hg^{2+} + 2HS^- = Hg(HS)_2$	38.64	Benoit et al., 1999
$Hg^{2+} + 2HS^- = HgS_2H^- + H^+$	33.0	Benoit et al., 1999
$Hg^{2+} + 2HS^- = HgS_2^{2-} + H^+$	23.8	Benoit et al., 1999

Chapter 4. RESULTS

4.1 Stability of Metal-Sulfide Solutions Exposed to Air

Chemical stability refers to a substance that does not easily decompose or is modified chemically. This is a desirable characteristic for materials used in preservation since it suggests an ability to resist chemical degradation over time and/or exposure to varying conditions. Chemical stability also refers to a material's chemical inertness and it's ability to resist chemical change (ALCTS Newsletter, 1990). The stabilities of metal-sulfide solutions exposed to air, as measured by the sulfide remaining in the solutions, are shown in Figures 4.1-4.7. Some metal-sulfide solutions (Zn-S, Pb-S, Cd-S, and Cu-S) were stable in oxic water for prolonged periods of time, often several weeks or months. However, sulfide solutions of Fe, Mn and Ni were much less stable in oxic waters; the sulfide concentration decreased to below the detection limit within 8 - 70 hours. The sulfide in the Zn-S, Fe-S, Mn-S and Pb-S solutions was measured by the direct MB method, whereas the sulfide in the Ni-S, Cu-S and Cd-S solutions was measured by the CVS method. Replicate measurements were individually performed with whole vessel analysis, so that any adsorption of sulfide on the vessel walls was taken into account.

EXPERIMENT #1: 10 μM Zn-S

As indicated in Figure 4.1, Zn-S solutions were stable in oxic water for several months. Approximately 30% of the nominal starting concentration of sulfide remained in the Zn-sulfide solution after being exposed to air for as long as 100 days. This long-term stability

of the Zn-sulfide solution in oxic water is in good agreement with that reported by Rozan *et al.* (2000) and Bowles *et al.* (2002). Both studies reported a half-life of more than 30 days for ZnS clusters.

EXPERIMENT #2: 10 μM Cu-S

Solutions of Cu-S remained stable in oxic waters for 50 days. As shown in Figure 4.2, After 50 days approximately 60% of the nominal starting concentration remained in solution.

EXPERIMENT #3: 10 μM Cd-S

Solutions of Cd-S remained stable in oxic waters for 50 days. Approximately 26% of the nominal starting concentration remained in solution (Figure 4.3).

EXPERIMENT #4: 5 μM Pb-S

Pb-S solutions remained stable in oxic waters for several days. After 7 days, less than 20% of the nominal starting concentration remained, while after 14 days less than 4% of the nominal starting concentration of sulfide remained in the Pb-sulfide solution after being exposed to air (Figure 4.4).

EXPERIMENT #5: 10 μM Fe-S

As indicated in Figure 4.5, solutions of Fe-S were not stable for more than a few hours. In fact, less than 10% of the nominal concentration remained after 48 hours and after 72 hours less than 2% of the nominal starting concentration remained in the solution. The

rapid oxidation of the Fe-S solution is consistent with that of synthetic FeS(s) (Nelson, 1978; Di Toro *et al.*, 1996).

EXPERIMENT #6: 50 μM Mn-S

Similar to Fe-S, Mn-S solutions also were not stable in oxic waters after a few hours (Figure 4.6). After 3 and 8 hours, sulfide concentrations were approximately 48% and 1% respectively of the nominal starting concentration.

EXPERIMENT #7: 10 μM Ni-S

As shown in Figure 4.7, Ni-S solutions were also not stable in oxic waters. After 2 and 8 hours, sulfide concentrations were approximately 10% and 2% respectively of the nominal starting concentration of sulfide remaining in the Ni-sulfide solution after being exposed to air.

Data was also used to calculate half-lives of the metal-sulfide species in solutions. Solutions of ZnS had a half-life of 38.72 days. This is in good agreement with other studies, which have reported a half-life of more than 30 days for ZnS species, as noted earlier (Bowles *et al.*, 2002, Rozan *et al.*, 2000). Solutions of FeS, and NiS had half-lives of 18.53 hours and 2.04 hours respectively. Solutions of MnS, PbS and CuS had half-lives of 0.3 hours, 0.41 hours and 0.031 hours respectively.

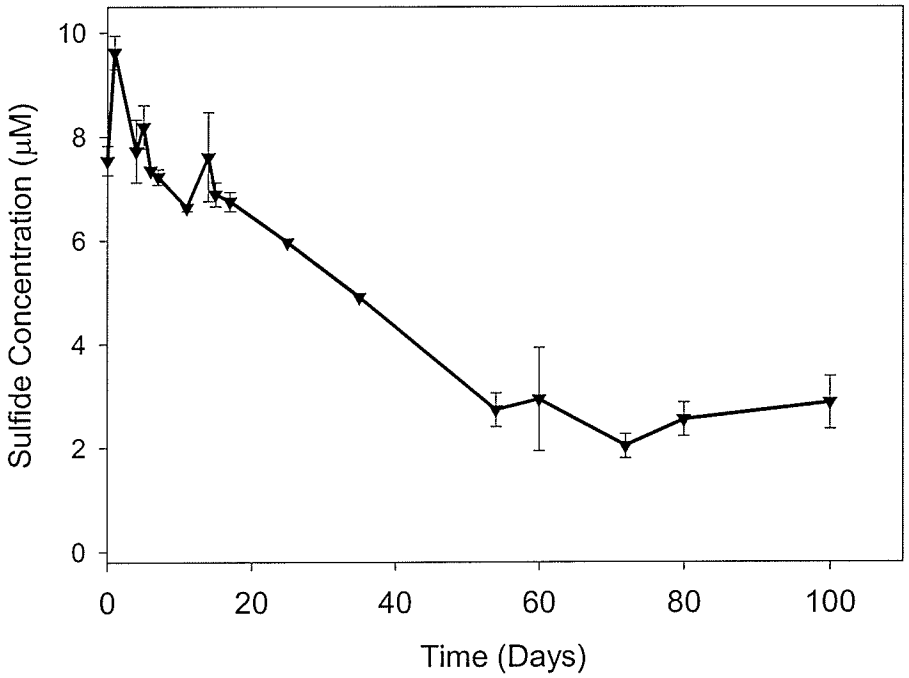


Figure 4.1: Stability of 10 μM Zn-S species exposed to air
(Error bars indicate the range of the two replicate measurements. Where there are no error bars, both replicates were identical)

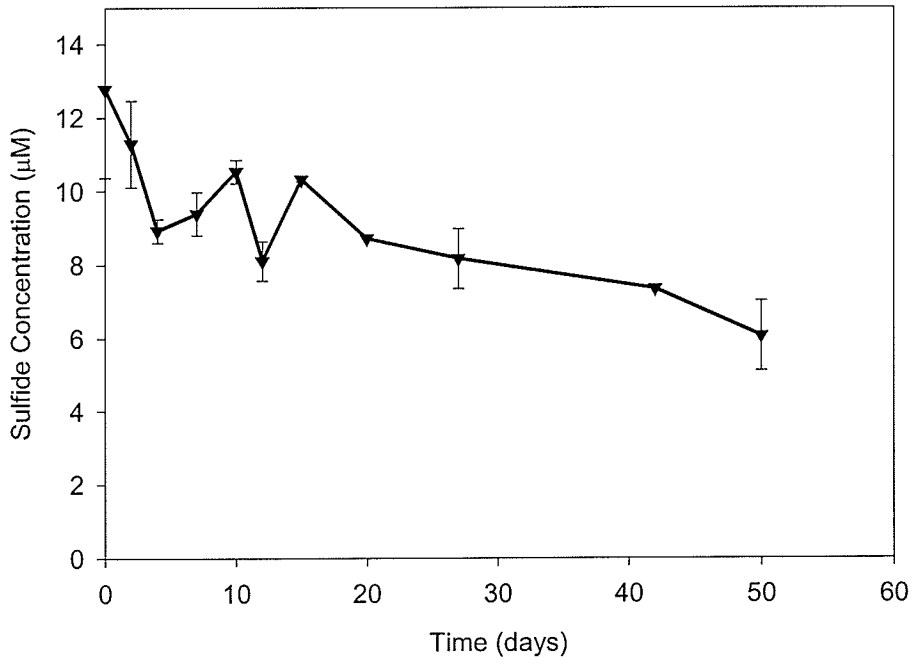


Figure 4.2: Stability of 10 μM Cu-S Species
(Error bars indicate the range of the two replicate measurements)

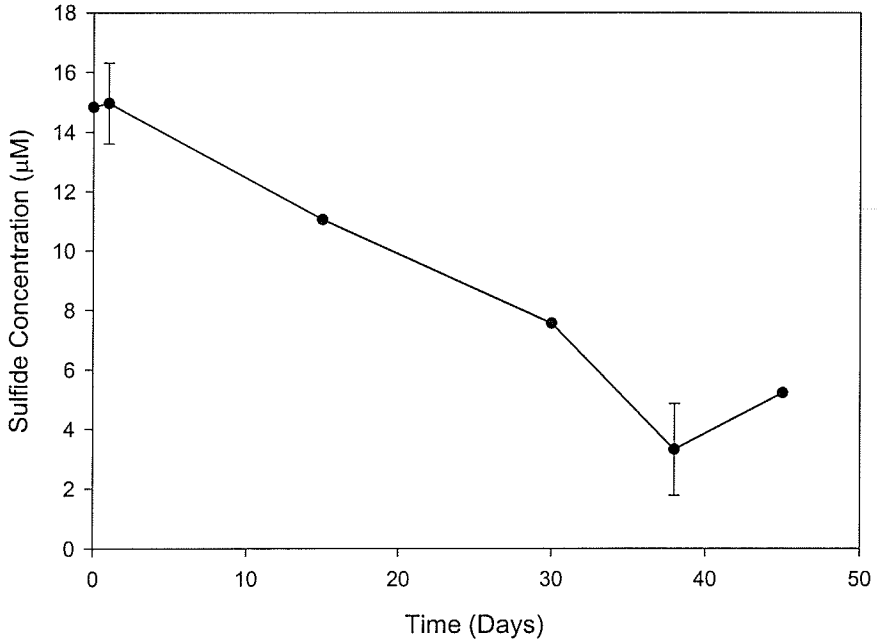


Figure 4.3: Stability of 10 µM Cd-S species exposed to air
(Error bars indicate the range of the two replicate measurements. Where there are no error bars, both replicates were identical)

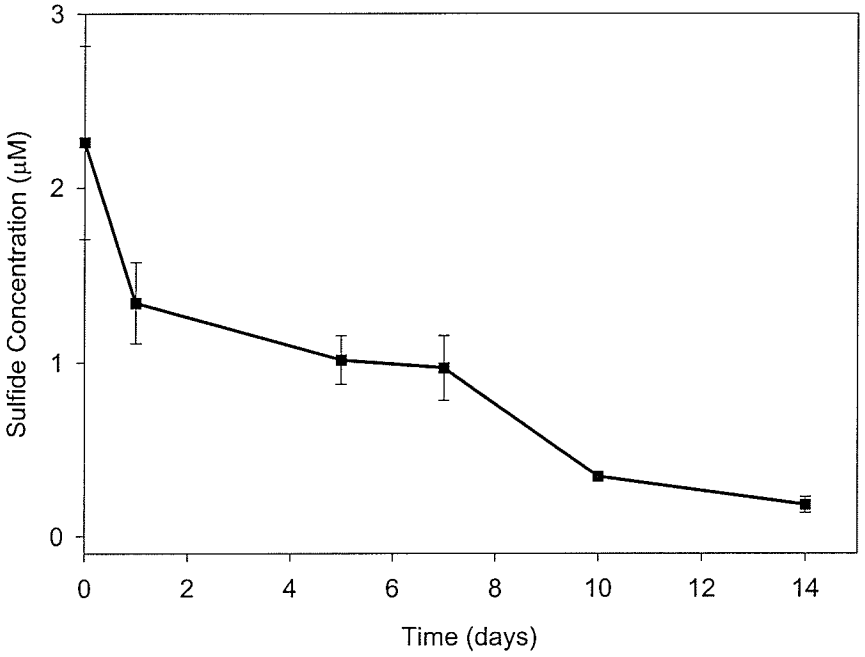


Figure 4.4: Stability of 5 µM Pb-S species exposed to air
(Error bars indicate the range of the two replicate measurements)

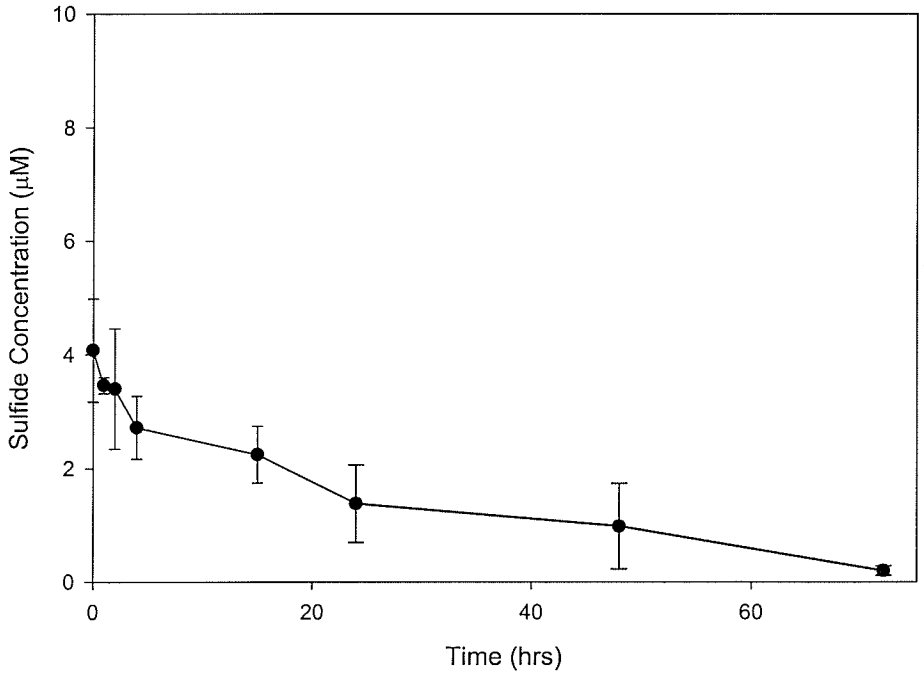


Figure 4.5: Stability of 10 µM Fe-S species exposed to air
(Error bars indicate the range of the two replicate measurements. Where there are no error bars, both replicates were identical)

