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Determination of the Mercury Complexation Characteristics of Dissolved Organic Matter in Natural Waters with "Reducible Hg" Titrations

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A new method for the determination of the concentration and conditional stability constant of dissolved organic matter that binds mercury (Hg) has been developed using an in vitro assay of reducible Hg. The technique is a wet chemical analogue to electrochemical approaches now in use for ligand studies of many other trace transition metals in natural waters. Ligand characteristics are obtained from additions of ionic Hg to buffered lake, river water, and seawater and determination of the wet chemically reducible fraction following equilibration of the spike. This approach is robust, as demonstrated by (i) analysis using three reducing agents of varying strengths, (ii) replicate analyses, (iii) comparison to well-characterized complexing species (chloride and EDTA) using a competitive ion-exchange resin, and (iv) kinetic studies. Results indicate that Hg-complexing equivalents are present in the dissolved phase (<0.2 μm) ranging from <1 to >60 nN concentrations and with log conditional stability constants (log K') in the range of 21−24. Only one ligand class was found in the natural waters analyzed. There was indirect evidence for a class of organic ligands that formed reducible complexes with Hg in freshwater. Such ligand characteristics indicate that the vast majority of ionic inorganic Hg dissolved in freshwater and coastal saltwaters is associated with organic complexes. Concentrations, affinities, and kinetics implicate multidentate chelation sites as the principal complexing moieties for Hg and discourage the use of humic carboxylic acids as a proxy for the ligands/functional groups.

Introduction

Complexation of mercuric (Hg^{2+}) ions by natural organic compounds has been posited as an influential and sometime controlling feature of the aquatic biogeochemical cycling of this toxic metal (*1*−*15*). While many of these studies suggest that the majority of Hg present in natural waters is complexed to organic ligands (*1*−*3*, *16*−*19*), little quantitative information regarding the bulk abundance and strength of the organic complexing agents currently exists. This is due in large measure to the lack of suitably sensitive and reliable techniques with which to study Hg speciation. At present, the "reactive Hg" determination is the only inorganic speciation assay in wide use. In this approach, additions of a reducing agent (usually Sn(II) or BH_4^-) are made to acid-preserved aqueous samples without destruction of the ambient organic matter, and the elemental Hg (Hg^0) so generated is quantified (*20*−*24*). This reactive concentration is often used as an estimate of the amount of Hg available for biogeochemical reactions such as in situ reduction or methylation. The reactive fraction is also generally assumed to represent Hg ions that are associated with inorganic or weak organic complexing agents. The reactive Hg determination is an operationally defined assay, however, whose results are a function of analytical conditions as well as the ambient aqueous chemistry (*24*).

Other approaches to determining Hg−DOC speciation have been employed, including tests performed on isolated fractions of DOC (*15*, *16*, *19*, *25*−*27*). These experiments are extremely valuable, particularly in gaining insight on the nature of Hg−DOC bonding. The process of isolation may favor some forms of DOC rather than others however, and there is therefore a need for bulk complexation information to provide a geochemical context for these more focused experiments.

Electrochemical techniques, similar to those employed in studies of other trace transition metals, have been applied to determining Hg concentrations and speciation (*17*, *18*) but may not be entirely reliable due to incomplete release of Hg° from electrodes (*28*). While these difficulties may be overcome with further development, the electrochemical approach is currently impractical for many studies of environmental Hg due mostly to a lack of suitable sensitivity.

The reactive Hg assay represents a potential wet chemical analogue to the electrochemical techniques and is a determination that many researchers currently making Hg measurements are capable of completing with little modification of their procedures or analytical facilities. We have therefore developed a new technique for determining the Hg complexation capacity and strength of dissolved organic matter in natural waters by adapting the operational "reactive Hg" assay for use in ligand titrations. In this approach, the labile fraction of Hg present in solution is assayed using the reactive or more precisely "reducible" determination and is assumed to represent only complexes of Hg that have stability constants that are too low to protect the Hg(II) from wet chemical reduction. Conversely, it is also possible that some complexes may be directly reducible but possess kinetics that prevent substantial reduction during the analytical time frame. The mathematical development of ligand titrations (*29*, *30*) in the context of natural waters is briefly described below. The complexation reaction between Hg(II) and an organic ligand is assumed to take the following form:

$$Hg^{2+} + L^{n-} \leftrightarrow HgL^{(2-n)+} \qquad K = \frac{HgL^{(2-n)+}}{\{Hg^{2+}\}\{L^{n-}\}} \quad (1)$$

where $HgL^{(2-n)+}$ is the Hg−ligand complex and the braces denote species activities in solution. Using the conventional modification of the thermodynamic stability constant (K) to a conditional form (based on concentrations rather than activities) gives

