MERCURY SPECIATION IN GALVESTON BAY, TEXAS:

THE IMPORTANCE OF COMPLEXATION BY NATURAL ORGANIC LIGANDS

A Dissertation

by

SEUNGHEE HAN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2004

Major Subject: Oceanography

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Approved as to style and content by:

Gary A. Gill (Chair of Committee)	Peter H. Santschi (Member)
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ABSTRACT

Mercury Speciation in Galveston Bay, Texas: The Importance of Complexation by Natural Organic Ligands. (December 2004)

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Chair of Advisory Committee: Dr. Gary A. Gill

The major goal of this research is the development of a competitive ligand equilibration-solvent solvent extraction (CLE-SSE) method to determine organically complexed mercury species in estuarine water. The method was applied to estuarine surface waters of Galveston Bay and the water column of Offatts Bayou. Thermodynamic equilibrium modeling estimated organically complexed mercury species in estuarine water using the conditional stability constants of mercury-organic complexes and the concentrations of organic ligands determined by CLE-SSE.

Two competing ligands, chloride and thiosalicylic acid (TSA), were used for CLE-SSE. Chloride ion competition determined conditional stability constants for 1 : 1 mercury-ligand complexes ranging from ~10^{23} to ~10^{24} with concentrations of organic ligands at low nM levels. TSA competition determined stronger mercury-binding ligands by manipulating the TSA concentration such that a higher binding strength was achieved than that for the mercury-chloride complex. TSA competition determined conditional stability constants for 1 : 1 mercury-ligand complexes ranging from ~10^{27} to ~10^{29}, with

ligand concentrations ranging from 10 to 100 pM. Mercury-organic binding strengths in these ranges are consistent with bidentate mercury complexation by low molecular weight organic thiols. A linear relationship was observed between log stability constants for the mercury-ligand complex and log ligand concentrations, supporting the hypothesis that there is a continuum of mercury binding site strengths associated with dissolved organic matter.

In Galveston Bay, organically complexed mercury accounted for > 95 % of the total dissolved mercury in surface water. Organic complexation of mercury coupled with mercury dissolution from particulate phases controls the filter-passing mercury distribution in surface waters of Galveston Bay. The estuarine distributional features of mercury-complexing organic ligands were similar to those of glutathione, supporting mercury complexation by a thiol binding group. In Offatts Bayou, a seasonally anoxic bayou on Galveston Bay, thermodynamic equilibrium modeling suggests that the speciation of dissolved mercury in anoxic systems is dominated by sulfide complexation rather than organic complexation.

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Finally, this dissertation is dedicated to my parents, my husband, and my daughter Chamin for their great support throughout the entire study period.

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CHAPTER I

INTRODUCTION

Background

Mercury in Natural Environments

[1]Mercury (atomic number 80), a Group IIB transition metal, has a filled electron shell configuration ($5d^{10}$ $6s^2$) along with zinc and cadmium. The oxidation potentials (E for $M = M^{2+} + 2e$) of zinc, cadmium and mercury are 0.762, 0.402 and -0.854, respectively. The very low oxidation potential of Hg represents its relative inertness to oxidation reaction. The physical and chemical properties of mercury are characterized by high surface tension, low electrical resistance, high specific gravity and constant volume of expansion over the temperature range of its liquid state (Lin and Pehkonen, 1999). Because of these unique physical and chemical properties, the utilization of mercury increased dramatically after the Industrial Revolution, which included expanded uses in industry, mining, metallurgy, manufacturing, medicine and dentistry. Various compounds of mercury have been used as a catalyst, fungicide, and pesticide. Elemental mercury has also been utilized as a flowing cathode in chloro-alkali plants (Schroeder and Munthe, 1998; Lin and Pehkonen 1999).

This dissertation follows the journal style of *Geochimica et Cosmochimica Acta*.

Globally, the major source of mercury to seawater is atmospheric deposition (Mason et al., 1994) which has both natural and anthropogenic sources. Natural sources of atmospheric mercury include outgassing of the earth's mantle/crustal material, evasion from surface soil, water, vegetation surfaces, volcanoes, and forest fire (Schoeder and Munthe 1998). Prior to 1970, the largest source of anthropogenic mercury was chloro-alkali plants. Since environmental restrictions and concerns effectively closed all chloro-alkali plants, the major sources of anthropogenic mercury are now coal combustion, waste incineration, and metal smelting and refining (Schoeder and Munthe 1998). A global mass balance study by Mason et al. (1994) argued that natural and anthropogenic sources of mercury account for one third (2000t/yr) and two thirds (4000 t/yr) of the global emission to the atmosphere, respectively.

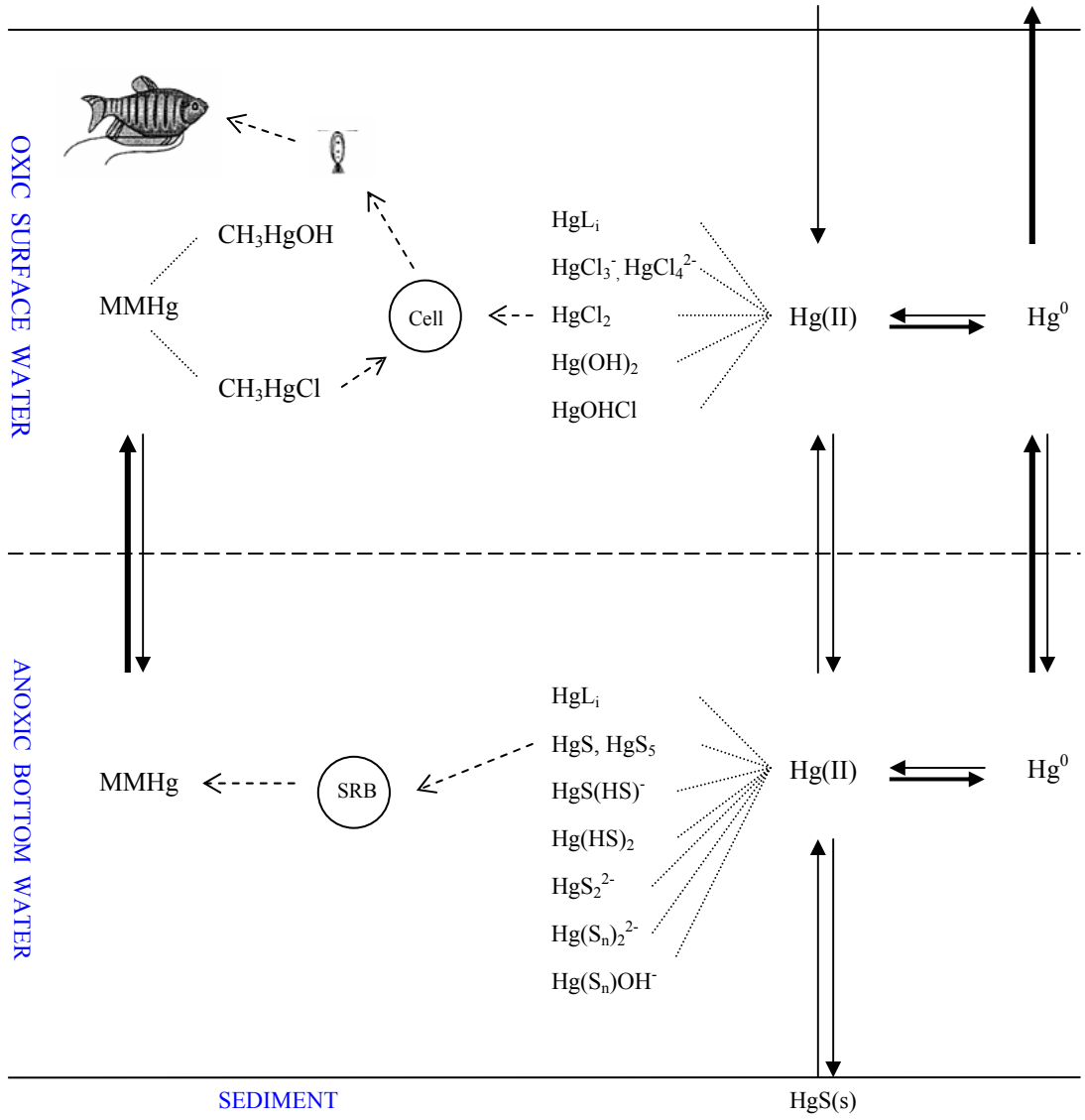
Mercury is able to exist in three different oxidation states: 0, +1 and +2. In nature, most mercury has an oxidation state of 0 and +2. The major chemical form of mercury emitted to the atmosphere is thought to be elemental mercury (Hg^0). The slow process of oxidation from Hg^0 to Hg^{2+}, resulting in a long residence time (1 - 2 yr) in the atmosphere, causes global circulation of mercury. After oxidation, Hg^{2+}, a highly surface reactive species, settles rapidly onto the earth surface through dry and wet deposition (Lin et al., 2001).

Speciation of Mercury in Oxic and Anoxic Environments

In oxic environments, aqueous mercury is partitioned into dissolved (< 0.45 μm) and particulate phases. The variation of particulate mercury in natural waters depends

principally on variations in suspended particulate matter (SPM) concentrations. The content of organic matter in SPM has been considered as an important factor to determine the binding mercury (Coquery et al., 1997; Cossa and Gobeil 2000, Laurier et al., 2003; Choe et al., 2003). Dissolved aqueous mercury in oxic environments is known to have several different chemical forms (Figure 1.1): elemental mercury (Hg^0), monomethylmercury (MMHg), dimethylmercury (DMHg), inorganic mercury (HgX_i), and organic associated mercury (HgL_i).

Experimentally, Hg^0, MMHg and DMHg are measured by cold vapor atomic fluorescence spectroscopy (CVAFS) after chemical separation (Mason and Fitzgerald, 1991; Mason et al., 1993; Mason et al., 1998). Inorganic mercury can be measured semi-quantitatively as a $SnCl_2$ reducible mercury, which would include inorganic and kinetically labile organic mercury. Thermodynamic calculations indicate that inorganic mercury species consists of hydroxyl-, chloro-, and hydrochloro- mercury depending on pH and salinity (Turner et al., 1981). Among the diverse mercury species, neutral $HgCl_2^0$ is considered to be a key species for determining cellular uptake of mercury based on its high octanol–water distribution coefficient (K_{ow} of $HgCl_2$ = 3.3; Mason et al., 1996). The possibility of complexation by S^{2-} and HS^-, which exist at low nM concentrations in oxic surface waters, is not yet clear in spite of high stability constants between mercury and sulfide species. Hg^0 is the chemical form of mercury evading from surface waters into the atmosphere. Photo-reduction, rather than microbial reduction, is known to be the principle mechanism for reducing Hg^{2+} to Hg^0 at natural levels of mercury (Morel et al., 1998).



SRB: Sulfate reducing bacteria
L_i: Mercury complexing organic ligands

Figure 1.1. Chemical speciation of aqueous mercury in oxic and anoxic waters modified from Morel et al., 1998.

The speciation of aqueous mercury in anoxic environments is mainly influenced by the high affinity between mercury and sulfide (Figure 1.1). In its solid state, mercury sulfide (HgS) exists in two different structures, a black form (metacinnabar) and a red form (cinnabar). Metacinnabar, detected in mercury contaminated soil by scanning electron microscope (Barnett et al., 1997), is metastable at room temperature and pressure and evolves into cinnabar in several days (Morel et al., 1998). Even though cinnabar has a very low solubility product (log K_{sp} = -38.9; Dyrssen and Kremling, 1990), the solubility of cinnabar increases in real sediment pore water conditions through the complexation of mercury by sulfide and bisulfide species (Paquette and Helz, 1997; Benoit et al., 1999a; Jay et al., 2000) as well as dissolved organic carbon (DOC) (Ravichadran et al., 1998, 1999). This phenomenon would control the mercury concentration in pore water. MMHg production has been proven to occur in anoxic sediment and water mainly by sulfate reducing bacteria through lab culture experiments (Gilmour and Henry 1991; Choi and Bartha, 1993; Choi et al., 1994) and field observation (Mason et al., 1993). In anoxic conditions, neutral monosulfide species (HgS^0) or polysulfide species (HgS_5) are predicted to be a bioavailable species by thermodynamic calculations and limited experimental evidence (Benoit et al., 1999a; Paquette and Helz, 1997; Jay et al. 2000).

An unknown fraction of Hg(II) is bound by organic acid in oxic and anoxic natural water (HgL_i). The quantitative and qualitative information of organic associated mercury in natural water is very limited due to its low concentration (pM level) and heterogeneous characteristics, in spite of its importance to the biogeochemical cycling of

mercury (Stordal et al., 1996a). This research focuses on investigating organic associated mercury in marine systems. Specifically, information will be sought on the concentrations, binding strengths, correlation with other biological factors and the role of Hg-organic matter in estuarine waters.

The Importance of Natural Organic Matter in Mercury Biogeochemistry

It is generally known that the chemical speciation of an element in natural water governs its biogeochemical behavior and bioavailability (Santschi, 1988; Buffle, 1990; Santschi et al., 1997). Like many other metals, mercury is readily complexed by natural dissolved organic matter (DOM). This interaction is important in controlling the solubility, mobility and bioavailability of aqueous mercury. In freshwater environments, dissolved organic mercury compounds have been reported through direct measurements (Gill and Bruland, 1990; Stordal et al., 1996a) and equilibrium calculations (Wu et al., 1997; Mason and Sullivan, 1998; Benoit et al., 2001a) to be a major portion of dissolved mercury present. Evidence of significant binding between mercury and natural organic matter in freshwater environment (Mierle and Ingram 1991; Watras et al., 1995; Driscoll et al., 1995; Cai et al., 1999) is provided by strong correlations between concentrations of dissolved mercury (including monomethyl mercury) and DOC. In freshwater, mercury-organic complexation has been suggested to increase the solubility of mercury, playing a role as a mercury transporter from terrestrial to aquatic environments (Mierle and Ingram 1991; Watras et al., 1995; Driscoll et al., 1994, 1995; Cai et al., 1999).

In estuarine environments, river borne mercury is commonly trapped in estuarine sediments, resulting in insignificant amounts being transferred to coastal waters (Cossa and Gobeil, 2000). Particulate mercury concentrations often show positive correlations with particulate organic matter concentrations in estuaries (Coquery et al., 1997; Cossa and Gobeil 2000, Laurier et al., 2003; Choe et al., 2003). A number of reports stress the governing role of dissolved organic matter in controlling the distribution of dissolved mercury in estuarine systems (Guentzel et al., 1996; Stordal et al., 1996a; Conquery et al., 1997; Bilinski et al. 2000; Choe et al., 2003; Conaway et al., 2003). The flocculation of DOM and associated mercury during estuarine mixing could explain apparent non-conservative behavior of dissolved mercury in estuarine waters. Evidence of flocculation comes from studies of the partitioning of mercury between dissolved and colloidal phases in different estuaries (Guentzel et al., 1996; Stordal et al., 1996a; Choe et al., 2003). Stordal et al. (1996b) demonstrated the colloidal pumping mechanism (Honeyman and Santschi, 1989), rapid and irreversible adsorption of particle-reactive mercury to colloids and subsequent coagulation of these colloids.

The role of DOM in mercury bioavailability is complex. Mercury and MMHg concentrations are frequently observed to increase in fresh water with the amount of DOC released from wetlands. As a consequence, the concentration of DOC can control the supply of total mercury and MMHg to the lower trophic level of food chain (Driscoll et al., 1994, 1995; Watras et al., 1998). However, high DOC concentrations in freshwater systems often do not result in higher mercury in fish. In one case, a lake having a very high concentration of DOC (DOC > 25 mg C/L) had declining mercury

levels in fish (Driscoll et al., 1994, 1995). Driscoll et al. (1995) suggested that diminished bioavailability of MMHg by complexation with DOC could be the reason. This observation agrees with the fact that the predominant species of mercury in fish is MMHg, which shows greater trophic transfer efficiency than inorganic mercury (Mason et al., 1996; McAloon and Mason 2003). Microcosm laboratory experiments proved that amphipods living in sediment and water with higher organic levels accumulate less MMHg and inorganic mercury than amphipods living in lower organic environments (Lawson and Mason, 2001). Similarly, the uptake of both inorganic mercury and MMHg decreased with increasing concentration of humic substances in dipteran (*Chaoborus)* larvae in a laboratory microcosm experiment (Sjöblom et al., 2000). In addition, higher concentrations of MMHg in zooplankton and fish were observed in a lake having lower DOC, while lower concentrations of MMHg were observed in a lake showing higher DOC and similar concentrations of total mercury (Gorski et al., 2003).

The previous discussion demonstrates that the chemical speciation of dissolved mercury strongly influences its biogeochemical cycling and bioavailability in an estuarine environment. Mercury complexation with natural organic matter could increase the solubility of terrestrial mercury hence it could control the amount of mercury in the freshwater end member. In the estuarine water column, loss of dissolved mercury through colloidal coagulation and particle settling could be related to the amount of mercury-complexing organic matter. In addition, the mercury complexed by DOM should control the bioavailability of mercury and MMHg mercury through competition with lipophilic species.

Binding of Mercury by Inorganic and Organic Reduced Sulfur

In saline systems, the inorganic speciation of mercury is highly dependant on the chloride ion content (Turner et al., 1981). At typical oxic seawater conditions, the chemical equilibrium program, MINEQL (Schecher and MaAvoy, 1992), estimates that > 95 % of the inorganic mercury exists as the trichloro and tetrachloro complexes. Reduced sulfur, however, has an even higher affinity for the B-type cations (Hg^{2+}, Cu^{+}, Ag^{+}, and Cd^{2+}) (Stumm and Morgan, 1996) but its concentration is usually much lower. Hence chloride, inorganic and organic reduced sulfur are likely complexants for controlling mercury speciation and its biogeochemical cycling in aquatic systems.

Most chemical speciation research on mercury-sulfide complexation is focused on high sulfidic environments related to the production of monomethyl mercury. Table 1.1 shows solubility information for cinnabar and formation constants for mercury sulfide and bisulfide species. Benoit et al. (1999a) examined the relationship between MMHg production and mercury speciation in sulfidic pore water: MMHg production is intracellular and HgS^{0}(aq) was assumed as a bioavailable species by passive diffusion model. They showed that when equilibrium involves only cinnabar dissolution, HgS^{0}(aq) was constant with varying sulfide concentrations, which did not agree with MMHg production observations. Assuming mercury adsorption onto solid phases, bioavailable HgS^{0}(aq) decreased with increasing sulfide concentration in the μM to mM range. This result is compatible with field observations (Benoit et al., 1999a), results from octanol–water partitioning coefficient (D_{ow}) (Benoit et al., 1999b), and pure culture experiments (Benoit et al 2001b. 2001c).

Table 1.1. Formation constants of mercury-(bi)sulfide complexes.

complex	Log K_f[a]
$Hg^{2+} + SH^- = HgS\,(s) + H^+$	38.0
$Hg^{2+} + SH^- = HgS^0\,(aq) + H^+$	26.5
$Hg^{2+} + SH^- = HgSH^+$	30.5
$Hg^{2+} + 2SH^- = Hg(SH)_2^0$	37.5
$Hg^{2+} + 2SH^- = HgS_2H^- + H^+$	32.0
$Hg^{2+} + 2SH^- = HgS_2^{2-} + 2H^+$	23.5

[a]Average of literature values rounded to the nearest 0.5 log unit by Benoit et al. (1999a).

The elemental sulfur often occurs near the oxic/anoxic redox boundary in sediments. By including elemental sulfur, the model predicted increased solubility of cinnabar and HgS_nOH^- was estimated as the major inorganic species at μM sulfide levels (Paquette and Helz, 1997 and Jay et al., 2000). Model results indicate a decreasing proportion of ring structured polysulfide species (i.e. HgS_5), possible lipophilic species, with increasing sulfide concentration, which also corresponds to the experimental observation of decreasing MMHg production with increasing sulfide concentration (Jay et al., 2000).

Stability constants between mercury and selected organic ligands are summarized in Table 1.2. In general, mercury-thiol complexes have stability constants up to 30 orders of magnitude higher than those for simple carboxylate and amine complexes. Organic compounds having double bonded sulfur (R=S) and thioether (RSR) show lower stability constants than those of thiols and higher than those of carboxylates and amines.

Table 1.2. Formation constants of mercury-organic complexes.

Complex	Log K_1[a]	Log β_2[b]	Reference
Mercaptoacetic acid ($HSCH_2COOH$)	34.5 (0.1)	43.8 (1.0)	Martell et al., 1998
2,3-Dimercaptopropanol ($SHCH_2CHSHCH_2OH$)	25.5 (0.7)	34.1 (0.7)	Smith and Martell, 1989
2-Mercaptobenzoic acid ($SHPhCOOH$)	24.8 (0.1)	33.4 (0.1)	Smith and Martell, 1989
Cysteine ($HSCH_2CH(NH_2)COOH$)	14.4 (0.1)		Martell et al., 1998
Thiourea (H_2NCSNH_2)	11.4 (0.1)	22.1 (1.0)	Smith and Martell, 1989
Diethylenetrithiodiacetic acid ($HO_2CCH_2SCH_2CH_2SCH_2CH_2SCH_2CO_2H$)		19.1 (0.5)	Smith and Martell, 1989
Ethylenedithiodiacetic acid ($HO_2CCH_2SCH_2SCH_2CO_2H$)		19.0 (0)	Smith and Martell, 1989
EDTA ($((HOOCCH_2)_2N(CH_2)_2N(CH_2COOH)_2$)	23.5 (0)		Morel and Hering, 1993
Formic acid ($HCOOH$)	5.4 (0)	7.1 (0)	Morel and Hering, 1993
Acetic acid (CH_3COOH)	6.1 (0)	10.1 (0)	Morel and Hering, 1993
Methylamine (CH_3NH_2)	8.7 (0)	17.9 (0)	Morel and Hering, 1993
Aniline ($PhNH_2$)	4.6 (1.0)	9.2 (1.0)	Smith and Martell, 1989

[a]$K_1 = [HgL^{2-n}]/([Hg^{2+}][L^{n-}])$; [b]$\beta_2 = [HgL_2^{2-2n}]/([Hg^{2+}][L^{n-}]^2)$; Values in parentheses represent ionic strength which the equilibrium constant was determined.

Dyrssen and Wedborg (1991) estimated the importance of mercury–thiol species in seawater by using conditional stability constants determined by titrations and linear free energy relationships ($Hg^{2+} + 2RS^- = Hg(SR)_2$: $10^{41.6}$; $Hg^{2+} + RS^- = Hg(SR)^+$: $10^{22.0}$; $Hg^{2+} + OH^- + RS^- = HgOHSR$: $10^{32.2}$). They suggested that the two most important Hg-

thiol species were $Hg(SR)_2$ and HgOHSR at pH = 8, pCl = 0.25 and $[RS^-]$ = 0.1 – 10 nM.

Recently, X-ray Absorption Spectroscopy (XAS) was used to obtain information on the bonding environment between mercury and soil-extracted humic matter (Xia et al., 1999; Hesterberg et al., 2001; Qian et al. 2002). The chemical identities of binding atoms (sulfur and oxygen in the first coordination shell and sulfur and carbon in the second coordination shell) and binding lengths were interpreted from extended X-ray absorption fine structure (EXAFS). The ratio of reduced to oxidized sulfur was obtained from X-ray absorption near edge structure (XANES). Thiol (RSH), disulfide (RSSR) and disulfane (RSSH) were proposed as the binding sites for mercury in humic acid (Xia et al. 1999). Similar results were obtained by Hesterberg et al. (2001) who showed that the fraction of increased Hg–S with increasing S/Hg ratio, which supports the possibility of a double sulfur bond at a very low Hg/S ratio. The same technique, applied to MMHg, demonstrates that MMHg prefers reduced sulfur rather than oxygen (Qian et al, 2002). Even though the most abundant functional groups in humic acid are carboxylic and phenolic acids, mercury shows a high affinity to coordinate with reduced sulfur in humic acid, which occurs at levels of 1 ‰ or higher (Buffle and De Vitre, 1994).

Occurrence of Sulfide and Thiols in Seawater

Hydrogen sulfide (H_2S) is known to be produced in anoxic environments where sulfate is used as an electron acceptor and reduced to sulfide by anaerobic bacteria. The presence of hydrogen sulfide in oxic environments has been reported despite its

thermodynamic instability. Hydrolysis of carbonyl sulfide (OCS), a well-known seawater biological decay product, is known as a major source of hydrogen sulfide in surface seawater (Elliott et al., 1987; Cutter and Krahforst, 1988; Radford-Knœry and Cutter 1994; Cutter et al., 1999). Sulfate reduction within macroscopic particles or large organic aggregates (marine snow) containing oxygen depleted interstitial water is another suggested source of sulfide in the oxic water column (Cutter and Krahforst, 1988).

In oxic environments, sulfides should be rapidly oxidized by IO_3^-, dissolved O_2, and H_2O_2 (Millero et al, 1989; Millero 1991a, 1991b). However, this reaction can be slowed if sulfide is stabilized by complexation with B-type metals. The importance of copper as a complexing metal for dissolved sulfide was suggested from the thermodynamic calculation using conditional stability constants between sulfide and several metals (Dyrssen, 1988; Al-Farawati and van den Berg 1999). Along with the thermodynamic consideration, kinetic inertness of copper-sulfide and zinc-sulfide complexes was explained by the electron configuration of the metal and ligand field effect (Luther III and Tsamakis, 1989).

Like sulfide, organic thiol (R-SH) is an important binding group for mercury complexation through the high affinity between mercury and reduced sulfur. Glutathione (a tripeptide, γGluCysGly) is the most prevalent intracellular thiol species in animals, plants and bacteria (Meister and Anderson, 1983). The importance of the thiol ligand in glutathione for copper complexation was shown by Leal et al. (1999) and Tang et al. (2001). The production of glutathione was increased at increased copper levels (Leal et

al., 1999), which could be related to the production of phytochelatin, a cysteine-rich polypeptide ((γGluCys)$_n$Gly; n = 2 - 11) produced by plants, algae, and fungi as a detoxifying ligand (Morel and Hering, 1993). Copper was reported as one of the most effective inducers for phytochelatin production (Ahner and Morel, 1995; Ahner et al., 1997) and lab culture experiments proved that phytochelatin production was induced even when algae are not under metal stress (Ahner et al., 1995).

A relation between glutathione and dissolved mercury has not yet been reported, though strong affinity between thiols and mercury suggests that glutathione can be an important mercury-binding organic ligand. This hypothesis is supported by the high concentration ratio of glutathione to mercury (~1000) in seawater.

Review of Metal Speciation Studies

Traditionally, two different voltammetric methods have been actively studied for copper complexation: differential pulse anodic stripping voltammetry (DPASV) and competitive ligand equilibration–cathode striping voltammetry (CLE–CSV). DPASV is useful to characterize weak organic ligands, since truly inorganic copper is measured during copper titration without competing ligand. The range of log conditional stability constants measured by DPASV was 8.5 to 13.5 with ligand concentrations of 1.8 – 46 nM for estuarine, coastal, and open ocean water (Coale and Bruland, 1988; Donat et al., 1994; Bruland et al., 2000).

CLE-CSV method, applying different competing ligands, is more effective in the adjustment of a window of binding strength than DPASV. The binding strength between

copper and the competing ligand controls the binding strength between copper and the organic ligand to be identified. Tropolone, catechol, salicylaldoxime, and 8-hydroxyquinoline have been used as common competing ligands of CLE–CSV for copper complexation. Overall, a wide range of copper complexing ligand concentrations and conditional stability constants were measured depending on the binding strength between copper and the competing ligand: the measured log conditional stability constants and ligand concentrations are 11.5 - 16.1 and 3.2 – 300 nM for salicylaldoxime (Campos and ven den Berg, 1994; Bruland et al., 2000; Laglera and van den Berg 2003), 11.0 - 14.9 and 13 - 196 nM for catechol (Apte et al., 1990a; van den Berg et al., 1990; Xue and Sigg, 1993; Tang et al., 2001), 11.0 - 15.8 and 4 – 150 nM for tropolone (Donat and van den Berg 1992; Xue and Sunda 1997), and 14 - 15 and 6 - 13 nM for 8-hydroxyquinoline (Bruland et al., 2000; van den Berg et al., 1990). Voltammetric methods successfully estimate organic complexation of copper, indicating dissolved copper in estuarine and open ocean water exists predominantly as an organically complexed form (99 – 100 %).

It has been thought that L_1, the strong copper–complexing ligand, has a source and behavior distinct from other classes of ligands. The chemical and distributional characteristics of the L_1 ligand, which shows a dominant effect on copper complexation, has been actively studied for the last decade (Zhou and Wangersky, 1989; Zhou et al., 1989; Xue and Sigg, 1990; Moffett et al., 1990; Gonzalez-Davila et al., 1995; Gerringa et al., 1995; Moffett and Brand 1996; Moffett et al., 1997). In-situ production of copper complexing organic ligands was suggested from the negative relationship between algal

blooms and free copper concentrations observed in a eutrophic lake (Xue and Sigg, 1990). In addition, culture experiments using marine phytoplankton have demonstrated the role of algal exudates in buffering free copper concentration (Zhou and Wangersky, 1989; Zhou et al., 1989; Gonzalez-Davila et al., 1995; Gerringa et al., 1995). The function of marine cyanobacteria which produce L_1 copper complexing organic ligands has been demonstrated through culture experiments and field observations (Moffett et al., 1990; Moffett and Brand 1996; Moffett et al., 1997).

In addition to copper, organic complexation of zinc (Bruland, 1989; Donat and Bruland 1990; Muller and Kester, 1991; Xue et al., 1995), cadmium (Bruland 1992; Xue and Sigg, 1998), iron (Rue and Bruland, 1995), nikel (Donat et al., 1994), cobalt (Zhang et al., 1990; Qian et al., 1998; Saito and Moffett, 2001), and lead (Capodaglio et al., 1990) were assessed by cathodic and anodic striping voltammetry.

