EXPERIMENTAL STUDIES OF HG(II)-HG(0) TRANSFORMATIONS AND THEIR EFFECTS ON HG ISOTOPE FRACTIONATION

By

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ABSTRACT OF THE THESIS

Experimental studies of Hg(II)-Hg(0) transformations and their effects on Hg isotope fractionation by THOMAS CHARNG-SHUEN WANG

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Understanding the redox transformations of inorganic forms of mercury (Hg) is necessary for understanding the fate of mercury in environmental systems. In this study, the interactions of Hg(0) and Hg(II) with organic and inorganic substances were characterized using mercury stable isotopes. Interactions of a mixed Hg(0)-Hg(II) solution with thiol and humic substances were observed, with no net changes to redox speciation. In the presence of mercaptoacetic acid (MCA), an equilibrium isotope enrichment factor (ϵ^{202} Hg = δ^{202} Hg_{Hg(II)} - δ^{202} Hg_{Hg(0)}) of 1.34‰ between the Hg(II) and Hg(0) fractions was observed, similar with previously published values for Hg(0) oxidation by thiols. The equilibrium ϵ^{202} Hg values similarly determined for 2mercaptopropionic acid (MPA) and Suwannee River humic acids were 2.03‰ and 1.50‰, respectively. Reduction of mercury by siderite (FeCO₃) was also characterized with respect to isotope fractionation over the course of the reaction. This reaction resulted in an 87% reduction of Hg(II) over 30 minutes, with change in mercury isotope ratios of the reactant. Kinetic ϵ^{202} Hg values for the reduction of Hg(II) by siderite (ϵ^{202} Hg = δ^{202} Hg_{Hg(0)} - δ^{202} Hg_{Hg(II)}) were determined by closed-system model (-1.59‰) and Rayleigh distillation model (-1.07‰; product enrichment in light isotopes). The final equilibrium state exhibited an equilibrium ϵ^{202} Hg of -0.67‰. The results from the experiments conducted suggest that there is equilibrium isotope exchange between thiolbound Hg(II) and dissolved Hg(0), and that the reduction by siderite is rapid and may involve multiple processes through the reaction.

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Chapter 1. Introduction

Mercury (Hg) is an element that has become a global contaminant in the environment. Mercury has become ubiquitous in systems across the world and interest in understanding the role mercury plays in the environment stems from its toxicity to living organisms. A neurotoxin, the potential potency of mercury's effects depends on the form to which organisms are exposed (Liu et al., 2011). Elemental mercury [Hg(0)] is often associated with its liquid metal form, which is relatively unreactive (Hsu-Kim et al., 2013), but is capable of generating Hg(0) vapor above the liquid. As a result, Hg(0) has limited direct toxicity to organisms, but its volatility and persistence plays a large role in its global distribution (Liu et al., 2011). The oxidized form, mercuric mercury [Hg(II)], is highly soluble in water and can bind strongly to organic materials in sediments and soil humus, and dissolved in surface and groundwater (Khwaja et al., 2006). The fixation of Hg(0) vapor into soluble forms also increases accessibility to organisms which then are able to re-reduce Hg(II) to volatile Hg(0), or methylate the Hg(II) into organic forms (Hsu-Kim et al., 2013). Methylmercury, in contrast to the inorganic forms' importance in transport of mercury, is most notable for its impact on macro-scale organisms. Readily capable of bioaccumulation in aquatic organisms and concentration biomagnification through environmental trophic structures, methylmercury has become known for its acute toxicity and the impacts on human health from direct exposure or consumption of exposed organisms (Hsu-Kim et al., 2013; Liu et al., 2011). It is primarily from the impacts that methylmercury has on humans and other living organisms that the need to understand the mechanisms that mercury undergoes in environmental systems is emphasized. As a result, a more complete characterization of mercury's interaction in

environmental systems is integral to understanding the fate of mercury that is exposed to almost every aquatic system on earth.