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

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where K' is the conditional stability constant. If we define $[Hg_r]$ as the sum of the concentrations of all organic and inorganic Hg complexes (HgX) that are wet chemically reducible as

$$[Hg_r] = [Hg^{2+}] + \sum_i^n [HgX_i] = [Hg^{2+}](1 + \sum_i^n K_i[X^i]^{x_i}) = \alpha[Hg^{2+}] \quad (3)$$

and substituting $[Hg_r]$ for $[Hg^{2+}]$ in eq 2 yields:

$$K'' = \alpha\frac{[HgL^{(2-n)}]}{[Hg_r][L^{n-}]} = \alpha K'' \quad (4)$$

where K'' is the conditional stability constant based on the reducible Hg assay. Thus, during a reducible Hg titration, the K'' value will be directly determined and then must be corrected by a sample-specific α value. The α value is determined using procedures described later. If organic complexation is occurring to a significant degree, a plot of Hg_r versus total Hg for a series of Hg additions to a sample containing organic ligands will lie below the 1:1 line and show a nonlinear, upward-facing curve as the equivalence point is approached. The data can be linearized when the ratio of $[Hg_r]/([Hg_T] - [Hg_r])$ is plotted versuss $[Hg_r]$. Ruzic (*30*) and van den Berg (*29*) demonstrated that this plot is fit by the following functional form under conditions where a single 1:1 (M:L) complex class is assumed to form:

$$\frac{[Hg_r]}{[Hg_T] - [Hg_r]} = \frac{[Hg_r]}{[L_T]} + \frac{1}{K''[L_T]} \quad (5)$$

The total concentration of organic ligands ($[L_T]$) and their Hg affinity (K'') can be found from this treatment by linear regression.

We define "organic" complexing agents as compounds that contain carbon−carbon bonds, but in practice, this term is taken to mean biogeochemically generated macromolecules. Inorganic complexing agents, conversely, contain no carbon−carbon bonds and most prominently include chloride, hydroxide, and sulfide/polysulfides. Initially, it is assumed that organic and inorganic Hg complexes behave very differently in the analytical system and that only the inorganic forms are wet chemically reducible. This is a good assumption in the case of seawater, as the abundance and strength of $HgCl_y^{2-y}$ complexes ($\log K_1$=7.2, $\log \beta_2 = 14.0$, $\log \beta_3 = 15.1$; $\log \beta_4 = 15.4$; *31*) are such that organic complexes must have large K' values in order to compete (as their concentration is biogeochemically limited to <μM), and thus the reduction potentials of Hg−organic complexes that can compete will be very different from those of the inorganic forms (*32*). Other organic ligands may be present but will not contribute a significant amount of Hg-binding capacity.

In circumneutral freshwater, OH^- is the principal inorganic ligand ($\log K_1 = 10.6$; $\log \beta_2 = 21.8$; $\log \beta_3 = 20.9$; *31*) but is not very abundant. Therefore, organic ligands that need not have very high K' values to compete for Hg and Hg−organic complexes and that are reducible and therefore not detectable by this approach could comprise some of the reducible Hg.

Assuming for the moment that the Hg−OH−Cl system does adequately represent the reducible fraction of dissolved Hg, the dotted line in Figure 1 describes the value of log α over the typical environmental salinity range at pH 7.5 and therefore indicates the correction to K'' that should be made. The effective α value in a real sample can be measured through the use of an ion-exchange resin of known strength (K'_{Rs}) by setting up a competition between the reducible Hg

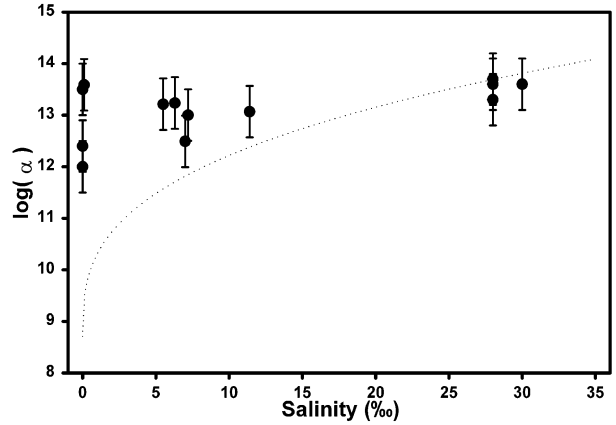

FIGURE 1. Dotted line is the theoretical value of α (ratio of reducible to free Hg) for the Hg−OH−Cl system at pH 7.5 over the range of ambient salinities. The single points with error bars are discrete α determinations made using a calibrated ion-exchange resin (Amberlite 200 C) in competition with the natural ligands. Note the significant deviation from the theoretical curve at lower salinities, indicating that ligands other than OH and Cl dominate the reducible pool speciation in under those conditions. These ligands are likely to be organic as well and have rapid release kinetics.

in solution and the resin phase. The α value for the ambient ligands can be determined using the following assuming Langmuir adsorption (heterogeneous-phase analogue to the aqueous equations mentioned above) by the resin:

$$\alpha = \frac{K'_{Rs}}{K''_{Rs}} = \frac{[Hg_r][Rs_T]^n}{[Hg_T] - [Hg_r] - [Hg_{nr}]}K'_{Rs} \quad (6)$$

where $[Hg_{Rs}]$ is the amount of Hg bound to the resin and $[Rs_T]$ is the amount of resin added to the treatment. A difference between the apparent and the theoretical α for a sample (Figure 1) indicates that the OH−Cl system does not accurately represent the Sn(II)-reducible pool of Hg and implicates other ligands as being important. These additional reducible ligands are likely to be organic as well. Thus, the initial assumption that organic and inorganic ligands behave different in our analytical apparatus may be tested.