Hypothesis

Organic complexation of mercury plays an important role in its biogeochemical cycling in an estuarine system, since transport, mobility, and scavenging processes of mercury are related to the coupling of organic complexation to adsorption-desorption processes. The main hypothesis being tested in this research is that the solution speciation of Hg(II) in surface estuarine water is dominated by complexation with an organic ligand of low concentration (~ pM) and high conditional stability constant ($K_{cond} > 10^{20}$).

Objectives

The proposed research seeks to determine the importance of mercury complexation with natural organic ligands in marine systems. The specific objectives are to:

1. Develop a method for the determination of mercury complexation with natural organic ligands in seawater based on competitive ligand equilibration-solvent solvent extraction (CLE-SSE) approaches.
2. Determine the concentrations of organic ligands that are complexing with dissolved mercury and determine the conditional stability constants of mercury-organic complexes by a CLE-SSE technique in surface water samples collected from Galveston Bay along a salinity gradient.
3. Measure the concentration of glutathione and other ancillary parameters such as, chlorophyll-*a*, nutrients, SPM, and DOC in surface water samples of Galveston Bay to assess their relationship to the organic complexation of mercury.
4. Measure the concentrations of mercury-complexing organic ligands and conditional stability constants of mercury-organic complexes in a hypoxic water column to determine the solution speciation of mercury in sulfidic environments.

CHAPTER II

DETERMINATION OF MERCURY-COMPLEXING ORGANIC LIGANDS: COMPETITIVE LIGAND EQUILIBRATION USING CHLORIDE

Introduction

Despite the importance of organic complexation in mercury biogeochemical cycling, direct assessments of the organic complexation of mercury and mercury speciation is very limited compared to that of other trace metals. Conventional methods used to determine organic complexation of metals, such as potentiometric and voltammetric methods, can not currently be used for mercury due to its low concentration and sensitivity limits associated with the methodologies. Recently several different methods for quantification of binding constants and concentrations of mercury complexing organic ligands have been reported (Wu et al., 1997; Skyllberg et al., 2000; Benoit et al., 2001a; Drexel et al., 2002; Haitzer et al., 2002, 2003; Hsu and Sedlak, 2003; Lamborg et al., 2003). The measured concentrations and conditional stability constants of mercury-binding organic ligands, previously mentioned, are summarized in Table 2.1. Overall, the stability constants between mercury and mercury binding organic ligands are similar to those of low molecular weight thiols. The data for natural water samples shown in Table 2.1 clearly illustrate the dominance of organic complexation over inorganic complexation in natural waters.

Table 2.1. Reported conditional stability constants of mercury-organic complex (K_{cond}) and concentrations of mercury binding organic ligand (L).

Reaction	$LogK_{cond}$	[L]	pH	Sample Type	Method	Reference
$HgX_i + L' = HgL$[a]	9.7 – 10.8	1.4 – 4.5 nM	7.2	Natural water	Anodic stripping voltammetry	Wu et al., 1997
$Hg^{2+} + L' = HgL$	21 – 23	0.3 – 60 nM	7.5	Natural water	Sn(II) reduction	Lamborg et al., 2003
$RSH^{n-} + Hg^{2+} = RSHg^{(n-1)-} + H^+$	11.6 – 12.4		6.0	DOM isolate	CLE–SSE	Benoit et al., 2001a
$Hg^{2+} + L' = HgL$	> 30	0.09 – 0.5 nM	7.2	Waste water, eutrophic lake water	CLE-SPE	Hsu and Sedlak, 2003
$Hg^{2+} + L^{2-} = HgL$	28.7	5 nmol/mg DOM		DOM isolates	EDLE	Haitzer et al., 2003
$Hg^{2+} + L^{2-} = HgL$	31.6 – 32.2		3.0–3.4	Soil organics	Hg sorption modeling	Skyllberg et al., 2000
$Hg^{2+} + dom_s^- = Hgdom_s^+$	22.8 – 23.2		6.0	DOM released from peat	Hg sorption modeling	Drexel et al., 2002

[a]HgX_i = inorganic mercury species, $L' = [Hg]_t - [HgL]$.

To date, only one study using voltammetry has been reported for conducting mercury complexation studies in natural waters: Wu et al. (1997) utilizing anodic stripping voltammetry achieved a detection limit of 0.1 nM and observed nM concentrations of mercury-binding organic ligands with log conditional stability constants of 9.8 – 10.8 (for $HgX_i + L' = HgL$, HgX_i = inorganic mercury, $L' = [L]_t - [HgL]$) in river, estuarine, and coastal water samples. Recently, Lamborg et al. (2003) developed an analogue of the voltammetric method to determine labile mercury species using $SnCl_2$ reduction. They found total ligand concentrations ranging 1 and 60 nM with log conditional stability constants between 21 and 24 (for $Hg^{2+} + L' = HgL$ $L' = [L]_t - [HgL]$) in natural water samples.

Another approach currently in use to determine metal speciation in natural waters is competitive ligand equilibration-solvent solvent extraction (CLE-SSE). Instead of measuring labile metal species by voltammetry, metal-ligand concentrations are calculated for hydrophilic or hydrophobic species following separation by solvent-solvent extraction. The advantages of the CLE-SSE method include low detection limit set by the metal-detection technique, reduced dissociation of organic complexes associated with electrochemical plating, and possible use of various concentrations of competing ligands. For example, acetylacetone was used as a competing ligand for copper complexation (Moffett and Zika, 1987; Miller and Bruland, 1994) and diethyldithiocarbamate was used as a competing ligand for silver complexation (Miller and Bruland, 1995).

Benoit et al. (2001a) applied CLE-SSE for mercury speciation to extracted dissolved organic matter (DOM) samples. In their method, neutral $HgCl_2^0$ was extracted into octanol, while the hydrophilic Hg-DOM complex remained in the water phase. Assuming ligand concentrations from the reduced S concentrations, log conditional stability constants of Hg-DOM were calculated from the known stability constants of $HgCl_2$: $\log K_{cond} = 11.6 - 12.4$ for $RSH^{n-} + Hg^{2+} = RSHg^{-(n-1)} + H^+$.

As an analogue of a solution extraction method, solid phase extraction (SPE) was developed as a separation technique for mercury speciation study (Hsu and Sedlak, 2003). Two competing ligands, glutathione (GSH) and diethyldithiocarbamate (DDC), were used and the concentrations and the conditional stability constants of Hg-DOM were calculated for waste and eutrophic lake waters: $\log K_{cond} > 30$ for $Hg^{2+} + L' = HgL$ and $0.07\ nM < [L] < 0.5\ nM$.

Another approach used to investigate mercury-organic complexation is the equilibrium dialysis ligand exchange (EDLE) method (Haitzer et al., 2003). This equilibrium model is based on the measurement of a conditional distribution coefficient of mercury between solutions inside and outside of a dialysis bag that separates Hg-DOM and Hg-EDTA complexes. The conditional distribution coefficients of mercury for DOM sites were determined using the known stability constant between mercury and EDTA at different pH conditions. Experimental results were explained by a simple discrete site model having bidentate binding sites: $\log K_{cond} = 28.7$ for $Hg^{2+} + site^{2-} = Hgsite$. The binding site concentration of 5×10^{-9} mol/mg DOM was estimated from the

separate experiment that determined the relationship between the [Hg]/[DOM] and the conditional distribution coefficient (K_{DOM}') of Hg-DOM complexes (Haitzer et al., 2002).

Surface complexation modeling coupled with mercury adsorption experiments has been used to determine formation constants between mercury and surface organic matter of soil. Surface formation constants were calculated to 10^{32} for $Hg^{2+} + L^{2-} = HgL$ using the reduced organic S concentration as a ligand concentration (Skyllberg et al., 2000). Drexel et al. (2002) reported a similar equilibrium model to fit the mercury K_d isotherm. A bidentate binding model for DOM released from peat explained the experimentally determined partition coefficient ([Hg] in peat/[Hg] in DOM): log K_{cond} = 22.8 - 23.2 for $Hg^{2+} + dom_s^- = Hg\text{-}dom_s^+$ and 7.3 - 8.7 for $Hg^{2+} + dom_w^- = Hg\text{-}dom_w^+$.

In summary, two different methods have been developed for the determination of organic complexation of filter-passing mercury in natural waters (Wu et al., 1997; Lamborg et al., 2003). Both methods indicate high ratios of HgL/HgX_i in natural water samples. The detection limits of both methods are still high for the determination of natural organic ligands in pM level. This chapter describes the development of a new CLE-SSE method to determine mercury-organic complexation in natural water. A linearization method following mercury titration of natural water samples was used to estimate concentrations of binding ligands and binding strengths of mercury-organic complexes. The calculated concentrations and binding strengths were compared to other speciation results performed for natural water samples.

Materials and Methods

Sample Collection

Filtered surface water samples were collected from Galveston Bay, Lavaca Bay and coastal Texas waters using a peristaltic pump system and polyethersulfone membrane filters (0.45μm) following ultra-clean sampling protocols (Gill and Bruland, 1990; Choe and Gill, 2001). Samples for ligand analysis were stored up to two months at 4˚C in the dark without acidification. Dissolved mercury samples were collected separately and acidified (0.06 N high purity HCl) within several hours of sampling. All bottles and tubing used for sampling and storage were made of Teflon® and were acid-cleaned with 6N HNO_3 and 4N HCl. Teflon® separatory funnels and vials used for SSE were cleaned with Citranox® detergent (Alconox) and were soaked in Micro® detergent (International Products Co.) and 4N HCl solution.

Reagents

A 1 M stock buffer solutions (pH = 7.0) was prepared using tris(hydroxymethyl)aminomethane (TRIZMA®, Sigma-Aldrich). HPLC grade toluene and reagent grade KCl were used for SSE without further purification. Bromine monochloride (BrCl) solution was prepared according to EPA Method 1631: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry. Low mercury contents in each reagent were verified with blank tests. Mercury standard solutions were prepared by dilution of a 1000 ppm stock standard

obtained from GFS Chemicals. The river water mercury standard, ORMS (National Research Council Canada), and sediment mercury standard, PACS (National Research Council Canada), were used to verify accuracy and recovery of cold vapor fluorescence spectroscopy (CVAFS) determination of mercury.

Potential interferences of the organic buffer on mercury speciation were verified to be insignificant by comparing the speciation results with those obtained with phosphate and borate buffers: The SSE was performed with Milli-Q® water containing mercury standard, thiosalicylic acid (as a model organic ligand), KCl (0.1 M), and buffer solution (either phosphate, borate or TRIZMA®). The concentration of water-extracted mercury was the same for each buffer solutions, demonstrating that the organic buffer, TRIZMA, does not form complex with mercury and does not have an effect on mercury speciation.

Water–Toluene Extraction

Mercury titrations were carried out by adding increasing amounts of inorganic mercury into a series of separatory funnels. In each separatory funnel, buffer solution (TRIZMA®, 0.01M), KCl, and mercury standard (generally 1 – 15 nM) were added to 100 mL of natural sample water, after which 10 mL of toluene was added. The mixture was allowed to equilibrate for 20 to 24 hours with intermittent shaking. After the last vigorous shaking, the water phase was drained from the funnel. A 30 mL aliquots of the water phase was acidified for the measurement of total mercury and the rest of the water was used for the measurement of pH.

Determination of Mercury

Cold vapor atomic fluorescence spectroscopy (CVAFS) with reduction using $NaBH_4$ was used to quantify mercury concentrations (Gill and Bruland, 1990; Choe and Gill, 2001). The precision of the CVAFS method was < 4 % (CV) and the detection limit (as 3 times the standard deviation of the method blank) was 0.16 pM. Recovery tests using a rive water standard (ORMS, National Research Council Canada) and a sediment standard (PACS2, National Research Council Canada) averaged 101 and 106 %, respectively.

In our laboratory, we typically treat natural water samples with UV-irradiation in acidic solution (0.06 N HCl, 24 hr.) prior to determination by CVAFS with $NaBH_4$ reduction (Gill and Bruland, 1990; Choe and Gill, 2001). However, applying the UV treatment step to the toluene-extracted water generated interference in the CVAFS determination that reduced sensitivity and reproducibility. Therefore an alternative digestion procedure was sought. For nM level mercury additions in natural water, three different acidification and digestion methods (0.06 N HCl only, 0.07 N HNO_3/0.06 N HCl, and 0.5 % BrCl solutions) showed good recovery (95 % for HCl, 100 % for HNO_3/HCl and BrCl) without UV-irradiation. At pico molar mercury additions in natural water, HCl digestion yielded poor recoveries (70 % at 10 pM and 86 % at 100 pM) while BrCl and HCl/HNO_3 digestion yielded 97 – 100 % recoveries. Therefore, the solvent extraction solutions were digested with a weak mixed acid (0.07 N HNO_3/0.06 N HCl).

Theory

An apparent conditional stability constant (K_{cond}') can be defined in terms of the concentration of free mercury ($[Hg^{2+}]$), the concentration of organic ligand that is not bound by mercury ([L']), and the mercury-organic complexes (HgL).

$$K_{cond}' = \frac{[HgL]}{[Hg^{2+}][L']} \tag{2.1}$$

$$[L'] = [L]_t - [HgL] \tag{2.2}$$

The concentration of HgL can be determined experimentally by linearizing the titration data as described in Ruzic (1982) and van den Berg (1984). This technique (2.3) permits the calculation of apparent conditional stability constants (K_{cond}') and total ligand concentrations ($[L]_t$) from the experimental determinations of $[Hg^{2+}]$ and [HgL].

$$\frac{[Hg^{2+}]}{[HgL]} = \frac{[Hg^{2+}]}{[L]_t} + \frac{1}{K_{cond}'[L]_t} \tag{2.3}$$

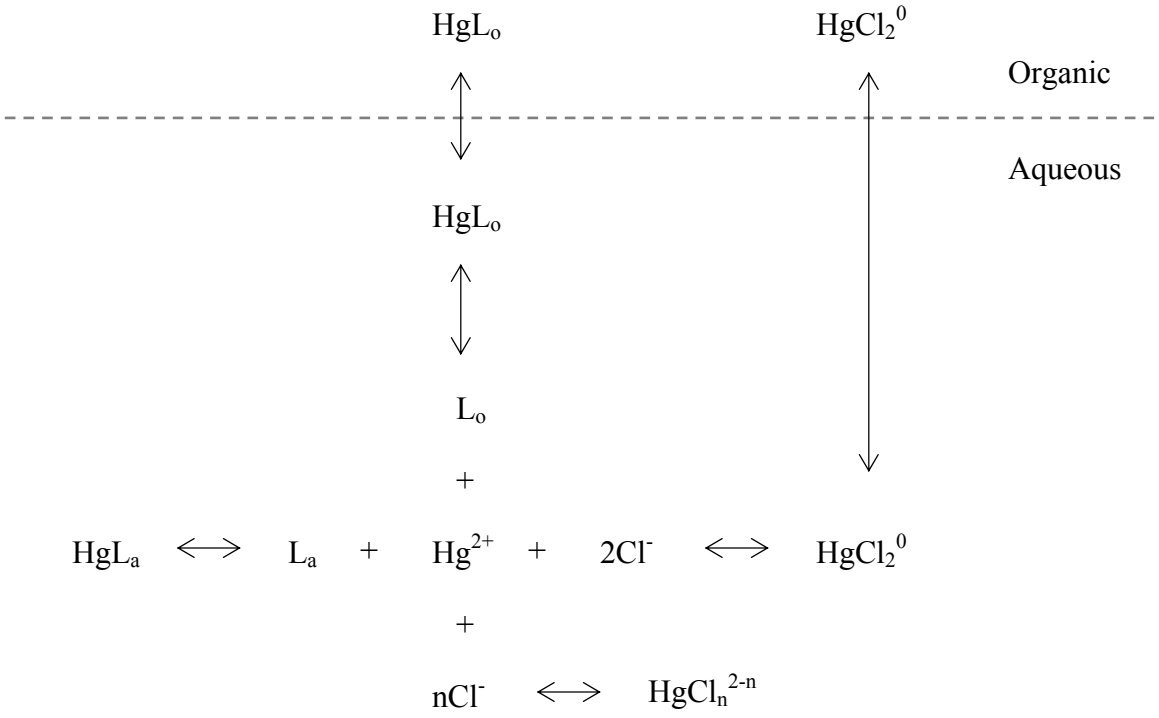
Once K_{cond}' is determined, conditional stability constants of free ions, K_{cond}, can be calculated using the ratio of unbound ligand to free ligand, $\alpha_L = [L']/[L^{n-}]$, at the titration condition.

$$K_{cond} = K_{cond}' \alpha_L \tag{2.4}$$

$$K_{cond} = \frac{[HgL]}{[Hg^{2+}][L^{n-}]} \tag{2.5}$$

Described below is the CLE procedure and theory to isolate and determine the concentration of HgL. The procedure is based on a series of inorganic mercury additions and water-toluene extractions using natural or artificially added chloride ion as a

competing ligand. Figure 2.1 illustrates the CLE that exists between aqueous mercury solution species and those that will partition into an organic solvent such as toluene in the experimental conditions (pH = 7.0, [Cl] > 0.1 M).



HgL_o = ΣHydrophobic organic mercury species
HgL_a = ΣHydrophilic organic mercury species
n = 3 and 4

Figure 2.1. CLE in water–toluene extraction using a competing ligand, chloride, at experimental conditions of pH = 7 and $[Cl^-] > 0.1$ M.

With experimental conditions, the total mercury concentration in natural water sample (Figure 2.1) can be expressed as (2.6) where V_o is the volume of the organic phase and V_a is the volume of aqueous phase.

$$[Hg]_t = [Hg^{2+}]_a + [HgCl_2{}^0]_a + [HgCl_2{}^0]_o \frac{V_o}{V_a} + [HgCl_3{}^-]_a + [HgCl_4{}^{2-}]_a + [HgL]_o \frac{V_o}{V_a} + [HgL]_a \quad (2.6)$$

The distribution of $HgCl_3^-$ and $HgCl_4^{2-}$ to the organic phase can be ignored because these species are hydrophilic at a 10:1 water to solvent ratio (Benoit et al., 2001a). The neutral species $Hg(OH)_2{}^0$ and $Hg(OH)Cl^0$ will only be important solution species when chloride levels are low and pH is high. By adding chloride to samples of low salinity and buffering the extractions at pH < 7.5, the amount of these solution species becomes less than 1 % of total mercury hence these species were not included in Figure 2.1 and (2.6).

The linearization of mercury titration requires the concentration of organic-complexed mercury, [HgL], in natural water. Equations from (2.7) to (2.11) explain the calculation of [HgL] from experimentally determined values. In equation (2.7), the concentration of aqueous mercury, $[Hg]_a$, is assumed as sum of $[HgCl_3^-]$, $[HgCl_4^{2-}]$, and aqueous $[HgCl_2{}^0]$. The concentrations of $[Hg^{2+}]_a$ and $[HgL]_a$ can be ignored due to their low concentrations.

$$[Hg]_a \approx [HgCl_3{}^-]_a + [HgCl_4{}^{2-}]_a + [HgCl_2{}^0]_a \quad (2.7)$$

The free mercury concentration, $[Hg^{2+}]_a$, is obtained from the experimentally determined $[Hg]_a$ using stability constants of $HgCl_n$ (n = 2,3, and 4) and chloride concentration. The stability constants of $HgCl_n$ ($\beta_2 = 10^{14.0}$, $\beta_3 = 10^{15.1}$, and $\beta_4 = 10^{15.4}$ at I = 0, 25°C) were obtained from Morel and Hering (1993). Free chloride concentration, $[Cl^-]_a$, is estimated

as the total chloride concentration resulted from the excess concentration of chloride. Corrections of stability constants for experimental ionic strength were given by Davies equation (Stumm and Morgan, 1996).

$$[Hg^{2+}]_a = \frac{[Hg]_a}{\beta_2[Cl^-]_a^{\,2} + \beta_3[Cl^-]_a^{\,3} + \beta_4[Cl^-]_a^{\,4}} \tag{2.8}$$

Once the free mercury concentration is determined, $HgCl_2^0$ in organic phase can be calculated from the toluene-water distribution coefficient (K_d) for $HgCl_2^0$ and $[HgCl_2^0]_a$. The determination of K_d for $HgCl_2^0$ is explained in the next section.

$$[HgCl_2^{\,0}]_a = \beta_2[Hg^{2+}]_a[Cl^-]_a^{\,2} \tag{2.9}$$

$$[HgCl_2^{\,0}]_o = K_d[HgCl_2^{\,0}]_a \frac{V_a}{V_o} \tag{2.10}$$

Equation (2.11), which is simplified from (2.6), explains the calculation of mercury-organic concentration, [HgL], in natural water sample.

$$[HgL] = [Hg]_t - [Hg]_a - [HgCl_2^{\,0}]_o \frac{V_o}{V_a} \tag{2.11}$$

The van den Berg/Ruzic plot (2.3), obtained from a series of [HgL] and $[Hg^{2+}]$ corresponding each mercury addition, gives the information of K_{cond}' and $[L]_t$.

Results and Discussion

Toluene-Water Distribution Coefficient for $HgCl_2$

The toluene-water distribution coefficient of $HgCl_2^0$, $K_d(HgCl_2^0)$, was determined from the relationship between the amount of mercury added ($[Hg]_t$) and the final concentration of mercury in the aqueous phase ($[Hg]_a$) in a series of water-toluene extractions of UV-irradiated seawater.

$$K_d(HgCl_2^0) = \frac{[HgCl_2^0]_o V_o}{[HgCl_2^0]_a V_a} \tag{2.12}$$

The concentration of the $HgCl_2^0$ species in each phase can be derived from mass balance expressions. The mercury concentration in UV-irradiated seawater is given by:

$$[Hg]_a = [Hg^{2+}]_a + [HgCl_2^0]_a + [HgCl_3^-]_a + [HgCl_4^{2-}]_a + [Hg(OH)_2^0]_a + [Hg(OH)Cl^0]_a \tag{2.13}$$

At a pH of 7 and when $[Cl^-] > 0.1$ M, the relative abundance of the Hg^{2+}, $Hg(OH)_2^0$, and $Hg(OH)Cl^0$ species is very small and the above expression can be reduced to:

$$[Hg]_a = [HgCl_2^0]_a + [HgCl^{3-}]_a + [HgCl_4^{2-}]_a \tag{2.14}$$

If it is assumed that only neutral species extract into the organic phase, then the mercury concentration in the organic phase at a pH of 7 and $[Cl^-] > 0.1$ M is given by:

$$[Hg]_o = [HgCl_2^0]_o \tag{2.15}$$

Also, the amount of mercury, which extracts into the organic phase, is given by the difference between the amount of mercury added in UV-irradiated seawater, $[Hg]_t$, and the final concentration of mercury remaining in the aqueous phase at equilibrium, $[Hg]_a$:

$$[Hg]_o = ([Hg]_t - [Hg]_a)\frac{V_a}{V_o} \quad (2.16)$$

Substituting (2.16) into the general expression for K_d (2.12) gives:

$$K_d(HgCl_2^0) = \frac{[Hg]_t - [Hg]_a}{[Hg]_a - ([HgCl_3^-]_a + [HgCl_4^{2-}]_a)} \quad (2.17)$$

By substituting the slope of titration curve (2.18) and the fractions of $HgCl_3^-$ ($f_{HgCl3} = [HgCl_3^-]/[Hg]_t$) and $HgCl_4^{2-}$ ($f_{HgCl4} = [HgCl_4^{2-}]/[Hg]_t$) determined from MINEQL+ into (2.17), K_d value is calculated by the equation (2.19).

$$S = \frac{[Hg]_a}{[Hg]_t} \quad (2.18)$$

$$K_d(HgCl_2^0) = \frac{1 - S}{S - (f_{HgCl_3^-} + f_{HgCl_4^{2-}})} \quad (2.19)$$

UV-irradiated seawater with salinity 9, 20, 29, 34 and 35 were used for conducting a series of titrations. Each individual titration consisted of three or more additions of mercury. On average, $K_d(HgCl_2^0)$ of 4.3 ± 2.7 was determined (n = 5).

Hydrophobic Natural Mercury Complexes

A fundamental requirement of successful application of CLE-SSE for speciation determinations is that a major fraction, or preferentially all, of the natural mercury complexes extract into one phase (Miller and Bruland, 1994). Water–toluene extractions of natural water samples to estimate lipophilicity of mercury complexes are given in Table 2.2. Natural mercury concentrations in filtered samples were determined by two

different methods: (1) acidification with 0.06 N HCl and 24 hours of UV irradiation; (2) acidification with a mixed acid solution (0.06 N HCl/0.07 N HNO_3).

For estuarine water samples, 38 – 55 % of the filter-passing mercury was detected without UV irradiation. The lower recovery in lower salinity water indicates higher organic complexation. While natural concentrations of filter-passing mercury are underestimated most likely due to incomplete sample digestion, this analytical bias becomes less significant at higher added mercury concentrations. At added mercury concentrations between 10 and 100 pM, mercury recovery was ~ 100 % using mixed acid digestion (see *Determination of Mercury* in MATERIALS AND METHODS).

The water-toluene extraction experiments suggest that the ligands that complex mercury at natural levels (i.e. < 10 pM) may be predominantly hydrophilic. Miller & Bruland (1995) reported high hydrophilicity of natural silver complexes which is also a B-type metal like mercury. As the concentration of mercury increases in natural waters, the hydrophilic ligand capacity is consumed and mercury complexation shifts to a mercury complex which is hydrophobic (Table 2.2). The hydrophobicity of mecury complexing organic ligands at nM concentrations was reported by Lamborg et al. (2003). In Galveston Bay, it appears that low concentrations of strong hydrophilic ligands control the complexation of mercury at the low pM levels, while hydrophobic organic ligands become more dominant at higher mercury concentrations.

Table 2.2. Extractability of mercury complexes in natural water samples from Galveston Bay at pH = 8.2 and 10 : 1 = natural water : toluene.

S	Filtered Hg (pM)		% Hydrophilic mercury complex[c]			
	UV Irradiation[a]	Mixed acid[b]	Added [Hg] = 0 pM	Added [Hg] = 10 pM	Added [Hg] = 100 pM	Added [Hg] = 1 nM
1	2.6	1.1	111	58	26	11
7	2.0	0.90	92	39	9.9	9.9
16	1.6	0.88	84	39	9.6	14

[a]Filtered mercury concentration determined following 24 hr of UV irradiation in 0.06 N HCl;
[b]Filtered mercury concentration determined following 24 hr digestion with a mixed acid solution (0.06 N HCl/0.07 N HNO_3);
[c]([Aqueous Hg]/[Total Hg])×100 determined following water-toluene extraction and 24 hr digestion of aqueous phase with a mixed acid solution (0.06 N HCl/0.07 N HNO_3).

Kinetic Experiments

The time required to reach equilibrium conditions between organic and inorganic mercury complexes was investigated. Concentrations of mercury extracted into the aqueous phase were determined in a series of extraction reactions using UV-irradiated seawater with reaction times up to 8 hours (Figure 2.2). Water-extracted mercury concentration reaches equilibrium within 4 hours. The kinetic experiments were extended to natural seawater to test the equilibration time of inorganic mercury in the presence of natural organic ligands. A rapid exchange was observed within 2 to 3 hours followed by a slow reaction for up to 8 hours (Figure 2.2).

Lamborg et al. (2003) reported kinetic experiments to determine the equilibration time between inorganic mercury and natural organic matter. They observed the Sn(II)-reducible mercury fraction to decrease fast in the first few hours (half-life 1.4 hrs) followed by a slow decrease up to 90 hours. They demonstrated that the slow reaction

resulted from Teflon-wall sorption and suggested 16 hrs as the equilibration time for the uptake of inorganic mercury by natural seawater (Lamborg et al., 2003). The results in Figure 2.2 can be explained by Teflon wall sorption of hydrophobic natural mercury complexes since only the natural seawater samples showed decreasing aqueous mercury with time. Based on the results in Figure 2.2 and Lamborg et al. (2003), about 20 hours of reaction time was used for natural water titrations and model titrations.

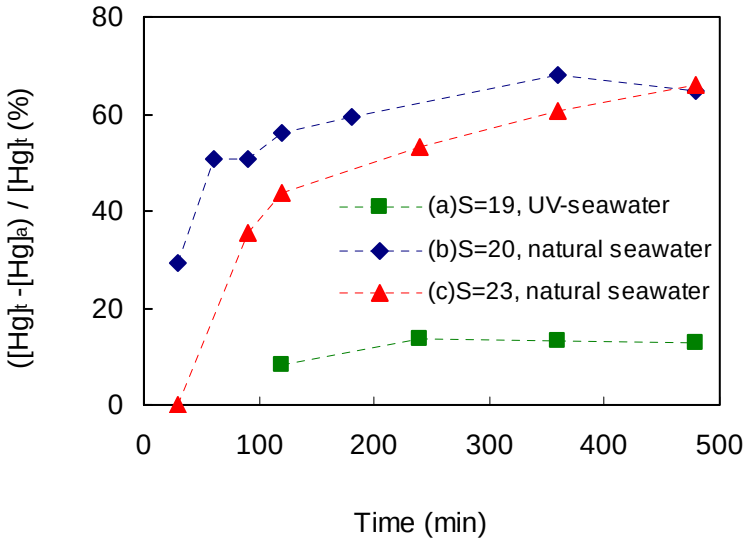


Figure 2.2. Kinetic experiments of CLE-SSE. (a) $[Hg]_t$ = 0.05 nM, $[TSA]_t$ = 1 nM, pH=8.2. (b)(c) $[Hg]_t$ = 0.01 nM, $[TSA]_t$ = 0.4 nM, pH=8.2.