In addition to biological transformations, cycling of mercury in the environment has been observed to be chemically mediated by a multitude of compounds. Reduction and oxidation are transformations that are strongly influenced by abiotic factors in environmental systems. A well-known chemical interaction with mercury involves thiols, reduced-sulfur ligand containing compounds formerly named "mercaptans", referencing the known strong, redox-capable bonding-affinity that mercury has with reduced sulfur (Khwaja et al., 2006; Wiederhold et al., 2010; Zheng et al., 2013). More complex organic compounds have since been observed to promote redox transformations of mercury, most notably humic substances derived from both aquatic sediments and soils (Allard and Arsenie, 1991; Skogerboe and Wilson, 2002; Zheng et al., 2013). Minerals have also begun to be characterized for potential contributions to mercury cycling in anoxic environments with low concentrations of organic matter. In addition to sulfide-containing pyrite (Bower et al., 2008), which as mentioned has an established affinity for mercury, iron minerals have been observed to play a role in mercury redox transformations. Specifically, iron minerals have been documented to promote Hg(II) reduction in anoxic environments (Ha et al., 2017; Richard et al., 2016; Wiatrowski et al., 2009). Addressing the role of cycling of inorganic forms of mercury, it would be beneficial to investigate interactions of different compounds with mercury, under conditions likely to be encountered in natural environments.

Mercury stable isotopes have been utilized to explore mercury transformations in both controlled experimental and natural systems. Many mercury-contaminated sites have been analyzed for isotopic signatures and there has also been increasing determination of fractionation factors for likely contributing processes (Bartov et al., 2013; Blum and Bergquist, 2007; Demers et al., 2013; Kritee et al., 2013). A notable finding from the study of mercury stable isotopes is a strong attribution of mass-independent fractionation (MIF) to photochemical reactions (Bergquist and Blum, 2009). However, for the more commonly observed mass-dependent fractionation (MDF), many mercury interactions and transformations have yet to be investigated and characterized.

In this master's thesis, I investigated reduction-oxidation reactions of mercury involving organic compounds and an inorganic ferrous mineral, and the extents of mercury stable isotope fractionation during these reactions. The objectives were: 1) to examine the kinetics and equilibration of reactions of thiol compounds and humic acids with mixed Hg(0)-Hg(II) solutions; 2) to determine mercury stable isotope fractionation factors for Hg(0)-Hg(II) in equilibrium with thiol compounds and humic acids; 3) to characterize mercury stable isotope fractionation during Hg(II) reduction by the ferrous carbonate mineral siderite (FeCO3).

Chapter 2. Methods

All glassware used for experiments was acid-cleaned sequentially with 4N and 3N HCl, and rinsed with Milli-Q water (18.2 M Ω cm, Millipore, Burlington, MA). The BrCl used for oxidizing and preserving Hg in samples was prepared following the procedure described in EPA Method 1631e, using TraceMetal-grade HCl. (Fisher Scientific, Hampton, NH)

2.1. Mixed Hg(0)-Hg(II) solutions prepared from metallic Hg(0)

A similar procedure to that of Zheng, et al., 2013 and Zheng, et al., 2019 was used to prepare solutions containing both Hg(0) and Hg(II), while limiting Hg-reactive ligands present in solution. A silicone permeation tube was prepared in oxic conditions by firmly knotting the ends of the silicone tubing (5/32" OD; Cole Parmer, Vernon Hills, IL) to contain a small droplet (0.2 - 0.4 g) of liquid Hg(0). The tubes were then submerged in deoxygenated Milli-Q water in 125 mL glass serum bottles (Wheaton Industries, Millville, NJ) in an N₂ atmosphere, sealed with bromobutyl stoppers (Wheaton) and covered completely with aluminum foil. Separate bottles were then allowed to equilibrate for 2 hours, 2 days (48 hours), and 2 months (>6 weeks) at 23-25°C. After the respective equilibration times, 5 mL samples were taken from each Hg solution for measuring total mercury (THg) and Hg(II). Samples for measuring THg were injected into sealed 40 mL septum-cap sample vials (BrooksRand Instruments, Seattle, WA) containing 1 mL concentrated BrCl (0.2 M) to oxidize all of the Hg in solution with excess to preserve the sample. Samples for measuring Hg(II) were purged with ultra-high purity N₂ gas (Airgas, Radnor, PA) for 3 min at approximately 20 mL/min, before 1 mL BrCl (0.2M) was added to preserve the remaining non-volatile Hg(II) in the sample.