A final important caveat to the wet chemical reduction−ligand titration technique is the kinetic stability of Hg−organic complexes. Nonreducible complexes that exchange Hg rapidly with reducible ligands will not be detectable. This is the result of the release of Hg from the nonreducible pool to the reducible pool through mass action inside the analytical apparatus as the reducible pool is removed (*24*). Such an effect is minimized in most electrochemical analyses as only a small percentage of the reducible metal pool is plated, resulting in a relatively small disturbance of equilibrium. In the proposed analysis, several percent of total Hg, and 100% of reducible Hg are removed from solution during a measurement, and thus significant perturbation occurs. Through kinetic release experiments, the magnitude of this effect can be gauged and corrections made so that complexes that release Hg on time scales that are slow relative to the sample processing time (5−10 min) will be accurately titrated. However, complexes which have large K' values but exchange Hg rapidly will still be undetectable, even with correction (e.g., EDTA, see below).

Methods

Natural water samples included in this study were collected from a variety of locations (Table 1). Collection was accomplished using ultra-clean techniques (*33*) either (i) by hand into a Teflon bottle from the front of a small boat while

TABLE 1. Location and Description of Sites of Natural Water Collections[a]

site	date(s) of collection	ligand (nN)	release rate const. (min^{-1})	log K' (log N^{-1})
humate		8 ± 3	<1 E−4	21.5
Arctic lakes	July 2000	13−30	ca. 1 E−3	21
MN bog	October 31, 2001	60 ± 10 (~1000 at $t = 0$ d)	2 E−3	21.2
MN lake	October 31, 2001	3.8 ± 0.2 (~74 at $t = 0$ d)	<5 E−4	22.2
CT river	December 6, 2001	6.4 ± 0.9	7.3 E−4	22.9
RI river	December 6, 2001	24.8 ± 0.8	9.7 E−4	21.6
sewage effluent	February 14, '02	3 ± 1	1.2 E−2	22.7
coast. embay	April 2000−December 2001	0.6−8	<5 E-4−3 E−2	23−23.9
ocean shelf	October 26, 2000	0.3	na	23

[a] All determinations made at pH 7.5. Ionic strength varies between samples. Humate is a 123 μM DOC solution of Fluka humate. Arctic Lakes are Toolik and Perfect Lakes, Alaska. MN bog is S6 Fen of Marcel Experimental Forest, MN. MN lake is Spring Lake, Marcel Experimental Forest, MN. CT river is the Connecticut River, Haddam, CT. RI River is the Pawcatuck River, Westerly, RI. Sewage effluent is from City of Groton Water Pollution Control Facility, Groton, CT. Coastal embayment is Long Island Sound, various locations. Ocean shelf is the Mid-Atlantic Bight, south of Montauk, NY. na, not available.

moving gently forward into the wind, (ii) into a Teflon bottle secured to a PVC pole for surface sampling from the bow of a medium-sized vessel, or (iii) from an acid-cleaned, Teflon-coated Go-Flo Niskin sampler (General Oceanics) when collecting subsurface samples or surface samples from larger ships. The sewage effluent sample was collected immediately upstream of the outfall by means of a Teflon dipping bucket. Samples were kept in the dark and near ambient temperature until processed.

In general, the samples were processed within 24 h of collection. The exceptions to this were the samples from the Marcell Experimental Forest, MN (samples supplied by D. Engstrom and colleagues). These samples were shipped cold and untreated and were filtered before analysis (15 d following collection). All samples were filtered to 0.2 μm using acid-cleaned polycarbonate membrane filters (Nucleopore). Immediately after filtration, 10−20-mL aliquots of the samples were buffered to ca. pH 7.5 using a phosphate buffer (final solution ~25 mM in P) in several small Teflon bottles that had been rigorously cleaned (see below). In Fe-rich waters, for example, pore fluids, the buffer should be avoided as it may form a precipitate. This particular pH was selected because it lies in the middle of the pH range for the samples analyzed, and phosphate was used because it buffers well in this range and does not form complexes with Hg. The potential impact on the titration results of normalizing all samples to the same pH is discussed in later sections. Following addition of buffer, 4−10 treatments were generated through spiking with a $Hg(NO_3)_2$ standard. The range in the final Hg concentrations depended on the anticipated ligand concentration but were typically between 0 and ca. 20 nM. The Hg standard was prepared fresh on each occasion by serial dilution of a concentrated standard (Fisher) with no additional acid. The spiked solutions were then typically allowed to equilibrate overnight (16 h) in the dark (usually with shaking) and were analyzed within 30 h of spiking.

