Environ. Sci. Technol. **2002**, *36,* 3564−3570

Binding of Mercury(II) to Dissolved Organic Matter: The Role of the Mercury-to-DOM Concentration Ratio

MARKUS HAITZER,*,†,‡
GEORGE R. AIKEN,† AND
JOSEPH N. RYAN‡

U.S. Geological Survey, Water Resources Division, 3215 Marine Street, Boulder, Colorado 80303, and Department of Civil, Environmental, and Architectural Engineering, University of Colorado, Boulder, Colorado 80309

The binding of Hg(II) to dissolved organic matter (DOM; hydrophobic acids isolated from the Florida Everglades by XAD-8 resin) was measured at a wide range of Hg-to-DOM concentration ratios using an equilibrium dialysis ligand exchange method. Conditional distribution coefficients (K_{DOM}') determined by this method were strongly affected by the Hg/DOM concentration ratio. At Hg/DOM ratios below approximately 1 μg of Hg/mg of DOM, we observed very strong interactions ($K_{DOM}' = 10^{23.2\pm1.0}$ L kg^{-1} at pH = 7.0 and I = 0.1), indicative of mercury−thiol bonds. Hg/DOM ratios above approximately 10 μg of Hg/mg of DOM, as used in most studies that have determined Hg−DOM binding constants, gave much lower K_{DOM}' values ($10^{10.7\pm1.0}$ L kg^{-1} at pH = 4.9−5.6 and I = 0.1), consistent with Hg binding mainly to oxygen functional groups. These results suggest that the binding of Hg to DOM under natural conditions (very low Hg/DOM ratios) is controlled by a small fraction of DOM molecules containing a reactive thiol functional group. Therefore, Hg/DOM distribution coefficients used for modeling the biogeochemical behavior of Hg in natural systems need to be determined at low Hg/DOM ratios.

Introduction

The binding of mercury(II) (denoted as Hg throughout the text) to dissolved organic matter (DOM) strongly affects mobility and bioavailability of Hg in aquatic ecosystems (*1*−*6*). While this fact is generally accepted, understanding the effect of DOM on aquatic Hg cycling on a quantitative basis has been limited due to a paucity of reliable Hg/DOM binding constants (*7, 8*). Literature values span many orders of magnitude and some of the published conditional binding constants lack an exact definition in the form of a chemical reaction equation together with a definition of the concentration basis for DOM used for the calculations (*9*−*14*).

Recent spectroscopic studies have found direct evidence of Hg binding to reduced organic sulfur (S_{red}) groups in organic matter (*15, 16*). Because interactions between Hg and compounds containing S_{red} can be very strong (*17*), and because the concentration of DOM-bound S_{red} ($DOMS_{red}$) in natural systems is generally much higher than the concentration of Hg (*15, 18*), strong interactions between Hg and $DOMS_{red}$ are expected under natural conditions. Most laboratory studies determining Hg/DOM binding constants used Hg concentrations in excess of S_{red} concentrations in DOM (e.g., refs *10* and *14*). Under these conditions, it is expected that a small fraction of the total Hg saturates all $DOMS_{red}$, while the majority of Hg binds to oxygen functional groups that are present in DOM at much higher concentrations than S_{red}. Because constants for Hg binding to oxygen functional groups are many orders of magnitude lower than constants for Hg−S_{red} interactions (*19*), the overall binding constants for Hg/DOM binding at high ratios of Hg to $DOMS_{red}$ are expected to be much smaller than at low ratios. This "metal concentration effect" has been described for the binding of other metals to DOM (*20, 21*), but according to the hard and soft acids and bases theory (*22, 23*), the effect is expected to be especially strong for Hg due to its pronounced "soft metal" character and the low concentration of "soft" S_{red} groups compared to the high concentration of "hard" oxygen functional groups in DOM.

We therefore hypothesized that the ratio of Hg to DOM strongly affects binding constants and that binding constants at low Hg-to-DOM ratios are in the range of binding constants for Hg−S_{red} interactions (i.e., much higher than literature values). Similar hypotheses have been discussed by Skyllberg et al. (*24*) and Hesterberg et al. (*16*) for the binding of Hg to soil organic matter, but to our knowledge the effect of the Hg-to-organic matter ratio has not been systematically investigated by comparing conditional binding constants measured at different Hg-to-organic matter ratios. To test the hypotheses stated here, we developed a method suitable for measuring conditional Hg−DOM binding constants over a wide range of Hg-to-DOM ratios, including low ratios.

Experimental Section

DOM Isolation and Characterization. This study used the hydrophobic acids fraction (humic and fulvic acids) of DOM isolated from water at the F1 site in the Florida Everglades in July 1997 (sample ID: F1HPoA; for details see ref *3*). In brief, surface water was collected, filtered through 0.3 μm glass fiber filters, acidified to pH 2 using HCl, and passed through XAD-8 resin. The hydrophobic acids fraction was retained on the XAD-8 resin and then back-eluted with 0.1 M NaOH. The eluate was hydrogen saturated, desalted, lyophilized, and stored for later use. For the experiments, 100 mg L^{-1} stock solutions were freshly prepared by dissolving a weighed amount of freeze-dried material in deionized water. The solution was filtered through a 0.45 μm PVDF membrane filter (Millex-HV, Millipore, Bedford, MA) to exclude any particles.

The number-average molecular weight of F1HPoA was determined by high-pressure size exclusion chromatography using a Protein-Pak 125 modified silica gel column (Waters, Milford, MA) and polystyrene sulfonate standards according to the method of Chin et al. (*25*). Elemental composition of the sample was determined by Huffman Laboratories (Golden, CO) after the method described by Huffman and Stuber (*26*). The relative content of reduced sulfur was measured by sulfur K-edge X-ray absorption near edge structure (XANES) spectroscopy (*27*). Carboxyl and phenol groups were quantified by titrating a hydrogen-saturated sample with base. Selected characteristics of F1HPoA are given in Table 1.

Reagents. Hg stocks used for experiments at low Hg concentrations were prepared by dissolving $HgCl_2$ (99.9995%, Alfa Aesar, Ward Hill, MA) in 10% (v/v) HNO_3. Stocks for

* Corresponding author phone: 303 541 3009; fax: 303 447 2505; e-mail: mhaitzer@usgs.gov; address: U.S. Geological Survey, 3215 Marine Street, Boulder, CO 80303.
† U.S. Geological Survey.
‡ University of Colorado.