Aliquots of the samples for analysis were diluted to 20 mL with Milli-Q water in analytical vials with septum caps (BrooksRand Instruments) to within operational range, before hydroxylamine hydrochloride (10% m/v) and tin (II) chloride (SnCl₂; Fisher Scientific) were added to neutralize oxidants and completely reduce the Hg in solution to Hg(0), respectively. Analysis was conducted by cold vapor atomic fluorescence spectroscopy (CVAFS) on a BrooksRand MERX-T Purge & Trap system (BrooksRand MERX-T), conforming to EPA Method 1631e. Hg(0) concentration was calculated as the difference in concentration measured between THg and Hg(II) in the same experimental solution:

$$C_{Hg(0)} = C_{THg} - C_{Hg(II)}$$
(Equation 1)

2.2. Equilibrium reaction of mixed Hg(II)-Hg(0) solutions with thiols and humic acids

The reactant Hg(II)-Hg(0) solutions were prepared as above, using the 48-hour (2 days) equilibration time. 6 mM stock solutions of mercaptoacetic acid (MCA) and 2mercaptopropionic acid (MPA) (Sigma-Aldrich, St. Louis, MO) were prepared in a Coy anaerobic glovebox (Coy Laboratory Products, Grass Lake, MI) in deoxygenated Milli-Q water, titrated to pH = 7 and maintained under an anoxic N₂ atmosphere. A 2 g/L (w/w) solution of Suwannee River humic acids ("Standard HA - Suwannee River II") obtained from the International Humic Substances Society (St. Paul, MN) was similarly prepared and maintained in anoxic conditions with deoxygenated Milli-Q water, titrated to a stable pH = 7.

50 mL serum bottle reactors (Wheaton) sealed with bromobutyl stoppers (Wheaton) and covered completely with aluminum foil, were filled completely with the 48-hour equilibrated Hg solution, leaving no headspace for Hg(0) volatilization. Sets of 5 or 6 reactors were injected with thiol solution to reach 100 μ M MCA or MPA, and inverted rapidly to mix the solution. At pre-set reaction times up to 12 hours, individual reactors were sacrificed for sampling. A series of 5 mL samples were withdrawn from each reactor and prepared for measuring THg and Hg(II), as presented above, with single samples prepared for THg measurement and triplicate samples prepared for Hg(II) measurement. The samples were similarly preserved at 17% (v/v) BrCl, and allowed to digest completely before analysis.

Humic acid experiments were conducted with a procedure similar to the thiol experiments. Three sets of reactors were prepared with 5 mg/L humic acids and the preset reaction times to sacrificially sample the reactors were staggered over 8 days. The reaction sampling times between the sets overlapped at 0 hr, 1 hr, 1 d, and 6 d, providing triplicate reaction samples at those reaction times. Single samples for THg and Hg(II) were taken from each reactor at each time point and prepared in the same manner as above.

The Hg concentrations were analyzed by CVAFS using the BrooksRand MERX-T. Isotopic analysis of the Hg samples was conducted at the University of Illinois at Urbana-Champaign on a Nu Plasma HR multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS, Nu Instruments, Wrexham, UK) using the double-spike method (Bartov et al., 2013). Samples were spiked with a calibrated ¹⁹⁶Hg/²⁰⁴Hg solution and allowed to equilibrate prior to analysis. The samples were introduced into the MC-ICP-MS using a cold vapor generator with SnCl₂ as reductant. Measurements were corrected for mass-bias using measured ²⁰⁴Hg/¹⁹⁶Hg ratios. Interferences from ¹⁹⁴Pt⁺, ²⁰³Tl⁺, ²⁰⁶Pb⁺ and HgH⁺ were negligible (Bartov et al., 2013). Isotope measurements were calculated to δ-notation using NIST 3133 as the mercury isotope standard:

$$\delta^{X} Hg = \left(\frac{\frac{X_{Hg}}{_{198}_{Hg}}}{\frac{X_{Hg_{std}}}{_{198}_{Hg_{std}}}} - 1\right) * 1000 \%_{0}$$
(Equation 2)

The primary isotope value presented for the experiments conducted here is δ^{202} Hg.