In addition to the bulk titration experiments, some treatments were analyzed following the addition of a granular chelating cation-exchange resin with a sulfonic acid-exchange site (Amberlite 200C—A200C—Na form available from Fluka). This resin was thoroughly washed with 18 MΩ·cm^{-1} water, air-dried at 50 °C, and stored in an acid-cleaned and pyrolyzed glass scintillation vial with a Teflon lid liner (Qorpak). In the resin experiments, ca. 10−200 mg of this dried form was weighed into titration bottles containing the sample solution and other ingredients as described above.

Samples were analyzed in an acid-cleaned borosilicate glass sparging system (custom-made at University of Connectiut), which allows for aliquot addition at the top while maintaining a flow of inert sparging gas (N_2) through the sparger head. Prior to addition of an aliquot of equilibrated sample to the sparger, 50 mL of deionized water was blanked of Hg by purging for 5 min in the presence of a reducing agent (ca. 5 g of Cu^0 shot which was positioned around the sparging frit, 0.1 mL of 50% $SnCl_2$ in ~2 M HCl or 5% $NaBH_4$). Then, 0.1−5.0 mL of each titration treatment was added to the sparger and purged for a specific time (5 min for Sn(II); 10 min for Cu^0 and BH_4^-) onto a Au-coated glass bead trap at a nominal gas flow rate of 0.5 L min^{-1}. The purge times were found to be sufficient to recover ~100% of an aqueous standard. Detection of the collected Hg was by cold vapor atomic fluorescence spectrometry (CVAFS; *20*−*22*). The analytical system was calibrated primarily with a saturated Hg^0 vapor standard injected using gastight syringes, and calibration was checked with a working aqueous standard added to the sparger that is routinely compared to certified reference materials.

Titration of a humic acid solution was also completed. This material was prepared by adding ca. 50 mg of Fluka humic acid (#53680) to 1 L of 18 MΩ·cm^{-1} water, shaking the mixture for 24 h, and then filtering to 0.2 μm. The dissolved organic carbon content of this stock solution was determined on a Shimadzu TOC 5000A analyzer and found to be 2640 ± 40 μM C. The resulting solution was diluted ca. 20-fold for titration and subsequently handled in the same way as ambient samples in resin addition titrations.

A major part of maintaining quality and precision during the titrations was combating adsorption of Hg to the walls of the treatment bottles. We found that the loss of Hg to the bottle walls was greatly aggravated by adsorption of hydrophobic organic matter to the bottles from previous samples (see the results of the Marcell sample analysis below). Acid cleaning did not appear to adequately remove this organic film. We found that routine cleaning of the titration bottles involving a 12-h soak in 1 M KOH followed by a 24-h soaking in warm (50 °C) concentrated HNO_3 to be absolutely necessary for high-precision results.

Results

Except in the cases where noted, [Hg_r] values reported below are from measurements made using Sn(II) as the reducing agent. The minimum time required for a spiked sample to reach equilibrium with added Hg was assessed by following the reducible Hg content of deionized water, river water, and seawater samples over time. The results were similar to that of Rolfhus (*13*), who demonstrated that the loss of spiked Hg_r from waters of Long Island Sound and the Connecticut River followed similar kinetics in both media. Figure 2 depicts the results of a similar experiment where Hg was added to pH 7.5 buffered seawater as well as buffered deionized water.

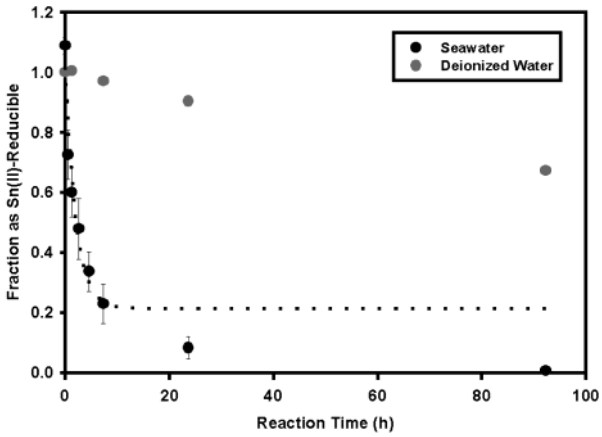

FIGURE 2. Uptake of reducible Hg by natural organic matter following addition of a spike. The behavior of this sample (UConn dock; $S = 25$ ppt) is representative of others. Decrease in reducible Hg from the deionized water treatment is taken to be the result of adsorption of Hg to the walls of the sample bottle (Teflon). Over longer time periods, the uptake rate of Hg in the seawater sample appears similar to that of the deionized water treatment, suggesting that decreases in reducible Hg during that time were the result of wall adsorption as well. The dotted line represents the seawater uptake curve corrected using the deionized water uptake curve to account for wall adsorption. The half-life of corrected uptake in this case was 1.44 h ($k = 0.008$ min^{-1}). This demonstrates that equilibration is relatively slow and that titration should be conducted after about 16 h of equilibration. This experiment also suggests that holding samples too long may result in an overestimate of the influence of ligands due to wall adsorption.