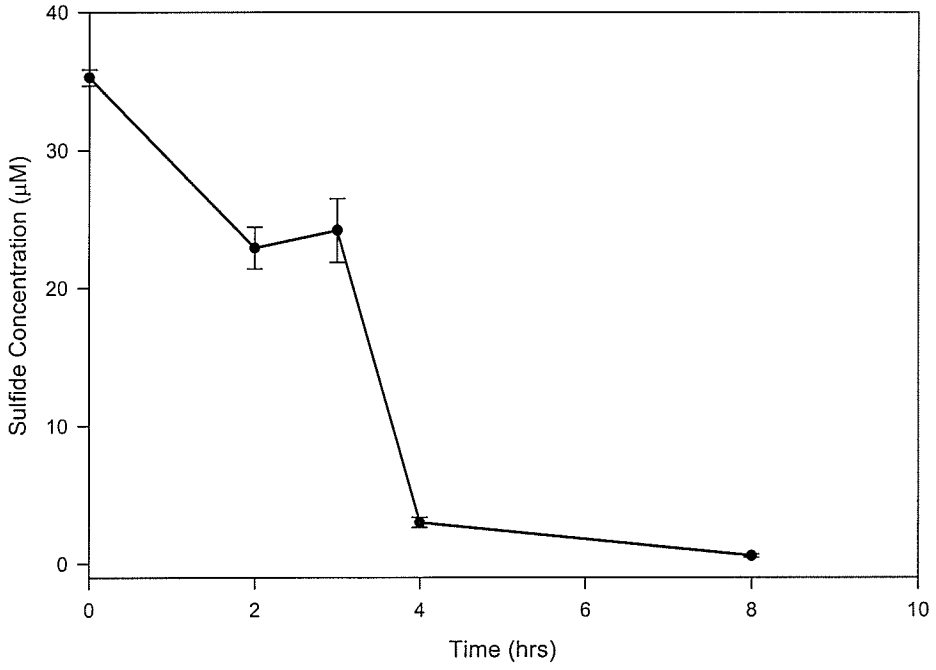


Figure 4.6: Stability of 50 µM Mn-S species exposed to air
(Error bars indicate the range of the two replicate measurements)

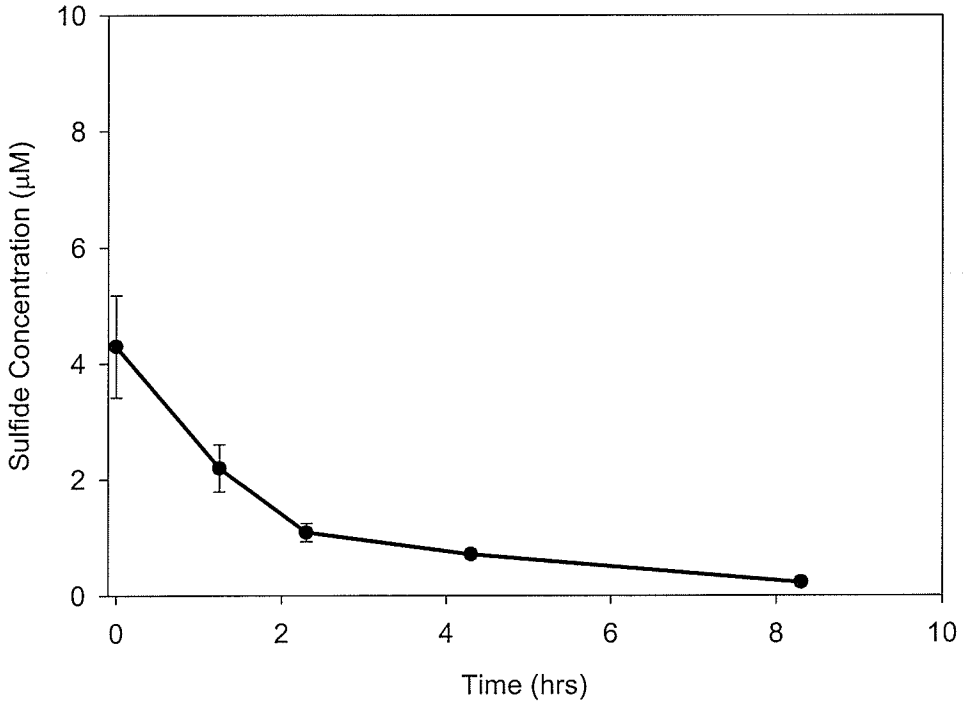


Figure 4.7: Stability of 10 μM Ni-S species
(Error bars indicate the range of the two replicate measurements. Where there are no error bars, both replicates were identical)

4.1.2 Adsorption of Metal-Sulfide Species onto Glass Walls

Bowles *et al.* (2002) reported that the synthesized ZnS clusters adsorb strongly to plastic and borosilicate glass beakers. To test these results in our system and to determine if sulfide adsorption onto the walls of the borosilicate reactors was important, the stability study was performed in 500 mL borosilicate glass beakers as well as in 60 mL glass amber jars. A comparison of the concentrations of sulfide obtained by adding the reagents directly to the reactors containing Zn and Pb sulfide solutions ("whole-system analysis"; any adsorption to the glass wall will be included in the measurement) with that obtained when a sub-sample of the solution ("sub-sample analysis"; any adsorption on the glass wall will not be included in the measurement) was analyzed. For sub-sample analysis, samples were prepared in 500 mL borosilicate glass beakers, and 5-mL sub-samples of the solution were removed from the beaker and analyzed for AVS or CVS. On the other hand, for samples prepared in 60 mL glass amber jars (whole-system analysis), the appropriate AVS or CVS reagents were added directly to the beaker.

As shown in Figures 4.8 and 4.9, the sulfide measured in the sub-sample study was significantly less than that in the whole-system study, demonstrating that loss to the glass walls did occur, even though beakers had previously been soaked in 10% (v/v) nitric acid for cleaning. Over time, greater sulfide loss occurred, hence a greater amount of species adsorbed as sulfide concentrations decreased. Such adsorption may occur because species bind to –OH sites on vessel walls (Stumm and Morgan, 1996; Bowles, et al., 2002).

The finding that metal sulfide species adsorb to a variety of materials is consistent with studies on the adsorption of colloidal ZnS and thin films of ZnS onto substrates such as glass and silicon (Duran *et al.*, 1999; Simpson *et al.*, 1998). At concentration ranges relevant to environmental metal concentrations, problems have been reported with the storage of soft metals in sulfide-containing waters. Simpson et al. (1998) have found that analytical losses of copper occur in sulfide-containing waters during storage.

Because of this strongly adsorption of the metal-sulfide species onto the experimental apparatus, these results indicate that proper storage and treatment of natural samples containing metal-sulfides must be considered while loss of metals for toxicity experiments may result when using instrumentation. Additionally, adequate precautions must be exercised when sampling and analyzing metal-sulfide solutions in oxic waters to minimize the loss of sulfide during sampling. In situ dialysis samplers are preferred. Peepers collect truly dissolved and fine colloidal samples via an equilibrating process, and any loss of metal-sulfide species to the peeper should be compensated by the diffusion equilibrium. Significant amounts of metal-sulfide species can be lost on membranes and containers using traditional techniques such as filtration and centrifugation.

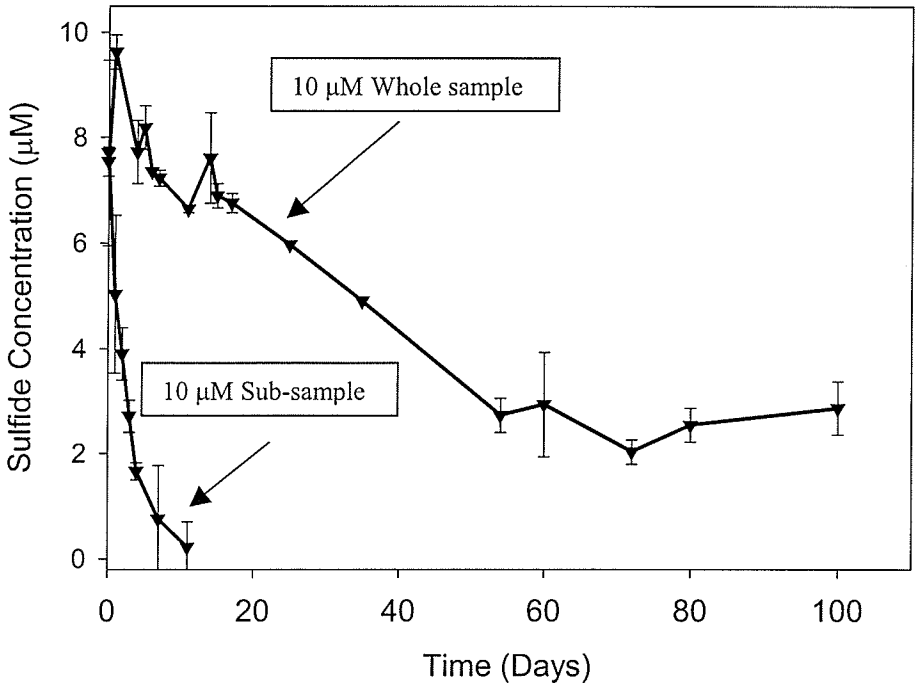


Figure 4.8: Adsorption of Zn-S species onto glass wall
(Error bars indicate the range of the two replicate measurements)

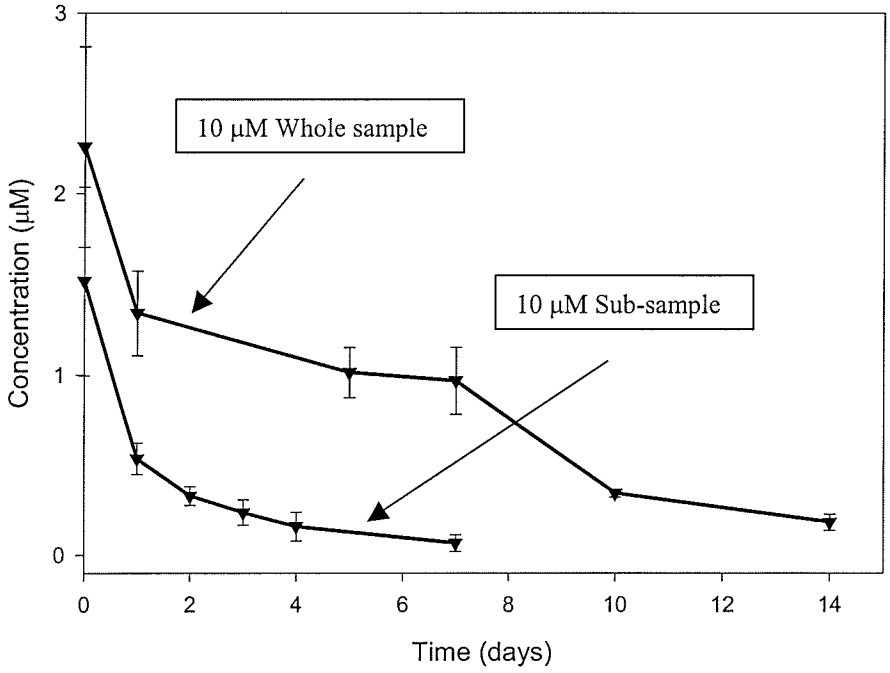


Figure 4.9: Adsorption of Pb-S species onto glass wall
(Error bars indicate the range of the two replicate measurements)

4.1.3 Evidence of Metal-Sulfide Formation

Indication of complex formation was obtained from UV-Visible absorption spectra of metal-sulfide solutions between 190nm and 350nm. As shown in Figures 4.10 and 4.11, there was a significant difference between the arithmetic sum of the metal solution spectrum and the sulfide solution spectrum, and the actual metal-sulfide solution spectrum. This indicates that new metal-sulfide species were formed. Solutions were transparent with no significant scattering of light at higher wavelengths. Similar conclusions were drawn by Luther *et al.* (1999) using electrochemical techniques.