Model Titrations

The accuracy of the developed method was tested by conducting a model titration using a known organic ligand in UV-irradiated seawater. Thiosalicylic acid (TSA) was used as the model organic ligand since its stability constants with mercury (Table 2.3) are in the same range as those of the natural organic ligand and the complex of $Hg(TSA)^0$ expects to have high liposolubility, like the natural organic ligands that bind mercury.

Table 2.3. Stability constants of thiosalicylic acid (TSA) complexes for ionic strength 0.1.

Complex	Log K_1	Log K_2	Reference
H-TSA	8.2	3.6	Budesinsky and Svec, 1971
Hg-TSA	24.84	8.64	Koul & Dubey, 1973

Model titrations were carried out with chloride as the competing ligand and TSA as the model organic ligand in UV digested seawater (Table 2.4). The van den Berg/Ruzic linearization technique was used to calculate concentrations and conditional stability constants for the model organic ligand. Theoretical log K_{cond}' at reaction condition was calculated using the stability constants in Table 2.3, reaction pH, and reaction ionic strength. Given the reproducibility associated with established values for conditional stability constants and [L], the results obtained for the model titration provide reasonable estimates of the concentration and stability constants of the model organic ligands.

Table 2.4. Titration of thiosalicylic acid (TSA) as a model ligand (L) with chloride as a competing ligand.

[Cl]	Measured [L]	Measured log K_{cond}'	Added [TSA]	Theoretical log K_{cond}'	pH
M	pM		pM		
0.39	35	24.5	49	24.4	8.4
0.38	190	24.2	200	24.2	8.0

Ligand Spectrums

Summarized in Table 2.5 are titration results of estuarine water samples determined using the natural chloride ion content as a competing ligand. Given in Table 2.5 are the slopes (slope = $[Hg]_a/[Hg]_t$) from the experimental titrations and the theoretical slopes determined by chemical equilibrium program using $[Cl]_t$, $[Hg]_t$, and pH. The calculated slope should correspond to the theoretical titration slope in the absence of any mercury-binding organic ligand. Determined slopes less than 1 represent partitioning of $HgCl_2^0$ into the solvent phase.

The presence of a spectrum of hydrophobic or hydrophilic organic binding sites which successively exist in larger concentrations, but with weaker binding strengths (van den Berg and Donat 1992; Bruland et al., 2000; Town and Fillera 2000; 2002), may cause the decrease or increase of the titration slope from the theoretical value. A change of the titration slope by organic interference has been observed in the CLE titration of copper (Miller and Bruland, 1994) and the voltammetric titration of mercury (Wu et al., 1997). From the Galveston Bay samples, deviation from the theoretical slope was higher

for the lower salinity samples, indicating higher organic interference for higher DOM samples.

The use of natural chloride as a competing ligand results in reduced window strengths for low chloride samples. Commonly, a window strength, which determines the detection range of conditional stability constants for ML, is represented by a complexation coefficient between metal and competing ligand (van den Berg et al., 1990). The complexation coefficients between mercury and chloride, log α_{HgCl} ($=\beta_2[Cl^-]^2 + \beta_3[Cl^-]^3 + \beta_4[Cl^-]^4$), given in Table 2.5 demonstrate the decreased window strength with decreasing salinity. The reduced window strength may be responsible for the reduced conditional stability constants of HgL for lower salinity samples of Galveston Bay surface water.

Table 2.5. Titration results of estuarine water samples with natural chloride as a competing ligand.

	S	[Cl]	Log α_{HgCl}[a]	[Hg][b] (pM)	[L] (nM)	Log K_{cond}'[c]	Exp. slope	Theor. slope[d]	pH
Lavaca Bay	17	0.27	12.9	2.8	2.9	22.3	0.57	0.65	7.5
	20	0.32	13.1	3.7	3.6	22.3	0.58	0.70	7.5
Galveston Bay	7.2	0.12	11.8	2.2	1.0	21.8	0.60	0.42	7.0
	12	0.19	12.4	1.6	0.38	21.9	0.61	0.55	7.0
	22	0.35	13.2	1.4	0.59	22.4	0.76	0.73	7.0
	29	0.46	13.6	0.90	1.9	23.0	0.79	0.82	7.0

[a] $\alpha_{HgCl} = \beta_2[Cl^-]^2 + \beta_3[Cl^-]^3 + \beta_4[Cl^-]^4$; [b] Filter-passing mercury concentration; [c] $K_{cond}' = [HgL] / ([Hg^{2+}][L'])$, $[L'] = [L]_t - [HgL]$; [d] The theoretical slope is the slope that would exist in the absence of any complexation of mercury by organic ligands. Slope less than 1 represents partitioning of $HgCl_2^0$ into the solvent phase.

The importance of chloride concentration on the determination of mercury speciation is shown in Figure 2.3 and Table 2.6. Chloride ions were added as KCl to estuarine water samples of initial salinity of 10. The higher conditional stability constants were obtained for higher chloride concentrations resulted from the increased window strength. The results of Table 2.6 suggest that lower binding strengths determined for lower salinity seawaters are analytical artifacts rather than true ligand characteristics. It also suggests that the same class of organic ligand can be determined to have different conditional stability constants depending on the binding strength between mercury and competing organic ligand.

Conducting titrations at an increased chloride concentration is in agreement with Miller and Bruland (1997)'s suggestion that titrations should be carried out from slightly higher competition strength than inorganic complexation for the saturation of any important weak organic ligand.

Table 2.6. Titration results of estuarine water samples (S = 10) with increasing chloride concentrations at pH 7.0.

[Cl] M	Log α_{HgCl}	Log K_{cond}'	[L] nM
0.17	12.3	21.8	4.22
0.38	13.4	22.9	5.10
0.54	13.9	23.0	5.15

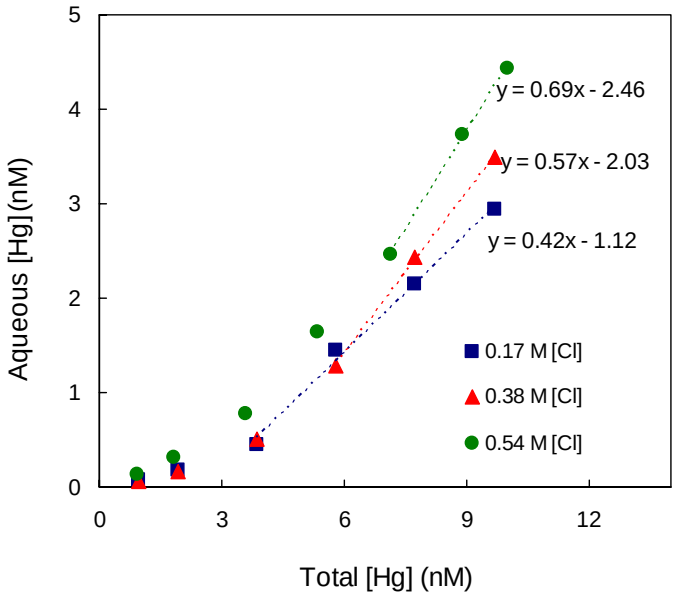


Figure 2.3. Titration curves of estuarine water sample (S = 10) with increasing chloride concentrations (by KCl) at pH 7.0.

Natural Water Titrations

Competitive ligand equilibration titrations with increased chloride concentrations were conducted on water samples collected from Galveston Bay and Texas coastal waters. An example titration curve and linearization plot is shown in Figure 2.4. Titration of UV-irradiated samples shows mercury-complexing ligands are digested as evidenced by the linear titration curve at all concentrations (Figure 2.4.a). Because the van den Berg/Ruzic plot is linear, this suggests that a 1 : 1 complexation exists between mercury and the natural organic ligands present.

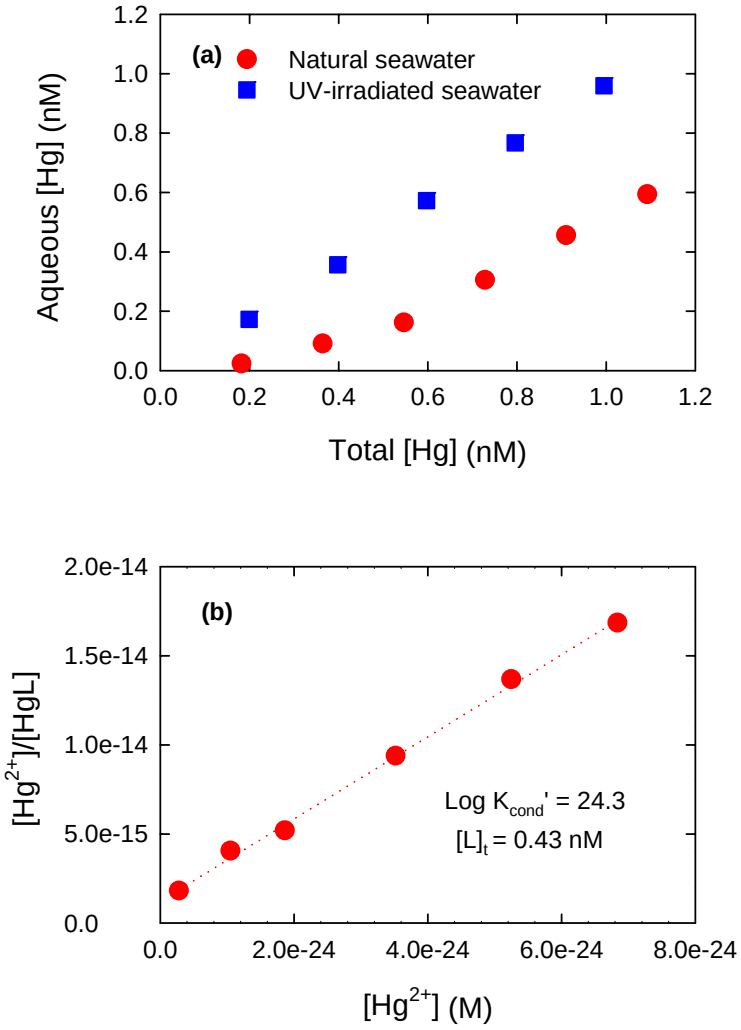


Figure 2.4. (a) Titration of coastal water from the Gulf of Mexico (S = 35) by CLE-SSE; (b) Linearization of titration data by van den Berg/Ruzic plot.

The results of mercury titrations are summarized in Table 2.7. Mercury-binding organic ligand concentrations ranged from 0.4 to 9 nM, and corresponding log conditional stability constants ($\log K_{cond}'$) ranged from 23.0 to 24.3, which agrees well with those of Wu et al. (1997) and Lamborg et al. (2003). Higher conditional stability constants were obtained compared to the results of similar salinity given in Table 2.5 due to an increased detection window through increased chloride. Conditional stability constants showed little or no variation with salinity. Total organic ligand concentrations are generally higher in low salinity water, suggesting river water origin for natural organic ligands.

Table 2.7. Concentrations of mercury binding organic ligand and conditional stability constants from CLE titrations with 0.52 – 0.55 M chloride as a competing ligand.

	S	[Hg][a] (pM)	Log α_{HgCl}[b]	[L] (nM)	Log K_{cond}'[c]	Log α_L[d]	Log K_{cond}[e]	pH
Coastal water	35	0.5	13.9	0.43	24.3	2.8	27.1	7.5
	33	1.1	13.8	0.79	23.7	2.8	26.5	7.5
Galveston Bay water	0.1	5.9	13.9	9.4	23.0	3.4	26.4	7.0
	5.9	3.1	13.9	8.1	23.1	3.4	26.5	7.0
	15	2.4	13.9	3.9	23.3	3.4	26.7	7.0
	27	1.2	13.8	1.4	23.0	3.4	26.4	7.0

[a]Filter-passing mercury concentration;
[b]$\alpha_{HgCl} = \beta_2[Cl^-]^2 + \beta_3[Cl^-]^3 + \beta_4[Cl^-]^4$;
[c]$K_{cond}' = [HgL] / ([Hg^{2+}][L'])$, $[L'] = [L]_t - [HgL]$;
[d]$\alpha_L = [L']/[L^{2-}]$;
[e]$K_{cond} = [HgL^0]/([Hg^{2+}][L^{2-}]) = K_{cond}' \times \alpha_L$.

The conditional stability constants reported in Table 2.7 were corrected to free ion equilibria, K_{cond}, using deprotonation constants (pK_{a1} = 6.3, pKa_2 = 10.3) of mercury binding groups in DOM as suggested by Haitzer et al. (2003). Their estimation of deprotonation constants results from the best fit of model data to experimental data for the plot of pH vs. Log K_{DOM}'. Conditional stability constants based on free ion equilibria range from $10^{26.4}$ to $10^{27.1}$. These values are similar to the stability constants between mercury and low molecular weight thiols (Table 1.1 in chapter I).

The importance of organic complexation of dissolved mercury in Galveston Bay waters can be demonstrated by calculating the % of the dissolved mercury present which exists as an organic complex using conditional stability constants, total ligand concentrations, and inorganic side reaction coefficients of mercury (α_{HgXi} = $[HgX_i]/[Hg^{2+}]$): $\frac{K_{cond}'[L]_t}{\alpha_{HgXi}} = \frac{[HgL]}{[HgX_i]}$ (2.20)

Using the data in Table 2.7, 78 to 100 % of the dissolved mercury exists as an organic complex at salinities between 0.1 and 27, with higher organic complexation at lower salinity. However, higher organic complexation is predicted at the natural pH of estuarine water (pH ~8.2) resulting from a reduced proton competition from mercury toward organic acid binding sites (Yin et al., 1997; Haitzer et al. 2003).

Samples of salinity 11 ppt were repeatedly titrated to determine the method precision and kinetic stability of the mercury complexing organic ligands (Table 2.8). A reduction in the concentration of mercury complexing organic ligands of 14 % was observed following 80 days of storage; the precision was < 9 % (CV).

Table 2.8. Kinetic stability of mercury complexing natural organic ligands.

Storage time (days)	[L]	Log K_{cond}'
4	5.70 ± 0.2	22.9 ± 0.06
40	5.10	22.5
80	4.89 ± 0.4	23.2 ± 0.04

Limitations of the Speciation Method

The CLE-SSE speciation method has several limitations in its ability to comprehensively understand ligand characteristics as well as mercury speciation. First, small amounts of (~10 %) hydrophilic organic ligands which exist in natural water sample (Table 2.2) can not be determined. Second, even by increased window strength through additions of chloride, the slopes of natural water titration curves were generally lower than those of UV-treated samples, suggesting that mercury complexation results from the spectrum of organic ligands rather than a discrete organic ligand. Third, the concentrations and binding strengths of organic ligands were determined for pH 7.0 instead of at natural pH. To make corrections for pH independent values requires making assumptions about the acid-base character of the mercury-binding ligands and careful assessment of this assumption is nonetheless warranted. Fourth, the lower concentrations of natural organic ligands with higher binding strength may exit since the determined organic ligand concentrations constants are three orders of magnitude higher than natural mercury concentrations. The higher concentrations of stronger mercury-binding organic ligands can be more important for natural mercury speciation.

Summary

This chapter describes a newly developed CLE-SSE method, employing chloride ion as a competing ligand, to determine stability constants and concentrations of natural organic ligands that complex mercury. The level of chloride competing ligand used was critical to the detection window. At low salinities, it was necessary to add chloride in order to maintain a consistent detection window with that of full strength seawater.

The log of conditional stability constants between mercury and natural organic ligands that complex mercury ranged between 23.0 and 24.3. These results are consistent with the range of previously determined values for natural waters and correspond to that of low molecular weight thiols. The determined organic ligand concentrations increase with decreasing salinity, suggesting a river water origin of mercury-complexing organic ligands.

The measured concentrations of mercury-complexing organic ligands are ~1000 times higher than natural mercury concentrations, indicating the possible existence of lower concentrations organic ligands with higher binding strengths. The titration with multiple competing strengths is required for a more detailed understanding of mercury-binding organic ligands as well as speciation of dissolved mercury in natural water.

CHAPTER III

DETERMINATION OF MERCURY-COMPLEXING ORGANIC LIGANDS: COMPETITIVE LIGAND EQUILIBRATION USING THIOSALICYLIC ACID

Introduction

The organic complexation of dissolved mercury controls the biogeochemical cycling of mercury in natural water. The measurement of stability constants and concentrations of mercury-complexing organic ligands is required to quantify complexation of dissolved mercury by natural organic ligands. Those parameters began to be reported recently using isolated organic matter (Skyllberg et al., 2000; Benoit et al., 2001a; Drexel et al., 2002; Haitzer et al., 2002, 2003) and natural water samples (Wu et al., 1997; Hsu and Sedlak 2003; Lamborg et al., 2003).

Copper is one of the metals which have been actively studied for metal-organic complexation. A systematic investigation of the detection window effect on copper-organic speciation revealed that the measured stability constants between copper and copper-binding organic ligands vary depending on the detection window strength (van den Berg et al., 1990; van den Berg and Donat 1992). A series of organic ligands in seawater, forming various strengths of copper complexes, explains the variation of α_{CuL} ($= K_{CuL} \times [L]$, L: copper-complexing organic ligand, K: conditional stability constant of copper-organic complex) as a function of the detection window. In addition, detected ligand concentrations were found to be decreased by the increasing detection window

strength. Intercomparison experiments using distinct methods of competitive ligand equilibration/adsorptive cathodic stripping voltammetry (CLE/ACSV) with different competing ligands support previous results of a continuum of copper-binding ligands (Bruland et al., 2000). As the analytical competition strength was increased, the copper-binding natural ligand concentrations were found to be decreased with increasing stability constants.

The analysis of compiled data for metal complexation extended the continuum of copper-binding organic ligand to other trace metals such as, Zn (II), Pb (II), and Cd (II) (Town and Filella, 2000; 2002). Comparing various metal complexing organics using detection window correction, the linear plot of log [L] vs. log K does not show a significant difference between various metals, indicating that behaviors of Cu, Zn, Pb, and Cd are not significantly different in terms of organic complexation. In addition, data points of log L vs. log K for natural water samples were located between those of biota and isolated aquatic fulvic acids, indicating that metal complexants in natural water are a combination of dissolved organic matter derived from a terrestrial source and formed in situ in the water column (Town and Filella, 2000; 2002).

In the previous chapter, a newly developed competitive ligand equilibration-solvent solvent extraction (CLE-SSE) method, employing chloride ion as a competing ligand, was used to determine stability constants and concentrations of organic ligands that complex mercury in natural waters. The conditional stability constants between mercury and natural organic ligands ranged between 22.9 and 24.3 and the organic ligand concentrations ranged between 0.4 nM and 9 nM in Gulf of Mexico and

Galveston Bay water samples. The concentrations of mercury-complexing organic ligands are ~1000 times higher than natural mercury concentrations, indicating that the complexation coefficient ($\alpha_{HgL} = K_{HgL} \times [L]$) from chloride competition can underestimate true mercury-organic complexation ratios. As discussed previously, mercury-binding organic ligands at lower concentrations and of higher binding strengths can be dominant for the natural mercury speciation. Therefore, the accurate calculation of mercury speciation requires at least two competing ligands including a strong organic ligand that exists at natural mercury concentrations (van den Berg and Donat, 1992).

The necessity of a competing ligand that has a stronger binding strength with mercury requires the development of a new competing ligand. This chapter describes a CLE-SSE method using thiosalicylic acid (TSA). TSA is an appropriate ligand to determine stronger classes of natural organic ligands that exist at concentrations close to the mercury concentrations. With this method, the window strength of mercury titration is increased by two orders of magnitude over chloride competition. The obtained data for conditional stability constants and ligand concentrations from TSA competition are compared to those of chloride competition in Chapter II and to previously reported values for natural water samples.

Materials and Methods

Sample Collection and Reagents

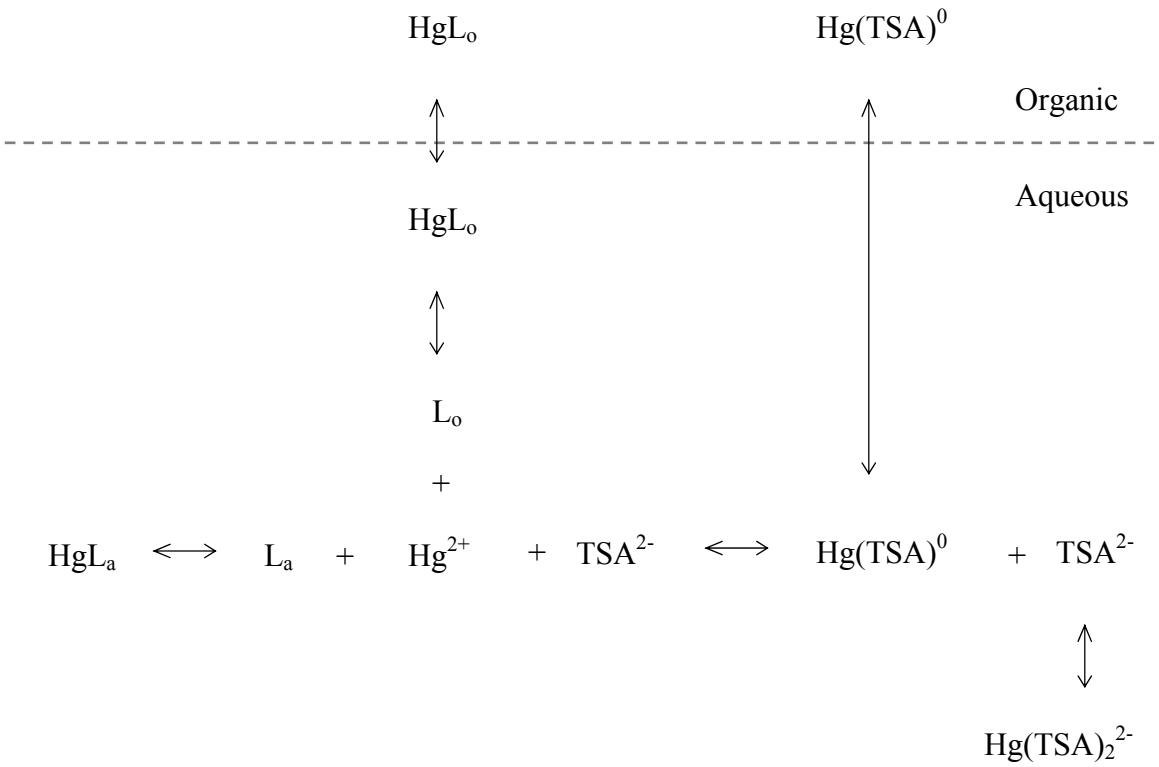
The same set of samples described in chapter II was used in the analytical development work described in this chapter. Ultra-clean sampling protocols (Gill and Bruland 1990, Choe and Gill, 2001) were also used for the sampling process. A pH 10 buffer solution was made using 2-amino-2-methyl-1,3-propanediol (Sigma-Aldrich) to 1M stock concentration. The competing ligand, TSA, was prepared fresh for each titration in an acetonitrile solution using Milli-Q® water. Other reagents used in this procedure were described previously in chapter II.

Water–Toluene Titrations and Determination of Mercury

The titration method is described in chapter II. Mercury measurements were conducted using cold vapor atomic fluorescence spectroscopy (CVAFS) (Stordal et al., 1996a; Choe and Gill, 2001). Natural water samples to determine filtered mercury concentration were treated with UV-irradiation in acidic solution (0.06 N HCl, 24 hr.) prior to determination by CVAFS with $NaBH_4$ reduction (Gill and Bruland, 1990; Choe and Gill, 2001). Sample pretreatment with 0.06 N hydrochloric acid or 0.5 % bromine monochloride (EPA Method 1631) was used for the determination of extracted mercury. Hydrochloric acid oxidation was not strong enough to recover all the mercury in natural water (See MATERIALS AND METHODS in chapter II). Therefore the results of

recovery tests for each sample were applied to determine the concentration of mercury in extracts.

Theory



TSA = Thiosalicylic acid
L_a = Hydrophilic organic ligands
HgL_a = ΣHydrophilic mercury-organic complexes
L_0 = Hydrophobic organic ligands
HgL_o = ΣHydrophobic mercury-organic complexes

Figure 3.1. CLE in water–toluene extraction using competing ligand, thiosalicylic acid (TSA) at pH = 10. Inorganic mercury species are not presented considering their low percentage of total mercury.

Illustrated in Figure 3.1 is the proposed mercury equilibria established in a CLE titration with inorganic mercury using the competing ligand, TSA. TSA complexes with many divalent metals, including Cu(II), Zn(II), Cd(II), Ni(II) (Suffet and Purdy, 1966; Al-niaimi and Al-saadi, 1974), Mn(II) (Bodidi and Valle, 1990) and Hg(II) (Koul and Dubey, 1973; Al-Niaimi and Al-Saadi, 1974) through two coordination sites, sulphhydryl (-SH) and carboxyl (-COOH). Stability constants for the equilibria involved in Figure 3.1 are summarized in Table 3.1.

Table 3.1. Stability constants of inorganic and organic mercury complexes for thiosalicylic acid (TSA) competition.

Complex	Log β_i[a]	Reference
$HgCl_2$	14.0 (0)[b]	Morel and Hering, 1993
$HgCl_3$	15.1 (0)	Morel and Hering, 1993
$HgCl_4$	15.4 (0)	Morel and Hering, 1993
$Hg(OH)_2$	21.8 (0)	Morel and Hering, 1993
HgOHCl	18.1 (0)	Morel and Hering, 1993
HgTSA	24.84 (0.1)	Koul and Dubey, 1973
$HgTSA_2$	33.47 (0.1)	Koul and Dubey, 1973

[a] $\beta_i = \frac{[ML_i]}{[M][L]^i}$;

[b]Values in parentheses represent ionic strengths at which the equilibrium constants were determined.

The methodology is based on the assumption that the neutrally charged $Hg(TSA)^0$ extracts into the toluene, while all other charged mercury species remain in the aqueous phase. Total dissolved mercury per unit volume of sample water can be expressed by:

$$[Hg]_t = [Hg^{2+}]_a + [HgX_i]_a + [HgX_i]_o \frac{V_o}{V_a} + [Hg(TSA)^0]_a + [Hg(TSA)^0]_o \frac{V_o}{V_a} + [Hg(TSA)_2{}^{2-}]_a + [HgL]_a + [HgL]_o \frac{V_o}{V_a} \quad (3.1)$$

In equation (3.1) and below, HgX_i denotes inorganic mercury species including $HgCl_2{}^0$, $HgCl_3{}^-$, $HgCl_4{}^{2-}$, $HgOH_2{}^0$, and $HgOHCl^0$ complexes. HgL denotes mercury-natural organic complexes. V_o and V_a represent the volume of the organic phase and the aqueous phase, respectively. For the experimental TSA concentration (1 - 2 nM), inorganic mercury species represent < 0.1 % of the total mercury present. That inorganic mercury portion which is neutrally charged and potentially extractable into organic solvent is an even smaller fraction. This assessment can be made by comparing values for the product of the equilibrium constant and the ligand concentration: $\alpha_{HgXi} = [HgX_i]/[Hg^{2+}] = 10^{14.0}$; $\alpha_{HgTSAi} = [HgTSA_i]/[Hg^{2+}] = 10^{17.3}$ at S = 35, $[Hg]_t$ =0.1 nM, $[TSA]_t$ = 2 nM, and pH = 10.