As can be seen for deionized water, buffering to a neutral pH can result in a small but measurable decrease of Hg_r in solution, presumably in this particular case due to adsorption to the walls of the Teflon bottle in which the experiment took place. By contrast, the seawater sample shows a dramatic decrease of Hg_r during the first few hours following spiking. Following this period, Hg_r decreases at a rate similar to that observed in deionized water, suggesting that further decreases of this fraction of Hg (after ca. 10 h) are due to adsorption to the bottle walls as well. When the seawater decay curve is corrected for the wall adsorption (by fitting the seawater data with the function $[Hg_r]_t = [Hg_r]_0 e^{-(k'+w)t}$, where k' is the pseudo-first-order rate constant for complex formation and w is the rate constant for adsorption by the bottle walls and found from the deionized water treatment), an exponential decay to a constant level of about 20% reducible Hg is evident (Figure 2, dashed line). The implication is that uptake of Hg(II) by organic ligands is not instantaneous and appears to occur with a pseudo-first-order rate constant with the value of $k = 0.01$ min^{-1} estimated from the initial part of the curve. The net Hg_r decay constant was 0.008 min^{-1}, suggesting that a period of no less than about 9 h should be allowed for equilibration of an inorganic spike before titration analyses can take place. Hering and Morel (*34*) have noted similarly slow complexation reactions for Cu with EDTA in seawater and cautioned that complexation studies must make allowances for such behavior. The data presented in Figure 2 also caution against holding samples too long after spiking. While some of the data presented here equilibrated for as long as 30 h, no attempt has been made to correct the results for additional reducible Hg decay that occurred after the optimal time.

Figure 3 illustrates the details of titration analyses using two river water samples as examples. The left panel shows the result of reducible Hg determinations versus total Hg and displays the nonlinear curve shape predicted by theory. The right panel shows the result of linearization of the data

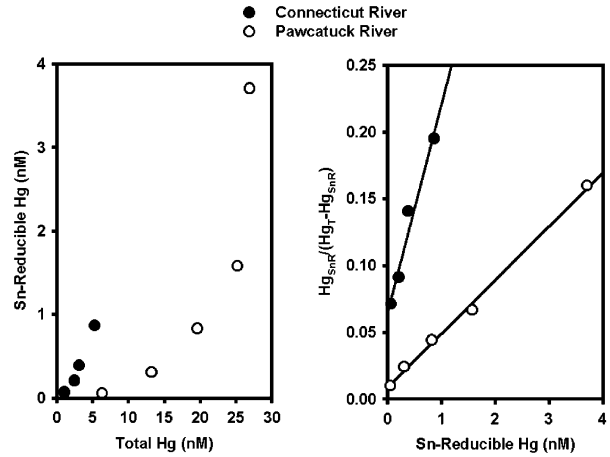

FIGURE 3. Example of titration data. These samples are from the Connecticut and Pawcatuck Rivers. The right-hand panel is the transformed data (van den Berg/Ruzic plot) used to determine the complexation characteristics of the organic matter.

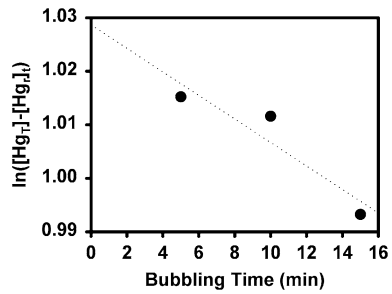

FIGURE 4. Changes in reducible Hg as a function of bubbling time for the Connecticut River sample of Figure 3. The $\ln(Hg_T - Hg_r)$ is plotted in accordance with eq 7.

following Ruzic (*30*) and van den Berg (*29*). The linearity of these analyses suggest that 1:1 complexation models are appropriate and that only one ligand class was observed.

As mentioned above, determinations of Hg complex release rates also were made routinely. The results of sparging a sample of Connecticut River water for a variety of times is shown in Figure 4. The amount of reducible Hg recovered from the sparger increased steadily with time. This is not due to incomplete removal from the sparger, as 1.5 min at the nominal flow rate should have been sufficient to remove Hg^0 quantitatively (*35*), but instead was due to the release of Hg from the nonreducible to the reducible pool. This was demonstrated by the consistently complete removal of reducible Hg from a control (18 MΩ·cm^{-1} water) spiked to the same concentration over all bubbling times. Assuming that reducible Hg is generated by release from the nonreducible pool following first-order kinetics, the time rate of change of reducible Hg may be described by the following equation:

$$\frac{d[Hg_r]}{dt} = k[Hg_{nr}] = k([Hg_T] - [Hg_r]) \qquad (7)$$

where Hg_r is reducible Hg and Hg_{nr} is nonreducible Hg. This equation has the following solution:

$$[Hg_r]_t = [Hg_T] - ([Hg_T] - [Hg_r]_0)e^{-kt} \qquad (8)$$

where the subscripts t and 0 refer to time. The value of k may be determined from the slope of $\ln([Hg_T] - [Hg_r]_t)$ against bubbling time for any one of the treatments (Figure 4). With the k value determined, corrections for mass action can be made to the reducible Hg determinations. These corrections

were sometimes large (factor of 2). A range of k values have been observed, from $<10^{-4}$ to 3×10^{-2} min^{-1}, with some particular values summarized in Table 1. Figure 4 is a somewhat unusual example of the release rate measurements in that the data could be easily viewed as exhibiting a nonlinear behavior. The bubbling times were chosen to be both similar to the usual time to be appropriate for extrapolation and to cover enough of a range to see a significant change in the reducible Hg collected. Most cases were more linear than Figure 4, and this particular example suggests that there may be more than one kinetic class of ligand present in the sample. We are currently investigating this possibility in greater detail.