Hg(0) concentrations were calculated as the difference between measured THg and Hg(II) concentrations (Eq. 1). δ^{202} Hg values for Hg(0) were calculated using a twocomponent mixing model using the concentrations and δ^{202} Hg values measured for THg and Hg(II):

$$\delta_{Hg(0)}C_{Hg(0)} = \delta_{THg}C_{THg} - \delta_{Hg(II)}C_{Hg(II)}$$
(Equation 3)

Equilibrium isotope enrichment factors (ϵ) were calculated for the thiol and humic acid experiments from observed δ^{202} Hg for Hg(II), and calculated δ^{202} Hg for Hg(0) at equilibrium using the equation as follows:

$$\varepsilon^{202} Hg = \{\delta^{202} Hg \text{ of } Hg(II)\} - \{\delta^{202} Hg \text{ of } Hg(0)\}$$
(Equation 4)

2.3. Reduction of Hg(II) by siderite (FeCO₃)

A siderite (FeCO₃) stock solution was prepared using FeSO₄ (Sigma-Aldrich) and Na₂CO₃ (Fisher Scientific) in an anaerobic glovebox, following the procedure presented by Ha et al. (2017). The siderite precipitate was rinsed with Milli-Q water to pH 7, producing a final suspension of 59.56 g/L siderite, determined from Fe²⁺ concentration by ferrozine assay, and maintained under anoxic conditions (N₂). With a $K_{sp} = 10^{-10.89}$, almost all (>99.99%) of the Fe²⁺ is expected to be in siderite mineral form. A 100 µM HgCl₂ solution was also prepared in an anaerobic glovebox using powdered HgCl₂ (Fisher Scientific) in deoxygenated Milli-Q water.

A series of independent reactors was prepared with 30 mL of 0.1 g/L siderite (approximately 0.86 mM FeCO₃) in deoxygenated Milli-Q water, transferred anoxically as a suspension in water, in 50 mL glass serum bottles (Wheaton) sealed with bromobutyl stoppers (Wheaton) and covered completely with aluminum foil. Each reactor was injected with 0.07 mL HgCl₂ solution to 50 ppb (0.25 μ M) to initiate the reaction. After the set reaction time had passed, reactors were sacrificed and acidified with 1 mL 5 N H₂SO₄ (TraceMetal-grade, Fisher Scientific) to dissolve the siderite, stabilize the Fe(II) and stop the reaction. Acidified reactors were purged with ultra-high purity N₂ gas for 5 mins, then emptied into BrooksRand sample vials. 5 mL concentrated TraceMetal-grade HNO₃ (15 M, Fisher Scientific) was used to rinse the empty reactors and added to the sample. Reagent blank reactors were prepared with omission of the HgCl₂ reactant, and sampled with the same procedure as the reduction reactors. Hg-reactant controls were also prepared with omission of siderite, and similarly sampled with the same procedure as the reduction reactors.

The samples were analyzed by CVAFS using the BrooksRand MERX-T for Hg concentration. The Hg isotopic compositions were analyzed at the University of Illinois at Urbana-Champaign using double-spike method as described above, on a Nu Plasma HR MC-ICP-MS. Hg(0) concentrations were determined as the difference between measured THg and Hg(II) concentrations (Eq. 1). δ^{202} Hg values for the Hg(0) pool were calculated using Eq. 3.

As the siderite experiment addressed the reduction of Hg(II) by siderite, mercury isotope enrichment factors were calculated as follows:

$$\varepsilon^{202} Hg = \{\delta^{202} Hg \text{ of } Hg(0)\} - \{\delta^{202} Hg \text{ of } Hg(II)\}$$
(Equation 5)

Chapter 3. Results

3.1. Hg solutions from metallic Hg⁰ in a silicone permeation tube

Liquid Hg beads placed in silicon tubing were capable of producing solutions of

mixed Hg(0) and Hg(II). THg measurements showed that Hg(0) readily dissolved in water, as the silicone is gas-permeable and allows for diffusion of Hg(0) vapors to pass from the metallic bead into solution. As depicted in Figure 1, THg concentrations in solution increased with longer exposure to the Hg permeation tube. Hg(II) was also measurable at all three equilibration times and also showed an increase with equilibration time. There was 3.41 ppb of total dissolved Hg after 2 hours of equilibration, of which only 0.13 ppb was Hg(II). After 2 days of equilibration, the total dissolved concentration was 6.75 ppb (34 nM) and contained the highest relative Hg(II) content of the three equilibration times tested, at 31.8% (2.15 ppb). The 2-month equilibration time was intended to generate the maximum Hg concentration under the experimental laboratory conditions. This solution reached a total Hg concentration of 28 ppb, below saturation of dissolved Hg(0) in water at the same temperature (60 ppb, 0.30 μ M), with 2.81 ppb Hg(II). Most of the observed Hg(II) generation occurred after 2 days of equilibration. Between 2 days and 2 months of equilibration, the Hg(II) concentration increased by only 0.66 ppb while Hg(0) concentration increased by 21.1 ppb. Subsequent experiments using mixed Hg(0)-Hg(II) solutions were conducted with 48-hour (2-day) equilibration times for Hg reactant solutions.