Water-toluene extraction experiments were conducted to show that $Hg(TSA)_2{}^{2-}$ is highly hydrophilic. Speciation modeling predicts that in a solution of 200 nM [TSA] and 0.1 nM [Hg] in pH 10, all the mercury exists as $Hg(TSA)_2{}^{2-}$. When this solution was subjected to organic extraction, > 98 % of the mercury remained in the aqueous phase. In addition, hydrophilic property of natural organic complexation was determined from

the water-toluene extractions of natural mercury complexes (see RESULTS AND DISCUSSION in Chapter II). These results allow simplification of equation (3.1) to (3.2).

$$[Hg]_t \approx [Hg(TSA)^0]_a + [Hg(TSA)^0]_o \frac{V_o}{V_a} + [Hg(TSA)_2^{2-}]_a + [HgL]_a \quad (3.2)$$

Once $[HgTSA^0]_o$ is determined from the mercury concentration extracted into the organic phase, each species in equation (3.2) can be determined using following relationships:

$$[Hg(TSA)^0]_a = \frac{[HgTSA^0]_o V_o}{K_d V_a} \quad (3.3)$$

$$[Hg^{2+}]_a = \frac{[Hg(TSA)^0]_a}{K_1 [TSA^{2-}]_a} \quad (3.4)$$

$$[Hg(TSA)_2^{2-}]_a = \beta_2 [Hg^{2+}]_a [TSA^{2-}]_a^2 \quad (3.5)$$

The toluene-water distribution coefficient (K_d) for $Hg(TSA)^0$ is explained in the next section. The concentration of TSA^{2-} in equation (3.4) and (3.5) can be assumed as total added [TSA], since TSA is highly selective for mercury and TSA concentration is excess of mercury concentration. The K_1 and β_2 of Hg-TSA are given in Table 3.1. Corrections of stability constants for experimental ionic strength are given by the Davies equation (Stumm and Morgan, 1996). Then, equation (3.6) allows the determination of [HgL] which is required for linearization of the titration data.

$$[HgL]_a = [Hg]_t - [Hg(TSA)^0]_a - [Hg(TSA)^0]_o \frac{V_o}{V_a} - [Hg(TSA)_2^{2-}]_a \quad (3.6)$$

Linearization of the titration data (Ruzic, 1982: van den Berg, 1984) to determine the conditional stability constant between mercury and organic ligand (K_{cond}') and the total concentration of mercury-binding organic ligand ($[L]_t$) is described in Chapter II and given by:

$$\frac{[Hg^{2+}]}{[HgL]} = \frac{[Hg^{2+}]}{[L]_t} + \frac{1}{K_{cond}'[L]_t} \tag{3.7}$$

In equation (3.7), conditional stability constant of mercury-organic complex denotes:

$$K_{cond}' = \frac{[HgL]}{[Hg^{2+}][L']}, \; [L'] = [L]_t - [HgL] \tag{3.8}$$

Results and Discussion

Toluene-Water Distribution Coefficient for Hg(TSA)

The toluene-water distribution coefficient (K_d) of $Hg(TSA)^0$ is determined from the slope of the mercury titration curve of seawater sample which contains appropriate amounts of TSA for the formation of $Hg(TSA)^0$ and $Hg(TSA)_2^{2-}$. The toluene-water distribution coefficient of $Hg(TSA)^0$ is given by:

$$K_d = \frac{[Hg(TSA)^0]_o V_o}{[Hg(TSA)^0]_a V_a} \tag{3.9}$$

The concentration of the $Hg(TSA)^0$ species in each phase can be derived from mass balance expressions. The mercury concentration in the aqueous phase is given by:

$$[Hg]_a = [Hg^{2+}]_a + [HgCl_2^0]_a + [HgCl_3^-]_a + [HgCl_4^{2-}]_a + [Hg(OH)_2^0]_a + [Hg(OH)Cl^0]_a + [Hg(TSA)^0]_a + [Hg(TSA)_2^{2-}]_a \quad (3.10)$$

At a pH 10 and when [TSA] = 1 - 2 nM and [Cl] < 0.56 M, the relative abundance of the Hg^{2+}, $HgCl_2^0$, $HgCl_3^-$, $HgCl_4^{2-}$, $Hg(OH)_2^0$, and $Hg(OH)Cl^0$ species is very small and the above expression can be reduced to:

$$[Hg]_a \approx [Hg(TSA)_2^{2-}]_a + [Hg(TSA)^0]_a \quad (3.11)$$

Substituting (3.11) into the general expression for K_d (3.9) gives:

$$K_d(HgTSA^0) = \frac{[Hg(TSA)^0]_o V_0}{([Hg]_a - [Hg(TSA)_2^{2-}]_a)V_a} \quad (3.12)$$

If it is assumed that only neutrally charged species extract into the organic phase, then the mercury concentration in the organic phase at the experimental condition (pH 10, [Cl] < 0.56 M, and [TSA] = 1 - 2 nM) is given by:

$$[Hg]_o = [Hg(TSA)^0]_o \quad (3.13)$$

The amount of mercury which extracts into the aqueous phase can be given by the difference between the amount of mercury added in seawater, $[Hg]_t$, and the amount of removed mercury from the aqueous phase at equilibrium:

$$[Hg]_a = [Hg]_t - [Hg]_0 \frac{V_0}{V_a} \quad (3.14)$$

Using the slope of titration curve (3.15) in x-axis of total mercury ($[Hg]_t$) and y axis of removed mercury from aqueous phase ($[Hg]^*$), and the fractions of $Hg(TSA)_2^{2-}$ in total mercury ($f_{Hg(TSA)2} = [Hg(TSA)_2^{2-}]/[Hg]_t$) determined from MINEQL, K_d value is calculated by the equation (3.16) which is deduced from (3.12), (3.13), (3.14) and (3.15).

$$S = \frac{[Hg]^{*}}{[Hg]_t} = \frac{[Hg]_o V_o}{[Hg]_t V_a} \tag{3.15}$$

$$K_d(HgTSA^0) = \frac{S}{1 - S - f_{HgTSA2}} \tag{3.16}$$

Galveston Bay samples were used for conducting a series of mercury titrations. On average, $K_d(HgCl_2^0)$ of 5.8 ± 2.5 was determined (n = 13). As discussed in Miller and Bruland (1997), determination of K_d for metal-organic complex by natural water titration method would include a calibration effect which accounts for uncertainties of stability constants and variations in seawater salinities.

Equilibration Conditions

An optimal reaction pH for the TSA competition was determined by carrying out a series of water - toluene extractions of $Hg(TSA)_2$ in DI water (I = 0.1 using KCl) at different pH's. At 200 nM [TSA], 0.10 nM [Hg], and pH > 9.5, 98 - 100 % of the mercury was observed in the aqueous phase, which is in agreement with formation of a stable hydrophilic compound, $Hg(TSA)_2^{2-}$. Moreover, this observation agrees with the pKa_2 reference value for TSA of 8.2 ± 0.1 (I = 0.1, 25°C) (Smith and Martell, 1989). Koul and Dubey (1973) also confirmed full ionization of TSA above pH 9.0 through the increase of pHg^{2+} as a function of pH in a reaction mixture of $Na_2[Hg(TSA)_2]$ and Na_2TSA.

A determination of proper reaction time for the equilibration of each mercury species is described in RESULTS AND DISCUSSIONS of chapter II. The same reaction

time of 20 to 24 hours was used for this method as used in chloride competition experiments.

Model Titration

The TSA competition method was evaluated by model titration using the well characterized ligand, diethyldithiocarbamate (DDC), in DI water. The titration curve was linearized by Scatchard (Mantoura and Riley, 1975) and the van den Berg/Ruzic plot (Ruzic, 1982; van den Berg 1984). Scatchard and van den Berg/Ruzic equations are rearranged for HgL_2 type model ligand instead of HgL type ligand since $Hg(DDC)_2^0$ is the major Hg-DDC complexes to compete with $Hg(TSA)_2$ in model titration. The conditional stability constant of HgL_2 complex is given by:

$$\beta_{2\,\mathrm{cond}}' = \frac{[HgL_2]}{[Hg^{2+}][L']^2} \tag{3.17}$$

In the above equation, a ligand concentration which is not bound by mercury (L') is given by (3.19) from (3.18).

$$Hg + 2L = HgL_2 \tag{3.18}$$

$$[L'] = [L]_t - 2[HgL_2] \tag{3.19}$$

The quadratic equations which correspond to Scatchard plot (3.20) and van den Berg/Ruzic plot (3.21) are determined by substituting (3.19) into (3.17):

$$\frac{[HgL_2]}{[Hg^{2+}]} = 4\beta_2[HgL_2]^2 - 4\beta_2[L]_t[HgL_2] + \beta_2[L]_t^2 \tag{3.20}$$

$$\frac{[Hg^{2+}]}{[HgL_2]} = \frac{-4\beta_2[L']^2}{[L]_t^2}[Hg^{2+}]^2 + \frac{4}{[L]_t}[Hg^{2+}] + \frac{1}{\beta_2[L]_t^2} \tag{3.21}$$

One of two linearization methods showing higher accuracy for ligand concentration was used to calculate the results in Table 3.2. The relative error in determining the [DDC] was 9 % and that for the log conditional stability constants was 6 % (Table 3.3). The model titration successfully demonstrates that the developed method provides a reliable estimation of the stability constant and concentration of mercury-complexing organic ligand. The titration plot for 52 nM DDC at an ionic strength of 0.1 and a pH of 9.8 is shown in Figure 3.2.

Table 3.2. Titration of diethyldithiocarbamate (DDC) as a model ligand with thiosalicylic acid (TSA) as a competing ligand at pH = 9.8, I = 0.1.

Added [DDC]	Added [TSA]	Measured [DDC]	Measured Log $\beta_{2,\,cond}$'[a]	Theoretical Log $\beta_{2,cond}$'[b]
nM	nM	nM	I = 0.1	I = 0.1
10.4	106	11.8	35.8	33.9
52.0	106	49.5	36.0	33.9

[a] $\beta_{2,cond}' = [HgL_2] / ([Hg^{2+}][L']^2)$, $[L'] = [L]_t - 2[HgL_2]$;
[b]Corrections made with pK_a(HDDC) = 3.4 (Hsu and Sedlak, 2003).

Table 3.3. Stability constants between mercury and diethyldithiocarbamate (DDC) determined at I = 0.1.

Reaction	Log β_2[a]	Reference
$Hg^{2+} + 2DDC^- = Hg(DDC)_2^0$	40.6	Yeh et al., 1980

[a]Two phase solvent extraction constants for $[Hg^{2+}]_a + 2[DDC^-]_a = [Hg(DDC)_2^0]_o$.

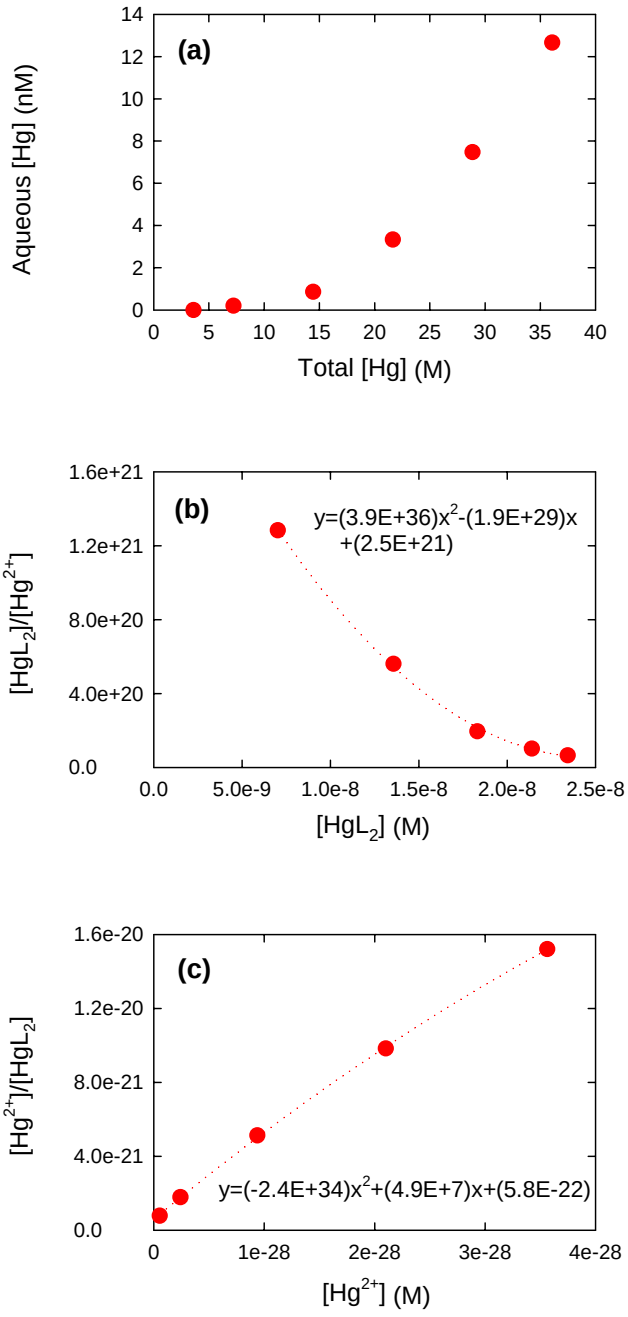


Figure 3.2. Model titration with diethyldithiocarbamate (DDC) 52.0 nM at pH 9.8 and I = 0.1. (a) Titration with inorganic mercury (b) Scatchard linearization plot for a 1 : 2 mercury–ligand complex. (c) van den Berg/Ruzic linearization plot for a 1 : 2 mercury–ligand complex.

Natural Water Titrations

Competitive ligand equilibration titrations were conducted on natural water samples collected from Galveston Bay, Lavaca Bay, and Texas coastal water using TSA as a competing ligand. Figure 3.3 shows an example titration curve for the determination of conditional stability constants and concentrations of natural organic ligands. Generally, van den Berg/Ruzic plots showed better linearity than Scatchard plots. This may be the result of higher analytical errors at lower mercury concentrations since the Scatchard plot depends more on the lower levels of mercury. The straight line of the van den Berg/Ruzic plot justifies the 1:1 (Hg : ligand) complexation assumption between mercury and natural organic ligands.

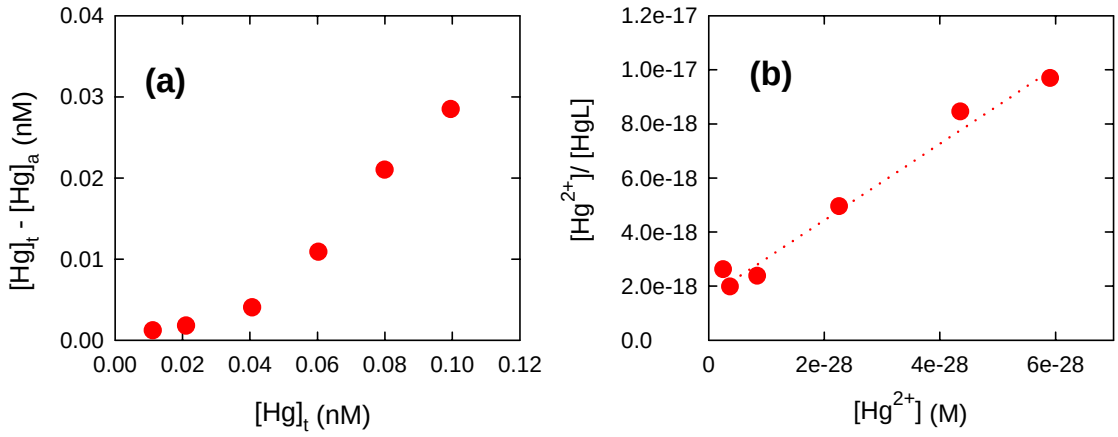


Figure 3.3. (a) Mercury titration of an estuarine water sample from Galveston Bay (salinity = 7) with 2 nM TSA as the competing ligand at pH = 9.8 ($[Hg]_t$ = total mercury, $[Hg]_a$ = aqueous mercury). (b) Linearization of the titration data using van den Berg/Ruzic approach.

A summary of the titration results for natural water samples collected from marine sites along the Texas coast is given in Table 3.4. The concentration of hydrophilic organic ligands ranged from 11 to 93 pM. Generally, low concentrations of mercury complexing ligands were detected in higher salinity waters. This trend is consistent with the previous results using chloride competition (Table 2.7) and other studies measuring of estuarine waters (Wu et al., 1997; Lamborg et al., 2003). The conditional formation constants between mercury and the complexing organic ligands range from $10^{26.6}$ to $10^{28.9}$ at pH of ~10.

Although the same concentrations of TSA were added for each titration of Galveston Bay samples, the lower ionic strength of lower salinity water resulted in increased window strengths. The same trend was observed for copper complexation in the Scheldt estuary (Laglera and van den Berg., 2003). From their test experiments, it was concluded that the detection window increase by decreasing salinity is partially responsible for increased conditional stability constants of CuL for lower salinity samples. The higher conditional stability constants determined for the lower salinity Galveston Bay samples may partially result from the same artifacts of competition strength change as copper.

The precision of the analysis was investigated by performing replicate measurements on one of the Galveston Bay samples. Relative standard deviations were 5 % for [L] and 0.2 % for log K_{cond}' from triplicate measurements. The accuracy associated with the determination of ligand concentrations and stability constants would rely on the accuracy of mercury determination in extracted water samples. One potential source of

error is associated with incomplete digestion of the extracts. Because recovery corrections were required, the potential for analytical error resulting from this treatment may exist.

Table 3.4. Concentrations of mercury binding organic ligand and conditional stability constants from CLE titrations using thiosalicylic acid (TSA) as a competing ligand.

Sampling Region	Salinity	[Hg] pM	Log $\alpha_{Hg(TSA)i}$ [a]	[L] pM	log K_{cond}' [b]	pH [c]
Texas coast	31	0.9	15.6	23	27.4	9.5
	34	1.1	15.6	11	27.8	9.5
	35	0.5	15.5	17	27.5	9.5
Lavaca Bay, TX	17	2.8	15.7	93	26.6	9.7
	20	7.7	15.7	72	26.9	9.7
	20	3.7	15.7	36	27.4	9.7
Galveston Bay, TX	1	2.6	16.7	78	28.9	9.8
	7	2.2	16.2	71	27.9	9.8
	12	1.7	16.2	83±4	27.7±0.06	9.8
	22	1.4	16.1	35	27.6	9.8
	26	1.1	16.0	23	27.5	9.8

[a] $\alpha_{Hg(TSA)i} = K_{1,Hg(TSA)}[TSA^{2-}] + \beta_{2,Hg(TSA)_2}[TSA^{2-}]^2$; [b] $K_{cond}' = \frac{[HgL]}{[Hg^{2+}][L']}$; [c] Titration pH.

Organic Mercury Complexation in Natural Waters - A Continuum Binding Model

Mercury has a strong tendency to bind with inorganic and organic reduced sulfur (Dyrssen and Wedborg, 1991; Benoit et al., 1999a; Xia et al., 1999; Hesterberg et al., 2001; Qian et al. 2002). Recent advances in technology allow us to have a molecular level view of bond information between mercury and soil humic acid. Xia et al. (1999) and Hesterberg et al. (2001) revealed the importance of RSH (thiol), RSSR (disulfide) and RSSH (disulfane) in mercury binding with soil humic substances using X-ray absorption spectroscopy (XAS). The thermodynamic information for mercury-complexing natural organic matter implies the importance of organic thiol group, which was recently reported for dissolved organic matter (DOM) isolates (Benoit et al., 2001a; Haizer et al., 2002; 2003; Drexel et al., 2002).

To date, only two studies have reported thermodynamic information for mercury-complexing organic ligands in natural water (Wu et al., 1997; Lamborg et al., 2003). A comparison of these published data is shown in Table 3.5. To allow direct comparison of the equilibrium constants, the data reported by Wu et al. (1997) were corrected to reflect conditional stability constants based on free mercury concentration. The Sn(II)-reducible mercury coefficients (α_{Hgr} = [Sn(II)-reducible Hg]/[Hg^{2+}]) determined for estuarine samples by Lamborg et al. (2003) were used for the corrections in Table 3.5: Log α_{Hgr} = 13.0 for salinity 0 and 19; Log α_{Hgr} = 13.5 for salinity 35. Wu et al. (1997) used inorganic mercury concentration approximated by anodic stripping voltammetry (ASV), which measures true inorganic mercury and labile organic mercury, in van den Berg/Ruzic plot. Therefore, a Sn(II)-reducible mercury coefficient would give an

appropriate conversion to allow the estimation of the equilibrium constant based on free mercury concentration.

Table 3.5. Reported stability constants and concentrations of mercury complexing organic ligands in natural water.

Reaction	S	Log K_{cond}'	Corrected Reaction	Corrected Log K_{cond}'[b]	[L] (nM)	Reaction pH	Reference
HgX_i + L' = HgL[a]	0	9.7	Hg^{2+} + L' = HgL	22.7	2.25	7.2	Wu et al., 1997
	0	10.2		23.2	4.47		
	19	10.6		23.6	2.67		
	19	10.8		23.8	2.51		
	35	9.8		23.3	1.35		
Hg^{2+} + L' =HgL	0	21.5			8.0	7.5	Lamborg et al., 2003
	0	21.0			20		
	0	21.2			60		
	0	22.2			3.8		
	0	22.9			6.4		
	0	21.6			24.8		
	0	22.7			3.0		
	~35	23.5			4.0		
	~35	23.0			0.3		

[a]HgX_i=inorganic mercury, L' = $[Hg]_t$ – [HgL]; [b] Corrections made with α_{Hgr} (= [reducible Hg]/[Hg^{2+}]) = $10^{13.0}$ for salinity 0 and 19, and α_{Hgr} = $10^{13.5}$ for salinity 35 from Figure 1 in Lamborg et al., 2003.

Plotted in Figure 3.4 are the ligand concentrations from these previous works (Table 3.5) as well as the new information from current work as a function of the conditional stability constants (Table 2.7 and 3.4). This treatment is similar to that used by Town and Fillela (2000; 2002) to assess the nature of the metal binding ligands. The data in Figure 3.4 are fairly consistent despite the fact that the methodologies and reaction conditions differ ($7.0 < pH < 9.8$). The continuous binding characteristics of natural organic ligands observed in Figure 3.4 agree with those of other metals (Town and Fillela, 2000; 2002). This conceptualization argues that stronger binding sites are utilized at lower metal concentrations and progressively weaker binding sites complex with higher metal concentrations. The binding coefficient ($\alpha_{HgL} = [HgL]/[Hg^{2+}]$) calculated from $K_{cond}' \times [L]$ in Figure 3.4 indicates that lower concentrations of organic ligands having higher stability constants are more important for mercury speciation than higher concentrations of weaker binding ligands: for example, when log [L] = -7, log $\alpha_{HgL} = \sim 13$; when log [L] = -11, log $\alpha_{HgL} = \sim 18$. Therefore, organic ligands that exist in the concentration range of natural mercury (few pM) would control the organic complexation of dissolved mercury in natural water.

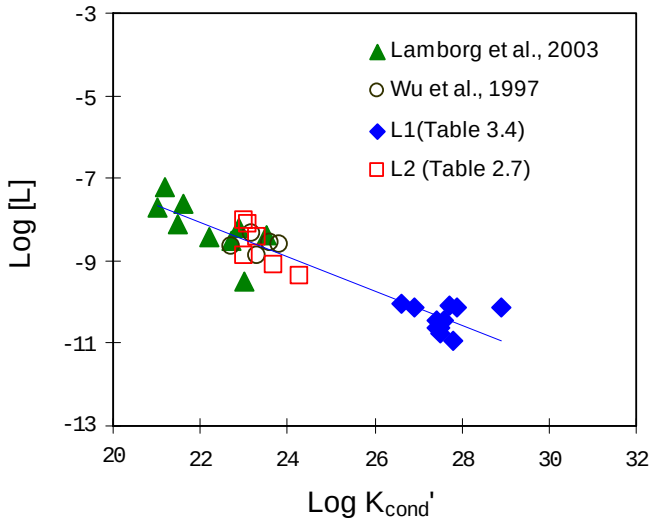


Figure 3.4. Relations between concentration of mercury-binding organic ligands and conditional stability constant of mercury-organic complexes reported for natural water samples. Data are shown in Table 2.7, 3.4, and 3.5. Log $K_{cond}' = [\Sigma HgL]/([Hg^{2+}][L'])$.

Estimation of Formation Constants for HgL

The value determined for a conditional stability constant can vary depending on the pH at which the determination is made. An increase of log K_{cond}' as a function of pH was shown by Haizer et al. (2003) and Averyt et al. (2004) for mercury complexing organic ligands and copper complexing organic ligands, respectively. This arises because metal competes with proton for organic binding site.

The stability constants and ligand concentrations obtained by CLE-SSE are given by:

$$K_{cond}' = \frac{[\Sigma HgL]}{[Hg^{2+}][L']} \qquad (3.22)$$

Where [ΣHgL] represents the sum of all the bound mercury at various sites on DOC, and L' represents the ligand concentration not bound by mercury:

$$[L'] = [L]_t - [\Sigma HgL] \quad (3.23)$$

If the amount of HgL is small compared to $[L]_t$ then,

$$[L'] \approx [L]_t \quad (3.24)$$

The conditional stability constants can be normalized to pH by assuming that: 1) The mercury-complexing organic ligand, L, has bidentate binding groups (H_2L) as mercury generally prefers two coordination site environments (Cotton et al., 1999):

$$[L]_t = [H_2L] + [HL^-] + [L^{2-}] \quad (3.25)$$

The acidity constants (pK_a) of 6.3 and 10.3 are assumed for H_2L as suggested by Haitzer et al. (2003). This assumption also agrees with the results of extended X-ray absorption fine structure (EXAFS) for soil humic matter, which shows that one electron donor in the first coordination shell is reduced sulfur ($8 < pK_a < 11$) and the other may be an oxygen, nitrogen, or sulfur ($pK_a < 10$) functional group (Xia et al., 1999; Hesterberg et al., 2001); 2) The sum of organic bound mercury, ΣHgL, is assumed to be a simple species of HgL^0. This assumption ignores other acid-base species of organic mercury complexes such as, HgHL and HgOHL which are not considered important species. Then pH normalized formation constant of HgL is given by:

$$K = \frac{[HgL^0]}{[Hg^{2+}][L^{2-}]} \quad (3.26)$$

The formation constants based on free ion concentration (3.26) are given in Figure 3.5 as a function of mercury-binding ligand concentration. The formation

constants based on free ion concentration range from $10^{23.8}$ to $10^{29.5}$. The formation constants in this range are consistent with stability constants of mercury complexation by thiol groups. The linearity in Figure 3.5 indicates that the observed continuous binding characteristics of ligands rely on the true heterogeneous characteristics of mercury complexing organic ligands rather than reaction conditions associated with the measurement. Generally, lower concentrations of stronger binding ligands dominate natural mercury speciation.

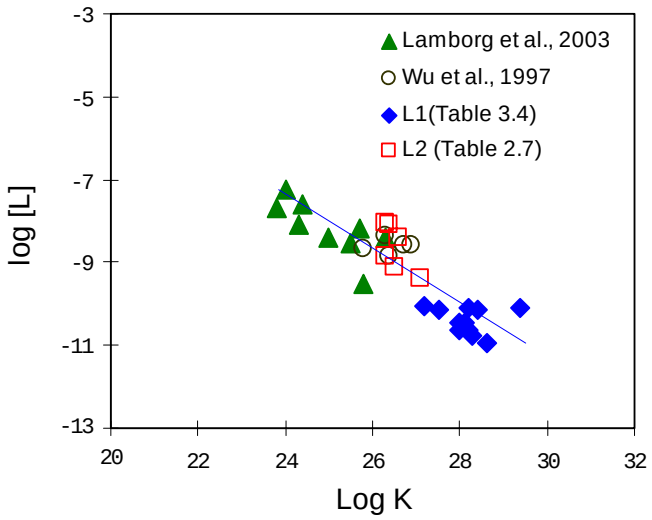


Figure 3.5. Relations between concentration of mercury binding organic ligand and conditional stability constant of mercury-organic complex reported for natural water samples. Log K = $[HgL^0]/([Hg^{2+}][L^{2-}])$.

Summary

Whereas complexation of dissolved mercury by inorganic ligands is well known, complexation by organic ligands is less understood primarily due to a lack of a proper detection method. In this work, a competitive ligand equilibration method using TSA as a competing ligand was successfully applied to dissolved mercury in natural water. Hydrophilic organic ligands which exist at 11 – 93 pM concentrations with log conditional stability constants of 26.6 - 28.9 were detected in coastal and estuarine water samples at pH 9.5 – 9.8.

The higher concentrations of mercury-complexing organic ligands in lower salinity samples agree with previous results determined by chloride competition. After the correction to the free ions equilibrium constant, the mercury-binding constants correspond to those of low molecular weight organic thiols. A linear relationship was observed between log K and log [L], supporting the hypothesis of continuous binding characteristics of metal-complexing organic ligands rather than the existence of discrete ligand class. The CLE-SSE method using TSA competition extends the detection limit of natural organic ligands to the pM molar level, which permits a detailed investigation of organic complexation of dissolved mercury in natural water.

CHAPTER IV

COMPLEXATION OF MERCURY BY ORGANIC LIGANDS IN GALVESTON BAY ESTUARY

Introduction

It is well recognized that the chemical speciation of mercury plays an important role in its estuarine cycling, influencing its evasion, dissolution, adsorption, and bioavailability. In estuarine environments, rivers are major sources of mercury with substantial additions from the atmosphere and sediments depending on sedimentary and biogeochemical conditions (Coquery et al., 1997; Mason et al., 1997, 1999). Most of the river-borne and estuarine mercury has been reported to be associated with suspended particles (Cossa and Noel 1987; Coquery et al., 1997; Mason et al., 1999; Lawson et al., 2001; Domagalski et al., 2001; Choe et al. 2003; Conaway et al., 2003). Mobilization of mercury from the particulate phase has been suggested in turbid estuarine waters through the co-variation between dissolved mercury and suspended particulate matter (SPM) (Cossa and Noel, 1987; Cossa and Martin 1991). In addition, the microbial degradation of sedimentary organic matter is known to release mercury into sediment pore water in relation to the reduction/oxidation behavior of iron and manganese oxide (Gobeil and Cossa, 1993).

To date, few studies have reported the chemical speciation of mercury in esturine systems. Some specific dissolved mercury species can be determined experimentally

including elemental mercury (Hg^0), dimethylmercury (DMHg), monomethylmercury (MMHg), and the operationally defined reactive mercury (Mason et al., 1993; Coquery et al., 1997; Mason et al., 1998; Mason et al., 1999; Lacerda et al., 2001). The concentration of organic mercury was determined as the differences between total mercury and reactive mercury based on determination using $SnCl_2$ reduction (Gill and Bruland, 1990).

Thermodynamic calculations have also been used to estimate the complexation of mercury. Leermakers et al. (1995) estimated conditional stability constants between mercury and humic matter ($K' = 10^{19}$, [humics] = 10 % of total DOC) corresponding to the experimental determination of reactive mercury. Their modeling results suggested that the mercury-humic fraction decreases from 100 % of the total solution concentration of mercury at the river water end-member to < 5 % at salinity 30. Guenzel et al. (1996) used experimentally determined colloidal mercury fractions to estimate the stability constants of mercury-organic complexes and concentrations of mercury-complexing organic ligands. HgL_2 complexation through two thiol functional groups ($\log K_1 = 22.1$; $\log K_2 = 41.6$; [L] = 1 - 5 nM) was shown to give a good estimation of colloidal mercury concentrations in estuarine water.

Furthermore, a surface complexation model (MOCO) was applied to simulate chemical and physical speciation of mercury in turbid estuarine environments (Laurier et al., 2003). Independently determined surface complexation parameters (such as, specific surface area, site density, surface acidity constant, surface complexation constant with mercury, and exchangeable particulate fraction) and estimated concentrations of

dissolved thiol ligands and stability constants of Hg-thiol complexes reproduced the phase and solution partitioning of mercury in the Seine estuary, France (Laurier et al., 2003).