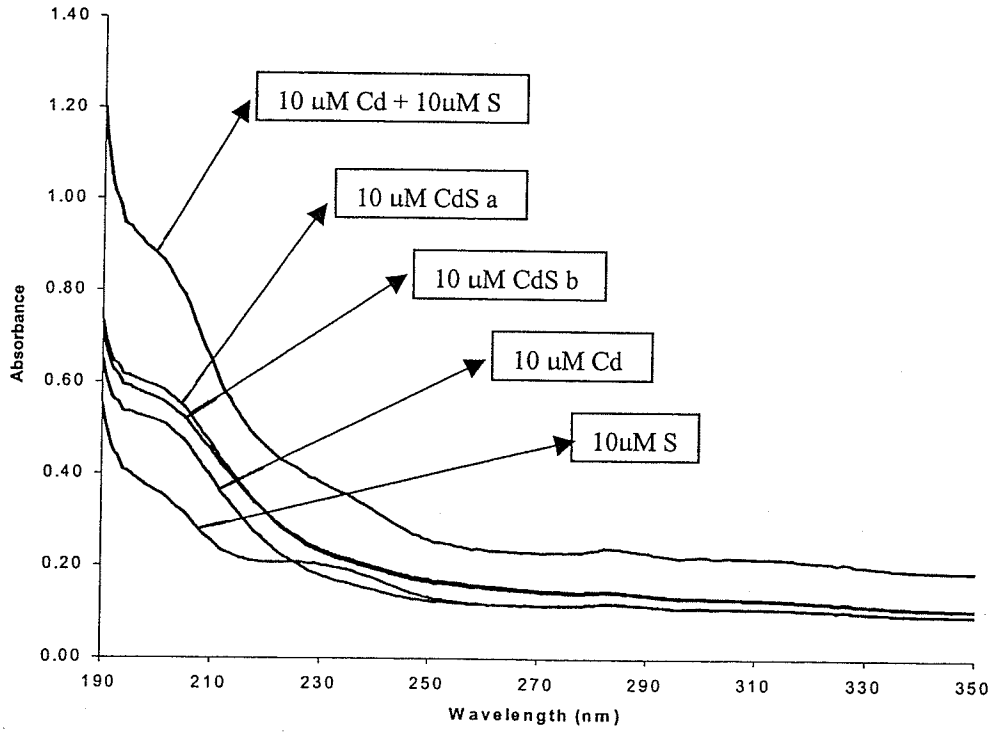


Figure 4.10: UV-VIS absorption spectra of 10 μM Cd(II) solution ($Cd(NO_3)_2$), 10 μM HS^- solution (Na_2S) and two 10 μM Cd-S solutions (a and b)

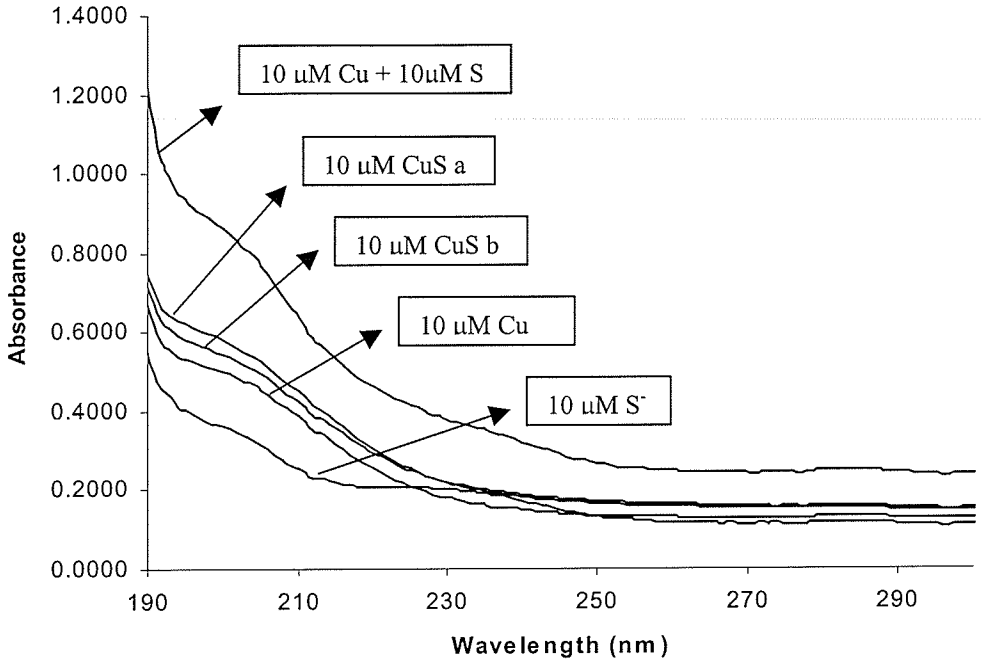


Figure 4.11: UV-VIS absorption spectra of 10 µM Cu(II) solution ($Cu(NO_3)_2$), 10 µM HS^- solution (Na_2S) and two 10 µM Cu-S solutions (a and b)

4.2 Characterization of Metal-Sulfur Species in Oxic Waters

4.2.1 UV-Visible Absorption Spectra

After completing the stability analysis of all the metals, further UV-visible absorption spectroscopic experiments were conducted on a few selected metal sulfides (Zn-S, Cu-S and Cd-S solutions) to detect any changes in the absorption spectra over time as well as to identify the form in which the species are present (*e.g.,* dissolved complexes or nanoclusters).

The peaks observed in the UV-visible absorption spectra (from 190 to 230 nm) of nominal 10 μM sulfide solutions each containing separate equimolar concentrations of Zn, Cd, or Cu as a function of time (0 – 65 days; Figures 4.12 to 4.16) showed absorption shoulders at wavelengths 195-210 nm. Absorption shoulders of similar shape have been reported for metal-sulfide nanoclusters synthesized in the presence of protective agents (e.g., Sooklal et al., 1996). However, for our solutions, the wavelengths of the absorption shoulders are at much lower wavelengths than those for metal-sulfide nanoclusters (usually > 250 nm; Sooklal et al., 1996). Our results for Cu are in agreement with those of Luther *et al.* (1999) who reported no absorption peak other than that for sulfide in a Cu-S solution. However, our results for Zn differ from those of Luther *et al.* (1999) who observed an absorption peak at 230, 264 and 290 nm in a Zn-S solution and attributed it to ZnS nanoclusters. These peaks were not observed in this study.

For nanoclusters it is well known that the absorption maximum at the absorption shoulder corresponding to the exciton energy shifts to shorter wavelengths with decreasing particle diameter, known as the quantum size effect (Wang and Herron, 1991). UV-visible absorption spectra (Figures 4.12 to 4.16) showed negligible shifts in wavelengths (or energy) over time. For example, solutions of 10 μM Zn-S had absorption shoulders for Day 0, 30 and 65 occurring at approximately 206.4 nm, 206.5 nm and 206.6 nm respectively. This indicates that solutions were composed of dissolved metal-sulfide particles or complexes, not nanoclusters. Although the spectra obtained had similar shapes of the spectra for metal sulfide particles (Bowles, et al., 2002; Hosokawa, et al., 1999; Sooklal, et al., 1996; Korgel and Monbouquette, 1996), characteristic peaks for nanoclusters at 290 nm were absent. This further suggests the absence of nanoclusters. Additionally, it was evident that Day 0 had the greatest absorbance, and decreased over time, corresponding with a decrease in concentration. This is indicative of changes in the solution, and consequently the solution losing stability over time, in agreement with our previous results. Also, the effect of time on absorbance to the solutions appears to weaken as the differences between day 30 and 65 lessens.

As the UV-VIS spectra approach 190 nm, a strong absorbance was measured, as shown in Figures 4.10 to 4.16. This is a result of the charge-transfer transition (or the broad band effect) exhibited in charge-transfer bands or spectra. In the ultraviolet and/or visible sections of a spectrum, it is common for coordination compounds to exhibit strong charge-transfer absorbances. For metal complexes, there are often intense charge transfer bands occurring in the ultraviolet region. If two charge-transfer bands exist, it is common

not to observe low energy absorbances in some regions of the spectrum (Cotton and Wilkinson, 1962; Miessler and Tarr, 1998). The board band effect may act as an interference during metal-sulfide characterization.