From release rate measurements, the value of k was found to be 7×10^{-4} and 1×10^{-3} min^{-1} for the Connecticut and Pawcatuck River samples, respectively. When corrections for mass action (release from the nonreducible pool) are made to the reducible values in Figure 3, the resulting total ligand and affinity values found were as follows: Connecticut: $[L_t] = 6.4 \pm 0.9$ nN, $\log K'' = 9.4 \pm 0.1$ N^{-1}; Pawcatuck: $[L_t] = 24.8 \pm 0.8$ nN, $\log K'' = 9.7 \pm 0.1$ N^{-1} (also shown in Table 1). The higher ligand concentration in the Pawcatuck River is consistent with that river being highly colored and draining the Great Swamp area of southern Rhode Island. Normality units are used because the data linearization approach assumes 1:1 complexes and therefore determines complexation equivalents that may or may not correspond to moles of a particular molecule or functional group. The concentration of "ligand" also must be characterized as a ligand class, as multiple ligands that share similar properties could be responsible for the bulk characteristics described by this method (*29*, *30*).

The uncertainty values associated with the determinations are those from the regression analysis. In the case of the stability constant, the regression uncertainty is likely to be less than the degree to which the value can be said to be known (see the results of multiple analyses below). It can be stated, however, that there is no evidence for multiple ligand classes in these samples given the level of analytical precision. No evidence for multiple nonreducible ligand classes has been found in any of the samples we have analyzed to date in freshwaters or saltwaters.

The value of the α term was determined using competition between the natural ligands and a granular ion-exchange resin (A200C). This particular resin was selected after screening several others because it (i) is available in a Na form, making it readily usable without extensive preparation; (ii) has an adequately low Hg blank; (iii) has a large mesh size and does not float (so that filtration is not required); and (iv) displays a moderate Hg affinity. Some of the other resins tested (e.g., Duolite GT-73, chelating thiol designed for Hg binding) have such high Hg affinities that they are impractical for this application because they out-compete the natural ligands. The use of the resin was necessary because we were unable to identify a competing ligand of known strength that that formed Hg complexes that were not Sn(II) reducible. Those compounds tested included ethylenediaminetetraacetic acid (EDTA), *trans*-1,2-cyclohexylenedinitrilotetraacetic acid (CDTA), nitrilotriacetic acid (NTA), diethylenetrinitrilopentaacetic acid (DTPA), mercaptoacetic acid, and glutathione. This was an interesting finding as it indicates that some compounds with large K values for Hg complexation (as with most of the compounds mentioned above) are Sn(II) reducible even at the millimolar concentrations tested. This indicates that the release kinetics of these particular Hg complexes must be rapid, as described in the Introduction, such that the complexed Hg may quickly reequilibrate with the reducible pool as the Hg^0 product is stripped out. Therefore, we first calibrated the A200C against EDTA (in simulation of freshwater) and Cl^- (in simulation of estuarine and seawaters) and then used the resin as a transfer standard to determine the K' value for the sample ligands.

TABLE 2. Results of Amberlite 200C Calibration

salinity	log K' for Hg−resin$_2$ complex[a]
0	6.1 ± 0.6
17	7.0 ± 0.3
35	7.3 ± 0.1

[a] Each value is the average ± 1 SD of three determinations.

Calibration of the resin was accomplished using the following approach. Known amounts of EDTA or synthetic seawater were added to three titration bottles, buffered to pH 7.5, and spiked with Hg. Varying amounts of resin were added to the bottles, and the mixtures were allowed to equilibrate as with ambient samples. Additional titration bottles were prepared identically but without resin to establish the exact concentration of the Hg spike (and compared to calculated values from the serial dilution) and bottles without a Hg spike to estimate the native Hg present on the resin. In all the experiments with A200C, no detectable native Hg was observed even following the addition of BrCl to the treatment. In the remaining treatments, the amount of $[Hg_r]$ was determined on an aliquot of the supernatant drawn off by pipet. As the Hg−EDTA/Hg−Cl_y complexes are reducible, a determination of the $[Hg_r]$ in the liquid was equivalent to a determination of the total dissolved Hg. Thus, as the complexation affinities of EDTA and Cl^- are documented ($\log K_{HgEDTA} = 21.5$) (*31*), a K'_{resin} value could be determined in contrast to these ligands. Associations between Hg and the resin were modeled as Langmuir adsorption (eq 9 used in the Cl treatments and eq 10 used in the EDTA treatments):