10.1021/es025699i CCC: $22.00
Published on Web 07/17/2002

TABLE 1. Selected Characteristics of the F1HPoA Isolate (after (40))

number-average molecular wt (Da)	C[a] (%)	total S[a] (%)	reduced S[b] (% of total S)	carboxyl content[a] (meq/g)
1030	52.2	1.73	60.0	5.45

[a] Analyzed on dried samples and reported here on an ash-free basis. [b] The relative content of reduced sulfur was measured by sulfur K-edge X-ray absorption near edge structure (XANES) spectroscopy (*27*).

experiments at high Hg concentrations were prepared from $Hg(NO_3)_2$ (99.99+%, Aldrich, Milwaukee, MI) to avoid chloride interference. Stock solutions of buffers, $NaClO_4$ (inert background electrolyte used to adjust ionic strength), and EDTA were filtered through 0.45 μm membrane filters to exclude the presence of any particles. The pH of EDTA solutions was adjusted to 7.0 using NaOH.

Hg Analysis. Hg was analyzed using a Millennium Merlin mercury analyzer (PSA Analytical, Kent, U.K.). Samples were oxidized overnight by a mixture of KBr, $KBrO_3$, and HCl to break down all organic Hg complexes. After oxidation, the samples were prereduced with $NH_2OH{\cdot}HCl$ to destroy excess bromine, and then Hg was reduced to volatile Hg(0) with $SnCl_2$. Hg(0) was separated from solution by purging with high purity argon gas, dried in a semipermeable dryer tube, and detected by cold vapor atomic fluorescence spectrometry (CVAFS). Duplicates and standards prepared from a certified reference material (NIST-3133) were analyzed frequently to assess precision and accuracy of the method. Analysis results for a batch of samples were regarded acceptable if results for standards showed 80−120% recovery and duplicates were within 20% relative difference. Generally, standard recovery was 90−110%, and duplicates were within 10% relative difference.

DOM Analysis. Concentrations of dissolved organic carbon (DOC) were measured using an OI 700 (OI Analytical, College Station, TX) total organic carbon analyzer. DOC-based concentrations were converted to DOM-based concentrations using the organic carbon content of F1HPoA (Table 1).

Measurement of Hg−DOM Binding. Conditional distribution coefficients for Hg−DOM interactions were measured using an equilibrium dialysis ligand exchange (EDLE) method that was developed to determine distribution coefficients for radionuclide−DOM interactions at nanomolar metal concentrations (*28*) and later modified to take into account some leakage of DOM across the dialysis membranes (*29*−*31*). This technique was adapted to measure the equilibrium distribution of Hg between DOM retained inside a dialysis bag and an outside solution containing no DOM. To avoid Hg depletion in the outside compartment due to strong binding to DOM in the inside compartment and sorption to the container walls, Hg concentrations on both sides of the dialysis membrane were buffered by an auxiliary ligand whose Hg complexes can pass through the membrane. Stability constants for the interactions of the auxiliary ligand with Hg^{2+} are known, so conditional distribution coefficients (K_{DOM}') for the reaction of Hg^{2+} with DOM

$$\mathrm{Hg} + \mathrm{DOM} = \mathrm{HgDOM} \tag{1}$$

(charges omitted, 1:1 stoichiometry assumed) could be calculated from the distribution of Hg between inside and outside of the dialysis bag. K_{DOM}' is defined by

$$K_{DOM}' = \frac{[\mathrm{HgDOM}]}{[\mathrm{Hg}](\mathrm{DOM})} \tag{2}$$

where square brackets denote concentrations of HgDOM and Hg in molar (M). The equilibrium concentration of DOM is denoted by round brackets and is equivalent to the measured concentration of DOM (in kg L^{-1}) under the condition that the concentration of Hg is much smaller than the concentration of DOM. K_{DOM}' is a mass-related conditional distribution coefficient (units of L kg^{-1}) that is free from any additional assumptions (e.g., about the molar concentration of DOM or the molar concentration of Hg binding sites). K_{DOM}' was calculated from experimental data, taking into account some leakage of DOM from the inside to the outside compartment, following the equation (*29*)

$$K_{DOM}' = \frac{Q\alpha_{Hg}}{(\mathrm{DOM})_{in} - (\mathrm{DOM})_{out}(Q+1)} \tag{3}$$

where $(DOM)_{in}$ and $(DOM)_{out}$ are the concentrations (kg L^{-1}) of DOM in the inner and outer analysis compartment (DOM_{in} was always more than 10 times greater than DOM_{out}). Q is a measure for the concentration of Hg in the inner and the outer compartment ($[Hg]_{in}$ and $[Hg]_{out}$) and is defined by

$$Q = \frac{[\mathrm{Hg}]_{in} - [\mathrm{Hg}]_{out}}{[\mathrm{Hg}]_{out}} \tag{4}$$

α_{Hg} is a measure of complexation of Hg by the auxiliary ligand L and OH^-. Under the condition of $[Hg]_{total} \ll [L]_{total}$, α_{Hg} can be written as

$$\alpha_{Hg} = 1 + \sum_{i=1}^{n}\beta_i^{L}[L]^i + \sum_{k=1}^{o}\beta_k^{OH}[H]^{-k} = 1 + \sum_{i=1}^{n}\beta_i^{L}\left(\frac{[L]_{total}}{1 + \sum_{j=1}^{m}[H]^j\beta_j^{H}}\right) + \sum_{k=1}^{o}\beta_k^{OH}[H]^{-k} \tag{5}$$

where β_i^{L} and β_j^{H} are the overall stability constants for the stepwise binding of L to Hg and H (protons) to L, respectively. β_k^{OH} is the overall hydrolysis constant referring to the formation of $Hg(OH)_k$ from $Hg + kH_2O$. A detailed derivation of the calculation of K_{DOM}' is given in Glaus et al. (*29*).

The EDLE method requires an auxiliary ligand suitable to compete with DOM so that meaningful Hg distribution ratios (Q = [HgDOM]/[HgL]; see eq 4) are reached. In this study, the acceptable range for Q was set to >0.1 (reliably measurable Hg binding to DOM) and <4.0 (approximate diffusion limit of the system after 48 h; see initial slope of curves in Figure 1, parts A and B). Additionally, the precautions must be followed: (1) the ligand should not interfere with Hg analysis; (2) the possibility of mixed-complex formation (DOM−Hg−L) (see Glaus et al. (*30*)) should be avoided; (3) the ligand should be usable at pH values relevant for most freshwaters (pH 6−8); and (4) reliable binding constants for Hg−L interactions should be available from the literature. Table 2 summarizes the suitability of different ligands for EDLE experiments with Hg and shows that EDTA is the only ligand fulfilling all preconditions for experiments at low Hg/DOM ratios. The disadvantage of EDTA is that it is only strong enough to effectively compete for Hg with low concentrations of DOM (about 1 mg L^{-1} for the F1HPoA isolate used in this study). For EDLE experiments at high Hg/DOM ratios, hydroxide was found to be a suitable ligand. Relevant reactions of these ligands and binding constants for these reactions are summarized in Table 3.