In this chapter, the complexation of mercury by organic ligands in Galveston Bay was investigated. The concentrations and stability constants of mercury-complexing organic ligands were measured and were used to calculate the solution speciation of filter-passing mercury. The speciation measurements were investigated in relation to the physical and biogeochemical conditions of estuarine waters. The salinity, temperature, and SPM concentrations were measured to estimate hydrosedimentary conditions of Galveston Bay along with other geochemical conditions such as nutrients, DOC, chlorophyll-a and glutathione concentrations.

Study Area

Galveston Bay in Texas is the second largest estuary along the coastline of the Gulf of Mexico. It encompasses 1554 km^2 of surface area and is surrounded by 526 km^2 of marshland (Pinckney et al., 2002). The major source of freshwater input to Galveston Bay is the Trinity River (~ 83 %) and minor sources include the San Jacinto River (8 %) and Chocolate Bayou (< 1 %) (Orlando et al., 1993). Even though Galveston Bay is located next to many industrial complexes, especially the petroleum industry, the concentrations of trace metals in Galveston Bay with the exception of the San Jacinto

River and upper Houston Ship Channel are low and similar to other pristine estuaries along the Texas coast (Wen et al., 1999).

As a shallow (average depth of ~2 m) and turbid estuary, Galveston Bay has a high riverine and sedimentary flux of nutrients and trace metals. The cycling of phosphate in Galveston bay is controlled by the sedimentary flux as well as phytoplankton uptake and release as the distribution as a function of salinity is non-conservative (Santschi 1995). The measurement of sediment-water exchange flux of several trace metals showed that benthic flux can be a primary input in Galveston (Wen et al., 1999; Warnken et al., 2001). Tang et al. (2002a) recently reported that the distribution of some of trace metals (Cd, Cu, Ni, Pb and Zn) showed mid-estuary maxima as well, which indicates the role of sedimentary fluxes in Galveston Bay as sources of those metals. Stordal et al. (1996a) determined concentrations of filter-passing mercury between 0.3 and 6.8 pM in the surface water of Galveston Bay estuary. Like other trace metals, mercury concentrations are elevated in the upper Houston Ship Channel (~ 6 pM).

Galveston Bay can be divided into four regions depending on geographic locations: Trinity River, Trinity Bay, Upper Galveston Bay, and Lower Galveston Bay. The locations of each region are shown in Figure 4.1.

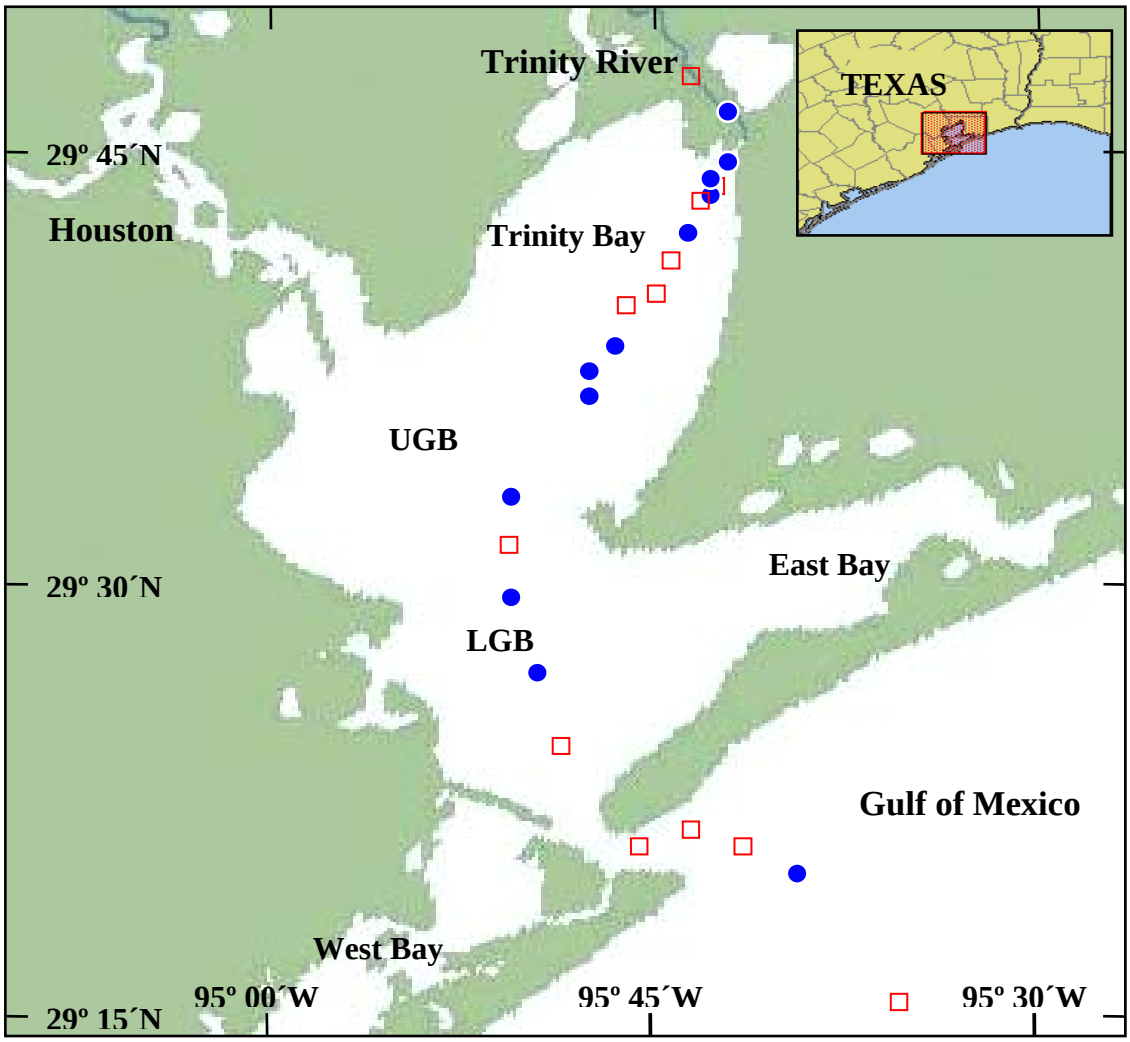


Figure 4.1. Sampling locations in Galveston Bay estuary on June 3 and 4, 2003 (circle) and October 13 and 18, 2003 (square). UGB: Upper Galveston Bay. LGB: Lower Galveston Bay.

Materials and Methods

Sample Collection

Surface water samples in Galveston Bay were collected along a salinity gradient (Figure 4.1) on June 3 (Station 1 – 6) and 4 (Station 7 – 12), 2003 and on October 13 (Station 1 - 6) and 18 (Station 7 – 12), 2003 using ultra-clean sampling protocols (Gill and Bruland 1990; Stordal et al., 1996a; Choe and Gill 2001). Unfiltered samples were collected at approximately one meter depth using Teflon® tubing connected to a peristaltic pump. Filtered samples were collected by the same method by placing a polyethersulfone membrane filter (0.45μm) on the exit tubing. Suspended particulate matter (SPM) and chlorophyll-a samples were collected in 1L polyethylene bottles: sample filtrations were performed within 24 hours after sampling. Samples for nutrients and dissolved organic carbon (DOC) analysis were frozen immediately after collection using dry ice and were stored frozen until analysis. Samples collected for glutathione (GSH) analysis were stored in Teflon® vials and were acidified with methanesulfonic acid (1mM) within several hours after sampling. Filtered samples collected for mercury-complexing ligand analysis were stored in 2 L Teflon® bottles at 4˚C without any preservation for up to two months. Filtered and unfiltered mercury samples were collected in 500 mL Teflon® bottles and were acidified (0.06 N high purity HCl) within 12 hours of collection. Teflon® bottles and tubing used for sampling and storage were all acid-cleaned following ultra-clean sampling protocols (Gill and Bruland, 1990; Stordal et al., 1996a; Choe and Gill, 2001).

Sample Analyses

The concentration of suspended particulate matter (SPM) was determined by dry weight of particles following vacuum filtration using polycarbonate filters (0.45 μm). Chlorophyll-a samples were filtered using GF/F (Whatman) filters, after which acetone extractions and measurements by a fluorometric method were performed. Nutrients were analyzed by a flow-injection spectrophotometric method (Armstrong and Stearns, 1967). A catalytic high temperature organic carbon analyzer (Shimadzu TOC 5000) was used to determine the concentrations of organic carbon in filtered water samples. Filter-passing glutathione (GSH) concentrations were determined by HPLC using a fluorescence detection method (Tang et al., 2000, 2002b).

Filtered and unfiltered mercury concentrations were analyzed by $NaBH_4$ reduction, gas-phase stripping onto gold, and detection by cold vapor atomic fluorescence spectroscopy (CVAFS) following UV digestion (Choe et al., 2003). The precision of the CVAFS method was < 4 % (CV) and the detection limit (as 3 times the standard deviation of the method blank) was 0.16 pM. The concentrations and stability constants of mercury-complexing organic ligands were determined by mercury titrations using CLE-SSE as described in chapters II and III. Two different detection windows of chloride competition and thiosalicylic acid (TSA) competition were applied to the October samples, while only thiosalicylic acid competition was applied to the June samples.

Ultrafiltration

Cross-flow ultrafitrations (Wen et al., 1996; Guo et al., 2000a, 2000b) were carried out on six of the October samples using a 10 kD membrane (Amicon® Miniplate). Filtrations were performed within 24 hours after the sampling to minimize phase change of mercury due to coagulation (Choe and Gill, 2001). The ultrafiltration method is described in Choe and Gill (2001). Concentration factors > 15 (see Table 4.1) were used based on the results in Choe and Gill (2001) in which they found effective retention for molecules larger than the nominal pore size and less retention for molecules smaller than the nominal pore size. Prior to use, membrane performance was tested using standard organic molecules: vitamin B-12 (mw 1350) and dextran (mw 4400 and 19500) (Table 4.1). The colloidal concentrations in Table 4.1 were calculated as described below. When ultrafiltration was completed, the system was rinsed with permeate solution (~70 mL) for ~ 5 min. under low back pressure (5 psi) to improve the recovery of retained material. The [wash] in (4.1) denotes the concentration of solute in this fraction.

$$[\text{colloidal}] = \frac{[\text{retentate}] - [\text{permeate}]}{\text{CF}} + \frac{[\text{wash}] - [\text{permeate}]}{\text{CF}_w} \tag{4.1}$$

$$\text{CF} = \frac{\text{initial volume}}{\text{retentate volume}};\ \text{CF}_w = \frac{\text{initial volume}}{\text{wash volume}}$$

Retention coefficients were 0.014, 0.53, and 1.1 for vitamin B12 (mw 1350), dextran of molecular weight 4400, and 19500, respectively. The retention of organic molecules smaller than the nominal pore size have been reported by Guo et al. (2000) and Choe et al. (2001) in which the higher retention was observed when the CF is lower

and standard molecules are larger.

Table 4.1. Retention coefficient (R_c) and permeation coefficient (P_c) of organic macromolecules by 10 kD membranes.

Molecule	MW	CF	Initial Conc. mM	Permeate Conc. mM	Colloidal Conc. mM	Pc[a]	Rc[b]	Mass Balance[c] %
Vitamin B12	1350	17	2.8	2.6	0.040	0.93	0.014	94
Dextran	4400	19	1.6	0.77	0.84	0.49	0.53	102
Dextran	19500	18	0.51	0.046	0.55	0.05	1.1	116

[a] $P_c = \frac{[\text{permeate}]}{[\text{initial}]}$; [b] $R_c = \frac{[\text{colloidal}]}{[\text{initial}]}$; [c] $\text{Mass balance} = \frac{[\text{colloidal}] + [\text{permeate}]}{[\text{initial}]} \times 100$.

Results and Discussion

Biogeochemical Environments of Galveston Bay

Figure 4.2 shows Trinity River discharge as a daily mean gage height (ft) at Romayor, Texas (http://waterdata.usgs.gov/tx/nwis). This site is considered representative of flow of Trinity River into Galveston Bay (Warnken and Santschi, 2004). The average daily discharges rate for the June sampling period (May 3, 2003 - June 3, 2003) was 62 m^3/sec and for the October sampling period (September 13, 2003 - October 13, 2003) was 119 m^3/sec. Discharge during both sampling periods was lower than the average of 311 m^3/sec during the recent decade (1992-2001).

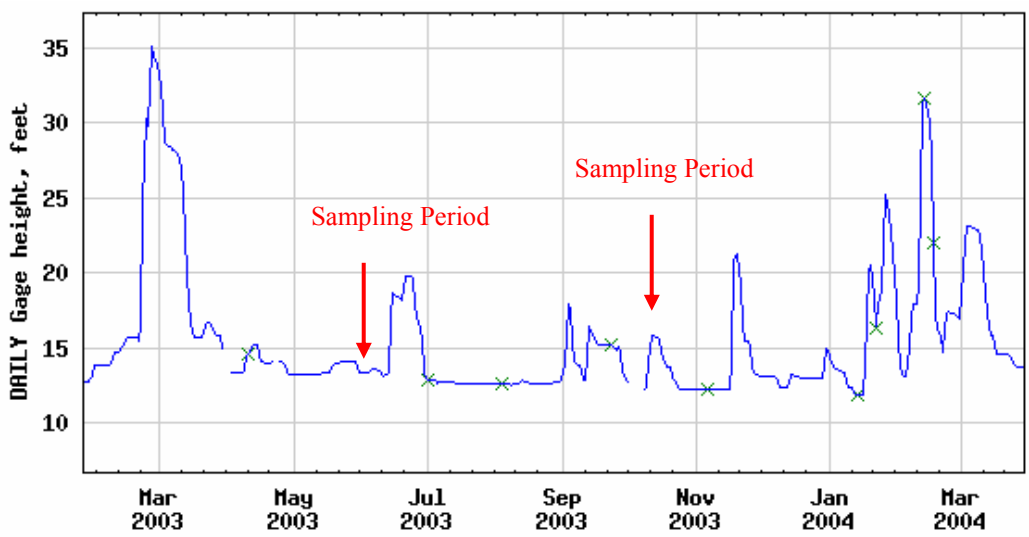


Figure 4.2. Daily mean gage height of Trinity River measured at Romayor, Texas (http://waterdata.usgs.gov/tx/nwis).

The salinity, temperature, SPM, chlorophyll-a, and nutrients are shown in Table 4.2 and Figure 4.3. The high turbidity zone (HTZ) occurring at the Trinity River and upper Trinity Bay has been reported in other studies (Wen et al., 1999; Hung et al., 2001; Tang et al., 2002a). Salinities increased considerably with depth at stations two to five during both transects, supporting the generation of an HTZ by density-driven circulation at the head of Trinity Bay. The resuspension of bottom sediments due to high hydrodynamic energy may shift the turbidity maximum to Upper Trinity Bay in June (the low flow season) from Trinity River in October (the high flow season). The HTZ occurring in Upper Galveston Bay (Station 8 and 9) in June and coastal regions (Station 11) in October may be attributed to the local hydrodynamic conditions such as, tidal- or strong wind-driven resuspension.

Table 4.2. Temperature, salinity, suspended particulate matter (SPM), chlorophyll-a, and dissolved nutrients concentrations in Galveston Bay estuary in June and October 2003.

Station Number	Temp. (ºC)	Salinity	SPM (mg/L)	Chl-a (μg/L)	Phosphate (μM)	Silicate (μM)	Nitrate (μM)	Ammonium (μM)
Jun 2003								
1	28.7	0	45	13	1.2	58	19	2.3
2	28.9	0.9	61	8.2	2.3	31	14	6.9
3	29.2	3.6	56	7.7	1.6	22	9.6	8.2
4	-	5.4	47	13	2.5	22	5.9	2.2
5	29.5	7.2	38	9.3	2.4	20	nd	0.26
6	29.2	9.8	20	6.5	2.6	29	nd	0.35
7	30.5	12.2	18	5.1	3.5	51	0.10	0.51
8	28.2	16.2	54	11	2.5	25	nd	0.72
9	-	18.2	46	7.3	2.6	14	0.29	1.4
10	28.0	21.8	14	5.6	1.2	9.4	nd	0.61
11	-	25.7	17	6.1	0.80	7.2	0.094	0.76
12	26.7	28.6	5.0	2.6	0.29	7.1	0.079	0.70
October 2003								
1	24.6	0.1	71	8.4	2.5	68	9.3	1.1
2	26.0	1.3	57	8.4	2.1	40	2.2	2.9
3	25.7	3.7	49	6.0	2.2	49	2.4	4.1
4	26.4	5.9	17	15	3.1	37	0.45	0.48
5	25.7	7.3	5.7	12	3.7	30	2.6	1.3
6	25.7	10.6	7.4	8.2	3.8	49	nd	0.47
7	25.6	12.6	11	15	4.1	64	nd	0.42
8	-	15.3	14	9.1	3.1	60	nd	0.61
9	25.0	17.3	29	12	2.3	52	0.67	0.64
10	24.5	19.5	33	8.8	1.9	46	0.84	0.93
11	23.8	23.3	54	5.3	1.2	30	0.89	1.7
12	25.3	26.8	1.3	1.9	0.36	12	0.094	0.61

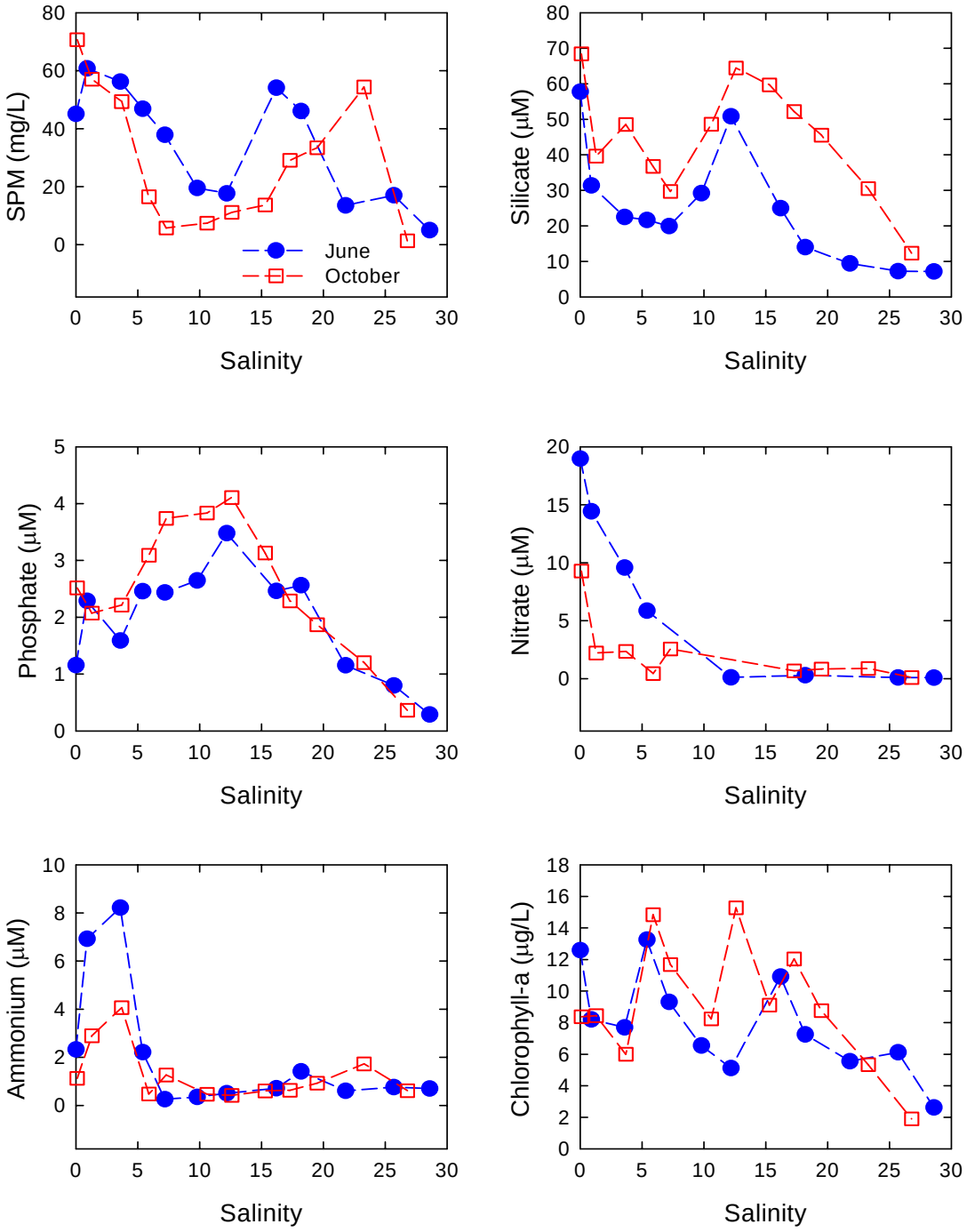


Figure 4.3. Estuarine distributions of suspended particulate matter (SPM), dissolved nutrients (nitrate, ammonium, silicate, and phosphate), and chlorophyll-a in Galveston Bay estuary in June and October of 2003.

The distributions of silicate and phosphate showing a mid-estuary maxima at salinity 12, which agrees on the previous reports (Santschi 1995; Wen et al., 1999; Hung et al., 2001; Tang et al., 2002a), suggest the importance of benthic fluxes of those nutrients. The regeneration of phosphorous from anaerobic diagenesis of iron minerals was suggested by Santschi (1995) based on the accumulated nutrients data of Galveston Bay. The distributions of nitrate showing non-conservative mixing behaviors indicate removal of nitrate by biological activities such as photosynthesis and denitrification. The low concentrations of nitrate occurring in Lower Trinity Bay and Galveston Bay may indicate nitrate-limiting primary production which has been observed in Galveston Bay during all seasons from May 1999 to July 2000 (Örnólfsdóttir, 2002). Higher chlorophyll-a concentrations were observed at Trinity Bay and Upper Galveston Bay where waters are calmer and nutrient fluxes are higher (Wen et al., 1999; Hung et al., 2001; Tang et al., 2002a).

Distribution of Organic Carbon

Dissolved organic carbon concentrations ranged from 82 to 221 μM in June and from 97 to 290 μM in October (Table 4.3 and Figure 4.4). Previous observations range between 100 and 500 μM in Galveston Bay (Wen et al., 1999; Guo et al., 2000a; Tang et al., 2002a). The higher DOC in October than June at the river water end-member is in agreement with Warnken and Santschi (2004) who reported DOC in the Trinity River linearly increasing with increasing river water discharge. The same authors estimated that the benthic DOC flux accounts to 20 – 80 % of the total DOC flux to Trinity Bay

during low flow conditions.

Table 4.3. The concentrations of organic carbon in Galveston Bay surface waters.

Station Number	Salinity	DOC[a]	COC[b]		UOC[c]		Mass Balance	CF
		(μM)	(μM)	%[d]	(μM)	%[e]	%	
June 2003								
1	0	209						
2	0.9	213						
3	3.6	221						
4	5.4	208						
5	7.2	218						
6	9.8	211						
7	12.2	-						
8	16.2	178						
9	18.2	146						
10	21.8	95.9						
11	25.7	90.7						
12	28.6	82.0						
October 2003								
1	0.1	266	49.3	19	243	91	110	14.1
2	1.3	289						
3	3.7	290	27.8	10	243	84	93	17.7
4	5.9	252						
5	7.3	261	21.9	8	213	82	90	16.4
6	10.6	236						
7	12.6	192	20.8	11	158	82	93	16.1
8	15.3	213						
9	17.3	195	12.2	6	179	92	98	16.4
10	19.5	136						
11	23.3	119	12.0	10	108	91	101	15.2
12	26.8	97.2						

[a]Dissolved organic carbon (<0.45 μm);
[b]Colloidal organic carbon (10 kD – 0.45 μm);
[c]Ultrafiltrate organic carbon (<10 kD);
[d][COC]/[DOC]×100;
[e][UOC]/[DOC]×100.

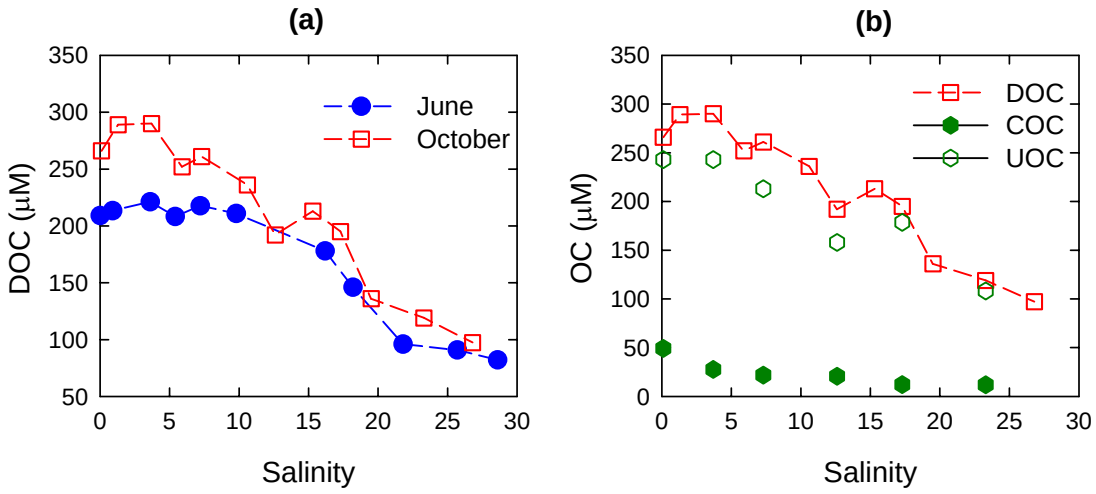


Figure 4.4. Distributions of organic carbon in Galveston Bay. (a) DOC < 0.45 μm. (b) October samples, DOC <0.45 μm, 10 kD < COC < 0.45 μm, UOC <10 kD.

Ultrafiltration was carried out on six of the October samples using 10 kD membranes. The ultrafiltration results for DOC are shown in Table 4.3 and Figure 4.4. An average of 11 ± 4 % of the filter-passing organic carbon was colloidal-size (10 kD – 0.45 μm) organic carbon. These results agree with previous determinations in Galveston Bay: 23 ± 9 % (10 kD – 0.45 μm) in July, 1995 (Wen et al., 1999) and 6.4 ± 1 % (10 kD – 2 μm) in July, 1993 (Guo and Santschi, 1997). While DOC exhibits nearly conservative estuarine mixing behavior, COC shows a non-conservative estuarine distribution, suggesting there is an in-situ removal of COC by flocculation.

Distribution of Glutathione

Glutathione (GSH) concentrations in surface waters of Galveston Bay ranged

from 18 to 79 nM for the June samples and from 20 to 111 nM for the October samples (Table 4.4). The estuarine distribution exhibits nearly conservative mixing in October during higher flow conditions, while a slight estuarine source is observed in June during low flow conditions (Figure 4.5). The distribution of GSH along the salinity gradient co-varies with the distribution of DOC (Figure 4.6). The correlation factor (r^2) between [GSH] and [DOC] was 0.92, suggesting that Trinity River may be the primary source of GSH in surface waters of Galveston Bay with a smaller contribution from sediment flux during low flow conditions.

Table 4.4. Glutathione (GSH) concentrations in filter-passing (< 0.45 μM) surface waters of Galveston Bay estuary.

Station Number	Salinity	GSH (nM)	Station Number	Salinity	GSH (nM)
Jun-03			Oct-03		
1	0	73.8	1	0.1	104 ± 1.5 [a]
2	0.9	77.4	2	1.3	111 ± 3.8
3	3.6	78.6	3	3.7	107 ± 2.1
4	5.4	78.2	4	5.9	92.2 ± 1.4
5	7.2	70.1	5	7.3	79.8 ± 1.1
6	9.8	64.0	6	10.6	75.8 ± 4.4
7	12.2	61.6	7	12.6	67.2 ± 3.2
8	16.2	57.0	8	15.3	57.7 ± 0.1
9	18.2	50.9	9	17.3	52.4 ± 1.5
10	21.8	36.6	10	19.5	48.0 ± 1.9
11	25.7	27.6	11	23.3	35.6 ± 0.5
12	28.6	18.1	12	26.8	20.1 ± 0.4

[a]Standard deviation determined from the duplicate measurements.

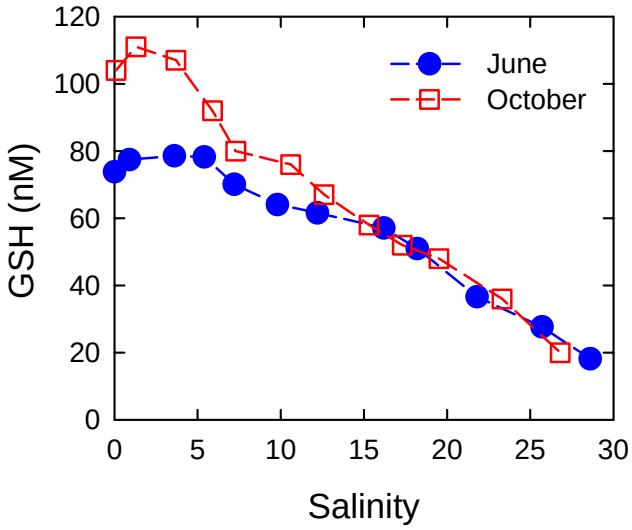


Figure 4.5. Estuarine distribution of filter-passing glutathione in surface waters of Galveston Bay.