Figure 1. Composition of Hg solutions equilibrating with metallic Hg through silicone permeation tubing. Hg(II) concentrations are represented by dark grey bars and Hg(0) concentrations are represented by white bars.

3.2. Equilibrium reaction of mixed Hg(II)-Hg(0) solutions with thiols and humic acids

Reactions of Hg solutions with reduced-thiol compounds MCA and MPA, reached chemical equilibrium within 12-hours. The MCA reaction (Fig. 2a) maintained a steady approximate 27% across the independent reactors through the experiment, the same as the initial Hg solution (t = 0 hr). In the MPA reaction (Fig. 2b), the Hg(II) measurements for these reaction times were less consistent than those observed in the MCA reaction, and the Hg(II) content varied by about 23% after the initial time point. The time zero (0 hr) reactor for the MPA experiment, intended to reflect the initial Hg solution concentration and composition, contained an approximate 30% lower THg concentration than the other reactors for this experimental set.

The measured δ^{202} Hg for the MCA and MPA experiments are presented in Figures 2c and 2d, respectively. The initial Hg solutions in both experiments contained Hg(II) that was isotopically heavier (higher 202 Hg/¹⁹⁸Hg ratio) than THg in the reactors, and remained heavy for the duration of the reaction. In the MCA experiment, a 0.52‰ decrease in δ^{202} Hg of Hg(II) was measured after 12 hours of reaction (Fig. 2c). Compared to the initial Hg(II) isotopic composition, the equilibrium δ^{202} Hg of Hg(II) had a lighter Hg(II) isotopic signature. In the MPA experiment (Fig. 2d), δ^{202} Hg of Hg(II) varied from -1.16‰ to 1.86‰ (mean = -1.26‰), with no systematic trend over the course of this reaction.

Reactions of Hg solutions with humic acids showed no net reduction or oxidation of the Hg over 8 days (Fig. 3a). The concentration of Hg(0) and Hg(II) remained constant compared to the initial Hg solution. The THg concentration varied between 9.69 and 12.5 ppb (48.5 and 62.5 nM). The Hg(II) content in the reactors varied between 7% and 18% of THg. No evidence of Hg(0) oxidation or Hg(II) reduction were observed in the humic acid experiment.

The δ^{202} Hg values for the humic acid experiment also exhibited no change over the course of the reaction (Fig. 3b). The average δ^{202} Hg of THg was approximately -1.45‰. The average δ^{202} Hg of Hg(II) was approximately at 0.00‰. No systematic changes in the δ^{202} Hg of Hg(II) were observed over the course of the experiment.

Equilibrium isotope enrichment factors (ϵ^{202} Hg) were calculated for the three compounds from the observed δ^{202} Hg measurements for Hg(II) and calculated δ^{202} Hg values for Hg(0) in Equation 4, using the longest reaction times to represent each reaction's equilibrium conditions (Table 1). As an example depicted in Figure 4, the observed equilibrium ϵ^{202} Hg for the MCA reaction was 1.34‰ (Table 2), from the difference between δ^{202} Hg of Hg(II) and δ^{202} Hg of Hg(0). The observed equilibrium ϵ^{202} Hg for MPA was 1.76‰ and for the Suwannee River humic acids was 1.50‰, which are similar to the equilibrium ϵ^{202} Hg for the initial (2 day) Hg solution.



Figure 2. Reaction of Hg solutions with MCA and MPA. a) Hg reacted with MCA; Hg(II) fraction of THg represented by solid squares; b) Hg reacted with MPA; Hg(II) fraction of THg represented by solid triangles and white triangles, respectively; c) δ^{202} Hg of Hg(II) during Hg reaction with MCA; d) δ^{202} Hg of Hg(II) during Hg reaction with MPA. Measured δ^{202} Hg of THg in (c) and (d) are represented by horizontal dashed black lines for the respective reactions. Error bars in Hg concentration represent 1 σ of triplicate samples. Error bars in δ^{202} Hg represent analytical uncertainty from the double-spike method.