Method Development. To adapt the EDLE technique for Hg as the target analyte, extensive method development was necessary. Mainly, a suitable type of dialysis membrane and auxiliary ligand had to be found. Regenerated cellulose membranes (Spectra/Por SP 6 and SP 7, molecular weight

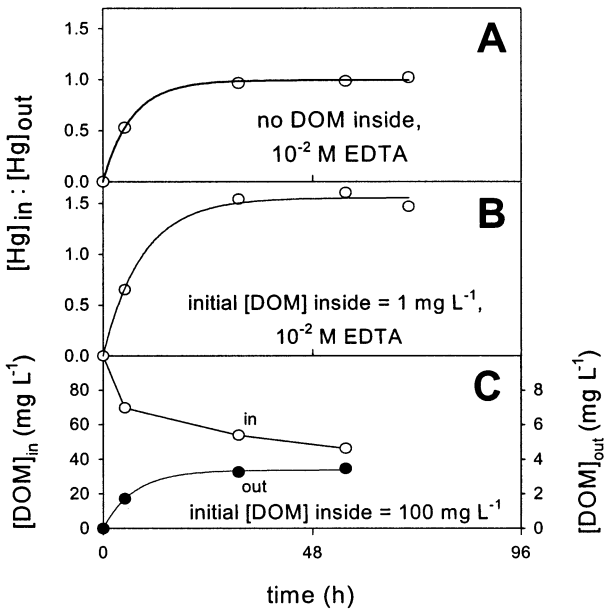

FIGURE 1. Partitioning of Hg (1.0 μg L^{-1}) spiked in the outside compartment in the absence (A) and presence (B) of DOM in the inside compartment. (C) Partitioning of DOM spiked in the inside compartment. Conditions for all experiments: RCBT 3500 membrane, pH 7.0, ionic strength 0.1 M.

TABLE 2. Suitability of Different Auxiliary Ligands for EDLE Experiments[a]

	low Hg: DOM				high Hg/DOM
	bromide	iodide	cysteine	EDTA	hydroxide
strong Hg binding	+	++	++	0+	−
Hg recovery	0	−	+	+	+
no DOM−Hg−L	?	?	?	+	+[b]
suitable for pH 6−8	−[c]	0	+	+	+
reliable log K (Hg−L)[d]	0	0	−	++	+

[a] (+) = good, (0) = fair, (−) = bad, (?) = not known. [b] Chelate formation with DOM is regarded more likely than formation of DOM−Hg−OH. [c] $HgBr_n$ is only competitive with HgDOM at low pH, where H^+ competition decreases Hg−DOM binding but does not affect Hg−Br binding. [d] Based on number of literature values, agreement between data, and IUPAC recommendation.

TABLE 3. Summary of Binding Constants Used for the Calculation of K_{DOM}'

reaction	log β[a]	ionic strength, temperature[b]	ref
$Hg^{2+} + EDTA^{4-} = HgEDTA^{2-}$	21.8[c]	0.1 M, 20 °C	(*41*)
$HgEDTA^{2-} + H^+ = HgEDTAH^-$	3.1[c]	0.1 M, 20 °C	(*41*)
$EDTA^{4-} + H^+ = EDTAH^{3-}$	10.2[d]	0.1 M, 20 °C	(*41*)
$EDTA^{4-} + 2H^+ = EDTAH_2^{2-}$	16.4[d]	0.1 M, 20 °C	(*41*)
$EDTA^{4-} + 3H^+ = EDTAH_3^-$	19.1[d]	0.1 M, 20 °C	(*41*)
$EDTA^{4-} + 4H^+ = EDTAH_4$	21.1[d]	0.1 M, 20 °C	(*41*)
$Hg^{2+} + OH^- = HgOH^+$	10.2	0.1 M, 25 °C	(*42*)
$Hg^{2+} + 2OH^- = Hg(OH)_2$	21.2	0.1 M, 25 °C	(*42*)

[a] Overall binding constants. [b] Corrections for the experimental temperature of 22 °C using the van't Hoff equation are not significant. [c] IUPAC tentative recommendation (*19*). [d] IUPAC recommendation (*19*).

cutoff (MWCO) 1000 Da, Spectrum Laboratories, Rancho Dominguez, CA), as used by Glaus et al. (*31*), strongly sorbed Hg because of sulfide residues left in the membrane from the manufacturing process. As an alternative, cellulose ester membranes without sulfide residues (Spectra/Por CE Biotech, MWCO 1000 Da) were tested. However, despite the nominal MWCO of 1000 Da, diffusion of Hg in the presence of the auxiliary ligand of choice (EDTA, molecular weight 292 Da) was much too slow. Experiments checking the membrane diffusion of EDTA and other small organic acids with different amounts of negative charge at pH 7 ($acetate^-$, $oxalate^{2-}$, $citrate^{3-}$) revealed that the diffusion of these small molecules across CE membranes decreased with increasing negative charge, explaining the very slow diffusion of EDTA (mainly $EDTAH^{3-}$ at pH 7) and $EDTAHg^{2-}$. Finally, regenerated cellulose "Biotech" membranes (Spectra/Por RCBT, MWCO 3500 Da) were tested. These membranes are free from sulfides and allowed for fast diffusion of Hg−EDTA complexes across the membrane (Figure 1, parts A and B). However, the lowest available molecular weight cutoff is 3500 Da, which raised concern about the ability of these membranes to sufficiently retain DOM. Figure 1 shows that the Hg partitioning equilibrium in an EDLE experiment reached a plateau (Figure 1B) well before the concentration of DOM in the outside compartment reached 10 % of the inside concentration (Figure 1C). A comparison of Figure 1, parts A and B, also indicates that the presence of DOM in the inside compartment did not slow the establishment of diffusion equilibrium. This demonstrates that the rate-limiting step in the system was the diffusion across the dialysis membrane and not Hg−DOM complex formation. As shown by Glaus et al. (*29*), the EDLE method generally allows for reliable measurements of K_{DOM}' under such conditions. Therefore, RCBT 3500 membranes were chosen for the experiments, although these membranes are very fragile and break more easily than RC or CE membranes.