$$K'_{Rs} = \frac{[Hg_{Rs}]}{[Hg_{Diss}]} \frac{[Cl^-]^x}{[Rs_T]^n} K_{HgCl_x} \tag{9}$$

$$K'_{Rs} = \frac{[Hg_{Rs}]}{[Hg_{Diss}]} \frac{[EDTA_T]}{[Rs_T]^n(1 + 10^{11,12-pH} + 10^{17,8-2pH})} \frac{\gamma_{Hg}\gamma_{EDTA}}{\gamma_{HgEDTA}} K_{HgEDTA} \tag{10}$$

where Rs represents the resin, $HgRs_n$ is Hg bound to the resin, $[Rs_T]$ is the total mass concentration (mg L^{-1}) of resin in the titration bottle, and γ is the activity coefficient for a particular species. Note that in eq 9, the value of x depends on which Hg−Cl complex is the dominant form (either 3- or 4-chloro). The value of n (stoichiometric coefficient of the resin in complexing Hg) can be determined from examination of the calculated K'_{resin} values over the range of added resin (K' should be resin mass independent). This mathematical treatment assumes that Rs_T is equivalent with "available" resin. As the adsorption capacity is large as compared to the amount of Hg added and the resin starts in a Na-saturated form, this is a reasonable construct.

It was found that a stoichiometry of 1:2 (Hg:resin, i.e., $n = 2$) provided the best fit to these data in all cases. The values of K'_{resin} for three different salinity conditions (zero salinity from calibration against EDTA) are shown in Table 2. The results show a slight salinity dependence and indicate that K'_{resin} is determined to within about an order of magnitude. The value of K' increases with increasing salinity, contrary to the expectation that increased cation activity would lower the number of available binding sites for Hg and therefore lower K'. Turner et al. (*36*) recently found a similar trend in sediment−water partitioning of Hg in estuaries and attributed

TABLE 3. Results of Triplicate Analyses of Natural Samples[a]

date	Sn(II) reducible $[L_T]$ (nN)	Sn(II) reducible log K' (M^{-1})	Cu^0 reducible $[L_T]$ (nN)	Cu^0 reducible log K' (M^{-1})	BH_4 reducible $[L_T]$ (nN)	BH_4 reducible log K' (M^{-1})
April 2000	8.1 ± 0.8	24.7 ± 0.5	8 ± 1	24.4 ± 0.5		
January 2002	0.32 ± 0.07	24.7 ± 0.5			0.09 ± 0.02	>25

[a] Seawater collected from the UConn dock. Also, analysis using different reducing agents is shown.

this counter intuitive phenomenon to a coupled "salting out"/sorption mechanism. Given the uncertainty in the data and without a more detailed understanding of the resin itself, we have adopted this empirical data and not pursued its explanation at this time. For use with samples at any salinity, the value of K'_{resin} was interpolated from these data using a linear fit: $\log K'_{resin} = (6.2 + 0.03 \times \text{salinity}) \pm 0.5$.

Interestingly, the observed α values are significantly larger than the theoretical values under low salinity (<ca. 15 ppt) conditions (Figure 1). For example, the Connecticut and Pawcatuck River samples described above showed log(α) values of 13.5 and 12.0, respectively. This implies that a ligand other than OH^- or Cl^- dominates the reducible-Hg pool in freshwaters and is most likely organic as well. The candidates need not be exotic, for as demonstrated, there are several compounds that strongly complex Hg but that are still Sn(II)-reducible. By performing titrations in the presence of resin and examining the reducible Hg versus resin bound in addition to the reducible versus nonreducible distribution, it should be possible to gain complexation data on the reducible ligand pool as well. However, the precision of our analyses have so far precluded this, and we are therefore only able to report that $K'_r \times [L_r]$ must approximately equal α under freshwater conditions (ca. $10^{12}-10^{14}$). Under salinity >15 ppt conditions, the theoretical α curve appears to be a good predictor of the ambient α value. This implies that the Hg−L−Cl system is an appropriate speciation model and that no reducible organic ligands were able to compete with Cl^-.

In the case of titrating isolated humic materials, no Sn(II)-reducible Hg was found in the dissolved phase following mass action correction. However, titration versus the ion-exchange resin was still possible by plotting the ratio of the total dissolved to the adsorbed Hg versus the total dissolved Hg in accordance to the following equation (translated version of eq 5):

$$\frac{[Hg_{Diss}]}{[Hg_{Rs}]}[Rs_T]^n = \frac{K'_L[L_T]}{K'_{RS}} + \frac{K'_L[Hg_{Diss}]}{K'_{RS}} \qquad (11)$$

Results from titration of a prepared humic acid solution are shown in Table 1.