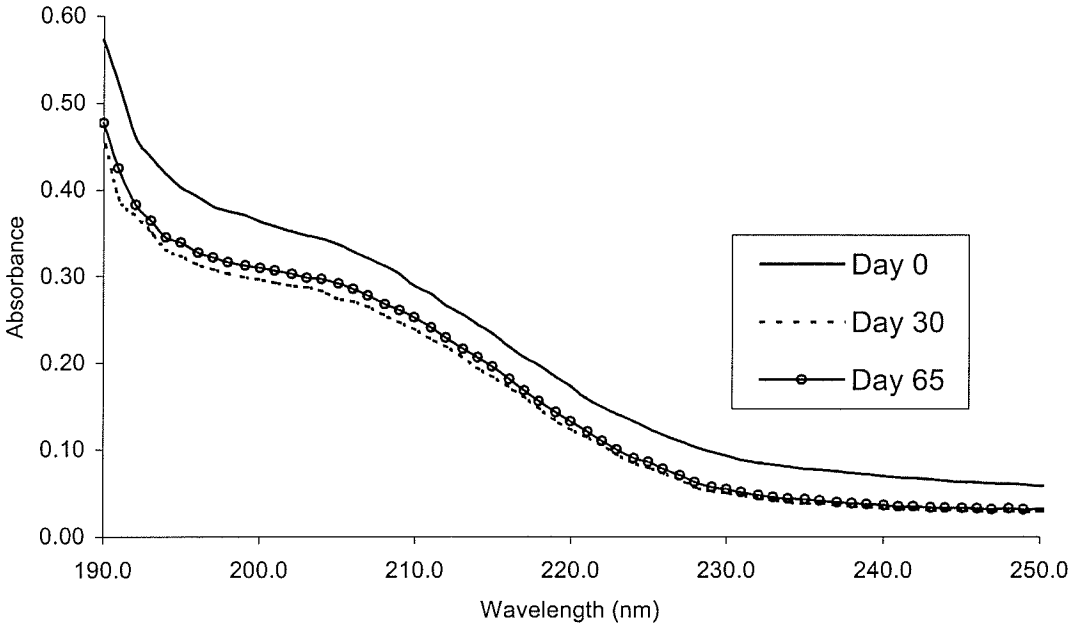


Figure 4.12: UV-VIS spectra of 10 μM Zn-S species

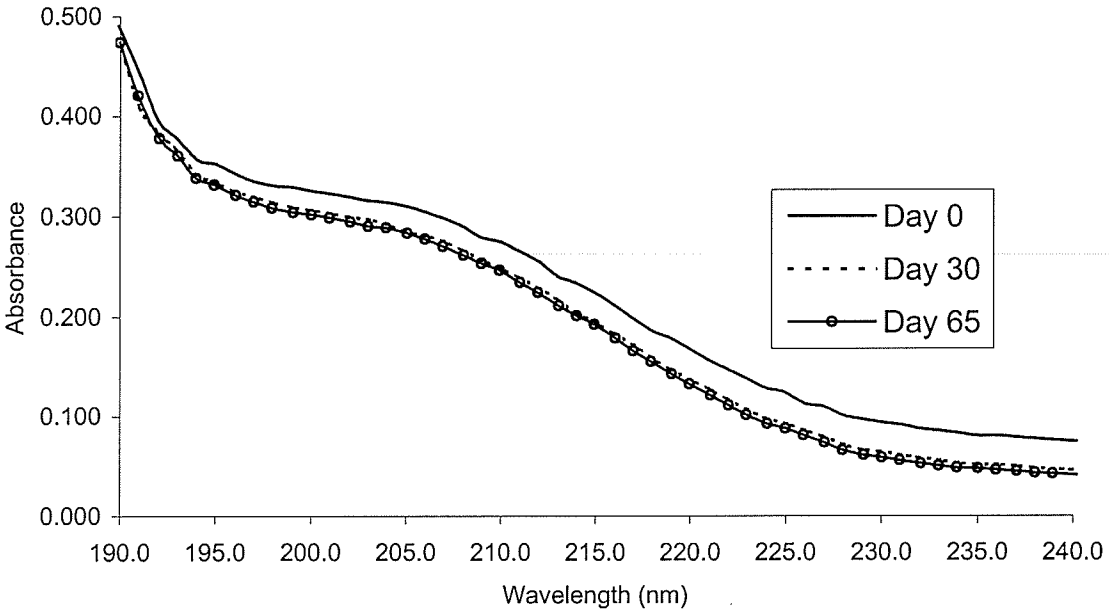


Figure 4.13: UV-VIS spectra of 10 μM Cu-S species

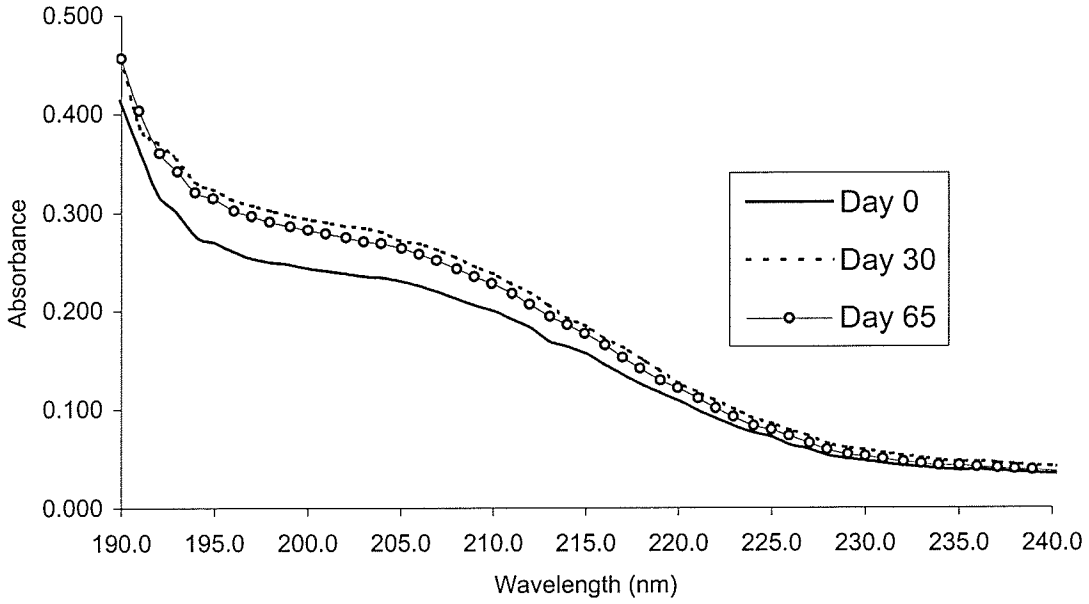


Figure 4.14: UV-VIS spectra of 10 μM Cd-S species

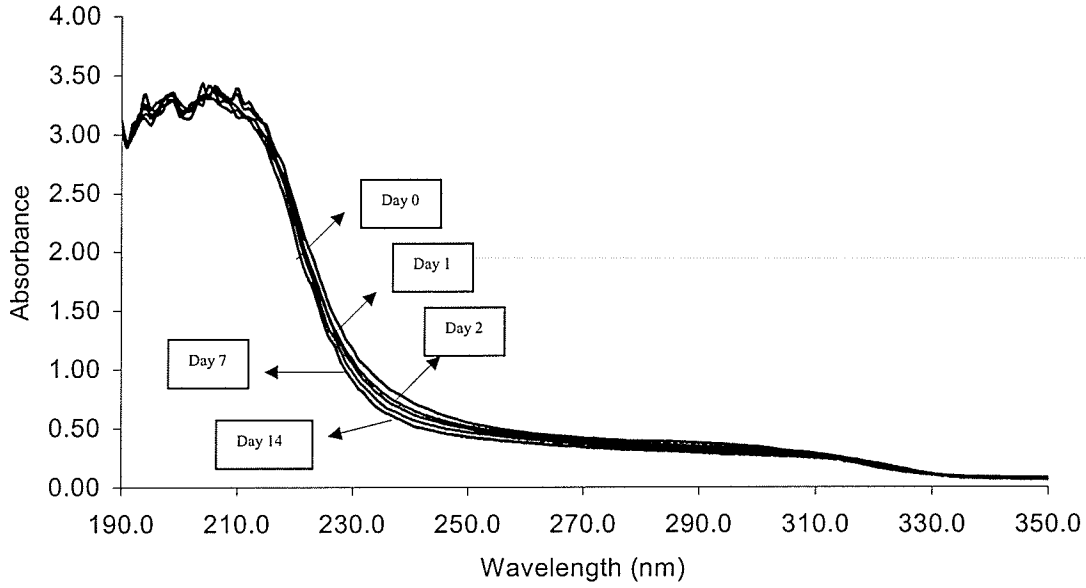


Figure 4.15: UV-VIS spectra of 200 μM Zn-S species

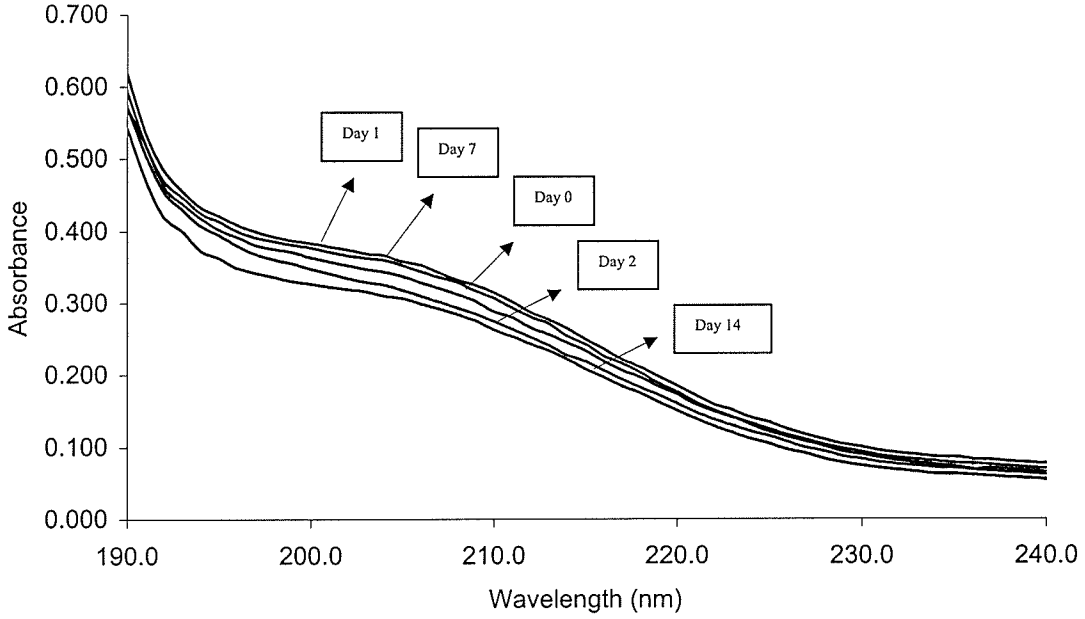


Figure 4.16: UV-VIS spectra of 10 μM (Diluted from 200 μM) Zn-S species

4.2.2 MS Characterization

Detectable peaks were observed in the low mass range m/z of 0 – 500 that may be assigned to simple mononuclear metal-sulfide complexes. Unfortunately, we were not able to determine the stoichiometries and concentrations of these metal-sulfide species, due to the insufficient mass resolution of the instruments at the low mass ranges. However, contrary to Rozan *et al.* (1999) and Luther *et al.* (2002), we did not observe any significant peaks at high mass ranges (e.g., > 200 Da) that could be assigned to metal-sulfide clusters of more than one metal atom (i.e., Me_xS_y; x>1). Our results nevertheless suggest that the clusters (Me_xS_y; x>1) in the previous studies (Rozan *et al.*, 1999; Luther *et al.* 2002) were likely artifacts resulting from freeze-drying or vacuum drying of their samples before mass spectrometry analysis.

When using ESI-MS, it is sometimes difficult to obtain good resolution to distinguish isotopic ratios. Inspection of the spectra revealed that isotopes of Zn^{2+} were not present, while isotopes of Cd^{2+} and Pb^{2+} were indistinguishable. When Zn^{2+} solutions were analyzed with MS, isotopes were present, but absent when ZnS solutions were examined, suggesting that peaks are in fact polysulfides, not ZnS species. However, the quantification of analytical results and the determination of isotope ratios are often difficult in all inorganic mass spectrometric studies due to incomplete knowledge of ion formation processes (Becker and Dietze, 2000). Experiments done by Micallef, *et al.*, found a loss of radioisotopes (^{65}Zn) during HPLC separation. This does not mean there was a loss to the column, but a metal exchange in the system could be occurring. They

also found problems when separating metal binding proteins with HPLC. In addition, metal complexes may be labile during HPLC separation (Micallef, *et al.* 1992).

EXPERIMENT #1: Zn-S SOLUTION

Solutions of 10 μM ZnS were prepared for analysis by ESI-MS and LC-MS. As shown in Figure 4.17, peaks were obtained at m/z of 65 and 97, possibly corresponding with the species S_2^- and ZnS, respectively. Additionally, after tandem MS-MS were performed on both peaks, it was determined that fragments occurred at m/z of 33.3 for the m/z = 65 peak and m/z = 33.3 and 65 for the m/z = 97 peak. This is indicative of HS^- present in the sample. However, this leads one to believe that the peak at m/z of 97 may also be in fact S^-_3 and not ZnS.

At low cone voltages, experiments by Løver *et al.* (1997) showed that some metal-sulfide bonds (ZnS and CdS) remained intact during observed fragmentation. Fragmentation of the metal-sulfide core unit occurs only at very high voltages. However, ions containing Zn show a decreased resistance to fragmentation compared to Cd. Also, the degree of fragmentation decreases with decreasing cone voltage (Løver *et al.*, 1997). Lack of significant fragmentation in these experiments may be explained by this phenomenon (Løver *et al.*, 1997).

EXPERIMENT #2: Cd-S SOLUTION

Using mass spectrometry to characterize Cd-S species was inconclusive. Although isotopic ratios of Cd were not clearly observed, peaks in the appropriate m/z range (m/z

of 106, 110, 111, 112, 113, 114 and 116 respectively) were present. However, their intensities were low, often comparable to background noise while other peaks predominated. Also, higher mass peaks were not observed, indicating that Cd_xS_y clusters were most likely not present (See Figure 4.18).

EXPERIMENT #3: Pb-S SOLUTIONS

Solutions of 5μM PbS were prepared for analysis by ESI-MS. Spectra reveal that PbS_2 and $Pb(HS)_3$ species may be present in solution. However, isotopic peaks of Pb (m/z of 206, 207 and 208 respectively) were difficult to quantify due to low resolution of the mass spectra.