Experimental Setup. The experimental system for measuring binding constants consisted of duplicate or triplicate sets of acid cleaned 125 mL borosilicate glass bottles with Teflon-lined caps containing 93 mL of outside solution and 7 mL of inside solution. To separate the inside solutions, RCBT 3500 dialysis bags (Spectra/Por regenerated cellulose "Biotech" membranes, MWCO 3500 Da), sealed by tying knots on both sides, were used. The pH value in both compartments was buffered using 5 mM phosphate buffer (pH 7), 5 mM acetate buffer (pH 5), or a 10 mM mixture of both buffers (pH 6), and ionic strength was set at 0.1 M with $NaClO_4$, taking into account ionic strength effects of EDTA and the buffer. Inside solutions additionally contained 1.0 mg L^{-1} of DOM (F1HPoA isolate) at the start of the experiments. Because of leakage of DOM through the membrane, inside DOM concentrations at the end of the experiments were approximately 0.5 mg L^{-1}. Parameters that were varied (by order of magnitude steps) were total concentration of Hg (0.01−1000 μg L^{-1}) and concentration of the ligand (EDTA: 10^{-5}−10^{-1} M; hydroxide: 10^{-9}−10^{-7} M). At the start of the experiments, 10 or 100 μL of a Hg stock solution (0.01−1000 mg L^{-1} in 10% HNO_3) was added to the outside compartment, and then samples were rotated end-over-end at 22 ± 2 °C. After 48 h, aliquots were taken from the inside and outside compartments and analyzed for Hg. The pH value was measured in the outside solution. Because DOM concentrations in the actual samples were too low to be measured reliably and because EDTA interferes with dissolved organic carbon (DOC) analysis, DOM concentrations were extrapolated from parallel partitioning experiments using an initial inside concentration of 100 mg L^{-1} DOM (see Figure 1C). This approach gave final DOM concentrations (after 48 h) for the EDLE experiments of approximately 0.5 mg L^{-1} (inside compartment; 7 mL) and 0.04 mg L^{-1} (outside compartment; 93 mL).

Results and Discussion

To better understand the partitioning of Hg in the experimental system, we first consider a hypothetical EDLE experiment that uses an "idealized" form of DOM having a single type of Hg-binding site at a concentration in excess

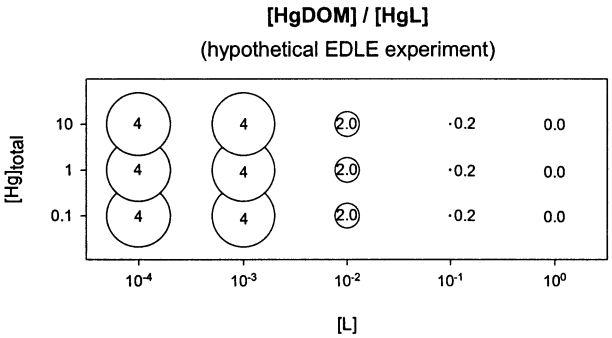

FIGURE 2. Distribution ratios between HgDOM and HgL in a hypothetical EDLE experiment, where a single type of Hg-binding site is assumed for DOM, the concentration of Hg is smaller than the concentration of the Hg binding site, and the auxiliary ligand L forms 1:1 complexes with Hg. Bubble size and numbers represent Hg distribution ratios (Q = [HgDOM]/[HgL]).

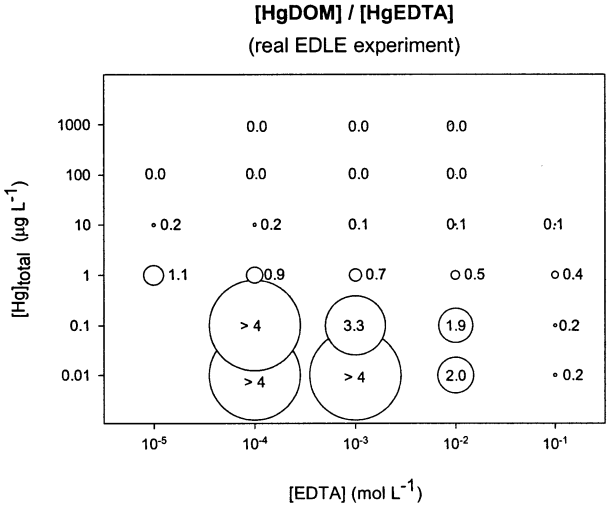

FIGURE 3. Distribution ratios between HgDOM and HgEDTA in a real EDLE experiment using order of magnitude concentration steps for [EDTA] and total Hg. Bubble size and numbers represent Hg distribution ratios (Q = [HgDOM]/[HgEDTA]). Nearly "ideal" behavior (see Figure 2) is only observed at $[Hg]_{total} \leq 0.1\ \mu g\ L^{-1}$. At $[Hg]_{total} \geq 1\ \mu g\ L^{-1}$, the HgDOM/HgEDTA distribution ratios are lower than expected under "ideal" conditions, suggesting a limitation of the amount of Hg that can be bound by DOM under these experimental conditions.

of [Hg], and an auxiliary ligand that forms 1:1 complexes with Hg (Figure 2). In such a system, almost all Hg is bound to L at very high concentrations of L so that the distribution ratio Q between Hg bound to DOM and Hg bound to the auxiliary ligand L (Q = [HgDOM]/[HgL]) is indistinguishable from zero (i.e., [HgL] ≫ [HgDOM]). Within a certain range of [L], Q is inversely proportional to the concentration of the ligand (i.e., order of magnitude decreases in [L] cause order of magnitude increases in Q). At low [L], the auxiliary ligand is much less effective in binding Hg than DOM, resulting in distribution ratios limited to a constant value because the amount of Hg diffusing from the outside to the inside compartment during the experimental time is limited. The total amount of Hg added to the system does not affect the distribution ratio Q. This is explained by the fact that, over the entire range of Hg concentrations, Hg is competitively bound to both the auxiliary ligand L and the Hg binding sites on the DOM (assuming that [L] and [sites] are always > [Hg]).