The effects of thermodynamics as well as an assessment of the overall reproducibility of this technique are illustrated in Table 3, which details the complexation characteristics of water collected from the UConn dock (a: April 2000; b: January 2002) and determined in triplicate. Additionally, the results when using Sn(II) and two other reducing agents (Cu^0 and BH_4^-) are shown. The reproducibility of $[L_T]$ as determined by Sn reduction in the two treatments were rsd = 13 and 22%, respectively. The observed ligand activity and complex stability follow sensible thermodynamic trends: with Cu^0, which is weaker than Sn(II), identical ligand concentrations and affinities; with BH_4^-, which is stronger than Sn(II), lower ligand concentrations with higher affinities. It should be noted that the rsd values of the titration slope and intercept determined with BH_4^- were large, making some of the K' values statistically undefined (e.g., the third set minimum value is shown without error bars). These results suggest that the organics binding Hg occupy a spectrum of abundances and strengths, but that essentially no organic complexation capacity lies below the reducing potential of Sn(II) that is stable on a 5-min bubbling time. By placing the constraint that ΔG must be less than 0 for the reduction reaction between agent and complex in order to proceed, the Cu/Sn experiment allows a lower end value for K' spectrum to be placed on seawater samples. This value is about 10^{19} when side reactions of Sn(II) with chloride are also included (*37*). If one were to apply the same principle to the Sn/BH_4^- experiment, then up to 30% of the complexation capacity could have log K' values above about 60. Such a value is unrealistically high and suggests that BH_4^- should reduce all the Hg complexes present but does not do so as a result of kinetic limitations. Therefore, Sn appears the best choice of reducing agent of the three tested because of its fast reaction kinetics and the position of its reduction potential relative to the ambient complexation affinity spectrum.

TABLE 4. Decay of Ligand Concentration in a Sample of Water from the Marcell S6 Experimental Bog

days after collection	$[L_T]$ (nN)
15	60 ± 10
25	3.9 ± 0.5
35	1.2 ± 0.1

Table 1 contains the results of some of the titrations completed. The range of ligand concentrations reported covers at least 2 orders of magnitude, from 0.2 to >60 nN. The $[L_T]$ in freshwater samples is general higher than in seawater samples. The samples from Long Island Sound rose to comparable freshwater levels at times and were generally higher during the spring and summer. The K' values were generally lower in freshwater than in seawater and have as yet shown no discernible seasonal trend.

The range of ligand concentrations and strengths found using this technique are in general agreement with other techniques (*16, 18, 38*). The K' values that we found are similar to the higher end of the range reported by Mantoura et al. (*16*). The K' values were also similar to the thermodynamic K for humate isolated from the Everglades, Florida (*15*). This is perhaps not surprising as the Hg affinity of the ligand equivalents is so high that the total ligand present behaves as if it were free in titration (i.e., H^+, Ca^{2+}, Mg^{2+}, and trace metals cannot compete with Hg for the complexation sites). We also found that, during extended storage time, a dramatic loss of complexation capacity to Teflon bottles can occur (Table 4). This implied that the majority of Hg−organic complexation was accomplished by hydrophobic compounds, an observation also made in the Everglades work (*14, 15*). It has also been found through spectroscopic investigation of isolated soil humic material that Hg binding was mostly accomplished through a bidentate chelation process involving sulfur- and probably oxygen-bearing functional groups (*19, 26, 39*). Our kinetics experiments came to a complementary conclusion for the bulk DOC as the complexes were slow to bind and release Hg. Furthermore, the ligand equivalents were exceptionally rare in the DOC pool showing an equivalent/mole ratio of 6.5×10^{-5} L/DOC

in the humate solution. These results further indicate that isolated terrestrial humate has many of the same binding characteristics as the bulk complexation equivalents examined in this study. It is also interesting to note the apparent significant seasonal variation in ligand concentration shown in Table 3. These variations could be associated with seasonal variations in the supply of terrestrial organic matter and primary production in Long Island Sound and is the focus of our ongoing research.

As has been noted by others (e.g., ref *40*), binding site titrations such as the ones present here, only reveal ligand data on those species that lie within the appropriate "window". For example, it is possible that there are ligands which are rarer than those determined here but are sufficiently strong to be relevant to Hg biogeochemistry. As the amount of Hg added to the samples was high relative to the ambient concentration, a rare/strong ligand class might have been saturated and been included in the overall complexation capacity. There are at least two approaches to resolve this potential problem. First, larger volume titrations would allow lower spiking concentrations, closer to the ambient range, to be used. Second, a competitive ligand could be added to increasingly render the ambient Hg more and more reducible, precluding the need to add any Hg spikes. We are currently pursuing both these refinements to the application of reducible Hg titrations.

Our data suggest that Hg complexation is quantitatively dominated by organic compounds. In freshwaters, >99.9% of Hg is to be found in organic complexes, including the reactive Hg reported in the literature. In saltwaters, the fraction in organic complexes varies, with coastal waters also dominated (>50%) by organic forms. This technique implies that offshore samples also have significant amounts of Hg–organics but are not likely to be the dominant form. These findings are significant as the organically complexed pool is likely to have much different biogeochemical reactivity and can therefore affect Hg biogeochemistry on local, regional, and global scales.

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