Figure 3. Reaction of Hg solutions with Suwannee River (SR-2) humic acids. (a) fraction of THg as a function of time; Hg(II) and Hg(0) fractions are represented by black squares and white squares, respectively. (b) δ^{202} Hg as a function of time; δ^{202} Hg of Hg(II) and Hg(0) are represented by black squares and white squares, respectively. Measured δ^{202} Hg of THg is represented by horizontal dashed black lines. Error bars in Hg concentration represent 1 σ of triplicate reactors, where available.

At equilibrium:					
	[Hg(II)] (ppb)	δ^{202} Hg(II)	[Hg(0)] (ppb)	δ^{202} Hg(0)	
MCA	2.54	-0.60‰	6.40	-1.95‰	
MPA	5.50	-1.49‰	1.59	-3.47‰	
Humic acids (Suwannee River–2)	1.29	0.03‰	8.40	-1.53‰	

Table 1. Equilibrium Hg concentrations and δ^{202} Hg from thiol and humic acid reactions.



Figure 4. Calculation of the equilibrium isotope enrichment factor (ϵ^{202} Hg) from δ^{202} Hg values for Hg(II) and Hg(0) in the MCA equilibrium reaction. δ^{202} Hg values for Hg(II) and Hg(0) are represented by solid black squares and white squares, respectively. δ^{202} Hg of THg is represented by a horizontal dashed black line. Error bars in δ^{202} Hg represent analytical uncertainty from the double-spike method.

	Observed ε ²⁰² Hg from mixed Hg(0)-Hg(II)	ε ²⁰² Hg measured by Zheng et al.
MCA	1.34‰	1.25‰
MPA (2-MPA)	2.03‰ª	1.10‰
Humic Acid	1.50‰ ^{a,b}	1.54‰

Table 2. Observed equilibrium isotope enrichment factors (ϵ^{202} Hg) for thiol and humic acid reactions, compared to ε^{202} Hg values found by Zheng et al. (2019).

^a δ^{202} Hg at equilibrium similar to initial composition ^bHumic acid used in these experiments was from the Suwannee River and that used by Zheng et al. was Elliott soil humic acid, both obtained by IHSS.

3.3. Reduction of Hg(II) by siderite (FeCO₃)

The reduction of Hg(II) by siderite occurred very rapidly (Fig. 5), with approximately 60% of the Hg being reduced in 4 minutes. At the shortest reaction time (t = 4 seconds), only 74% of the introduced Hg remained in solution, indicating that 26% of the Hg(II) was reduced to Hg(0) within four seconds. The chemical reaction reached equilibrium at approximately 30 minutes. At equilibrium there was approximately 13% Hg(II) remaining, corresponding to 87% reduction to Hg(0). Control experiments were conducted for quality control. The reagent blank reactor contained negligible amounts of Hg. The Hg-reagent controls at t = 0 hr contained the measured total initial injected Hg (48 ppb, 0.24 μ M), and at t = 4 hr showed a passive 24% loss of Hg over the duration of the reaction. One reactor (t = 52 min) had a reaction time that was longer than the time marking chemical equilibrium, but was measured to have a much higher Hg(II) concentration than the other reactors with similar reaction times. Apart from the outlier, all the other independent reactors exhibited agreement of the reaction progress.

The δ^{202} Hg of the Hg(II) (Fig. 6a) showed an overall isotopic composition that was heavier than the initial HgCl₂ stock (-0.37‰). During reaction with siderite, the δ^{202} Hg values of the remaining Hg(II) initially increased towards a heavier isotopic composition. At 30 min, while the reduction reaction was ongoing, δ^{202} Hg of Hg(II) peaked at 0.55‰. After 30 min of reaction, the δ^{202} Hg values started trending towards a lighter isotopic composition and reached an equilibrium value of 0.21‰ at 1 hour. The t = 52 min reactor was the outlier in Hg concentration and similarly showed an anomalous Hg(II) isotope value of -0.14‰ which was lighter than all of the other measured δ^{202} Hg values for Hg(II). The calculated δ^{202} Hg of the Hg(0) (Fig. 6b) had a lighter isotopic composition than the HgCl₂ stock. It was inferred that the δ^{202} Hg values of Hg(0) trended towards lighter isotopic composition in the first 20 minutes and then became heavier as the reduction reaction progressed towards chemical equilibrium. The δ^{202} Hg of Hg(0) was different before and after the chemical equilibration time of 30 minutes. After 30 min, when the reduction was at chemical equilibrium, δ^{202} Hg of the Hg(0) product remained steady at -0.48‰.