The result of a real EDLE experiment using EDTA as the auxiliary ligand is shown in Figure 3. A nearly "ideal" behavior of the system, as outlined in Figure 2, was only observed for total Hg concentrations $\leq 0.1\ \mu g\ L^{-1}$. At a Hg concentration

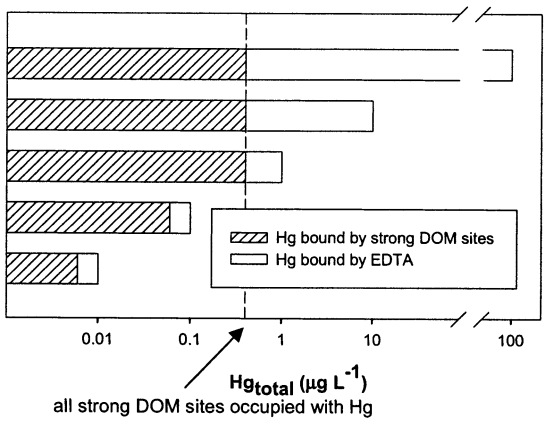

FIGURE 4. Graphical representation of the concept suggesting that the experimental distribution of Hg at concentrations above approximately $1\ \mu g\ L^{-1}$ was limited by the binding of Hg to a small number of strong binding sites on the DOM ([DOM] = 0.5 mg L^{-1}). All of the Hg that was not bound by the strong sites was bound by EDTA, which outcompeted weak Hg binding sites on the DOM. The nature of strong Hg binding sites on the DOM is oversimplified in this plot by assuming a certain concentration of one type of strong Hg binding site, whereas real DOM is a mixture of different types of strong binding sites.

of $1\ \mu g\ L^{-1}$, order of magnitude decreases in [EDTA] caused only small increases in DOM/ligand distribution ratios. At $10\ \mu g\ L^{-1}$ of Hg, distribution ratios were only slightly above zero. At Hg concentrations $\geq 100\ \mu g\ L^{-1}$, binding of Hg to DOM was no longer detectable by the EDLE method.

These results can be explained by assuming a limited number of strong Hg binding sites on the DOM, as outlined in Figure 4. This concept suggests that, at $\leq 0.1\ \mu g\ L^{-1}$ of Hg, the concentration of strong Hg binding sites on the DOM was higher than the concentration of Hg. Therefore, the distribution of low concentrations of Hg was controlled by competitive binding to DOM and EDTA. The behavior of the system at $\geq 1\ \mu g\ L^{-1}$ of Hg can be explained by assuming that the strong sites were saturated with Hg, and weak binding sites on the DOM were outcompeted by EDTA (even at 10^{-5} M) so that all Hg that was not bound to strong sites was complexed by EDTA. Therefore, the distribution ratios measured at 1 and $10\ \mu g\ L^{-1}$ of Hg do not represent an equilibrium competition between strong sites and EDTA because the Hg binding capacity of the strong DOM sites was exeeded. Small increases of Q with decreasing [EDTA] at 1 and $10\ \mu g\ L^{-1}$ of Hg (Figure 3) can be explained by making the reasonable assumption that, within the heterogeneous group of DOM molecules containing strong Hg binding sites, binding affinities for Hg were similar but not exactly the same. Thus, decreases in [EDTA] lead to increased binding of Hg to progressively weaker subfractions of strong sites, resulting in small increases of Q. At 100 and $1000\ \mu g\ L^{-1}$ of Hg, the small number of strong sites was saturated by an insignificant fraction of the total Hg and the largest part of the Hg present in the system was bound to EDTA, which even at a concentration of 10^{-5} M outcompeted the weak DOM binding sites. Therefore, under these conditions, Hg concentrations in the inside compartment were analytically indistinguishable from outside concentrations, resulting in a Q value of zero.

To actually quantify the binding of Hg to weak DOM sites, an additional EDLE experiment was carried out. This experiment had to use high concentrations of Hg to make sure that the binding of small amounts of Hg to strong sites did not significantly affect the result. Furthermore, a weak auxiliary ligand suitable to achieve competitive binding of Hg to weak DOM sites had to be used. Figure 5 shows the

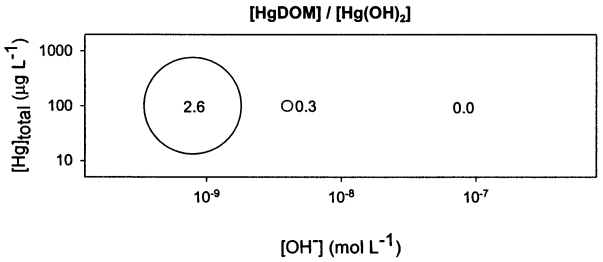

FIGURE 5. Distribution ratios between HgDOM and $Hg(OH)_2$ in an EDLE experiment using hydroxide as the auxiliary ligand. Bubble size and numbers represent Hg distribution ratios (Q = [HgDOM]/[$Hg(OH)_2$]). Competitive binding of Hg to DOM and OH^- under these conditions suggests interactions of Hg with weak DOM sites that are present at high concentrations.

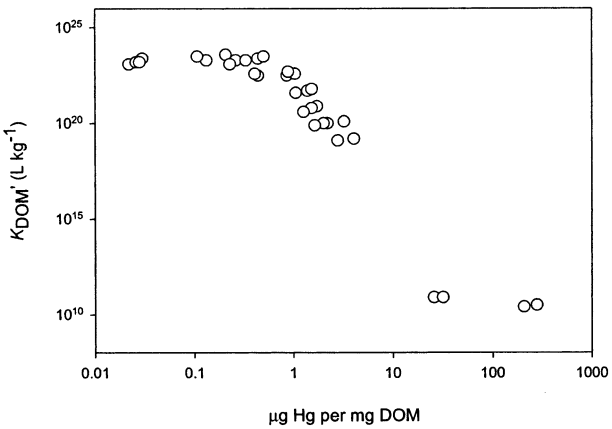

FIGURE 6. Relationship between Hg/DOM concentration ratio (measured in the inside compartment of EDLE experiments) and the conditional distribution coefficient K_{DOM}' calculated according to eq 3. Experimental conditions: $(DOM)_{inside}$ = 0.5 mg L^{-1}, $(DOM)_{outside}$ = 0.04 mg L^{-1}, I = 0.1 M, pH = 7.0 (EDTA experiments, < 10 μg of Hg/mg of DOM), pH = 5.6 (OH^- experiment, 10−100 μg of Hg/mg of DOM), pH = 4.9 (OH^- experiment, 100−1000 μg of Hg/mg of DOM). For exact numbers, see Table 1 in the Supporting Information.

binding of 100 μg L^{-1} of Hg to DOM in the presence of hydroxide as the auxiliary ligand. Competitive binding of Hg to DOM and OH^- under these conditions suggests interactions of Hg with weak DOM sites. The fact that a distribution ratio of 2.6 was reached at a total Hg concentration of 100 μg L^{-1} demonstrates that the binding of Hg to weak DOM binding sites was not limited by a small number of sites, as observed for Hg binding to strong DOM sites.