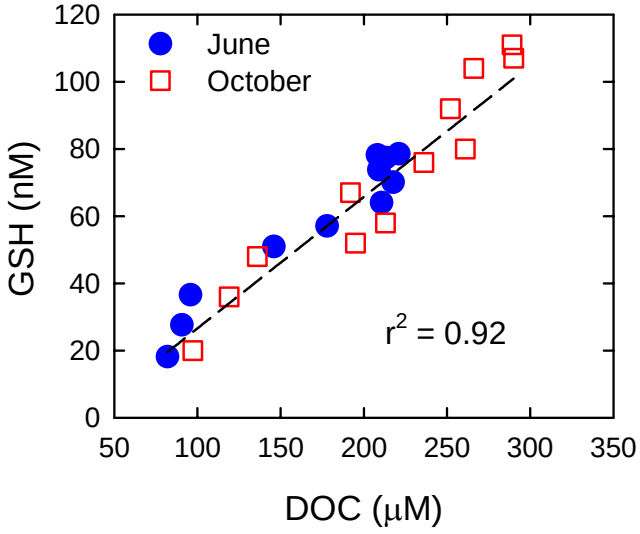


Figure 4.6. Relationship between the concentrations of filter-passing glutathione and filter-passing organic carbon in Galveston Bay surface waters.

GSH is known to be produced by the bacterial degradation of organic matter in anoxic pore water. Few nM to μM concentrations of glutathione have been reported in anoxic pore waters (Mopper and Taylor, 1986; Kiene et al., 1990). GSH in oxic surface water has recently been determined using cathodic stripping voltammetry and other approaches (Table 4.5). Cathodic stripping voltammetry was able to detect a sulfide-like peak in natural seawater and it was believed to be a thiol species due to its kinetic stability (Al-Farawati and van den Berg, 2001). Since the voltammetric method was not able to identify each thiol species, concentrations were estimated as GSH or thiourea equivalents (Christine et al., 1998; Leal et al., 1999; Al-farawati and van den Berg, 2001), or as total reduced sulfur concentrations (Tang et al., 2001). Reported thiol concentrations range from 0.7 to 15 nM for coastal waters and from 0.2 to 130 nM for estuarine waters (Table 4.5). Co-variations between thiol concentration and chlorophyll-a concentration have been observed in coastal waters of the Western North Sea and English Channel (Al-farawati and van den Berg, 2001), and in estuarine waters of Galveston Bay (Tang et al., 2001).

The GSH concentrations determined in this work agree well with previous determinations by cathodic stripping voltammetry and copper titrations (Tang et al., 2001; Laglara and van den Berg, 2003). However, an order of magnitude difference was observed between current results and Tang et al. (2000) who determined concentrations of each thiol species in surface waters of Galveston Bay in August, 1999.

Table 4.5. Reported concentrations of filter-passing thiols in estuarine, coastal and open ocean waters.

Sample Type	Sampling Region	Thiol concentration	Method	Reference
Open ocean water	North East Atlantic	1 - 15 nM (glutathione equivalents)	Cathodic stripping voltammetry	Christine et al., 1998
Coastal water	Western North Sea, English Channel	0.70 - 3.60 nM (thiourea equivalents)	Cathodic stripping voltammetry	Al-Farawati and van den Berg, 2001
Estuarine water	Galveston Bay	10 - 130 nM (glutathione equivalents)	Cathodic stripping voltammetry	Tang et al., 2001
Estuarine water	Scheldt Estuary	24 - 114 nM	Cu titration	Laglera and van den Berg, 2003
Estuarine water	Galveston Bay	0.2 - 6.2 nM (glutathione)	HPLC	Tang et al., 2000

GSH concentrations from ultrafiltration are shown in Table 4.6. The majority of the GSH was detected in the permeate fraction (<10 kD), attributable to the small molecular size of GSH (mw 307.33). About 7 % of the GSH in Trinity River water was retained by the 10 kD membrane, which is thought to be an artifact of the ultrafiltration processes (Guo et al., 2000b). With the exception of the river water end member, GSH concentrations in the colloidal size fraction are less than 2 % of total GSH.

Table 4.6. Filter-passing, colloidal, and ultrafiltrate glutathione (GSH) concentrations in surface water samples of Glaveston Bay collected in October, 2003.

Station Number	Filter-passing GSH[a]	CF	Colloidal GSH[b]		Ultrafiltrate GSH[c]		Mass Balance
	nM		nM	%	nM	%	%
1	104 ± 2	14.1	6.8 ± 1	6.6	90 ± 4	87	93
3	107 ± 2	17.7	0.81 ± 0.3	0.76	100 ± 4	93	94
5	80 ± 1	16.4	1.50 ± 0.2	1.9	74 ± 1	93	95
7	67 ± 3	16.1	0.80 ± 0.04	1.2	65 ±0.9	96	98
9	52 ± 2	16.4	0.54 ± 0.05	1.0	50 ± 0.3	95	96
11	36 ± 0.5	15.2	0.42 ± 0.07	1.2	32	90	91

[a] < 0.45 μm;
[b] 10 kD – 0.45 μm;
[c] < 10 kD.

Total and Particulate Mercury

Total mercury concentrations in unfiltered surface waters of Galveston Bay ranged from 1.7 to 13 pM in June and from 1.8 to 25 pM in October (Table 4.7 and Figure 4.7). These results are similar to previously measured values in Galveston Bay (Stordal et al., 1996a). Total mercury concentrations in Galveston Bay are dominated by the particulate mercury fraction due to the high affinity of mercury to bind with SPM as in many aquatic systems (Coquery et al., 1997; Mason and Sullivan, 1998; Mason et al., 1999; Domagalski, 2001; Lawson et al., 2001; Conaway et al., 2003; Choe et al., 2003; Laurier et a., 2003). Particulate mercury was 73 ± 12 % of the total mercury in June and 70 ± 17 % in October.

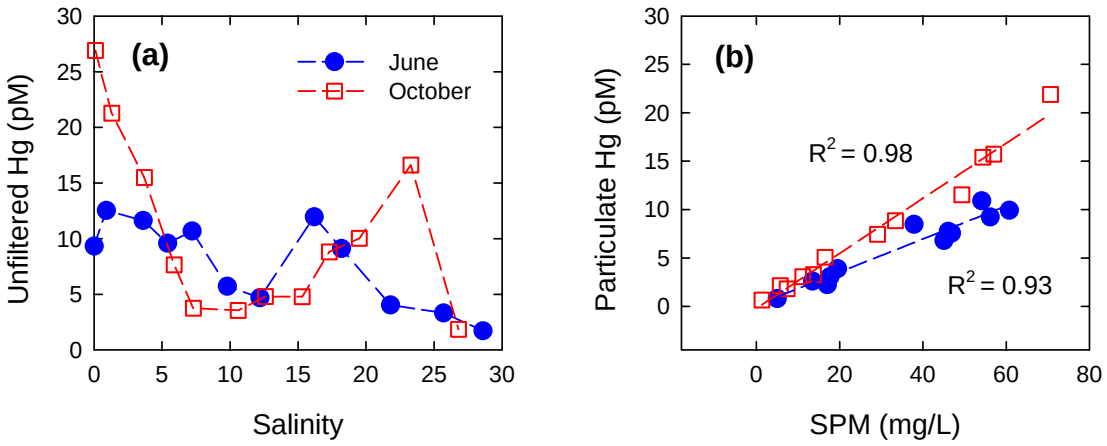


Figure 4.7. (a) Distributions of unfiltered mercury in surface waters of Galveston Bay. (b) Relationship between concentration of suspended particulate matter (SPM) and particulate mercury.

Table 4.7. The concentrations of unfiltered, filter-passing (< 0.45 μm), particulate (> 0.45 μm), colloidal (10 kD – 0.45 μm) and permeate (< 10 kD) mercury in surface waters of Galveston Bay.

St #	Salinity	Unfiltered Hg	Filter-passing Hg	Particulate Hg	Colloidal Hg		Permeate Hg		Mass Balance	CF
		(pM)	(pM)	(pM)	(pM)	%	(pM)	%	%	
Jun. 2003										
1	0	9.3	2.5	6.8						
2	0.9	13	2.6	9.9						
3	3.6	12	2.4	9.2						
4	5.4	9.6	2.0	7.6						
5	7.2	11	2.2	8.5						
6	9.8	5.7	1.8	3.9						
7	12.2	4.7	1.6	3.1						
8	16.2	12	1.1	11						
9	18.2	9.1	1.4	7.7						
10	21.8	4.0	1.4	2.6						
11	25.7	3.3	1.1	2.2						
12	28.6	1.7	0.9	0.8						
Oct. 2003										
1	0.1	27	5.0	22	2.6	51	3.3	65	117	14
2	1.3	21	5.5	16						
3	3.7	16	4.0	12	0.89	22	3.0	77	99	18
4	5.9	7.6	2.6	5.1						
5	7.3	3.7	1.6	2.1	0.18	11	1.3	83	94	16
6	10.6	3.5	1.7	1.8						
7	12.6	4.8	1.7	3.1	0.11	6	1.3	73	80	16
8	15.3	4.8	1.5	3.3						
9	17.3	8.8	1.4	7.5	0.12	9	1.0	71	80	16
10	19.5	10	1.2	8.8						
11	23.3	17	1.2	15	0.12	10	1.1	87	98	15
12	26.8	1.8	1.2	0.6						

SPM influence on total mercury concentration occurs through the interaction between particulate organic carbon (POC) and mercury in freshwater and estuarine water environments (Mason and Sullivan, 1998; Lawson et al., 2001; Choe et al., 2003; Laurier et al., 2003). SPM in October showed a higher enrichment of mercury than in June (Figure 4.7.b), which might be related to an organic carbon enrichment in October particles. The higher chlorophyll-a concentration in October SPM supports the organic carbon enrichment of October SPM (Figure 4.8). The role of phytoplankton biomass controlling particulate mercury concentration has been reported in other turbid estuaries (Coquery and Welbourn, 1995; Coquery et al., 1997; Laurier et al., 2003).

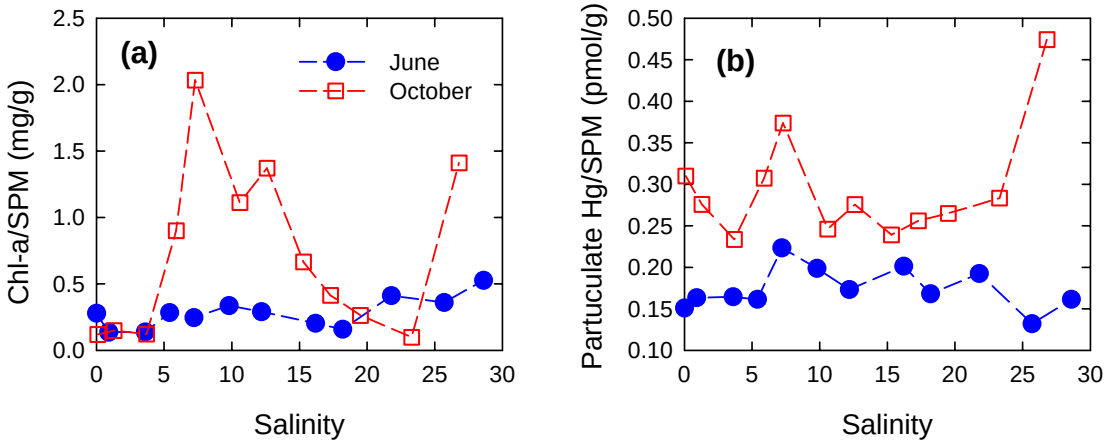


Figure 4.8. (a) Distributions of chlorophyll-a/SPM. (b) Distributions of particulate mercury/SPM.

Mercury concentrations in filtered (< 0.45 μm) surface water samples ranged from 0.90 to 2.6 pM in June and from 1.0 to 5.5 pM in October (Table 4.7) and were similar to previous results (Stordal et al., 1996a). A non-conservative estuarine mixing behavior was observed for dissolved mercury in October, but was not apparent in June (Figure 4.9.a). In-situ removal is hypothesized as a reason of the non-conservative mixing behaviors observed for dissolved mercury in October rather than a dilution of river water mercury concentrations because river water discharge rates are low. This hypothesis is supported by the positive correlation observed between particulate mercury (as [unfiltered mercury] – [filtered mercury]) and dissolved mercury (Figure 4.9.b). When data points corresponding to the HTZ in the mid-estuary, where dissolved mercury may not be in equilibrium with particulate mercury, were excluded, the correlation becomes even more significant (r^2 increases from 0.61 to 0.86). Figure 4.9.b indicates that particle-water interactions may control the concentration of dissolved mercury in Galveston Bay. Interaction between dissolved mercury and particulate mercury phases have been suggested in turbid estuaries where organic degradation occurs in surface and subsurface sediments (Cossa and Noel, 1987; Cossa and Martin 1991; Cossa and Gobeil, 2000; Laurier et al., 2003).

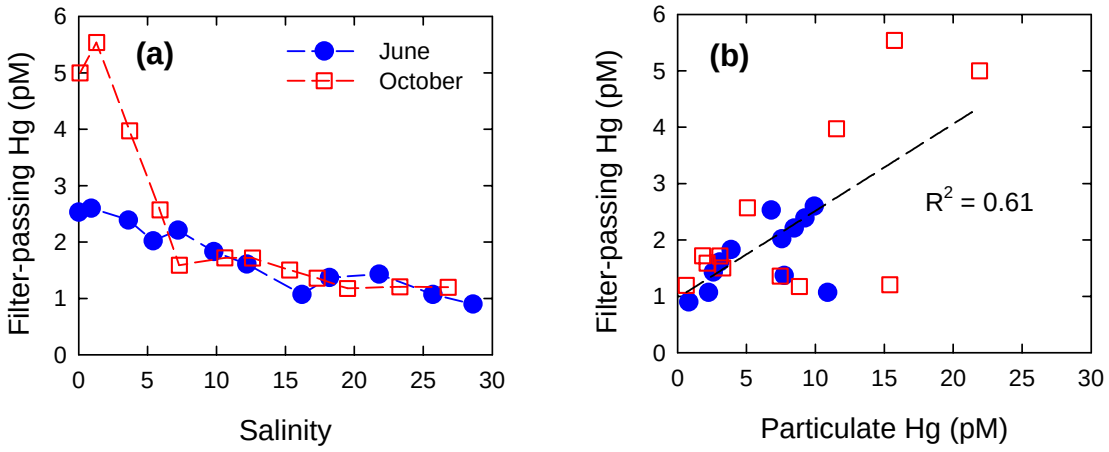


Figure 4.9. (a) Distribution of dissolved mercury in Galveston Bay surface waters; (b) Relationships between dissolved mercury and particulate mercury.

Ultrafiltration

Ultrafiltration results for mercury conducted on six of the October samples are shown in Table 4.7 and Figure 4.10. Six to 51 % of the filter-passing mercury was in the ≥ 10 kD size fraction. These results are similar to those obtained by Guentzel et al. (1996) for Ochlockonee Estuary: 3 - 40 %. Previous work in Galveston Bay by Stordal et al. (1996a) determined colloidal mercury to range between 12 to 93 % with an average of 57 ± 20 % using a 1 kD membrane. Colloidal mercury and colloidal organic carbon (COC) concentrations co-varied (Figure 4.10.b), demonstrating that COC controls the distribution of colloidal mercury in Galveston Bay (Guentzel et al., 1996; Stordal et al., 1996a; Choe et al., 2003). The colloidal coagulation and flocculation have been suggested as a removal process of dissolved trace metals (Honeyman and Santschi,

1988; Santschi et al., 1997) and dissolved mercury (Cossa et al., 1988; Stordal et al., 1996a; Stordal et al., 1996b).

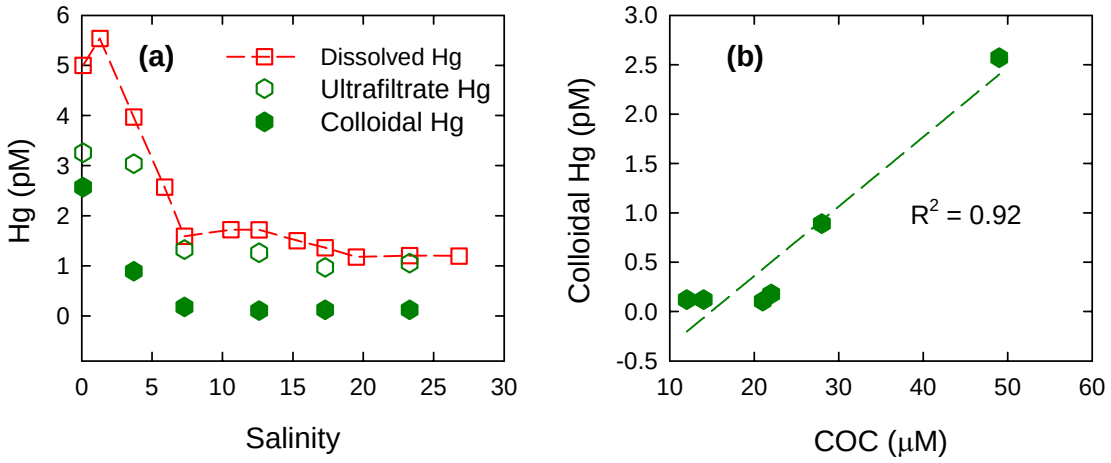


Figure 4.10. (a) Distributions of dissolved (<0.45 μm), colloidal (10 kD – 0.45 μm) and ultrafiltrate (<10 kD) mercury in Galveston Bay surface waters; (b) Relationship between colloidal organic carbon (COC) and colloidal mercury.

A particle concentration effect, a decrease in K_d as a function of SPM due to the presence of colloidal ligands (Benoit et al., 1994; Benoit 1995; Benoit and Rozan 1999), was observed in both seasons (Figure 4.11). The K_d's determined for Galveston Bay are similar to those found previously and in other estuaries (Stordal et al. 1996a; Mason and Sullivan 1998; Choe et al., 2003). A significantly reduced particle concentration effect is observed when the colloidal phase is taken into consideration (Figure 4.11.b).

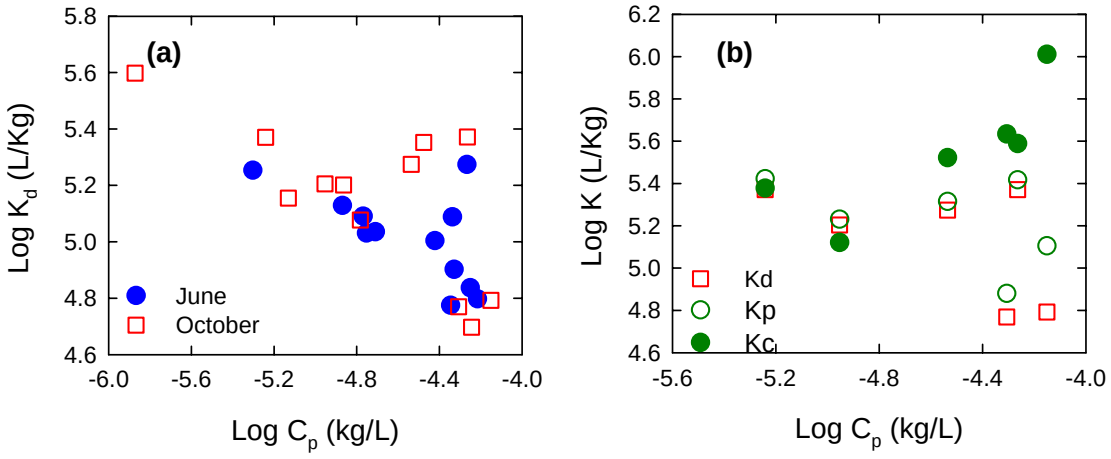


Figure 4.11. (a) Relationship between particle-water partition coefficients (K_d) and particle concentrations (C_p): K_d (L/kg) = [particulate mercury] (mol/kg) / [filter-passing percury] (mol/L). (b) Distributions of K_d, K_c, and K_p as a function of particle concentration (C_p): K_c (L/kg) = [colloidal mercury] (mol/kg) / [filter-passing mercury] (mol/L); K_p (L/kg) = [permeate mercury] (mol/kg) / [filter-passing mercury] (mol/L).

Distribution of Mercury-Complexing Organic Ligands

Measured concentrations of mercury-complexing organic ligands ([L]) and conditional stability constants (K') are given in Table 4.8 and Figure 4.12. The concentrations of strong mercury-complexing organic ligands (Ls) range from 23 pM to 78 pM in June and from 19 pM to 93 pM in October, with average log K_{Ls}' values of 28.1 and 27.9, respectively. The weak mercury-complexing organic ligands (Lw) concentrations range from 1.4 nM to 10 nM in October with an average log K_{Lw}' value of 23.1. The weak class ligand was not determined for the June samples. The Ls ligand shows esturine distributional features similar to those of silicate and phosphate, suggesting an importance of sediment–water exchange, while Lw ligand shows more

linear distributional features along a salinity gradient.

Table 4.8. Concentrations and conditional stability constants of mercury complexing organic ligands in filtered surface water samples of Galveston Bay.

Station number	Salinity	[a]L1			[d]L2		
		[b]Log α_{HgTSA}	[c]Log $K_{HgLs'}$	$[Ls]_t$ (pM)	[e]Log α_{HgCl}	[f]Log $K_{HgLw'}$	$[Lw]_t$ (nM)
Jun-03							
1	0	17.00	29.2	77.5			
2	0.9	16.71	28.9	77.5 [g]			
3	3.6	16.52	29.1	68.5			
4	5.4	16.42	28.7	71.9			
5	7.2	16.23	27.9	70.9 [g]			
6	9.8	16.23	27.8	76.9			
7	12.2	16.18	27.7	83.0 [g]			
8	16.2	16.13	27.6	58.5			
9	18.2	16.12	27.5	51.8			
10	21.8	16.12	27.6	35.1 [g]			
11	25.7	16.00	27.5	36.5 [g]			
12	28.6	16.00	27.6	22.9			
Oct-03							
1	0.1	16.91	28.8	86.2	13.94	23.0	9.35[g]
2	1.3	16.71	28.6	92.6	13.93	22.9	9.80
3	3.7	-	-	-	13.93	23.3	6.21
4	5.9	16.42	27.9	78.7	13.94	23.1	8.06 [g]
5	7.3	16.22	27.5	88.5	13.93	23.0	7.30
6	10.6	16.22	27.7	82.8	13.97	22.9	5.70
7	12.6	16.17	27.2	88.5	13.87	22.9	4.78
8	15.3	16.17	27.5	75.8	13.87	23.3	3.91 [g]
9	17.3	16.17	27.6	63.3	13.93	23.2	3.86
10	19.5	16.12	28.0	51.8	13.84	23.4	2.60
11	23.3	16.12	27.6	35.1	13.81	23.1	2.36
12	26.8	16.12	28.1	18.7	13.81	23.0	1.38 [g]

[a]Determined by TSA competitions; [b]$\alpha_{HgTSA} = K_{1,HgTSA}[TSA^{2-}]+\beta_{2,HgTSA2}[TSA^{2-}]^2$;
[c]$K_{HgLs'} = [HgLs]/([Hg^{2+}][Ls'])$, $[Ls']=[Ls]_t-[HgLs]$; [d]Determined by chloride competitions;
[e]$\alpha_{HgCl} = \beta_{2,HgCl2}[Cl^-]^2 + \beta_{3,HgCl3}[Cl^-]^3 + \beta_{4,HgCl4}[Cl^-]^4$; [f]$K_{HgLw'} = [HgLw]/([Hg^{2+}][Lw'])$
$[Lw']=[Lw]_t-[HgL]$; [g]Data are also shown in chapter II and III.

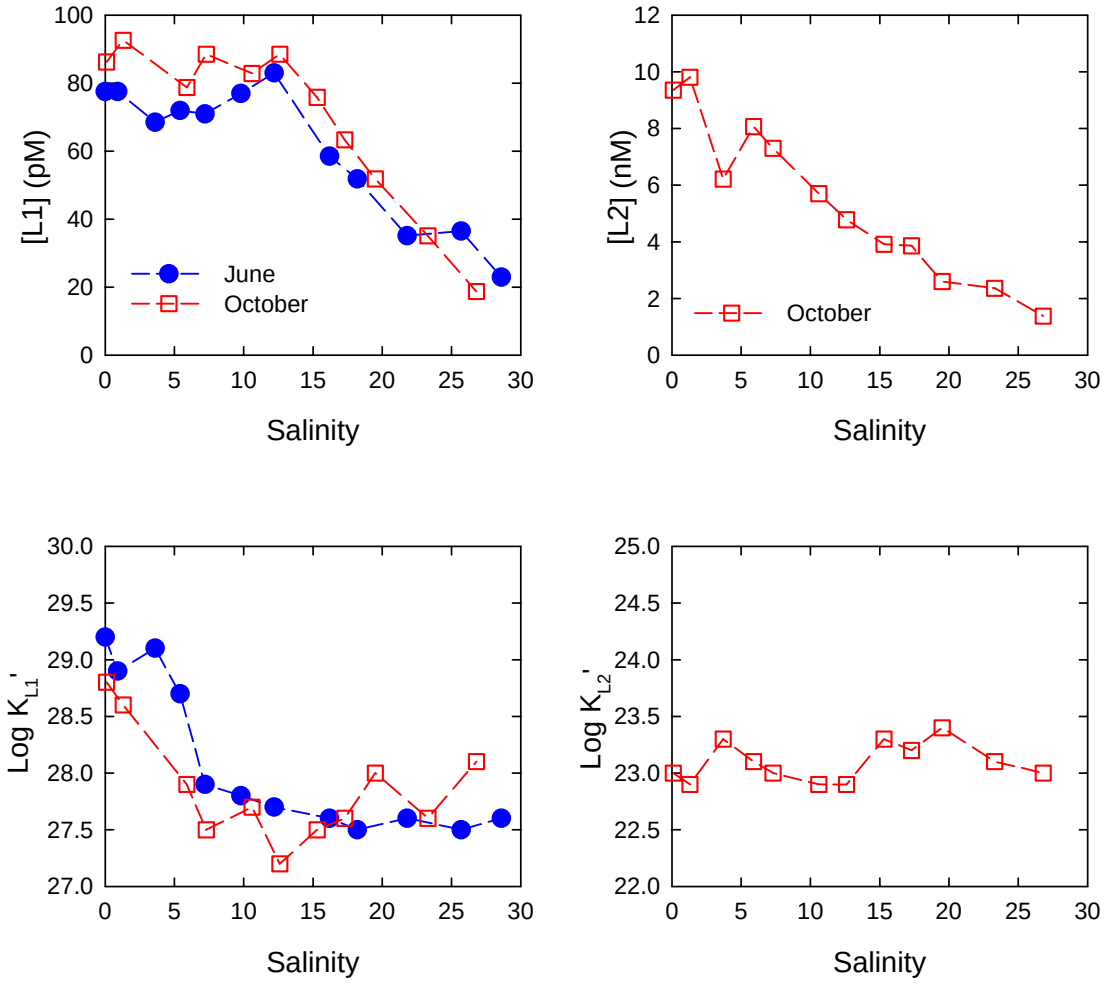


Figure 4.12. Distributions of concentration of mercury-complexing organic ligand ([L]) and conditional stability constant of HgL (log K') in Galveston Bay surface waters.

The concentrations and conditional stability constants determined for Lw are compatible to previous reports for natural waters (Wu et al. 1997; Lamborg et al. 2003). The gradual decrease of mercury-complexing organic ligand concentration with increasing salinity is in agreement with that observed for copper-complexing ligands in estuarine water by Apte et al. (1990a), Tang et al. (2001), and Laglera and van den Berg (2003).

Note that the numerical value of the conditional stability constant for Lw does not vary with salinity, while the conditional stability constant for Ls increases at lower salinities (Figure 4.12). The change in K_{HgLs}' with salinity may not be real, but rather a function of window strength of the determination (Apte et al., 1990b; Laglera and van den Berg, 2003). The constant distributions of log K_{HgLw}' along the salinity gradient are attributable to the constant window strength as a function of salinity as represented by log α_{HgCl} (Table 4.8). However, the log K_{HgLs}' increases approximately 1.5 log unit at zero salinity with the increase in log α_{HgTSA} of ~ 1. The ionic strength effect may result in an increase in the detection window for low salinity waters when constant concentrations of TSA were added (~ 2 nM) for the whole salinity range. A similar change in stability constants with salinity was reported for copper complexation by Laglera and van den Berg (2003). They observed an increased log α_{CuSA} (SA = salicylaldoxime) from 5.1 to 6.1 with salinity decreasing from 30 to 0.2 when [SA] is held constant. The log K' of the copper-organic complex was correspondingly increased from 14.5 to 15.9. Additionally, they demonstrated from the separate experiments that at a constant detection window, the variation of conditional stability constants by salinity

change was relatively small: for salinity 0.2 water, when log α_{CuSA} = 6.2, [L1] = 33 nM and log K' = 15.8; when log α_{CuSA} = 5.2, [L1] = 43 nM and log K' = 15.2. Their results indicate that the detected stability constants of low salinity waters are overestimated by the detection window increase resulted from an ionic strength change.

Relationship between Glutathione and Mercury-Complexing Organic Ligand

The concentrations of the Ls and Lw ligands show positive relationship with GSH concentrations (Figure 4.13), demonstrating that processes which influence GSH dynamics in Galveston Bay also influence the biogeochemical cycling of mercury-complexing organic ligands. A relationship between GSH and mercury-complexing organic ligands has not been reported to date, though a relationship between copper-complexing organic ligands and thiols have been reported in estuarine waters (Tang et al, 2001; Laglera and van den Berg, 2003).