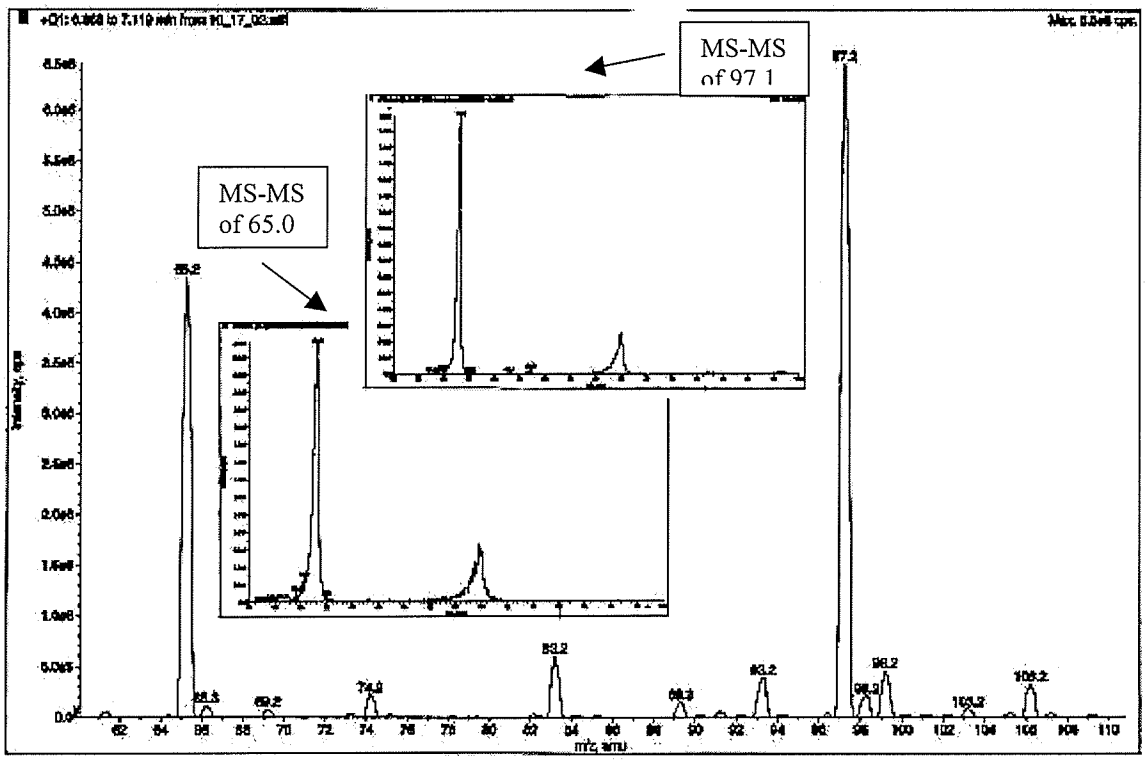


Figure 4.17: HPLC-MS spectra of 10 μM ZnS and MS-MS fragments

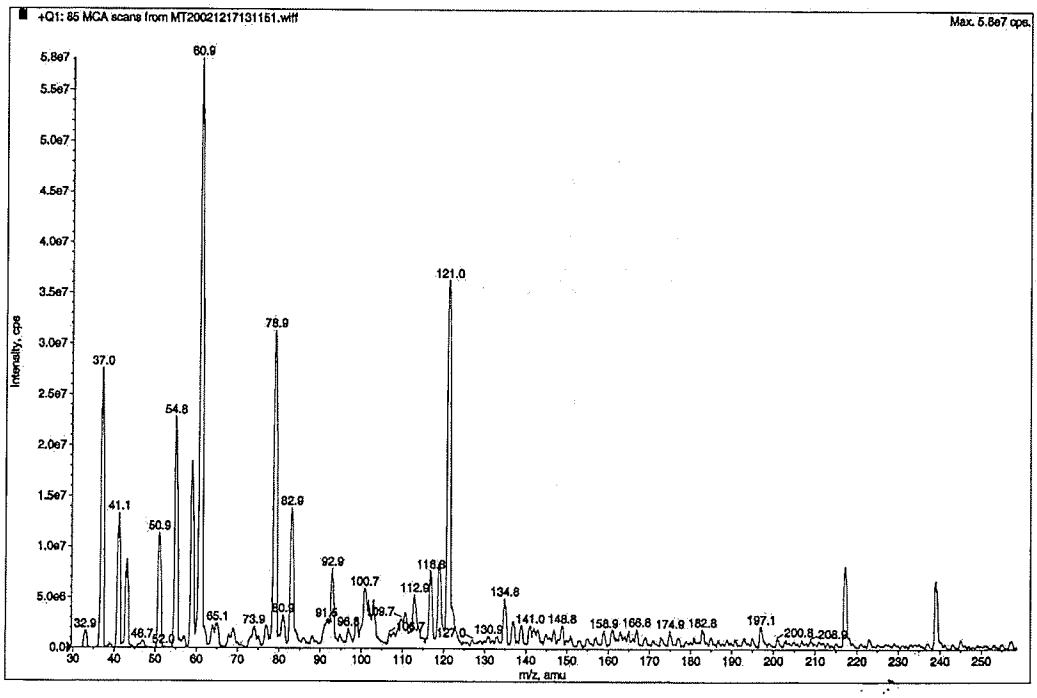


Figure 4.18*: ESI-MS spectra of 10 μM CdS*

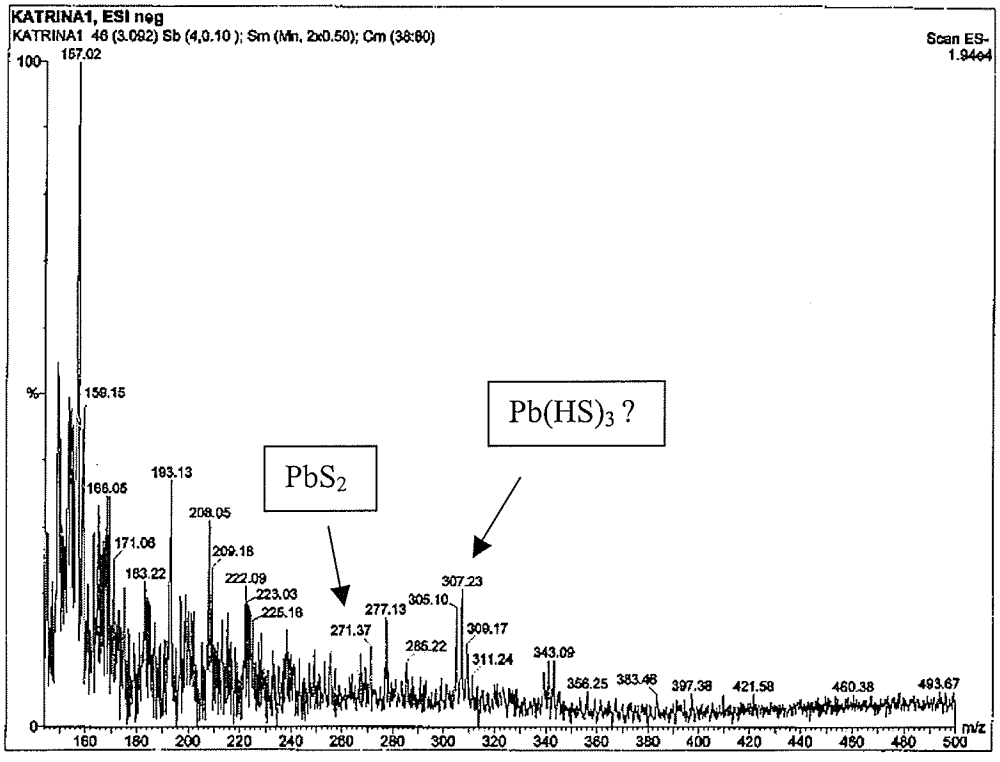


Figure 4.19: ESI-MS spectra of 5 μM PbS

4.2.3 TEM Characterization

To study the changes of the metal-sulfide clusters over time, TEM was used to observe morphological changes within the solutions over similar time periods used during the stability analysis. The images shown here are only a few examples of the images obtained, but do not represent all of the species in solution at that point in time. During the duration of the study, no visual precipitation of particles was observed in the solutions. Although the experiment was conducted for 64 days, bacteria were observed in samples analyzed on day 57, thus samples after this day were neglected due to impurities or other interferences with the metal-sulfide clusters. During the analysis, samples were sealed in airtight containers and stored in a refrigerator.

As shown in Figure 4.20 (A – C), Zn-S species appear in aggregates. *Day 1* shows a small aggregate of Zn-S particles, while *day 8* indicates an increase in the number of particles in each aggregate. By *day 15* Zn-S species appears as typical amorphous colloids.

Similar shape and size dynamics were observed for the Cu-S species (Figure 4.20 D - Figure 4.20 F). No image could be obtained for *day 1*. However, by *day 8*, small aggregate of Cu-S particles were present. By *day 15* Cu-S appears in typical colloids. Samples analyzed on day 43 and 50 contained both aggregates and colloids, but the samples were predominately Cu-S aggregates.

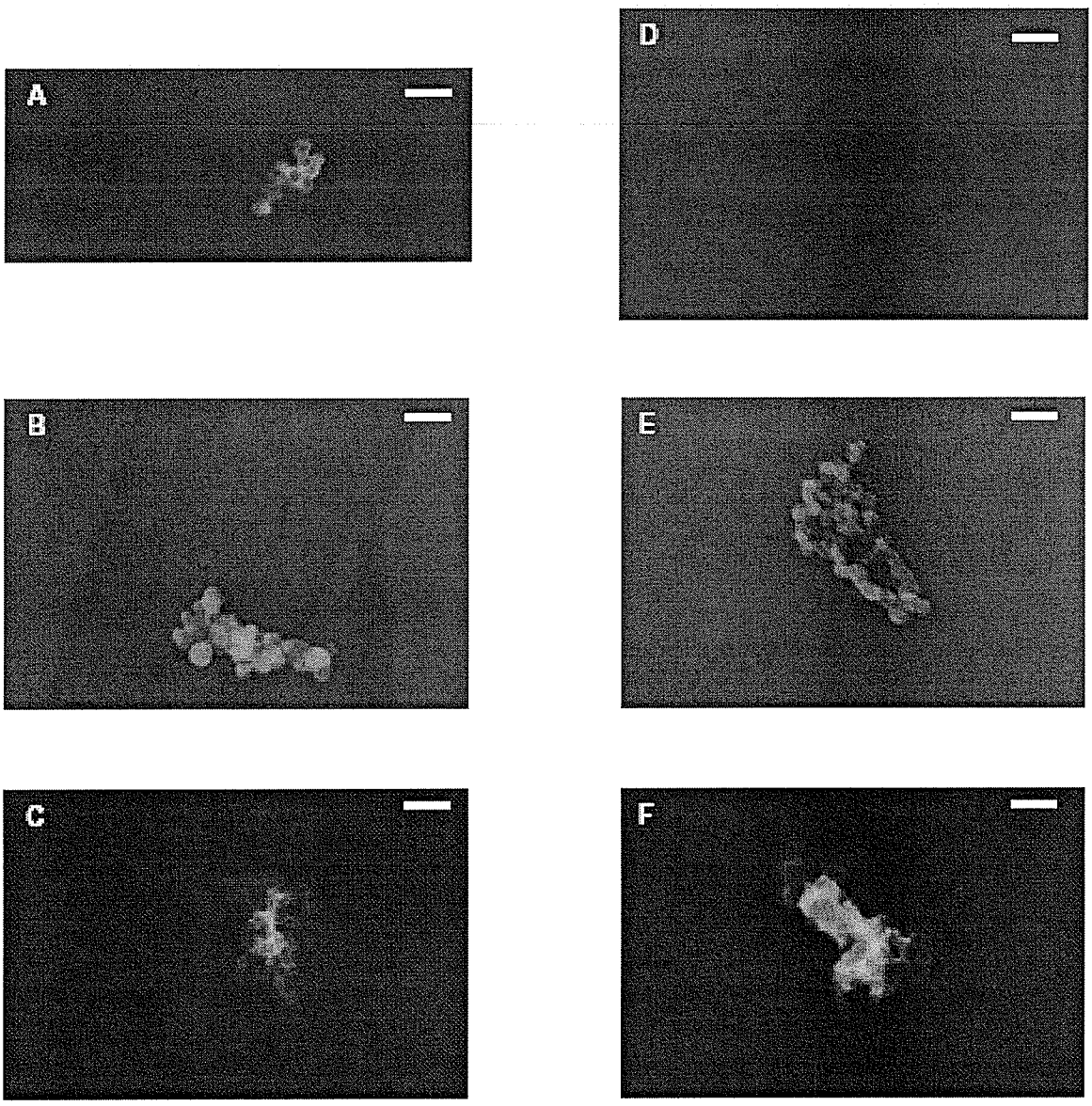


Figure 4.20: *TEM images of a nominal 10 μM sulfide solution containing Zn (A, B, C) and Cu (D, E, F) at Day 1 (A, D), 8 (B, E), and 15 (C, F) at I = 4×10^{-5} M. The white bar represents 100 nm.*

4.2.4 TEM Characterization: Metal-Sulfide Solutions at High Ionic Strength

It has been suggested (Luther *et al.*, 1999) that seawater (or high ionic solutions) may act as a protective agent, preventing nanocluster growth. Metal sulfide clusters of Zn-S and Cu-S were synthesized at high ionic strength (I = 0.7 M) and analyzed by TEM to study any morphological differences between solutions of low ionic strength ($I = 4\times10^{-5}$ M) with solutions of high ionic strength. A comparison of Figure 4.20 (ZnS and CuS) with Figure 4.21 (Ionic ZnS and CuS) does not support this hypothesis, since a similar dynamic nature of the metal-sulfide species was observed in all samples. Also, Table 4.1 illustrates the size distribution of ZnS and CuS solutions both in high ionic and low ionic solutions.

Metal-sulfide species appeared as particles or clusters initially (Day 1-8), but by day 8 appear as aggregates or colloids. They do not, however appear as nanoclusters as predicted. Also, the size of the clusters is similar in both solutions (See Section 4.2.6).

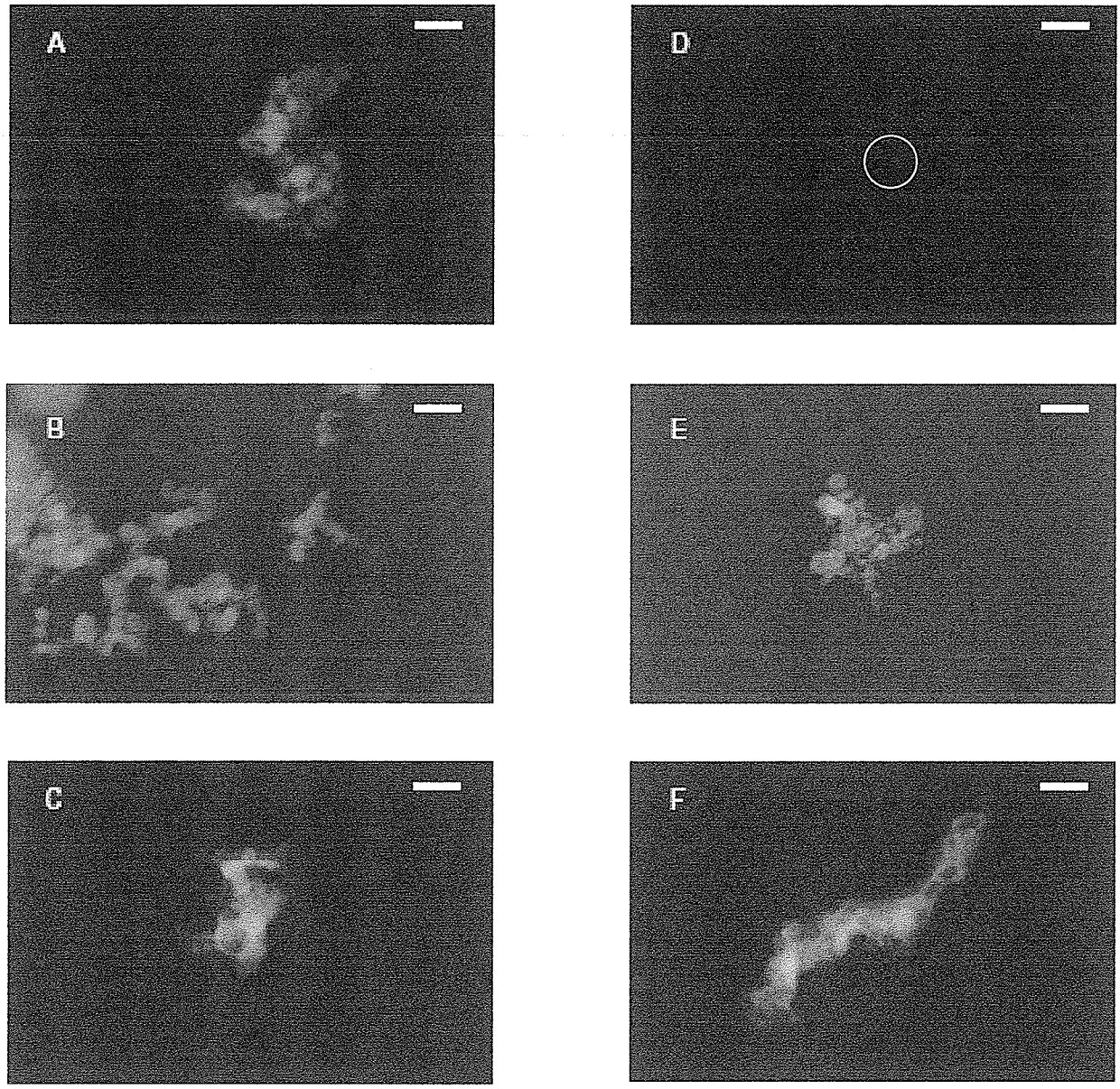


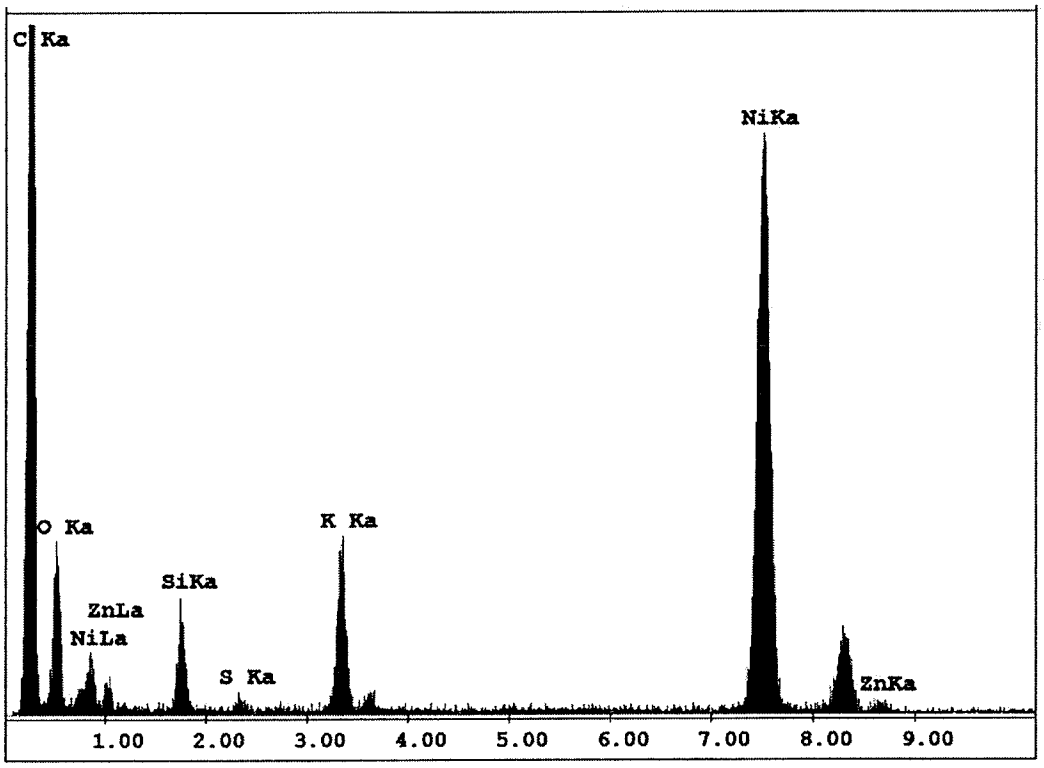
Figure 4.21: *TEM images of a nominal 10 μM sulfide solution containing Zn (A, B, C) and Cu (D, E, F) at Day 1 (A, D), 8 (B, E), and 15 (C, F) at I = 0.7 M. The white bar represents 100 nm.*