To obtain a comprehensive overview of the results, conditional distribution coefficients K_{DOM}' were calculated (eq 3, Table 3) from distribution ratios observed in the EDLE experiments and plotted against the concentration ratio of Hg to DOM (Figure 6). The overall uncertainty of the calculated K_{DOM}' values including statistical errors (i.e., replicate reproducibility) and systematic errors (mainly uncertainty of stability constants for complex equilibria of EDTA with Hg^{2+} and H^+) was estimated to be 1.0 log units. For an extended discussion of statistical and systematic errors in EDLE experiments, see Glaus et al. (*29*, *31*). Figure 6 shows very strong binding of Hg to DOM at Hg/DOM concentration ratios below approximately 1 μg of Hg/mg of DOM. At higher concentration ratios, we observed a sharp drop in Hg−DOM binding affinity, followed by relatively constant low values for K_{DOM}' at Hg/DOM concentration ratios above approximately 10 μg of Hg/mg of DOM. These data thus confirm our hypothesis of a pronounced effect of the Hg/DOM concentration ratio on the strength of the interactions between Hg and DOM.

Our second hypothesis was that reduced sulfur (S_{red}) groups are the dominant Hg binding sites at low Hg/DOM concentration ratios and that Hg is mostly bound to carboxyls at high ratios of Hg to DOM. To verify this hypothesis, we first compared the EDLE-derived concentration of strong Hg binding sites on the DOM to the concentration of S_{red} determined by X-ray absorption near edge structure (XANES) spectroscopy. The concentration of strong DOM ligands was estimated from Figure 6, which shows a constant K_{DOM}' value of approximately $10^{23.2}$ L kg^{-1} at low Hg/DOM ratios up to a concentration ratio of approximately 1 μg of Hg/mg of DOM. At higher Hg concentrations, a decrease in K_{DOM}' indicates the saturation of strong sites. We therefore estimated the concentration of strong DOM sites to be equal to the molar concentration equivalent of 1 μg of Hg/mg of DOM (i.e., 5×10^{-9} mol of Hg/mg of DOM). The concentration of S_{red} calculated from the total sulfur content of 1.73% and the fraction of S_{red} (60.0%) for the DOM isolate (Table 1) at a concentration of 1 mg L^{-1} was 3.2×10^{-7} M. Thus, the molar concentration of S_{red} determined by XANES was 64 times the molar concentration of strong Hg binding sites derived from EDLE experiments, suggesting that only a small fraction (1.6%) of S_{red} was involved in the strongest interactions between Hg and DOM. This finding agrees well with data reported recently by Amirbahman et al. (*32*) who, using an equilibrium dialysis method, estimated that only approximately 2% of S_{red} in Suwannee River humic acid took part in strong interactions with methylmercury.

Studies using extended X-ray absorption fine structure (EXAFS) spectroscopy found, however, that interactions between Hg and humic acids included a significant amount of Hg−S_{red} bonds, even at low molar S_{red}/Hg ratios of 0.3−4.0 (*15*, *16*). The difference between results from studies measuring strong mercury−DOM interactions (ref *32* and this study) and results from EXAFS spectroscopy may be explained by taking into account that these two methods measure different types of Hg−S_{red} interactions. EXAFS spectroscopy detects sulfur atoms in the vicinity of Hg atoms, without taking into account the binding strength between Hg and S_{red}. In contrast, the EDLE method measures Hg−DOM binding in the presence of a competitive ligand and, therefore, only detects Hg interactions with binding sites having the strongest affinity to mercury (probably thiol groups). A comparison of stability constants for Hg binding to thiols (RSH) versus sulfides (RSR) (e.g., cysteine vs methylcysteine or methionine) shows, for example, that constants for mercury−thiol interactions are always many orders of magnitude higher than constants for Hg interactions with similar molecules in which the thiol group is methylated (*19*). Thus, it is likely that under the competitive conditions of the EDLE experiments, Hg is bound only to a small fraction of the total S_{red} (e.g., thiol groups which are freely accessible), whereas under the conditions of the EXAFS measurements Hg additionally interacted with S_{red} groups that had a much lower affinity for Hg (e.g., sulfide, polysulfide, thiophene).

To further evaluate the hypothesis that thiol groups were the primary strong binding sites for Hg, we compared Hg−DOM binding constants measured at low ratios of Hg/DOM to literature values for binding constants describing Hg binding to simple thiol ligands. Because literature values for binding constants generally refer to the binding of a metal ion to a deprotonated ligand (*33*), the conditional distribution ratios K_{DOM}' calculated from EDLE experiments had to be converted to binding constants describing Hg^{2+} binding to deprotonated DOM−thiol ligands ($Hg^{2+} + RS^- = HgRS^+$). This conversion required assumptions about concentration and protonation constants of DOM−thiol ligands in order to be able to apply the concentration of unprotonated thiol groups instead of the concentration of DOM in eq 3. Using the concentration for strong Hg binding sites found in the

EDLE experiments (5×10^{-9} mol/mg of DOM; see the previous discussion) and an estimated average value of 10^{10} for the protonation constant of an organic thiol group (*19*), the calculated mean value for conditional binding constants of Hg to deprotonated thiol groups in DOM ($K' = [HgRS^+]/([Hg^{2+}] \times [RS^-])$ was $10^{28.5}$ (at $I = 0.1$ M). The overall uncertainty for K' is estimated to be approximately 2 log units, which is higher than the uncertainty of approximately 1 log unit for K_{DOM}' values, because of additional errors involved in estimating the concentration and an average protonation constant for thiol groups in DOM. The value of $10^{28.5}$ for K' is clearly higher than typical stability constants for Hg interactions with oxygen- or nitrogen-containing ligands (*19*). Binding constants reported in the literature for Hg binding to thiol ligands are somewhat inconsistent, with values describing the interaction of Hg with the same ligand sometimes spanning several orders of magnitude (*19*). However, most of the values are very high ($>10^{20}$), emphasizing strong interactions between Hg and thiol ligands, as expected for interactions between a soft metal and a soft ligand according to the hard and soft acids and bases theory (*22*, *23*). For example, binding constants of $10^{24.8}$, $10^{25.7}$, $10^{34.5}$, and $10^{37.5}$ were reported for 1:1 binding of Hg to thiosalicylic acid, 2,3-dimercaptopropanol, thioglycolic acid, and cysteine (*17*, *34*, *35*). A comparison of these data to the stability constant of $10^{28.5}$, we determined for Hg binding to DOM (assuming that strong DOM ligands were thiols) indicates that organic thiols in the DOM probably were the primary binding sites at low ratios of Hg to DOM.