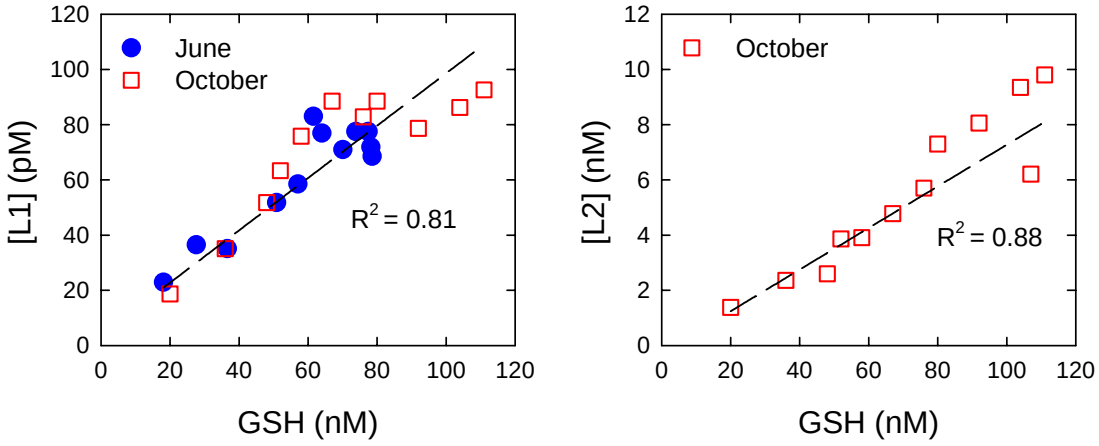


Figure 4.13. Relationship between mercury-complexing organic ligands (L) and glutathione (GSH) concentrations.

Concentrations and binding strengths of mercury-complexing ligands in filter-passing (< 0.45 μm) and permeate (< 10 kD) fractions of Galveston Bay waters are shown in Table 4.9. The conditional stability constants determined in permeate waters (average $10^{23.8}$) were slightly higher than those determined for filter-passing samples (average $10^{23.1}$) with the same detection windows (log α_{HgCl} = 13.9). An average of 53 % of the mercury-complexing organic ligands were partitioned into the permeate fractions (< 10 kD). However, direct comparison should be carefully considered due to the different conditional stability constants of each phase.

The weak organic ligand (Lw) occurring in concentrations an order of magnitude

lower than GSH concentrations show significant portions of macromolecules, which supports the hypothesis that Lw ligands are not simple small molecules like GSH. The inclusion of macromolecules (> 10 kD) in the titration resulted in weaker binding strengths, demonstrating that the complexation between mercury and low molecular weight ligands are stronger than the complexation between mercury and high molecular weight ligands.

The strong organic ligand (Ls) concentrations are 1000 times lower than GSH concentrations, suggesting that the Ls ligand is very specific and has highly selective binding strength with mercury. As described previously, its estuarine distribution shows a good correlation with GSH distributions, indicating that Ls, Lw, and GSH might have a common source and biogeochemical behavior in estuarine environments.

Table 4.9. Concentrations and conditional stability constants of mercury-complexing organic ligands in filter-passing (< 0.45 μm) and ultrafiltrate (< 10 kD) waters of Galveston Bay.

Station number	Salinity	Filter-passing		CF	Ultrafiltrate		
		[Lw] (nM)	Log K_{Lw}'		[Lw] (nM)	%	Log K_{Lw}'
Oct-03							
1	0.1	9.4	23.0	14.1	4.4	47	23.6
3	3.7	6.2	23.3	17.7	4.8	77	23.5
5	7.3	7.3	23.0	16.4	2.2	30	23.8
7	12.6	4.8	22.9	16.1	2.3	48	24.0
9	17.3	3.9	23.2	16.4	1.9	49	23.9
11	23.3	2.4	23.1	15.2	1.1	46	24.0

Continuum Binding Ligand Model

It has been postulated that natural organic matter has a continuum of binding sites with varying binding strengths (Bruland et al., 2000; Town and Filella, 2000; 2002). Evidence for the existence of this hypothesis is often demonstrated by plotting ligand concentrations against conditional stability constants. In a continuum model, ligand concentration decreases as the conditional stability constants increases. This relationship implies that a low concentration of high strength binding sites on DOC are responsible for binding metals, when metal concentrations are low, rather than a particular ligand (e.g. glutathione or EDTA) or ligand class (e.g. thiols).

The linear relationship between ligand concentrations and stability constants are presented in Figure 4.14 using the data compiled in Table 2.5, 3.4, 3.5, and 4.8. In Figure 4.14.a, the log K_{cond}' show linear relationship with log $[L]_t$ despite of different reaction conditions, which have been reported for other trace metals (Town and Fillela, 2000; 2001). The organic complexation of mercury observed in this work clearly suggests a continuous binding model. The conditional stability constants are corrected to free ion equilibria as described in Chapter III (Figure 4.13.b). Formation constants based on free ion concentrations show a smaller range of values ($10^{26.3}$ - $10^{29.8}$) than those of [L'] ($10^{22.9}$ -$10^{29.2}$), which is attributable to the normalization of pH variation associated with the measurement. Formation constants in this range are consistent with the stability constants of mercury complexation by thiol groups (see Table 1.2) and those determined by Haitzer et al. (2003). As described in chapter III, generally, lower concentrations of stronger binding ligands dominate natural mercury speciation.

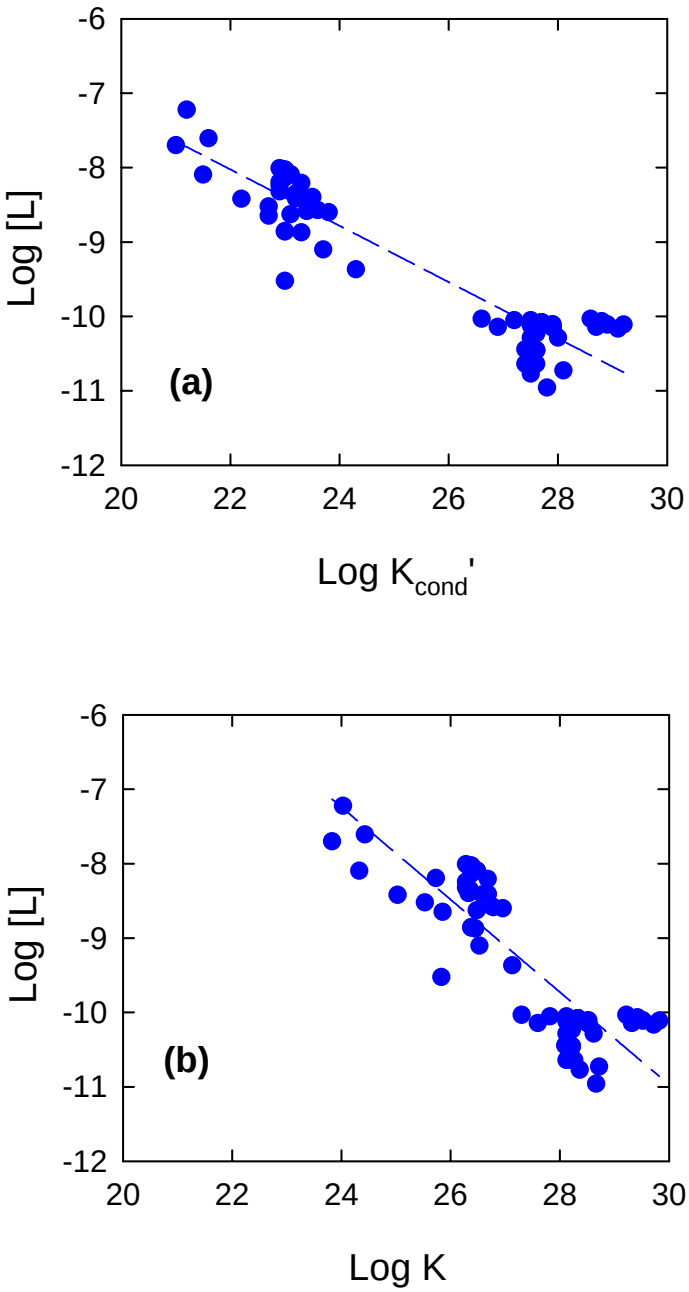


Figure 4.14. Relationship between the concentration of mercury-complexing organic ligand (L) and the stability constant (K) of HgL. Data are presented in Table 2.5, 3.4, 3.5, and 4.8. (a) $K_{cond}' = [\Sigma HgL]/([Hg^{2+}][L'])$, $L' = [L]_t - [\Sigma HgL]$. (b) $K = [HgL^0]/([Hg^{2+}][L^{2-}])$.

Chemical Speciation of Filter-Passing Mercury

The concentrations of organic and inorganic dissolved mercury species in Galveston Bay estuary were determined using a thermodynamic equilibrium modeling program, MINEQL (Schecher and McAvoy, 1992). The formation constants used for inorganic mercury species are summarized in Table 4.10. These values have been commonly used in other reports (Leermakers et al. 1995; Guenzel et al., 1996; Laurier et al., 2003). The concentrations of organic species (HgLs and HgLw) were calculated using the mercury and ligand concentrations presented in Table 4.7 and Table 4.8, respectively. The mercury-organic ligand stability constants given in Table 4.8 are conditional constants and are only usable at the pH of the determinations. A correction can be made for pH if an assumption is made about the pK_a for the ligand. Given in Table 4.11 are the corrected stability constants for the representative HgLs and HgLw when pKa_1 of 6.3 and pKa_2 of 10.3 are assumed (Haitzer et al., 2003). Assumptions and calculation process are described in Chapter III.

Table 4.10. Stability constants for formation of mercury inorganic complexes (at zero ionic strength and 25 ºC, Morel and Hering, 1993).

Complex	log β
HgOH	10.6
$Hg(OH)_2$	21.8
$Hg(OH)_3$	20.9
$HgCl_2$	14.0
$HgCl_3$	15.1
$HgCl_4$	15.4
HgOHCl	18.1

Table 4.11. Correction results of conditional stability constants of HgLs and HgLw using pK_a of H_2L: $pK_{a1} = 6.3$ and $pK_{a2} = 10.3$.

	Log K_{HgL}'[a]	pH[b]	Log K_{HgL}[c]
Ls	23.0	7.0	26.4
Lw	28.0	9.8	28.6

[a] $K_{HgL}' = [HgL]/([Hg^{2+}]([L_t] - [HgL]))$;
[b] Titration pH;
[c] $K_L = [HgL^0]/([Hg^{2+}][L^{2-}])$.

The solution speciation in Table 4.12 and 4.13 was calculated for seawater of pH 8.2 using the pH normalized conditional stability constants. MMHg concentrations are included in the October data based on separate determinations (Choe and Gill, 2003). MMHg and Lw ligand were not determined for June samples. Other mercury species such as elemental mercury and dimethylmercury included in total filter-passing mercury are generally less than 5 % of total dissolved mercury in estuarine environments (Mason

Table 4.12. Concentrations of filter-passing mercury species in surface waters of Galveston Bay collected on June 2003, T=29ºC, pH=8.2.

St #	S	Log K_{HgLs}	L_s (M)	HgLs (M)	$HgCl_2$ (M)	$HgCl_3$ (M)	$HgCl_4$ (M)	HgOHCl (M)	total Hg (M)
1	0	29.8	7.75E-11	2.53E-12	1.69E-22	1.06E-24	1.20E-27	6.86E-21	2.53E-12
2	0.9	30.0	7.75E-11	2.60E-12	8.46E-20	1.53E-20	5.79E-22	1.28E-19	2.60E-12
3	3.6	30.5	6.85E-11	2.39E-12	4.13E-19	2.99E-19	5.22E-20	1.69E-19	2.39E-12
4	5.4	30.2	7.19E-11	2.02E-12	1.45E-18	1.57E-18	4.35E-19	4.05E-19	2.02E-12
5	7.2	29.5	7.09E-11	2.21E-12	1.41E-17	2.04E-17	7.85E-18	3.02E-18	2.21E-12
6	9.8	29.5	7.69E-11	1.83E-12	1.93E-17	3.80E-17	2.09E-17	3.11E-18	1.83E-12
7	12.2	29.4	8.30E-11	1.61E-12	3.00E-17	7.37E-17	5.23E-17	3.96E-18	1.61E-12
8	16.2	29.4	5.85E-11	1.07E-12	4.87E-17	1.59E-16	1.57E-16	4.96E-18	1.07E-12
9	18.2	29.3	5.18E-11	1.37E-12	1.12E-16	4.10E-16	4.62E-16	1.02E-17	1.37E-12
10	21.8	29.5	3.51E-11	1.43E-12	1.56E-16	6.85E-16	9.51E-16	1.21E-17	1.43E-12
11	25.7	29.4	3.65E-11	1.07E-12	1.92E-16	9.93E-16	1.66E-15	1.27E-17	1.07E-12
12	28.6	29.5	2.29E-11	8.96E-13	2.53E-16	1.46E-15	2.76E-15	1.52E-17	9.00E-13

Table 4.13. Concentrations of filter-passing mercury species in surface waters of Galveston Bay collected on October 2003, T=25ºC, pH=8.2.

St #	S	Log K_{HgLs}	L_s (M)	Log K_{HgLw}	Lw (M)	HgLs (M)	HgLw (M)	$HgCl_2$ (M)	$HgCl_3$ (M)	$HgCl_4$ (M)	MMHg (M)	total Hg (M)
1	0.1	29.6	8.62E-11	26.6	9.35E-09	4.24E-12	4.83E-13	4.14E-21	8.33E-23	2.84E-25	2.76E-13	5.00E-12
2	1.3	29.7	9.26E-11	26.8	9.80E-09	4.67E-12	6.56E-13	5.31E-19	1.39E-19	7.14E-21	2.07E-13	5.54E-12
4	5.9	29.4	7.87E-11	27.4	8.06E-09	1.24E-12	1.28E-12	5.98E-18	7.10E-18	1.98E-18	5.35E-14	2.57E-12
5	7.3	29.1	8.85E-11	27.4	7.30E-09	5.87E-13	9.73E-13	7.54E-18	1.11E-17	3.95E-18	3.20E-14	1.59E-12
6	10.6	29.4	8.28E-11	27.4	5.70E-09	1.00E-12	6.98E-13	1.42E-17	3.03E-17	1.66E-17	1.96E-14	1.72E-12
7	12.6	29.0	8.85E-11	27.5	4.78E-09	6.25E-13	1.07E-12	2.89E-17	7.33E-17	4.90E-17	2.06E-14	1.72E-12
8	15.3	29.3	7.58E-11	27.9	3.91E-09	4.86E-13	1.00E-12	1.91E-17	5.87E-17	4.92E-17	1.07E-14	1.50E-12
9	17.3	29.4	6.33E-11	27.8	3.86E-09	5.27E-13	8.13E-13	2.50E-17	8.69E-17	8.40E-17	1.89E-14	1.36E-12
10	19.5	29.8	5.18E-11	28.0	2.60E-09	6.48E-13	5.22E-13	1.89E-17	7.42E-17	8.23E-17	1.44E-14	1.18E-12
11	23.3	29.5	3.51E-11	27.8	2.36E-09	5.04E-13	6.85E-13	6.11E-17	2.87E-16	3.90E-16	9.61E-15	1.20E-12
12	26.8	30.0	1.87E-11	27.7	1.38E-09	8.64E-13	3.35E-13	8.43E-17	4.54E-16	7.25E-16	5.35E-15	1.21E-12

et al., 1993; Leermakers et al., 1995; Mason et al 1998, 1999) hence any inaccuracy caused by ignoring these species would be minimal.

For the June transect, the organic-mercury species, HgLs, dominates (99.6 – 100 %) over the major inorganic mercury species, $HgCl_3^-$ and $HgCl_4^{2-}$, for the whole salinity range. Note that if the pKa_2 of H_2L would be higher, this would cause a decrease of % HgLs, but only at high salinity (data not shown). For the October transect, MMHg, separately determined, was 0.4 to 5.5 % of total filter-passing mercury, decreasing with increasing salinity. The organically complexed mercury species is calculated as a major mercury species (HgL > 94.5 %). Table 4.13 shows that HgLs competes with HgLw in the mid-salinity range, when both are assumned to be present. Even though conditional stability constants of HgLw are two orders of magnitude lower than those of HgLs, the greater in concentration gives a comparable complexation capacity to that of the Ls ligand. Similar observations were reported for copper-binding organic ligands, in which the α_{CuL} (= $K_{CuL}' \times [L]$) remains constant with varied conditional stability constants range and gives approximately constant CuL concentrations (Bruland et al., 2000).

The chemical equilibrium modeling of filter-passing mercury has several limitations in its interpretation. First, the other trace metals which compete with mercury for thiol binding sites were not included in the model calculations. However, since mercury is highly selective for thiol binding sites (for example, Log K_{HgDDC} = 31.9, Log K_{CuDDC} = 13.7, DDC = Diethyldithiocarbamates, Stary and Kratzer, 1968), any inaccuracy caused by ignoring these side reactions of thiol ligand could be ignored.

Second, the log conditional stability constants of HgL can increase to a range

from 30 to 33 when mercury-binding ligands concentrations extrapolate to natural mercury concentrations (1 - 10 pM) from Figure 4.14. In that case, the complexation coefficient of HgL (α_{HgL}) may be higher than reported values in Table 4.12 and 4.13.

Third, in addition to thermodynamic equilibrium, kinetics of reactions, such as, reduction to Hg^0, adsoption/desoprtion with particulate phase, and MMHg production, control the concentrations of dissolved mercury species in estuarine environments (Laurier et al., 2003). Covariation between dissolved reactive mercury and dissolved gaseous mercury (reduction product) was shown in the surface water samples of Seine Estuary, France (Laurier et al., 2003). Cossa et al. (2002) reported enhanced reduction of ionic mercury (up to 20 % of total dissolved mercury) during summer in the same estuary. While methylation of dissolved mercury has been reported to be ignorable (Laurier et al., 2003; Conaway et al., 2003; Choe et al., 2003), the reduction of Hg^{2+} to Hg^0 need to be considered for the understanding of dissolved mercury distributions in surface estuarine waters.

Summary

Two classes of ligands that complex mercury were determined in Galveston Bay estuary: a strong ligand (Ls), which exists at pM levels and is hydrophilic and a weak class ligand (Lw), which exists at nM concentrations and is hydrophobic. Both mercury-complexing organic ligands (Ls and Lw) show estuarine distributional features similar to GSH and DOC distributions, suggesting a common source and biogeochemical cycling in estuarine environments. Based on linear relationships determined for Log [L] and Log K, these ligands appear to be part of a continuum of binding sites on DOC fractions.

Almost all of filter-passing mercury in Galveston Bay exists as an organic complex ([HgLs] > 94.5 % of $[Hg]_t$) based on chemical speciation modeling using measured stability constants and concentrations of mercury-complexing organic ligands. Concentrations of MMHg ranged from < 1 to 6 % of total dissolved mercury with increasing concentrations by decreasing salinity.

Overall, the distribution of unfiltered mercury in Galveston Bay surface waters was controlled by different hydrodynamic and biogeochemical conditions of the bay. Particularly important is the resuspension of bottom sediments and organic carbon content in particles. Dissolution from particulate mercury and removal by sinking particles through colloidal coagulation/particle adsorption are hypothesized to be major production and removal processes of filter-passing mercury in surface waters of Galveston Bay. Galveston Bay appears to be a sink for filter-passing mercury during both seasons, trapping high concentrations of filter-passing mercury in the sediment.

CHAPTER V

MERCURY SPECIATION IN OFFATTS BAYOU – A SEASONALLY ANOXIC BAYOU ON GALVESTON BAY

Introduction

Biogeochemical behavior of mercury in a water column with varying redox conditions is determined by the different adsorption-desorption reactions involving physical and chemical speciation changes of mercury. Reducing conditions are known to increase the dissolution of Mn(IV) and Fe(III) oxyhydroxide as a consequence of the microbial degradation of organic matter and to change the speciation of dissolved trace metals (Dyrssen and Klemming, 1990; Perry and Pederson, 1993; Balistrieri et al., 1994; Cooper and Morse, 1996). The particulate and colloidal mercury adsorbed on Mn(IV) and Fe(III) oxyhydroxide (Tiffreau et al., 1995; Quemerais et al., 1998) and organic solid (Dmytriw et al., 1995) can be the source of dissolved mercury in anoxic water columns. Mobilization of mercury due to the dissolution of such mineral phases has been reported in anoxic sediment pore water (Gobeil and Cossa, 1993) and estuarine water (Mason et al., 1993).

Particulate FeS is thought to control dissolved trace metal concentrations in anoxic environments through the scavenging of metal sulfide complexes (Dyrssen and Kremling 1990). Cooper and Morse (1996) showed that the total reactive (HCl plus HNO_3 extractable) mercury fraction in anoxic sediments was dominantly associated with

pyrite. Mercury released by the dissolution of manganese and iron oxide can be re-adsorbed on the same phase while dissolved mercury diffuses to oxic layers or can be scavenged onto FeS (Mason et al., 1993). The precipitation to mercuric sulfide is also possible in high concentrations of mercury and sulfide: The pE-pH diagram of mercury speciation for Onondaga Lake ($[S^{2-}]_t$ = 2 mM, $[Cl^-]$ = 0.01 M, $[Hg]_t$ = 100 pM) predicted HgS precipitation under mild reducing conditions (-5 < pE < 5) and pH < 7.0 (Wang and Driscoll, 1995).

The solid HgS has an extremely low solubility product: $10^{-38.5}$ for $HgS + H^+ \leftrightarrow Hg^{2+} + HS^-$ (Dyrssen, 1989). Crystalline mercuric sulfide (HgS) in the form of metacinnabar and cinnabar has been found in mine impacted sediments (Kim et al., 2004) and mercury contaminated flood plains (Barnett et al., 1997). Mercury complexation by sulfide and bisulfide (HgS_2H_2, HgS_2H^-, HgS_2^-) in the presence of S^{2-} was shown to increase the solubility of solid mercuric sulfide through thermodynamic calculations (Morel et al., 1998). The existence of dissolved organic matter (DOM) in anoxic condition would drive competition between dissolution and adsorption/precipitation, resulting in an increased dissolved concentration of mercury. Organic matter isolated from Florida Everglade was shown to increase the solubility of cinnabar (Ravichandran, 1998) and precipitation/aggregation of metacinnabar was inhibited by humic fraction of DOM (Ravichandran, 1999).

According to Dyrssen and Wedborg (1991), mercury-thiol complex should be a dominant species of dissolved mercury as long as sulfide concentration does not highly exceed thiol concentration based on the higher stability constants of mercury-thiol

species than those of mercury-sulfide species ($Hg^{2+} + 2SH^- = Hg(SH)_2$: Log K = 37.7; $Hg^{2+} + 2RS^- = Hg(RS)_2$: Log K = 41.6). However, depending on the reaction conditions, such as concentration of DOM, concentration of sulfide, and pH, the competition between the organic-mercury and sulfide-mercury complexes can occur. Evidences supporting a possible competition between sulfide and DOM species for mercury are provided by the inhibitions of DOM from the nucleation of metacinnabar and by the enhanced dissolution of cinnabar in the existence of DOM (Ravichandran et al., 1998, 1999; Ravichandran 2004).

Offatts Bayou, a sub-estuary of Galveston Bay, is located on Galveston Island. During the early 1900's, this area was used as a borrow pit for major construction, resulting in a 1 km x 2 km size artificial basin. The depth of the basin is 2 - 5 times deeper than surrounding waters, resulting in a restricted water exchange between Offatts Bayou and adjacent coastal waters (Cooper and Morse 1996). In addition, there is a significant annual change in surface water temperatures which result in a seasonal thermohaline stratification of the water column. A highly sulfidic bottom layer develops every summer due to the intense oxidation of organic matter and limited mixing of water (Cooper and Morse, 1996).

In this chapter, the dissolved mercury speciation in a stratified water column, which has anoxic bottom water, is estimated by the thermodynamic calculations. The concentrations of mercury-complexing organic ligands and stability constants of mercury-organic complexes are experimentally determined. Literature values are used for

mercury-sulfide speciation and thermodynamic calculation are carried out using MINEQL.

Materials and Methods

Sample Collection

Samples were collected on September 9, 2003 at the eastern end of Offatts Bayou in a basin that is ~ 7 m depth. Dissolved oxygen (DO) concentrations were measured at 0.5 m depth intervals by DO meter and the measured concentrations of dissolved oxygen were used to decide sample collection depths. Teflon coated Go-Flo bottles which were cleaned with weak hydrochloric acid were used to collect water samples from four different depths: one oxic, two transitions, and one anoxic. Go-Flo bottles were developed two times for each depth and the first bottles were used for the in-situ measurements of dissolved oxygen, salinity, temperature, and pH. The second Go-Flo bottles were moved to the lab immediately after the sample collections, after which filtrations using peristaltic pump were carried out in the clean lab. The filtration process using ultraclean protocol is described in Chapter IV. The filtered water samples were stored separately for the analysis of sulfide, mercury, monomethylmercury (MMHg), glutathione (GSH), dissolved organic carbon (DOC), and mercury-complexing organic ligands. A CTD was deployed at the sampling site to obtain water column profiles of salinity, temperature, and DO.

Sample Analysis

Dissolved sulfide concentrations were analyzed by the methyleneblue spectrophotometric method (Okumura et al., 1999) modified from Cline method (Cline 1969). The preparation of reagents and standard sulfide solutions are described in Okumura et al. (1999). About 100 mL sample were filtered into two graduates syringes (50mL) connected to other syringes each of which is containing 4 mL mixed diamine solutions (0.08 g *N*,*N*-dimethyl-*p*-phenylenediamine sulfate, 0.6 g iron (III) chloride, and 4 g of magnesium chloride in 100 mL of 6 M hydrochloric acid). The two solutions were mixed through the tubing between two syringes and, after 20 minutes, the generated colored solution was passed through Sep-Pak® C18 cartridges (Waters). The absorbed methyleneblue complex was re-eluted with 3 mL of methanol/0.01 M hydrochloric acid solution. The absorbance of elute was measured at 659 nm. The pre-concentration step using Sep-Pak® C18 cartridge was not applied to bottom layer samples which show high sulfide concentrations (> 200 μM).

Dissolved copper concentrations were measured by GFAAS with Zeeman background correction (Wen et al., 1996; Tang et al., 2002a). The analysis method for mercury, MMHg, GSH, DOC, and mercury-complexing organic ligands were described in Chapter IV.

Results and Discussion

Temperature, Salinity, and Dissolved Oxygen

Water column profiles of temperature, salinity, and dissolved oxygen obtained using a CTD are shown in Figure 5.1. The water column is stratified with significant oxygen depletion (≤ 0.5 mg/L) in near bottom waters. A broad transition zone exists between approximately 3.5 m and 5.5 m depth. The salinity increases with depth over the upper and mid water column (~ 6 m), which is responsible for producing the stratified structure. The persistence of a thermohaline structure during summer and the shift to mixed water column structure during winter is a common feature of this system (Cooper and Morse, 1996).

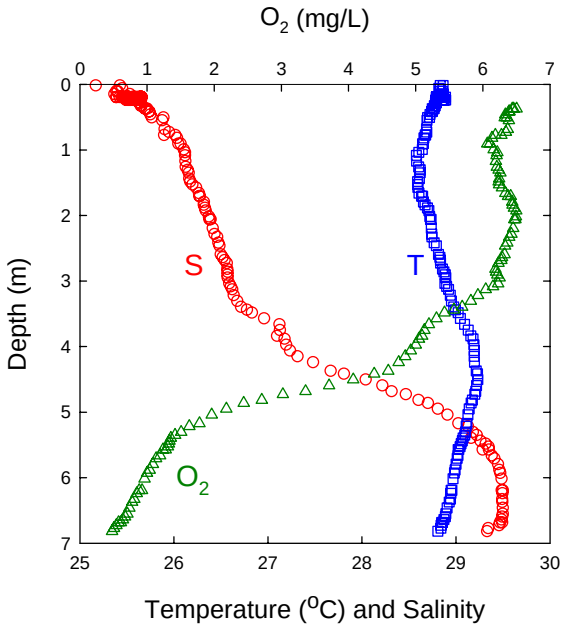


Figure 5.1. Profiles of temperature, salinity and dissolved oxygen in Offatts Bayou on September 9, 2003.

Water Column Chemistry

Measurement of salinity, DO, pH, suspended particulate matter (SPM), DOC, GSH, sulfide, and dissolved copper were made on discrete samples obtained from the water sample collections (Table 5.1).

Values of pH decrease by depth from 8.5 to 7.5, probably co-varying with ΣCO_2. The highest concentration of SPM at surface oxic layer suggests that major portion of SPM has a biological origin. DOC does not show a specific trend by depth. The highest GSH concentration at the bottom layer suggests that a sediment–water exchange flux is an important source of GSH. Dissolved copper concentration decreases with depth. Detailed investigation of copper distribution in an anoxic water column by Balistrieri et al. (1994) showed that decreased dissolved copper corresponds to an enrichment of Fe in particles at the same depth, which reflects the scavenging of Cu-sulfide species by particulate iron phases.

The total dissolved sulfide concentrations were determined to 26 nM - 234 μM which agrees with Cooper and Morse (1996) who reported 200 – 400 μM at the 6 – 7 m depth and < 5 uM at 0 – 5 m depth in September 1993. The total dissolved sulfide concentrations in bottom layer, 234 μM, rapidly decreased in the oxic and transition zone, showing that the stratified water column structure limits the mixing of dissolved sulfide between bottom layer and upper layers.

Table 5.1. Salinity, dissolved oxygen, pH, suspended particulate matter (SPM), dissolved organic carbon (DOC), glutathione (GSH), sulfide, and copper concentrations of the sampling site in Offatts Bayou (T = 29ºC).

Depth	S	O_2	pH	SPM	DOC[a]	GSH[a]	sulfide[a]	Cu[a]
m		mg/L		mg/L	μM	nM	nM	nM
0 - 1	25.9	6.6	8.5	90	166	47	35	15
3 - 4	26.8	5.0	8.2	28	145	42	55	8.2
4 - 5	28.0	2.1	7.9	29	175	44	180	9.2
6 - 7	29.5	0.49	7.5	18	165	61	230000	2.9

[a]In a filter-passing fraction (< 0.45 μm).