4.2.5 TEM-EDS Characterization

TEM alone provides only morphological information, but it may be coupled to a number of probes such as energy dispersive spectroscopy (EDS), allowing elemental and crystalline structure analysis on single particles (Buffle and Leppard, 1995). This allows for the determination of the metal to sulfide stoichiometric ratio. However, the complexes or colloids in the nominal 10 μM metal-sulfide solutions used to determine these ratios were low in mass and often smaller (200 –400 nm) than the electron beam used for precise and reliable results, thus quantifying the stoichiometric ratio could not consistently be obtained. Therefore, higher nominal concentrations were used (100 μM Zn-sulfide solution and 40 μM Cu-sulfide solution) for the EDS analysis.

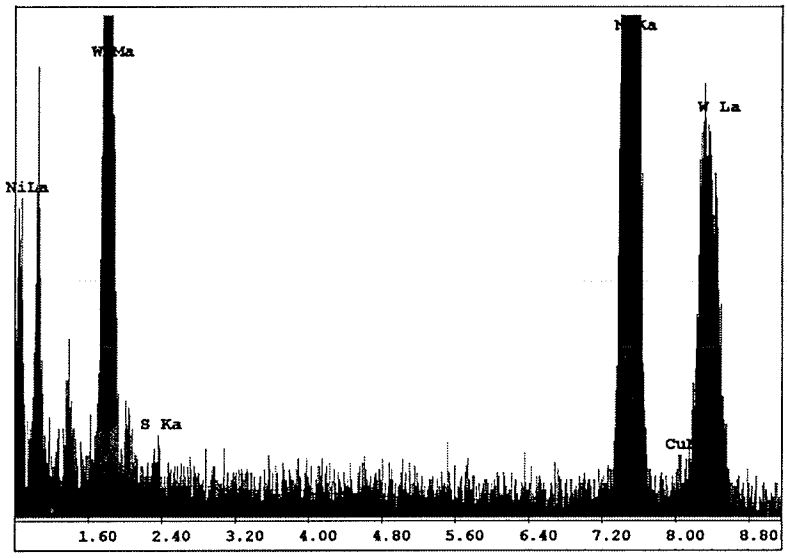
Analysis using TEM-EDS indicates that Zn-S occurs in a 1:1 Zn:S ratio (Figure 4.22). Other elements present in the TEM-EDS analysis include Ni (due to nickel TEM grids), W (due to phosphotungstunic acid, PTA, stain used on samples) and C (due to Formvar coating on grids).

Samples (40 μM CuS) analysed by TEM-EDS indicated that Cu-S may occur in various stoichiometric ratios including 1:1 Cu:S and 2:1 Cu:S (Figures 4.23 and 4.24 respectively), suggesting that at least some Cu(II) was reduced to Cu(I) by sulfide. Due to the lower mass (smaller complexes) of CuS, the atomic percent of Cu and S (2:1) were low (0.1 - 0.2%) and may be a source of uncertainty.



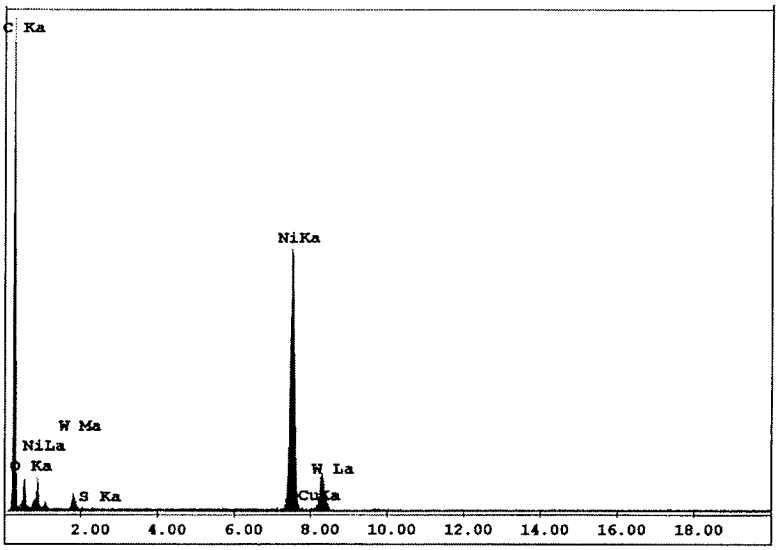
Element	C K	O K	Si K	S K	K K	Ni K	Zn K	Total
Atomic %	66.3	6.9	3.0	0.4	5.3	17.7	0.4	100.0

Figure 4.22: TEM-EDS of 100 μM ZnS



Element	C K	O K	S K	Ni K	Cu K	W L	Total
Atomic %	62.3	10.2	0.3	21.8	0.3	5.1	100.0

Figure 4.23: TEM-EDS of 40 μM CuS (1:1)



Element	C K	O K	S K	Ni K	Cu K	W L	Total
Atomic %	72.2	3.6	0.1	22.5	0.2	1.3	100.0

Figure 4.24: TEM-EDS of 40 μM CuS (2:1)

4.2.6 Analysis of ZnS and CuS Species: Size Statistics

Size analysis of metal-sulfide species was done using TEM. The size of ZnS species decreased over time while CuS species vary in size over time, providing no clear size trends, as shown in Figure 4.25. Additionally, the size distribution of ZnS and CuS solutions both in high ionic and low ionic solutions (Table 4.1) indicates very little difference between these two solutions.

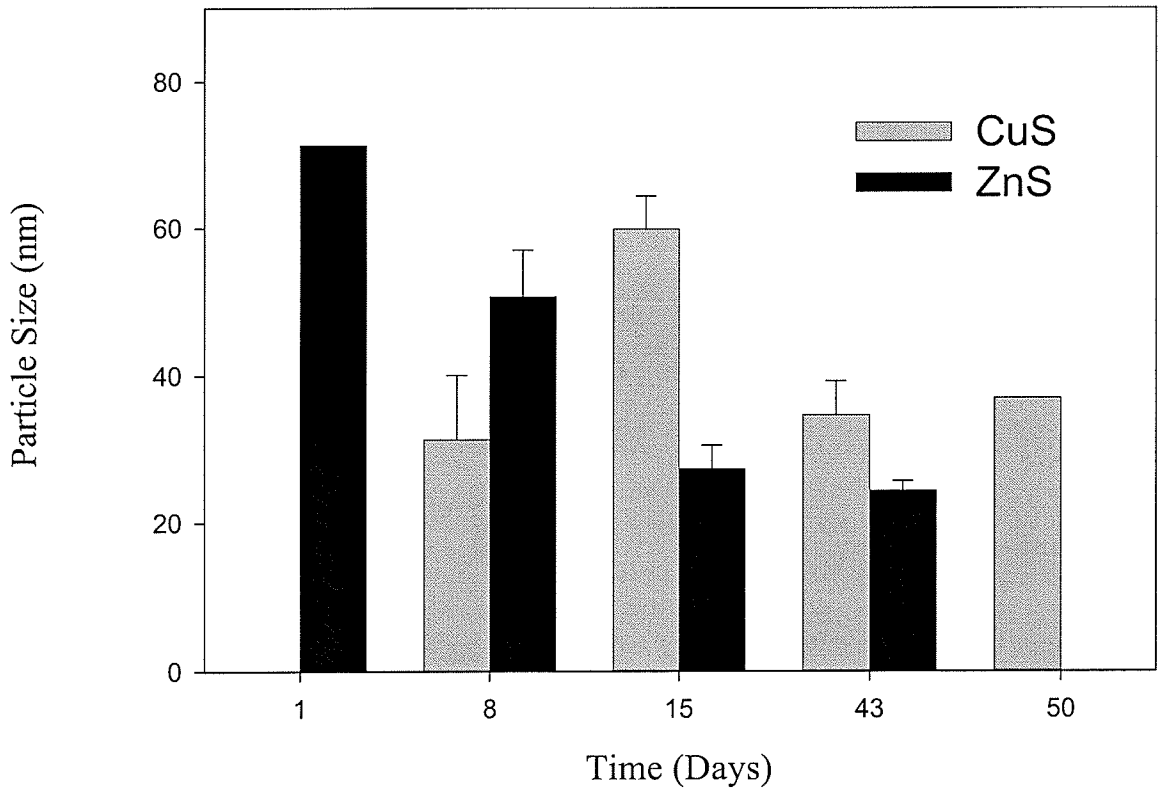


Figure 4.25: Average size distribution of ZnS and Cu_xS particles over time (calculated from TEM images)

Table 4.1: Size distribution of ZnS and CuS clusters (Calculated from TEM)

	Day	Size (nm)	Standard Deviation		Day	Size (nm)	Standard Deviation
CuS							(Whole
	8i	25.2	2.5	CuS (I = 0.7)	1	44.9	Complex)
	8ii	37.5	2.2		8i	51.6	3.2
	15i	56.6	12.4		8ii	17.2	0.8
	15ii	65.0	18.4		8iii	27.9	3.9
	15iii	58.1	5.7		8iv	19.5	3.5
	43i	37.9	7.8		15i	41.7	7.0
	43ii	31.5	8.8		15ii	31.8	8.3
	50	37.0	5.5				
ZnS	1i	26.7	3.4	ZnS (I = 0.7)	1i	34.2	4.6
	1ii	71.3	29.5		1ii	38.3	1.4
	8i	46.8	5.4		1iiia*	42.2	6.9
	8ii	47.3	7.1		1iiib*	16.6	3.7
	8iii	58.0	6.8		8	38.6	8.7
	15i	24.2	1.8		15	19.8	5.6
	15ii	30.6	2.7				
	43i	23.1	4.9				
	43ii	25.7	5.5				
	50i	77.3	8.6				
	50ii	24.9	2.6				

NOTE: Replicates are indicated by i, ii, and iii.
* *ZnS 1iiia and ZnS 1iiib (I = 0.7) were from the same cluster but indicated differences between large and small particles present*

4.3 Field Data: Sulfide in Surface Waters

The sulfide concentrations in the water samples collected from different depths of the two Canadian Shield lakes are shown in Table 4.2. Nanomolar to sub-micromolar levels of sulfide were measured in the waters. Sulfide levels were highest in the hypolimnion region of the lake, most likely due to the sulfide reduction processes occurring at the bottom of most lakes, and decreased up the water column, up to the epilimnion. Low concentrations of sulfide in surface water are likely the result of oxidation. CVS concentrations were greater than AVS concentrations due to a more aggressive analytical technique, allowing more sulfide to be liberated and measured. Also, samples collected using the portable environmental sampler tend to have higher sulfide levels than samples collected with peepers.

Table 4.2: Sulfide in water samples collected from Lake Wabigoon and Lake 658 (ELA)

Sample Location	Sampling Date	Sampling Method	Water Layer	AVS Concentration (nM)	CVS Concentration (nM)
Lake Wabigoon	July 2002	*In situ* filtration	Epilimnion	10.4	50.5
		Dialysis Peepers	Epilimnion	<DL	<DL
		Dialysis Peepers	Metalimnion	<DL	25.2
		Dialysis Peepers	Hypolimnion	9.7	31.4
ELA Lake 658	July 2002	*In situ* filtration	Epilimnion	2.5	8.2
		Dialysis Peepers	Epilimnion	2.7	9.1
		Dialysis Peepers	Metalimnion	18.2	9.3
		Dialysis Peepers	Hypolimnion	24.9	14.9
	September 2002	*In situ* filtration	Epilimnion	39.5	101.5
		Dialysis Peepers	Epilimnion	<DL	43.7
		Dialysis Peepers	Metalimnion	<DL	297.6
		Dialysis Peepers	Hypolimnion	37.9	443.9

DL = Detection limits

Chapter 5. DISCUSSION

5.1 Resistance of Metal-Sulfide Species to Oxidation

As shown in Figures 4.1 to 4.4, some solutions of metal sulfide species (Zn-S, Cu-S, Cd-S, and Pb-S) can be stable in oxic water for prolonged periods of time (weeks to months), whereas sulfide solutions of Fe, Mn and Ni (Figures 4.5, 4.6 and 4.7 respectively) were much less stable in oxic waters (hours to days). These results on sulfide resistance to oxidation are in general agreement with earlier reports (Rozan *et al.*, 2000; Rozan *et al.*, 1999; Rozan *et al.*, 2002; Bowles *et al.*, 2002) that certain metal sulfide species resist oxidation in synthetic solutions and that the resistance varies with the metal added to the solution. The observed order of oxidation resistance can be explained if we assume (i) the metal-sulfide species formed in the synthesized solutions are not dissolved species but metal-sulfide colloids (or fine particles), MeS(s) (the validity of this hypothesis will be further discussed below); (ii) the oxidation of metal-sulfide solids occurs according to the following 2-step process: MeS(s) is first dissociated to sulfide (fast step) followed by the oxidation of HS^- (slow step):

$$MeS(s) + H^+ = Me^{2+} + HS^-;\ K_{sp}$$

$$HS^- + O_2 \xrightarrow{k_1} \text{products}$$

where K_{sp} is the solubility product of the metal-sulfide solid, and k_1 is the rate constant for sulfide oxidation (Millero *et al.*, 1987).