To our knowledge, only one study has determined constants for Hg binding to organic matter at low Hg-to-organic matter ratios (*24*). Using soil organic matter (SOM), the authors found Hg−thiol stability constants on the order of 10^{32}. The constants measured in their study may have somewhat overestimated the strength of natural Hg−thiol interactions, because high concentrations of bromide were used in the experiments. This can cause the formation of mixed ligand complexes (e.g., SOM−Hg−Br), thus increasing the observed stability constants for Hg−SOM interactions (*30*). Nevertheless, the magnitude of binding constants reported in this study is in agreement with our data, indicating that Hg binding to organic matter at low ratios of Hg to organic matter is indeed controlled by strong interactions with reactive organic thiol groups.

For high Hg-to-DOM concentration ratios, as used in most studies measuring Hg−DOM binding constants (e.g., refs *10* and *14*), we had hypothesized that organic S_{red} groups are saturated by a small fraction of the total Hg and that carboxylic functional groups are the primary binding sites for Hg. The maximum Hg/DOM concentration ratio of approximately 240 μg of Hg/mg of DOM (1.2×10^{-6} mol of Hg/mg of DOM) reached in the EDLE experiment using hydroxide as the auxiliary ligand (Figure 5) shows that, under these conditions, Hg was bound to DOM ligands much more abundant than S_{red}. This suggests that carboxyls (5.45×10^{-6} mol/mg of DOM, see Table 1) were the main binding sites for Hg under these conditions. To further evaluate this assumption, binding constants for Hg interactions with weak DOM ligands were compared to literature values for mercury−carboxyl binding constants. For this purpose, K_{DOM}' values for interactions between Hg and weak DOM sites were converted to binding constants referring to the binding of Hg^{2+} to deprotonated carboxyl groups (see the previous discussion for thiols). Using a carboxyl concentration of 5.45×10^{-6} mol/mg of DOM (Table 1) and a mean protonation constant of $10^{4.5}$ for DOM−carboxyls (*36*) (although this assumption clearly oversimplifies the real protonation behavior of DOM), the mean K_{DOM}' value for Hg binding to weak sites ($10^{10.7}$ L kg^{-1}) was converted to an average conditional binding constant ($K' = [HgRCOO^+]/([Hg^{2+}][RCOO^-])$) of approximately 10^{10}. This value is within the range of Hg binding constants for simple organic ligands containing carboxyl groups (e.g., oxalate: $10^{9.7}$; citrate: $10^{10.9}$; salicylate: $10^{11.6}$ (*19*)), thus giving further indication that the major Hg−binding ligands at high ratios of Hg to DOM were indeed carboxyl groups.

Thus, our estimations for concentrations and binding strengths of strong and weak Hg−binding DOM ligands were consistent with the hypothesis that organic thiol groups are the primary binding sites for Hg at low ratios of Hg to DOM and that Hg is mainly bound to carboxyls at high Hg/DOM ratios. We also confirmed the hypothesis that Hg−DOM binding constants determined at low ratios of Hg to DOM are much higher than literature values (e.g., refs *10* and *12*−*14*). The concentration ratio of Hg/DOM in most natural systems is even lower than the lowest ratios used in this study. Assuming concentrations of dissolved Hg in natural waters between approximately 1 and 10 ng L^{-1} (*37*), a DOM concentration range of 1−100 mg L^{-1} (*38*) and 3×10^{-7} mol of S_{red}/mg of DOM (see the previous discussion), we can estimate that concentration ratios for Hg to DOM approximately range between 0.01 and 10 ng of Hg/mg of DOM. The corresponding molar Hg/S_{red} ratios range from 2×10^{-7} to 2×10^{-4} mol of Hg/mol of S_{red}. Therefore, it can be expected that only the strongest DOM binding sites will interact with Hg under natural conditions. Theoretically, it is possible that binding constants for Hg−DOM interactions at very low Hg/DOM ratios are somewhat higher than the values reported in this study, but the fact that K_{DOM}' values were constant over a range of Hg/DOM ratios from approximately 0.02 to 1 μg of Hg/mg of DOM (Figure 6) suggests that the upper limit of Hg−DOM binding strength was approached under the experimental conditions of the EDLE experiments.

Finally, it should be noted that Hg−DOM distribution coefficients determined in this study are only valid for a certain fraction of DOM from a certain environment under the given experimental conditions. Further EDLE experiments using different DOM fractions from a wide range of environments at variable pH and ionic strength are currently under way to evaluate the effect of environmental variables on Hg−DOM distribution coefficients. Results from such studies should allow for Hg−DOM interactions to be included in comprehensive metal−DOM binding models (e.g., ref *39*), which could eventually be used to estimate Hg speciation in natural waters.

Acknowledgments

This work was funded by the Deutsche Forschungsgemeinschaft (HA 3024/1-1), by the U.S. Geological Survey Place-Based Studies Program, and by the National Science Foundation's Environmental Geochemistry and Biogeochemistry Program (EAR-9807735). Martin Glaus (PSI, Switzerland) is gratefully acknowledged for a practical introduction into the EDLE technique. We thank Murthy Vairavamurthy (Brookhaven National Laboratory, NY) for XANES spectroscopy analysis.

Supporting Information Available

Exact numbers for the data shown in Figure 6 can be found in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Watras, C. J.; Bloom, N. S.; Hudson, R. J. M.; Gherini, S. A.; Munson, R.; Claas, S. A.; Morrison, K. A.; Hurley, J. P.; Wiener, J. G.; Fitzgerald, W. F.; Mason, R. P.; Vandal, G.; Powell, D.; Rada, R. G.; Rislov, L.; Winfrey, M. R.; Elder, J.; Krabbenhoft, D. P.; Andren, A. W.; Babiarz, C. L.; Porcella, D. B. In *Mercury pollution: integration and synthesis*; Watras, C. J., Huckabee, J. W., Eds.; CRC Press: Boca Raton, FL, 1994; pp 153−185.

(2) Stordal, M. C.; Gill, G. A.; Wen, L.-S.; Santschi, P. H. *Limnol. Oceanogr.* **1996**, *41*, 52−61.