Mercury and Monomethylmercury Concentrations

Mercury concentrations in unfiltered and filtered surface waters (Table 5.2) are comparable to those found in the Galveston Bay surface water transect (Chapter IV). Unfiltered mercury concentrations ranged from 1.9 to 2.2 pM in the upper layers and increase rapidly at the bottom layer to 6.7 pM. The filter-passing mercury showed a similar pattern, ranging from 0.73 to 1.1 pM in the upper layers, and increasing to 3.1 pM in the bottom layer. Unfiltered and filtered mercury concentrations show rapid increase at the sulfidic bottom layer, which is primarily caused by the increase of MMHg.

Higher MMHg concentrations were observed in the anoxic layer, which is often observed in stratified lakes (Bloom and Effler, 1990). MMHg is produced by sulfate-reducing bacteria in anoxic sediments (Compeau and Bartha, 1985; Gilmour et al., 1992). High MMHg concentrations in bottom water most likely result from the diffusion of MMHg from sediment pore water. However, Mason et al. (1993) reported higher concentrations of MMHg at the picnocline than anoxic layer, and the maximum MMHg

depth agreed with high bacterial populations. More detailed sample collections and analyses are needed to explain the relationship between MMHg distribution and redox cycle in a stratified water column.

Unfiltered mercury and MMHg concentrations do not covary with SPM concentrations. This stands in marked contrasts to the estuarine distribution where unfiltered mercury and MMHg concentrations covary with the distribution of SPM (chapter IV; Coquery et al., 1997; Mason and Sullivan, 1998; Mason et al., 1999; Domagalski, 2001; Lawson et al., 2001; Conaway et al., 2003; Choe and Gill, 2003; Choe et al., 2003; Laurier et a., 2003). In addition, filter-passing mercury and particulate mercury, which show linear relationship in Galveston Bay transect, do not show similar distributions in the sampling water column of Offatts Bayou (Table 5.2 and Table 5.3). This suggests that particle-water interactions that control the distribution of dissolved mercury in surface water of Galveston Bay do not control mercury cycling in stratified and oxygen depleted water column.

Table 5.2. Mercury and monomethyl mercury (MMHg) concentrations in Offatts Bayou.

Depth	Unfiltered Hg	Filter-passing Hg	Unfiltered MMHg		Filter-passing MMHg	
m	pM	pM	pM	%[a]	pM	%[b]
0 - 1	2.2 ± 0.07	1.0 ± 0.08	0.043 ± 0.02	2.0	0.021 ± 0.03	1.9
3 - 4	2.1 ± 0.02	0.73 ± 0.04	0.054 ± 0.01	2.6	0.030 ± 0.01	4.1
4 - 5	1.9 ± 0.03	1.1 ± 0.03	0.053 ± 0.02	2.8	0.046 ± 0.01	4.2
6 - 7	6.7 ± 0.3	3.1 ± 0.2	2.7 ± 0.3	40	2.0 ± 0.1	65

[a]% = [Unfiltered MMHg]/[Unfiltered Hg] x 100;
[b]% = [Filter-passing MMHg]/[Filter-passing Hg] x 100.

The filter-passing mercury fraction averaged 47 ± 9.6 % of the total mercury, which is higher than observed for surface waters of Galveston Bay (28 ± 14 %). A higher percentage of dissolved mercury in total mercury pool is also supported by a low particle-water partition coefficient (K_d, L/Kg) (Table 5.3). The K_d determined for Offatts Bayou averaged to 4.5 ± 0.3 (for Cp = 18 – 90 mg/L): the average for Galveston Bay was 5.1 ± 0.2 (for Cp = 1 - 71 mg/L). Lower particulate mercury fraction in total mercury agrees with Mason et al. (1993) who measured mercury concentrations in the stratified water columns of Pettaquamscutt estuary.

In Table 5.3, the particulate mercury/SPM (nmol/g) ratio is significantly lower in upper layers of Offatts Bayou compared to bottom layer and it was also lower than Galveston Bay samples (0.23 ± 0.08 nmol/g). Mercury enrichments of bottom water particles may relate to the organic-enriched SPM due to sediment organic degradation or to the presence of FeS precipitates scavenging dissolved mercury species.

Table 5.3. Concentrations of particulate mercury (Hg_p), particulate monomethylmercury ($MMHg_p$), and partition coefficients (K_d) in Offatts Bayou.

Depth	Hg_p	Hg_p / SPM	log K_d[a] of Hg	$MMHg_p$	$MMHg_p$ / SPM	log K_d[a] of MMHg
m	pM	nmol/g	L/kg	pM	pmol/g	L/kg
0 - 1	1.1	0.012	4.1	0.022	0.24	4.1
3 - 4	1.4	0.049	4.8	0.024	0.86	4.5
4 - 5	0.80	0.028	4.4	0.0070	0.24	3.7
6 - 7	3.6	0.20	4.8	0.70	39	4.3

K_d (L/kg) = [Particulate mercury] (mol/kg) / [Filter-passing mercury] (mol/L).

Concentrations of dissolved manganese and iron were not determined, however higher dissolved manganese and iron concentrations are expected at the oxic/anoxic interface (Gobil and Cossa, 1993; Mason et al., 1993). Dissolved Fe(II) and Mn(II) in the suboxic zone diffuse upward and are oxidized to Mn(IV) and Fe(III) at or above the oxic/anoxic interface. While dissolved Fe(II) and Mn(II) diffuse to oxic zone, highly insoluble phases such as MnO_2 and $Fe(OH)_3$ are formed and may resettle toward anoxic zone (Perry and Pedersen, 1993). The lowest particulate Hg and particulate MMHg concentrations occurred at 4 - 5 m depth (Table 5.3), which can be attributed to the mobilization of mercury by the dissolution of Mn and Fe solid phases.

Mercury-Complexing Organic Ligands

Mercury concentrations and conditional stability constants of mercury-complexing organic ligands were determined by competitive ligand equilibration-mercury titrations using thiosalicylic acid (TSA) as a competing ligand (chapter III). The window strengths ($\alpha_{HgTSA} = K_1[TSA^{2-}] + \beta_2[TSA^{2-}]^2 = 16.0 - 16.1$) were similar to those used with the Galveston Bay samples (chapter IV). The potential interferences of sulfide on the mercury titrations was considered by comparing titration slopes obtained for the Galveston Bay samples with those for the Offatts Bayou samples. The average slope for the Galveston Bay titration curves was 0.47 ± 0.07: slopes for Offatts Bayou samples were 0.44, 0.46, 0.47, and 0.39 for 0.5 m, 3.5 m, 4.5m, and 6.5 m samples, respectively.

Concentrations of mercury-complexing organic ligands were higher in Offatts Bayou (Table 5.4) than Galveston Bay surface water in the same salinity range (19 – 37

pM). The high ligand concentrations in Offatts Bayou may be attributable to the sediment-water exchange flux, resulting from an enhanced degradation of sedimentary organic matter in Offatts Bayou. The production of GSH due to bacterial degradation of organic matter (Mopper and Taylor, 1986; Kiene et al., 1990) might be related to the sedimentary flux of mercury-complexing organic ligands. Similar dynamics observed between GSH and mercury-complexing organic ligands in Offatts Bayou as well as Galveston Bay surface waters (chapter IV) support the previous hypothesis.

The stability constants of mercury-complexing organic ligands measured by the TSA competition at pH 9.6 condition were normalized to the pH independent conditional stability constants as described in chapter III. The corrected conditional stability constants are shown in Table 5.4.

Table 5.4. Concentrations of mercury-complexing organic ligands ([L]), conditional stability constants of mercury-organic complexes determined at pH = 9.6 (K'), and pH-corrected formation constants (K).

Depth (m)	α_{HgTSA}	[L] (pM)	Log K'[a]	[b]Log K[b]
0 – 1	16.1	68	27.3	28.1
3 – 4	16.1	81	27.3	28.1
4 – 5	16.0	121	27.0	27.8
6 – 7	16.0	162	26.8	27.6

[a] $K' = [HgL]/([Hg^{2+}][L'])$, $[L'] = [L]_t - [HgL]$;
[b] $K = [HgL^0]/([Hg^{2+}][L^{2-}])$.

Chemical Speciation of Filter-Passing Mercury in Stratified Water Column

The chemical speciation of filter-passing mercury was calculated using the equilibria described in Table 5.4 through 5.7 and the experimentally determined concentrations given in the previous section. The formation constants between mercury and dissolved sulfide shown in Table 5.5 were obtained from Benoit et al. (1999a; 1999b) who reported average literature values of formation constants between mercury and sulfide. The total sulfide concentrations ($[H_2S] + [HS^-] + [S^{2-}]$) measured by the methyleneblue method (Okumura et al., 1999) were used to approximate the concentration of hydrogen sulfide (HS^-) using the first dissociation constant for hydrogen sulfide ($pK_{a1} = 6.8$) taken from Dyrssen (1985) (Table 5.7).

Table 5.5. Formation constants between mercury and dissolved sulfide.

Reaction	Log K_f[a]
$Hg^{2+} + HS^- = HgS^0(aq) + H^+$	26.5
$Hg^{2+} + HS^- = HgS\ (s) + H^+$	36.5
$Hg^{2+} + HS^- = HgSH^+$	30.5
$Hg^{2+} + 2HS^- = Hg(HS)_2^0$	37.5
$Hg^{2+} + 2HS^- = HgS_2H^+ + H^+$	32.0
$Hg^{2+} + 2HS^- = HgS_2^{2-} + 2H^+$	23.5

[a]Benoit et al. 1999a; 1999b.

Table 5.6. Formation constants between copper and dissolved sulfide.

Reaction	Log K_f[a]
$Cu^{2+} + HS^- = CuHS^+$	14.1
$Cu^{2+} + 2HS^- = Cu(HS)_2^0$	21.6

[a]Dyrssen, 1988.

Table 5.7. Acidity constants of hydrogen sulfide and mercury-complexing organic ligand.

	pK_{a1}	pK_{a2}
H_2S[a]	6.88	14.15
H_2L[b]	6.3	10.3

[a]Dyrssen, 1985.
[b]Haitzer et al., 2003.

Solution speciation of mercury was conducted using the chemical equilibrium program MINEQL (Schecher and McAvoy, 1992). The results for the major mercury species are shown in Figure 5.2 and Table 5.8. Mercury saturation indexes were negative for all four depths, hence mercury-sulfide precipitation is not predicted. The modeling predicts that the dissolved mercury complexation is dominated by hydrogen sulfide rather than organic ligands in all depths. The mercury-organic (HgL) complexes are < 0.01 % of the total dissolved mercury, despite high stability constants between mercury and organic ligands.

Table 5.8. Concentrations of dissolved mercury-sulfide and mercury-organic species calculated by MINEQL using data in Table 5.4 - 5.7.

Depth	HgS_2^{2-}	$HgS(HS)^-$	HgS^0	HgL
m	M	M	M	M
0.5	1.4×10^{-14}	5.5×10^{-15}	1.1×10^{-12}	1.7×10^{-22}
3.5	1.2×10^{-14}	9.1×10^{-15}	6.8×10^{-13}	5.1×10^{-23}
4.5	3.1×10^{-14}	4.7×10^{-14}	9.8×10^{-13}	1.5×10^{-20}
6.5	2.2×10^{-13}	8.6×10^{-13}	1.3×10^{-14}	1.3×10^{-25}

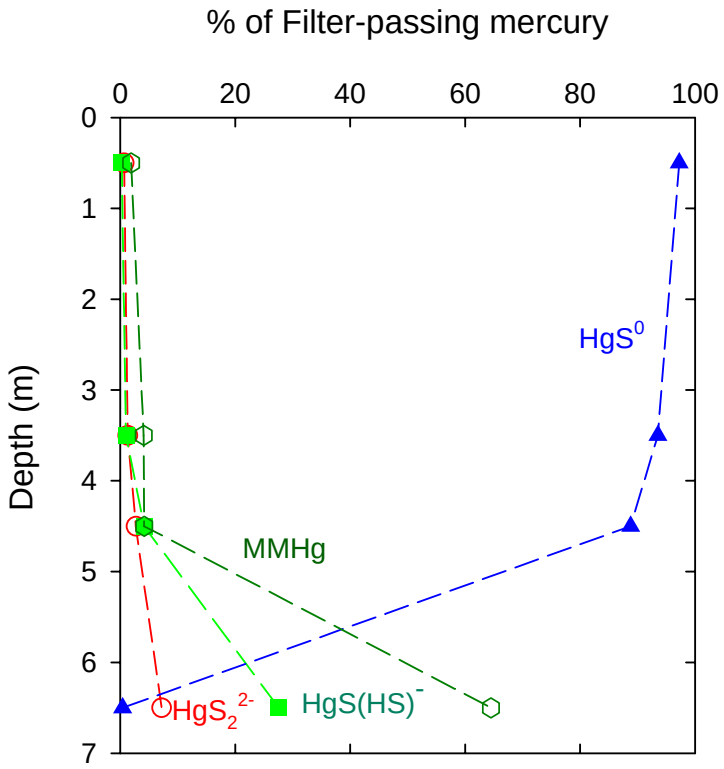


Figure 5.2. The chemical speciation of filter-passing mercury in the stratified water column of Offatts Bayou. Concentrations of each species are in Table 5.2 and 5.8.

The weak mercury-complexing organic ligands, L_2, were not determined here, however, their inclusion would not change the predicted mercury speciation since the $K_{HgL1}\times[L1]$ is similar to the $K_{HgL2}\times[L2]$ (chapter IV). The dominance of sulfide speciation on mercury in sulfidic environments is predicted from the concentrations each ligand and the stability constants of Hg-sulfide and Hg-organic. Since the stability constants between mercury and organics ($10^{27.6 - 28.1}$) are lower than those of mercury-sulfide ($10^{35.0}$ for $Hg^{2+} + HS^- = HgS^0(aq)$ at pH 8.5), and also concentrations of organic ligands (70 – 160 pM) are lower than those of sulfide (20 nM – 200 μM), the concentration of Hg-organic complex were determined to be much lower than Hg-sulfide concentration. The strong complexation of mercury by sulfide may be the reason for the low K_d values of mercury described in previous section.

The substrate for MMHg production in anoxic sediment is thought to be neutral mercury species such as HgS^0 (Benoit et al., 1999a, 1999b, 2001b). Such species are highly membrane permeable as evidenced by high octanol-water partition coefficient (Benoit et al., 1999b). Thermodynamic calculation predicts that the HgS^0 species decrease with increasing sulfide concentrations (Table 5.8), which agrees well with other thermodynamic calculations using cinnabar dissolution models (Benoit et al., 1999a; Jay et al., 2000). In Figure 5.2, the concentration of MMHg was highest at the lowest HgS^0 concentration, suggesting that that inorganic solution speciation of mercury is not only parameter that influences MMHg concentrations in anoxic systems.

The presence of elemental sulfur is known to increase the solubility of cinnabar through the complexation by polysulfide ligands, such as $Hg(S_n)SH^-$, $Hg(S_n)_2^{2-}$,

$Hg(S_n)OH^-$, and HgS_5 (Paquette and Helz, 1997; Jay et al., 2000). If elemental sulfur is present, then equilibrium calculations suggest that HgS^0 concentrations would decrease in bottom water in favor of the dissolved mercury species such as, $Hg(S_n)OH$, $Hg(S_n)_2^{2-}$. Similarly, in the upper layers, HgS^0 would decrease to < 1 % of total dissolved mercury by the formation of HgS_nOH^-. The existence of elemental sulfur can be related to the low concentrations of MMHg at the mild reducing conditions (4.5 m depth) though higher resolution sampling and analysis are required to test this possibility.

Summary

In this chapter, dissolved mercury speciation in the stratified water column of Offatts Bayou was investigated using a combination of chemical equilibrium modeling and experimentally determined thermodynamic data. Thermodynamic equilibrium modeling suggests that solution speciation of dissolved mercury in Offatts Bayou is dominated by inorganic sulfide-mercury species rather than organic-mercury species. The ratio of mercury-sulfide to mercury-organic was > 10^7. The modeling result suggests that different sorption/desorption from the surface estuarine water controls cycling of mercury in stratified water column.

The filtered mercury concentrations were similar in the upper layers and increased at the bottom water primarily due to an increased MMHg. Particulate mercury and particulate MMHg concentrations showed the lowest values at the oxic/anoxic interface, suggesting that dissolution of manganese and iron oxyhydroxide mobilizes mercury from

particulate into solution phase. Increased concentrations of particulate mercury and particulate MMHg in the bottom layer may be related to the scavenging of mercury-sulfide complexes by FeS. Overall, mercury cycling in stratified water column is related to the Fe and Mn redox cycle, particulate scavenging and sinking, and MMHg production.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Summary

Mercury Speciation Studies

Studies of organic complexation of mercury in natural water are very limited compared to those of other trace metals. Standard voltammetric methods using a mercury electrode can not be used for mercury speciation and low concentrations of mercury in natural water inhibit the simple application of the voltammetric method. Despite these complications, various detection methods to quantify mercury-complexing organic ligands and to determine conditional stability constants of mercury-organic complexes have been reported recently (Wu et al., 1997; Skyllberg et al., 2000; Benoit et al., 2001a; Drexel et al., 2002; Haitzer et al., 2002, 2003; Hsu and Sedlak 2003; Lamborg et al., 2003). What is most encouraging is that the methods employed are quite diverse and the results of obtained show good agreement for conditional stability constants.

Analytical Research Accomplishments

Among the most significant accomplishments of this research was the successful development of a new method to measure concentrations and stability constants of mercury-complexing organic ligands in natural estuarine water and fresh water.

Significant analytical details of the method include: (1) no extraction step is necessary to isolate dissolved organic matter from the sample matrix; (2) the method has the advantage of being easily applied across a salinity gradient; (3) the detection limit for determining natural organic ligand concentrations can be lowered to pM levels, which is being reported for the first time. This latter feature is a result of the combined effect of the low detection limit of cold vapor atomic fluorescence spectroscopy and use of a strong competing ligand, thiosalicylic acid.

Estuarine Mercury Cycling

Complexation of mercury by dissolved organic ligands influences its transport and distribution in estuarine water through a competition with other processes such as particle scavenging. Release of mercury from particulate matter during remineralization is enhanced by organic complexation through increased solubility. There have been several pieces of indirect evidence which suggest that dissolved organic matter is an important factor controlling mercury distributions in surface estuarine waters. While there have been reports which provide information on mercury-organic (HgL) complexation in estuarine waters, these studies are based on operationally defined analytical approaches and hence are not rigorously defined thermodynamically. As demonstrated by this research, thermodynamic calculations using measured stability constants and ligand concentrations can be utilized to determine [HgL] in estuarine waters.

Over 95 % of the filter-passing mercury in Galveston Bay exists as an organic

complex based on chemical speciation modeling. This result supports the hypothesis that dissolution from particulate mercury and removal by sinking particles through colloidal coagulation/particle adsorption are the major production and removal processes of filter-passing mercury in surface waters of Galveston Bay. This observation is also evidenced by the estuarine distribution of other factors such as SPM, dissolved and colloidal carbon, and particulate, dissolved, and colloidal mercury.

Thermodynamic Modeling of Mercury Speciation

To date, a number of publications have treated the interaction between mercury and natural organic ligands as unique complexes with unique conditional stability constants. However, my research suggests that mercury-binding ligands in Galveston Bay estuary should be characterized as a series of binding sites on natural dissolved organic matter. Hence, stability constants should be reported as a relative value of [L] or [Hg]/[DOM]. Recently, several reports (Skyllberg et al., 2000; Haitzer et al., 2002) pointed out that as the ratio of [Hg]/[DOM] changes, so does the strength of the binding between mercury and DOM. These reports showed that higher stability constants are determined at lower [Hg]/[DOM] ratios. This finding agrees with the results of this current dissertation research, that higher stability constants of HgL are observed at lower ligand concentrations.

The chemical speciation of dissolved mercury using thermodynamic calculations should be understood in relation to a continuous binding site model. In Chapter IV, competitive ligand equilibration-solvent solvent extraction (CLE-SSE) using thiosalicylic

acid competition determined the concentrations of mercury-complexing organic ligands (L_S), which were one to two orders of magnitude higher in concentration than typical natural mercury concentrations. The continuous binding site model argues that the dominant mercury-complexing organic ligand would exist at concentration level equivalent to that of natural mercury concentration. This treatment further suggests that mercury-binding organic ligands of higher K and lower [L] values than those found in this research using thiosalicylic acid may exist in estuarine waters.

Mercury Speciation in the Presence of Sulfide

The results of thermodynamic equilibrium modeling for the anoxic water column of Offatts Bayou suggest that the sulfide complexation of mercury can be important for surface oxic water. Tang and Santschi (2000) reported sulfide concentrations of Galveston Bay surface water of 4.3 ± 0.6 nM, which is only a factor of 8 lower than that observed in Offatts Bayou surface water. The calculated ratio of $[HgS^0]/[HgL]$ in the surface water of Offatts Bayou was $\sim 10^{10}$, which suggests a predominance of HgS^0 species over organically complexed mercury species. This finding implies a contradiction with the results of Chapter IV that the organic complex is the major form of dissolved mercury in fully aerobic surface water. One possible caveat is that the organic complexation coefficients ($K \times [L]$) determined underestimate the true binding that exists between mercury and dissolved organic matter. As described in the previous section, the complexation of mercury in Galveston Bay estuary should be modeled using a continuous binding site model. By extrapolating the data in Figure 4.14 to a ligand

concentration equivalent to typical concentrations of mercury in Galveston Bay estuary (~1 pM), the predicted log stability constant of HgL would be 31.7 for an average ionic strength of 0.5 and 33.0 for an ionic strength of 0 (Figure 6.1). Hence the continuous binding site model predicts a complexation coefficient (K×[L]) of $10^{21.0}$ (= $10^{33.0} \times 10^{-12}$).

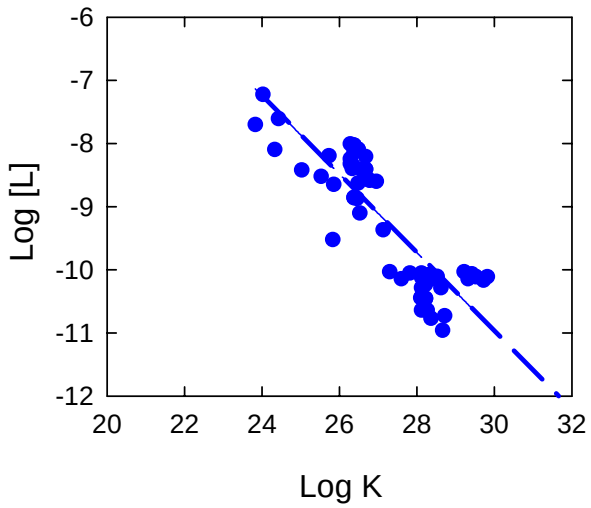


Figure 6.1. Extrapolation of linear relationship between the concentration of mercury-complexing organic ligand and the stability constant of HgL ($K = [HgL^0]/[Hg^{2+}]/[L^2]$) modified from Figure 4.14.

The impact of this organic complexation case in the presence of dissolved sulfide is illustrated in Table 6.1. Table 6.1 shows the competition for mercury between dissolved sulfide and mercury-binding organic ligands in surface water of Galveston Bay determined by chemical equilibrium modeling for a range of sulfide levels. The values of [L] and K_{HgL} used in Table 6.1 were obtained from figure 6.1 based on the assumption that organic ligands in natural mercury concentrations dominate the complexation of

dissolved mercury. The thermodynamic formation constant for HgS^0, also used in Chapter V, were obtained from Benoit et al. (1999b) who reported average literature values for the reaction: $Hg^{2+} + SH^- = HgS^0 + H^+$. It is important to include copper in this modeling since it is a dominant sulfide species ($CuHS^+$) in marine systems (Al-Farawati and van den Berg, 1999). The results in Table 6.1 demonstrate that sulfide, in the range of pM to nM, competes with mercury-complexing organic ligands, even in the presence of 10 nM of Cu which will bind most of the HS^- in that concentration range.

Table 6.1. Comparison between organic and sulfide speciation of dissolved mercury in surface waters of Galveston Bay based on thermodynamic equilibrium modeling.

Log K_{HgS}[a]	$[SH]_t$ (pM)	Log K_{HgL}[b]	$[L]_t$ (pM)	HgL^c (%)	HgS^c(%)
26.5	1	33.0	1	88	12
26.5	10	33.0	1	66	34
26.5	100	33.0	1	29	71
26.5	1000	33.0	1	5	95

[a]K_{HgS} for $Hg^{2+} + SH^- = HgS + H^+$ (Benoit et al., 1999b);
[b]K_{HgL} for $Hg^{2+} + L^{2-} = HgL^0$ determined from Figure 6.1 and corrected to zero ionic strength by Davies equation;
[c]Equilibrium conditions: pH = 8.2. [Cl] = 0.4 M, $[Hg]_t$ = 1 pM, $[Cu]_t$ = 10 nM, and log K_{CuSH} = 14.1 (Dyrssen, 1988).

There are two major caveats that must be recognized with assessing this modeling exercise. First, the reported values for the formation constant of HgS^0 vary over several orders of magnitude. Second, the formation constant of HgL in Figure 6.1 also varies over ~3 log units as a function of estuarine salinity. Generally, the lower salinity estuarine water shows higher stability constants than higher salinity estuarine

water. Hence, the results in Table 6.1 should be viewed somewhat as an approximation rather than an absolute. For example, if surface water has 1 nM [SH^-] and 1 pM $[Hg]_t$, the major mercury species is predicted to be HgS^0 (92 % of the dissolved mercury). However, if K_{HgS} is two orders of magnitude lower than the value used to generate concentrations in Table 6.1, then the major species of dissolved mercury will be HgL (75% of the dissolved mercury).

Clearly, dissolved sulfide can be an important ligand, competing with natural organic ligands to complex mercury in surface water. The dissolved mercury speciation in Galveston Bay surface water and Offatts Bayou surface water varied significantly depending on relatively small changes in the stability constants used for HgS^0 and HgL. Therefore, the determination of accurate stability constants of HgS^0 and HgL are required to estimate the relative importance between sulfide and organic ligands for the complexation of dissolved mercury in surface estuarine water.

Conclusions

Hypothesis

The main hypothesis that this study addressed was to test whether the solution speciation of Hg(II) in surface estuarine water was dominated by complexation with an organic ligand of low concentration (~ pM) and high conditional stability constant ($K_{cond} > 10^{20}$). CLE-SSE revealed that indeed there are organic ligands in Galvesyon Bay in low concentration (~pM) and high conditional stability constants ($K_{cond} > 10^{23}$). However, the

organic mercury complexation is not by a unique ligand but a series of organic binding sites on natural dissolved organic matter. Since the complexation coefficient (K × [L]) is higher at lower concentration of L, the most dominant binding sites will be those with concentrations equivalent to the natural mercury concentration (few pM). In this concentration range, the stability constants ($[HgL^0]/([Hg^{2+}][L^{2-}])$) between mercury and the binding sites on natural organic matter will range from 10^{30} to 10^{33} (Figure 6.1). This range of conditional stability constants agrees with that observed by Skyllberg et al. (2000) who reported surface complex formation constants between mercury and soil organic matter.

Future Research Efforts

The CLE-SSE method for determining the organic complexation of mercury developed in this study has a number of potential applications for future research on mercury. First, monomethylmercury production and the bioavailability of mercury is known to be related to the chemical speciation of dissolved mercury. Studies on mercury-organic complexation using CLE-SSE can be used as a tool to help elucidate the relationship between the methylation of mercury and the solution complexation of mercury. The relationship between the bioavailability of mercury and mercury-organic complexation can also be studied using the CLE-SSE method.

Second, the chemical speciation of dissolved mercury is an essential part of our understanding of the biogeochemical behavior of mercury, particularly the reduction of Hg(II) into Hg(0). The role of DOM on the direct and photochemical reduction of

mercury is important but poorly understood. Further chemical speciation studies need to be carried out to understand the role of DOM in mercury reduction.

Third, CLE-SSE can contribute to the investigation of the chemical speciation of dissolved mercury in open ocean water. In Galveston Bay estuary, mercury complexing organic ligand concentrations decreased during estuarine mixing, allowing the inorganic mercury species to become more significant. If ligand concentrations continue to decrease into the open ocean, then mercury speciation in surface open ocean water would show the competition between organic and inorganic complexation. This trend agrees with the results of an instrumental speciation study using $SnCl_2$ reduction (Cossa and Noel, 1987; Laurier et al., 2003).

Fourth, the CLE-SSE method can be used to understand the role of biota in the organic complexation of mercury in natural waters. The relationship between phytoplankton abundance and mercury-complexing organic ligands, and the relative importance between biogenic organic matter and refractory organic matter for mercury complexation can be studied in order to clearly understand the importance of biogenic organic matter in mercury biogeochemistry.

Final Remarks

The entire research process from the development of CLE-SSE method to the analyses of Galveston Bay samples was a challenging procedure, which requires complicated calculation processes and careful applications of recent techniques. Most of the research period was dedicated to develop the novel method of CLE-SSE and to

confirm the accuracy of the method. The CLE-SSE developed in this study can be a useful tool for the further study of mercury as discussed above. In addition, the result of this study highlights the potential importance of sulfide for the mercury complexation in natural waters.

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Han S. and Gill G. A. Determination of mercury complexation by competitive ligand equilibration using chloride, s*ubmitted to Environ. Sci. Technol.*

Han S., Gill G. A., Lehman R. D., and Choe K. -Y. Complexation of mercury by organic ligands in Galveston Bay estuary, Texas, *submitted to Mar. Chem.*

Han S., Gill G. A., Choe K. -Y, and Lehman R. D. Speciation of mercury in Offatts Bayou – a seasonally anoxic bayou on Galveston Bay, *submitted to Limnol. Oceanogr.*

Choe K., Gill G. A., Lehman R. D., Han S., Heim W. A., and Coale K. H. (2004) Sediment-water exchange of total mercury and monomethyl mercury in the San Francisco Bay-Delta. *Limnol. Oceanogr.* 49(5), 1512-1527.