The rate of sulfide oxidation in the metal-sulfide solution is then given by:

$$\frac{d[HS^-]}{dt} = -k_1[HS^-][O_2] = -\frac{k_1 K_{sp}[H^+]}{[Me^{2+}]}$$

Therefore, the higher the K_{sp} value, the higher the oxidation rate. For the 7 metals studied, the sulfide solids with Mn, Fe, and Ni have the highest K_{sp} values ($10^{2.98}$, $10^{-2.95}$, $10^{-5.25}$ for pink MnS(s), amorphous FeS(s) and amorphous NiS(s), respectively; NIST, 1997). As shown in Figures 4.5 – 4.7, sulfide solutions of these metals are indeed the least stable in oxic waters. Sulfide solutions with the other 4 metals (Zn, Pb, Cd, Cu) are less sensitive to oxidation (Figures 4.1 – 4.4), which is again in general agreement with the very low K_{sp} values of their corresponding sulfide solids ($10^{-11.47}$, $10^{-12.25}$, $10^{-14.36}$, $10^{-17.01}$ and $10^{-22.06}$ for ZnS(sphalerite), PbS(galena), CdS(greenockite), Cu_2S(chalcocite) and CuS(covellite) respectively; Daskalakis and Helz, 1993; Wang and Tessier, 1999). The above hypotheses alone however cannot explain the extremely slow oxidation rate of the Zn-S solution. According to the K_{sp} values, the oxidation rate of the Zn-S solution should be higher than that of the Pb-S solution, but the results determined from this study are reverse.

Such oxidation resistance is however at odds with studies on the sulfide oxidation kinetics (Zhang and Millero, 1994; Vazquez *et al.*, 1989; Millero *et al.*, 2001) which showed increased sulfide oxidation rate in oxygenated seawater in the presence of metal ions except Zn^{2+}. Millero (2001) and Vazquez *et al.* (1989) attributed the increase in oxidation rate to the formation of dissolved ion pairs (e.g., MHS^+). The other possible

explanation would be the loss of metal-sulfide species onto the labware (see Figures 4.8 and 4.9), which was not considered in Vazquez *et al.* (1989).

5.2 Identity of Metal-Sulfide Species in Oxic Waters

While our results agree with most of the recent studies (Rozan *et al.*, 2000; Luther *et al.*, 2002; Bowles *et al.*, 2002) that reduced sulfur species can persist in oxic waters, little was known about the chemical identities of these oxidation-resistant metal-sulfide species. As discussed in Chapter 2.2.2, these metal-sulfide species can be present in three possible forms: complexes (truly dissolved species in which the metal ion is the central ion and the sulfide ion acts as the complexing ligand), nanoclusters (M_xS_y), and colloids (dynamic particles, eventually becoming precipitates and minerals). Knowledge of the identity of the metal-sulfide species in oxic waters (synthesized or natural) is a prerequisite to determining their relative importance for metal speciation in these waters. For example, if the clusters reported in the literature are indeed colloids, they should be distinguished from aqueous metal-sulfide complexes that are truly dissolved species (i.e., for which a chemical potential can be defined; 1). Whereas the formation and concentrations of metal-sulfide complexes can be described by thermodynamic equilibria, those of metal-sulfide clusters and colloids cannot due to their dynamic nature.

Whereas previous studies in the literature have attributed the oxidation-resistance metal-sulfide species to the presence of "dissolved" nanoclusters (Rozan *et al.*, 2000; Bowles *et al.*, 2002; Luther *et al.*, 2003), our studies using a variety of analytical techniques (*e.g.*,

UV-visible absorption spectroscopy, MS, and TEM) do not suggest the presence of such nanoclusters in synthetic oxic metal-sulfide solutions. Instead, they are more likely a mixture of truly dissolved metal-sulfide complexes and dynamic metal-sulfide colloids. This is based on at least the following lines of evidence.

First, the metal-sulfide solutions were synthesized under conditions of oversaturation with respect to the corresponding metal-sulfide solid. For example, the IAP of the nominal 10 μM Zn-S solution at pH 7 is approximately $10^{-3.3}$, which is over 6 orders of magnitude higher than the solubility product of ZnS(s) ($10^{-9.02}$ and $10^{-11.47}$ for amorphous ZnS (Ball *et al.*, 1980) and sphalerite (Daskalakis and Helz, 1993), respectively). Although metal-sulfide nanoclusters or micro-clusters have been prepared in other studies from even higher degrees of oversaturation (millimolar metal and sulfide concentrations; e.g., Korgel and Monbouquette, 1996; Sooklal *et al*, 1996), they were stable in aqueous solution only when appropriate stabilizing or protective agents were present (e.g., surfactant vesicles; Korgel and Monbouquette, 1996; Sooklal *et al*, 1996). Otherwise, they would be present as dynamic colloids and tend to agglomerate, coagulate and form visible precipitates and eventually well-crystallized metal-sulfide minerals. The evolution of metal sulfide species is clearly demonstrated by the TEM images shown in Figures 4.20 and 4.21.

By comparing UV-visible spectra of filtered and nonfiltered samples, Luther *et al.* (1999) concluded that the Zn-S species in synthetic solutions of high ionic strength (I=0.7 M) were soluble molecular clusters with a size of 1.6 – 2.8 nm. They hypothesized that the

high ionic strength used in their study may have acted as a protective agent to prevent the nanoclusters from growing (Luther *et al.*, 1999). To test this hypothesis, we conducted TEM analysis of metal-sulfide solutions at an ionic strength of 0.7 M as a function of time (Figure 4.21). A comparison between Figures 4.20 and Figures 4.21 does not support this hypothesis, as a similar dynamic nature of the metal-sulfide species is observed at I = 0.7 M or 10^{-4} M.

The second line of evidence comes from the oxidation rate of the metal-sulfide solutions. As discussed earlier, the order of the oxidation rates agrees reasonably well (except for Zn-S solution which showed exceptional oxidation resistance) with that predicted by the K_{sp} values of the corresponding metal-sulfide solids, suggesting again the formation of fine metal-sulfide solids in the solution.

The finding that the species in the metal-sulfide solution strongly adsorb to glass walls (Figures 4.8 – 4.9), as well as to the walls of plastic containers (Bowles *et al.*, 2002), is also consistent with the behavior of colloidal metal sulfides that adsorb onto substrates such as glass and silicon (Simpson *et al.*, 1998), as pointed out by Bowles *et al.* (2002). The adsorptivity is indeed one of the major characteristics that differentiate dynamic unstable colloids from truly dissolved species (Stumm and Morgan, 1996).

If the metal-sulfide species were present as nanoclusters, the quantum confinement effect would be expected in their UV-visible absorption spectra (Wang and Herron, 1991). The extremely small size of nanoclusters results in quantum confinement of the

photogenerated electron-hole pair, resulting in a blue shift in the absorption spectrum; that is, the wavelength of the absorption shoulder should decrease with decreasing size of the clusters. Whereas this shift has been well documented for metal-sulfide nanoclusters synthesized in the presence of a protective agent (*e.g.*, Korgel and Monbouquette, 1996; Sooklal *et al.*, 1996), controversial results have been reported with aqueous metal-sulfide "clusters" in the absence of protective agents (Luther *et al.*, 1999; Luther *et al.*, 2002). As shown in Figure 4.12 – Figure 4.16, in this study we did not observe any absorption shoulders that lie in the wavelength range of metal-sulfide nanoclusters. Instead, the absorption shoulder wavelengths observed was much smaller than those of nanoclusters; this may be attributed to the truly dissolved metal-sulfide complexes that are readily formed in any solutions containing metal and sulfide ions (e.g., NIST 1997; Wang and Tessier, 1999).

So far the only direct evidence supporting the presence of metal-sulfide clusters in natural waters (Rozan *et al.*, 2000) and laboratory solutions (Luther *et al.*, 2002) has come from mass spectrometry. By analyzing vacuum or freeze-dried water samples, Rozan *et al.* (2000) and Luther *et al.* (2000) observed mass spectra that correspond to metal-sulfide clusters. However, our results indicate that the peaks attributed to these clusters were absent when the water samples are not vacuum or freeze-dried before their introduction to the mass spectrometer.

Although the above multi-lines of evidence do not support the presence of soluble metal-sulfide clusters in synthetic solutions, it is possible that stable metal-sulfide clusters may

occur in natural waters, provided that protective agents are present. Dissolved organic matter or other naturally occurring surfactants could act as protective agents. This however remains purely speculative until it is experimentally demonstrated.

5.3 Relative Importance of Dissolved Metal-Sulfide Complexes in Natural Surface Waters

At the present no analytical technique is sensitive and selective enough to measure the concentrations of different metal-sulfide species (complexes, clusters, or colloids) in natural waters. However, the relative importance of the truly dissolved metal-sulfide complexes in surface waters can be estimated using thermodynamic calculations.

The relative importance of truly dissolved metal-sulfide complexes can be estimated by assuming all the AVS are available for complexing metals such as Cd, Cu, Hg, and Pb. The Windermere Humic Aqueous Model (WHAM, Version 6.0) was used to model natural waters using field data and under the following conditions relevant to the surface water of a typical Canadian Shield lake: pH = 6.5, $[Ca]_T$ = 50 μM, $[Mg]_T$ = 20 μM, $[Na]_T$ = 20 μM, $[K]_T$ = 10 μM, $[SO_4]_T$ = 50 μM, $[Cl]_T$ = 10 μM, dissolved inorganic carbon (DIC) = 100 μM, dissolved organic carbon (DOC) = 10 mg C/L, $[Pb]_T$ = $[Cd]_T$ = $[Cu]_T$ = 1 nM, $[Hg]_T$ = 0.1 nM. WHAM 6 uses the Humic Ion Binding Model VI (Tipping, 1998) to calculate the binding of the metals to humic substances. Thermodynamic considerations are accounted for as well as the binding of protons and metal ions by

humic substances, which are important in many environmental problems. These results also provide hindsight on transport and bioavailability of heavy metals (Tipping, 1998). The results (Figure 5.1) indicate that dissolved metal-sulfide complexes are negligible in most surface waters. This was the case when AVS was in the range of nanomolars to submicromolars. It was also found that the environmental sampler provided similar results to peepers. In the waters of interest, the calculation shows that trace metal speciation is dominated by metal complexes with DOC (*e.g.,* fluvic and humic acids). However, the relative importance of metal-sulfide complexes increases with increasing AVS concentrations; in waters where AVS concentrations reach micromolar levels, dissolved metal-sulfide complexes may become the major metal species present.

It is important to note the limitations of thermodynamic modeling for natural waters. The choice of complexes to include in the models is limited to those for which equilibrium constants are available. Unfortunately, there is a general lack of understanding of the stoichiometries and equilibrium constants for many metal complexes with sulfide and with organic matter. In addition, the results from thermodynamic modeling represents the chemical composition of a system at equilibrium, which may not be attained in nature due to kinetic constrains.

The above estimation is for truly dissolved metal-sulfide complexes only. Since metal-sulfide colloids are dynamic particles, their relative importance can only be analytically measured. Unfortunately no sensitive technique is available at present to unambiguously identify and quantify metal-sulfide colloids in natural waters.