(3) Ravichandran, M.; Aiken, G. R.; Reddy, M. M.; Ryan, J. N. *Environ. Sci. Technol.* **1998**, *32*, 3305−3311.
(4) Ravichandran, M.; Aiken, G. R.; Ryan, J. N.; Reddy, M. M. *Environ. Sci. Technol.* **1999**, *33*, 1418−1423.
(5) Mason, R. P.; Lawrence, A. L. *Environ. Toxicol. Chem.* **1999**, *18*, 2438−2447.
(6) Sjöblom, Å.; Meili, M.; Sundbohm, M. *Sci. Total Environ.* **2000**, *261*, 115−124.
(7) Hudson, R. J. M.; Gherini, S. A.; Watras, C. J.; Porcella, D. B. In *Mercury pollution: integration and synthesis*; Watras, C. J., Huckabee, J. W., Eds.; CRC: Boca Raton, FL, 1994; pp 473−523.
(8) Ullrich, S. M.; Tanton, T. W.; Abdrashitova, S. A. *CRC Crit. Rev. Environ. Sci. Technol.* **2001**, *31*, 241−293.
(9) Mantoura, R. F. C.; Dickson, A.; Riley, J. P. *Estuar. Coastal Mar. Sci.* **1978**, *6*, 378−408.
(10) Lövgren, L.; Sjöberg, S. *Water Res.* **1989**, *23*, 327−332.
(11) Dryssen, D.; Wedborg, M. *Water, Air, Soil Pollut.* **1991**, *56*, 507−519.
(12) Yin, Y.; Allen, H. E.; Huang, C. P.; Sanders, P. F. *Anal. Chim. Acta* **1997**, *341*, 73−82.
(13) Benoit, J. M.; Mason, R. P.; Gilmour, C. C.; Aiken, G. R. *Geochim. Cosmochim. Acta* **2001**, *65*, 4445−4451.
(14) Lu, X.; Jaffe, R. *Water Res.* **2001**, *35*, 1793−1803.
(15) Xia, K.; Skyllberg, U. L.; Bleam, W. F.; Bloom, P. R.; Nater, E. A.; Helmke, P. A. *Environ. Sci. Technol.* **1999**, *33*, 257−261.
(16) Hesterberg, D.; Chou, J. W.; Hutchinson, K. J.; Sayers, D. E. *Environ. Sci. Technol.* **2001**, *35*, 2741−2745.
(17) Basinger, M. A.; Casas, J. S.; Jones, M. M.; Weaver, A. D.; Weinstein, N. H. *J. Inorg. Nucl. Chem.* **1981**, *43*, 1419−1425.
(18) Morra, M. J.; Fendorf, S. E.; Brown, P. D. *Geochim. Cosmochim. Acta* **1997**, *61*, 683−688.
(19) Pettit, L. D.; Powell, K. J. *IUPAC Stability Constants Database, version 4.11*; Academic Software: Yorks, U.K., 1999.
(20) Benedetti, M. F.; Milne, C. J.; Kinniburgh, D. G.; van Riemsdijk, W. H.; Koopal, L. K. *Environ. Sci. Technol.* **1995**, *29*, 446−457.
(21) Hummel, W.; Glaus, M. A.; Van Loon, L. R. *Radiochim. Acta* **1999**, *84*, 111−114.
(22) Pearson, R. G. *J. Chem. Educ.* **1968**, *45*, 581−587.
(23) Pearson, R. G. *J. Chem. Educ.* **1968**, *45*, 643−648.
(24) Skyllberg, U. L.; Xia, K.; Bloom, P. R.; Nater, E. A.; Bleam, W. F. *J. Environ. Qual.* **2000**, *29*, 855−865.
(25) Chin, Y.-P.; Aiken, G. R.; O'Loughlin, E. *Environ. Sci. Technol.* **1994**, *28*, 1853−1858.
(26) Huffman, E. W. D.; Stuber, H. A. In *Humic Substances in Soil Sediment and Water—Geochemistry, Isolation and Charakterisation*; Aiken, G. R., Wershaw, D. M., MacCarthy, P., Eds.; John Wiley & Sons: New York, 1985; pp 433−456.
(27) Vairavamurthy, M. A.; Maletic, D.; Wang, S.; Manowitz, B.; Eglinton, D.; Lyons, T. *Energy Fuels* **1997**, *11*, 546−553.
(28) Van Loon, L. R.; Granacher, S.; Harduf, H. *Anal. Chim. Acta* **1992**, *268*, 235−246.
(29) Glaus, M. A.; Hummel, W.; Van Loon, L. R. *Anal. Chim. Acta* **1995**, *303*, 321−331.
(30) Glaus, M. A.; Hummel, W.; Van Loon, L. R. *Environ. Sci. Technol.* **1995**, *29*, 2150−2153.
(31) Glaus, M. A.; Hummel, W.; Van Loon, L. R. *Appl. Geochem.* **2000**, *15*, 953−973.
(32) Amirbahman, A.; Reid, A. L.; Haines, T. A.; Kahl, J. S.; Arnold, C. *Environ. Sci. Technol.* **2002**, *36*, 690−695.
(33) Martell, A. E.; Motekaitis, R. J. *Determination and use of stability constants*; VCH: New York, 1992.
(34) Koul, M.; Dubey, K. *J. Inorg. Nucl. Chem.* **1973**, *35*, 2571−2573.
(35) Coates, R. L.; Jones, M. M. *J. Inorg. Nucl. Chem.* **1977**, *39*, 677−678.
(36) Perdue, E. M. In *Humic Substances in Soil Sediment and Water—Geochemistry, Isolation and Charakterisation*; Aiken, G. R., Wershaw, D. M., MacCarthy, P., Eds.; John Wiley & Sons: New York, 1985; pp 493−526.
(37) Morel, F. M. M.; Kraepiel, A. M. L.; Amyot, M. *Annu. Rev. Ecol. Syst.* **1998**, *29*, 543−566.
(38) Buffle, J. *Complexation reactions in aquatic systems: an analytical approach*; Ellis Horwood: Chichester, U.K., 1988.
(39) Tipping, E. *Aquat. Geochem.* **1998**, *4*, 3−47.
(40) Ravichandran, M. Interactions between mercury and dissolved organic matter in the Florida Everglades. Ph.D. Thesis, University of Colorado, Boulder, CO, 1999.
(41) Anderegg, G. *Critical survey of stability constants of EDTA complexes, IUPAC Chemical Data Series—No. 14*; Pergamon Press: Oxford, U.K., 1978.
(42) Gross, H.; Geier, G. *Inorg. Chem.* **1987**, *26*, 3044−3050.

Received for review April 2, 2002. Revised manuscript received June 5, 2002. Accepted June 13, 2002.

ES025699I