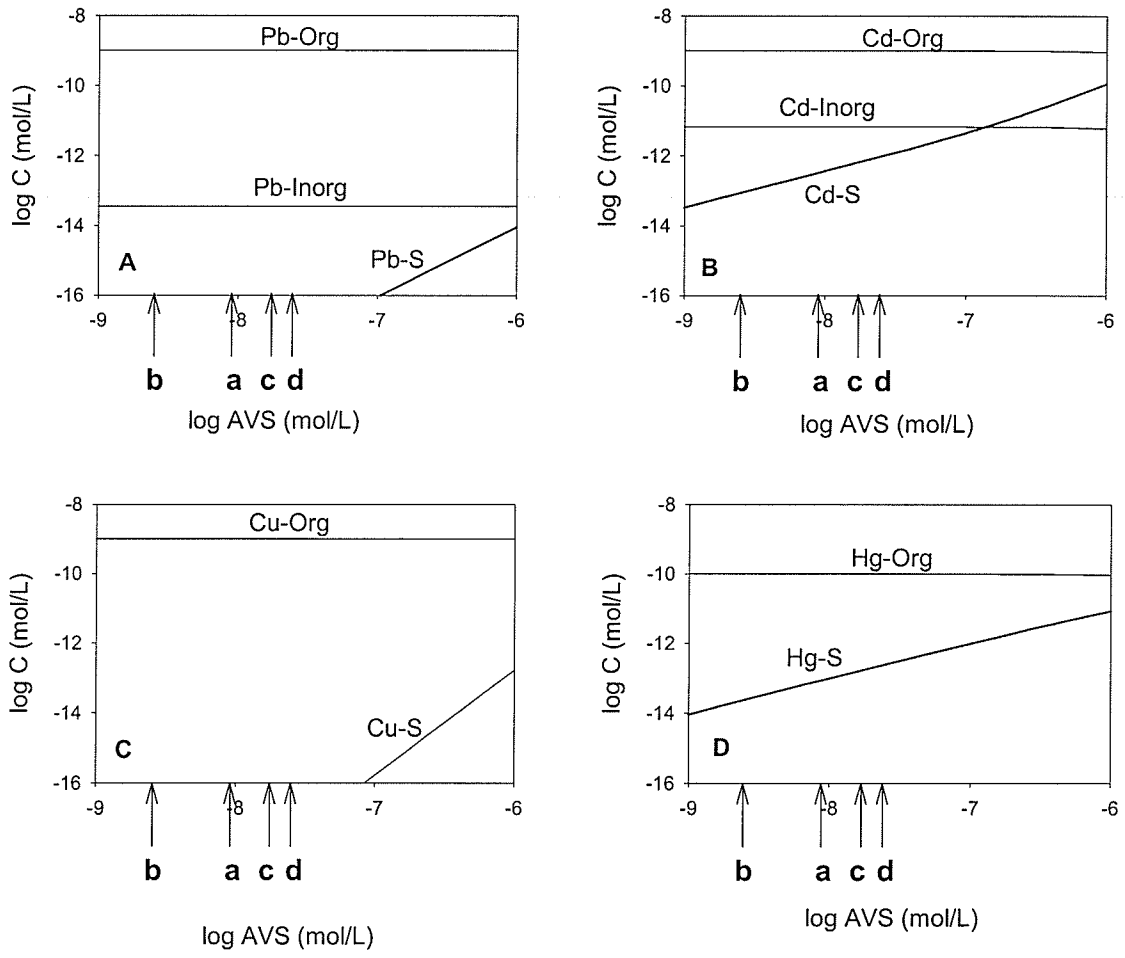


Figure 5.1. Metal speciation in the presence of AVS under conditions relevant to typical surface waters on the Canadian Shield

Me-S: metal complexes with sulfide; Me-Org: metal complexes with DOC; Me-Inorg: metal complexes with other inorganic ligands (e.g., H_2O, OH^-, CO_3^{2-}, Cl^-). a: Lake Wabigoon – hypolimnion (the AVS concentrations in the epilimnion and metalimnion were less than detection limits and are hence not shown in the figure); b: Lake 658 – epilmnion; c: Lake 658 – metalimnion; d: Lake 658 – hypolimnion.

5.4 Lessons Learned

Identifying and quantifying metal-sulfide species in oxic waters were extremely difficult when I started my project and continue to be problematic. The difficulties lie in (i) the very low levels of sulfide (nanomolars to micromolars; See Table 4.2) and metals (picomolar to sub-micromolars) in surface waters; (ii) the complexity of the sample matrix (e.g., DOC and other cations and ions); and (iii) the instability of some metal-sulfide species during sample handling and analysis. These analytical challenges will likely remain for at least the foreseeable future.

To study such a complex system without a single powerful technique, a weight-of-evidence (WOE) approach was used. A variety of analytical techniques were used, including mainly the UV-visible absorption spectroscopy, mass spectrometry, and transmission electron microscopy. The approached worked very well, as all the lines of evidence supporting the absence of metal-sulfide nanoclusters and the presence of metal-sulfide complexes and colloids.

During the course of the study, several important lessons were learned, which are summarized below.

5.4.1 TEM

Sample Preparation

Preparation of samples for TEM observation is often time consuming and on some occasions may result in the production of artifacts (Perret, *et al.*, 1991). When particles are isolated for characterization by TEM, two major problems may occur (Leppard, *et al.*, 1990): artifacts due to physico-chemical transformations of particles such as adsorption, coagulation or redox modification of the particles during sampling and sample handling, and artifacts due to shrinkage, aggregation/disaggregation of particles) during sample preparation.

In the TEM experiments conducted, bacteria ware observed on day 57, thus samples examined after this day were neglected due to impurities or other interferences which may occur with the metal-sulfide clusters. In addition, it is common to find inhomogenities in films that coat TEM grids, resulting in folds and fractures. These damaged surfaces are locations or interfaces for inorganic particles to accumulate, resulting in artifactual coagulation process (Perret, *et al.*, 1991). Thus, colloids or clusters near folds were not considered or obtained for analysis.

Particle collection by centrifugation is an alternative method of preparing non-living aquatic particles on TEM grids. Aqueous samples are centrifuged in conventional tubes, while specimen grids have been positioned at the bottom. This method allows a quantitative recovery of submicron particles onto the TEM grids, depending of the force

and time of centrifugation. However, direct centrifugation of samples onto the grid has restrictions: the procedure cannot be performed easily on the field, and grids are carbon coated which are delicate and easily damaged during centrifugation or while grids are air-dried. This may be a significant source of structural modifications (Perret *et al.*, 1991). This technique was used in this experiment, but carbon coated grids were readily damaged during centrifugation, while substantial sample loss occurred.

Because electrons are strongly absorbed in air, ultra-high vacuum conditions (10^{-7} - 10^{-10} Pa) are needed in TEM. Dehydration of colloidal material is required and must be done carefully to minimize artifacts. Other requirements for TEM include dehydration, fixation of physical structures, very thin slices or films and staining (Buffle and Leppard, 1995). In the technique used in these experiments, we did not let the grid air dry between applying the sample and the PTA stain, to minimize aggregation when concentrated to dryness.

Fixation minimizes the destruction of samples during sample preparation. This may be done physically using chemical reagents such as phosphotungstic acid (PTA) as was done in this experiment, or by physical stabilization of the structure in resin network during slow resin hardening. Also, staining is often necessary to allow observation of very fine, weakly electron-dense structures. Various chemical stains (often metal based) bind to the object of interest, may be used to increase electron density of organic material (Buffle and Leppard, 1995).

Future Perspectives

Varying opinions, sometimes opposing, exist in this area of TEM (including staining, stabilization, etc.) research. Much of it is actually hypothesis, as some of these things are hard to prove. Each individual researcher often has their own methodologies that they have found work for them in their lab, but for sometimes unknown reasons, the same method may not work for others. Some of these discrepancies are sample or tissue dependent.

New techniques, such as hydrophilic resins (*e.g.,* Nanoplast), have allowed the resolution of environmental problems and a rapid preparation of specimen grids, allowing statistical analysis of aqueous and sediment colloid characteristics (Buffle and Leppard, 1995). Further developments will be useful for aquatic colloid studies are also in progress: 3D reconstruction of objects feasible with new TEM apparatus; molecule specific markers are now available, allowing improved image analysis and statistical quantitative interpretations of chemical composition and fractal dimensions of colloid aggregates (Buffle and Leppard, 1995). However, sampling, sample processing, chemical contamination, and minimizing physical and/or microbial transformations (of colloidal material) must still be considered.

5.4.2 Mass Spectrometry

Laser Ablation Mass Spectrometry (LA-MS):

Rozan *et al.* (2000) are the first to report mass spectra of metal-sulfide precipitates, minerals, and metal-sulfide species in natural waters. By using Fourier transform mass spectrometry, they were able to achieve very good mass resolution. However, the natural water samples have to be freeze-dried and then laser ablated before the mass spectra can be obtained. As mentioned earlier, it remains unknown whether the metal-sulfide clusters they observed were artificially formed during the freeze drying or laser ablating process.

ESI-MS

ESI-MS is a soft ionization technique normally used for large biomolecules (*e.g.,* proteins, oligonucleotides) but is gaining application in the characterization of inorganic and organometallic systems (Løver *et al.*, 1997). One of the most obvious advantages is that aqueous samples do not need to be dried; instead, they can be directly introduced into the system. ESI-MS can be particularly useful in investigating clusters. While investigating cluster nuclearities, one may study the transition between clusters and colloids. Clusters with nuclearities of approximately 20 are not far from being colloidal in size. Colloids often contain poorly defined metal polyhedra, nuclearities and charges often not as precise as those of clusters. These differences may be studied using ESI-MS to characterize compounds at the transition between clusters and colloids.

Unfortunately, as described earlier in the thesis, good results for low concentrations of metal-sulfide species were not obtained in this study. One reason might be due to the relatively low mass resolution of the instruments that were available for this study. Both instruments (Quattro LC ESI-MS, Micromass and API 2000 triple quadrupole LC-MS/MS, MDS Sciex) were designated for large organic molecule studies; none were equipped with the Fourier transform functions.

It has been pointed out that changing experimental parameters (*e.g.*, solvent, desorption temperature, etc.) may have little effect on mass spectra (Dyson *et al.*, 2000). In some cases, however, solubility of species in the mobile phase can be important. Acetonitrile is commonly used as the mobile phase because of the high solubility of metal-sulfide clusters in solvent (Løver *et al.*, 1997). In this study, both acetonitrile and water were used as the mobile phase, but the results were not significantly different. Changing electronic parameters (*e.g.*, focusing potential, entrance potential, etc.) also had little effect on the appearance of the mass spectra, resulting only in small increases of resolution in our results.

Similar to potential artifacts with LC-MS, it remains unclear whether the ESI-MS spectra are representative of the true identities in the original samples (Dyson *et al.*, 2000). Dyson *et al.* (2000) reported that when studying metal species (clusters) with ESI-MS, variation of clusters results in different proportions of ions, resulting in difficulties when analyzing clusters by mass spectrometry. If various clusters with varying stoichiometry are present in solution, clear evidence of cluster proportions may not easily be acquired.

In addition, when using ESI-MS ambiguity exists as to whether the complex is present in an equilibrated neutral solution but is too labile to "survive" the ESI-MS experiment. In addition, some species may be unstable and readily form simpler species during the analysis (Spasojević *et al.*, 2001). This may be verified using TEM. Results found in this study indicated various species morphologies present in the sample at any given time. This observation may explain the difficulties in obtaining good mass spectrometric data.

HPLC-ICP-MS

Inorganic mass spectrometric methods are commonly used for multielemental analysis at trace and ultra-trace levels for isotope ratio measurements in different materials (Becker *et al.*, 2000). Inductively coupled plasma – mass spectrometry (ICP-MS), particularly the recently developed multiple collector ICP-MS (MC-ICP-MS) have enabled the measurement of isotope ratios with the highest precision, accuracy and sensitivity. Unfortunately, ICP-MS itself can only reveal elemental and isotopic composition and is not useful for analyzing molecular species such as metal-sulfide species, as any molecules will be ionized at high plasma temperature.

However, when coupled with a pre-separation technique such as high performance liquid chromatography (HPLC), ICP-MS can provide a promising technique for determining metal speciation at trace and ultra-trace levels that are encountered in natural waters. Low detection limits (nmol/L) may be achieved by on-column matrix removal and sample pre-concentration using HPLC-ICP-MS, and is comparable to voltammetric methods

(Ammann, 2002). Also, use of microbore columns allows better resolution power, increased separation efficiencies and enhanced detection limits. Microcolumns also provide increased mass sensitivity, use smaller sample volumes with no sample dilution, allowing the detection of smaller quantities, while consuming less solvent (Garraud *et al.*, 1997; Shum *et al.*, 1992).

The challenges in using HPLC-ICP-MS for metal-sulfide speciation, however, are mainly on the availability of appropriate columns and on the understanding of the equilibrium and kinetic processes of metal-sulfide species in the column. While it might be possible to use HPLC-ICP-MS to identify certain metal-sulfide complexes/clusters, it would be difficult to use it for quantitative purposes, as chemical equilibria between those species may shift along their movement in the column.

Chapter 6. CONCLUSIONS

Knowledge of the structure and chemistry of metal-sulfide species is crucial to understanding how metals behave in highly dilute environments which they naturally occur (Bell and Kramer, 1999). Metal-sulfide species control the solubilities of trace metals in sulfidic waters and are thought to play a significant role in the stabilization of hydrogen sulfide in surface oxic waters. The experiments conducted in this research project have demonstrated that these complexes do contribute to the stability of sulfide in oxic waters. Some metal-sulfide species (*e.g.,* Zn-S, Cu-S, Cd-S, Pb-S species) may remain stable in oxic waters for prolonged periods (weeks to months) of time, whereas other metal-sulfide species (*e.g.,* Fe-S, Mn-S, Ni-S species) are much less stable (sulfide decreased to below the detection limits within hours) in oxic waters.

Several approaches were used to further determine the actual form in which metal-sulfide species occur under oxic conditions. UV-visible absorption spectra of the metal-sulfide solutions did not show any peaks corresponding to dissolved metal-sulfide nanoclusters as suggested previously in the literature. Direct mass spectrometric analysis and transmission electron microscopic analysis further confirm that the metal-sulfide species resistant to oxidation in oxic waters are not soluble molecular nanoclusters; instead, they are a mixture of truly dissolved metal-sulfide complexes and dynamic metal-sulfide colloids. The morphology and size of the colloids change significantly with time.

Analysis of field samples becomes more cumbersome for the identification of metal-sulfide species. It is difficult to perform direct identification of these species analytically due to the complicated system and interferences that exist. In addition, current analytical techniques are not sensitive enough to detect or distinguish between the various metal-sulfide species. Analysis of acid volatile sulfide and chromium volatile sulfide in two lakes on the Canadian Shield, nevertheless, do indicate that metal-sulfide species can exist in natural surface waters. Although the results from the laboratory synthesized metal-sulfide solutions suggest that the metal-sulfide species in natural waters are likely to occur as dissolved complexes and colloids, it is possible that metal-sulfide clusters may also be present in natural surface waters, provided that stabilizing agents are present.

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