Observed isotopic fractionation during the reduction of Hg(II) by siderite was characterized by determining the enrichment factors for the reduction and final equilibrium reactions. The Hg stable isotope data up to 48 minutes were used to determine the isotopic fractionation of the reduction reaction, modeled to closed system and Rayleigh distillation models (Fig. 7). An isotope enrichment factor (ϵ^{202} Hg) for the closed system model was determined from extrapolation from a linear regression of δ^{202} Hg of Hg(0) as a factor of Hg(0) formation ($f_{Hg(0)}$; Fig. 7a), calculating the ϵ^{202} Hg of the reduction reaction as the difference between the intercept ($f_{Hg(0)} = 0$) and the initial reactant δ^{202} Hg. Using this approximation, the Hg reduction by siderite had an ϵ^{202} Hg of -1.59‰ (Eq. 5). An isotope enrichment factor (ϵ^{202} Hg) for the Rayleigh distillation model was determined from a linear regression to δ^{202} Hg of Hg(0) (Fig. 7b) using the following Rayleigh distillation linear fit equation:

$$\delta^{202} Hg_{Hg(0)} = \delta^{202} Hg_{Hg(II),0} - \varepsilon \left[f_{Hg(II)} / (1 - f_{Hg(II)}) \right] \ln f_{Hg(II)}$$
(Equation 6)

Using this approximation, the Hg reduction by siderite had an ϵ^{202} Hg of -1.07‰ (Fig. 8). The Hg isotope data after 48 minutes were used to determine the equilibrium isotopic fractionation after both redox and isotopic equilibrium were established, -0.67‰ (Eq. 5).



Figure 5. Reduction of Hg(II) by siderite (FeCO₃). Fraction of remaining Hg(II) of total in solution is represented by black squares. Reagent blank controls are represented by white diamonds. Hg-reagent controls are represented by black triangles. Error bars in $f_{\rm Hg(II)}$ represents an analytical uncertainty of 2 σ .



Figure 6. Hg isotope fraction during Hg(II) reduction by siderite. a) Measured δ^{202} Hg of Hg(II) (black squares); b) calculated δ^{202} Hg of Hg(0) (white squares). δ^{202} Hg of HgCl₂ source is represented by a horizontal dashed black line in both graphs. 30 minutes of reaction time is indicated by a vertical dotted line in both graphs. Error bars in δ^{202} Hg represent analytical uncertainty from the double-spike method (0.06‰).



Figure 7. Determination of enrichment factors of Hg(II) reduction by siderite from isotopic fractionation models, using Hg(0) product. a) equilibrium fractionation in a closed system model; b) kinetic fractionation in a Rayleigh distillation model. δ^{202} Hg of HgCl₂ source is represented by a horizontal dashed black line in both graphs. Linear regressions for fractionation models are shown as a grey line, with corresponding equations and R² for the regressions. Enrichment factor (ϵ^{202} Hg) for the closed system model (a) is approximately the difference between the intercept of the regression and the δ^{202} Hg of HgCl₂ (0-48 min; -1.59‰). Enrichment factor for the Rayleigh distillation model (b) is approximate to the slope of the regression (Eq. 6; 4 – 48 min; -1.07‰). Error

bars in $f_{\text{Hg}(0)}$ and -{[f_{\text{Hg}(II)}/(1-f_{\text{Hg}(II)})]Inf_{\text{Hg}(II)}} represents an analytical uncertainty of 2σ . Error bars in δ^{202} Hg represent analytical uncertainty from the double-spike method (0.06‰).



Figure 8. Rayleigh distillation model fit to calculated isotope composition of Hg(0) from reduction by siderite. δ^{202} Hg of Hg(0) predicted from Rayleigh distillation model are calculated from values determined in Figure 7b, and are shown by the grey line. δ^{202} Hg of HgCl₂ source is represented by a horizontal dashed black line. Error bars in $f_{Hg(0)}$ represents an analytical uncertainty of 2σ . Error bars in δ^{202} Hg represent analytical uncertainty from the double-spike method (0.06‰).

Chapter 4. Discussion

4.1. Hg solutions from metallic Hg⁰ in a silicone permeation tube

The Hg solution that formed from equilibration with metallic Hg(0) through the silicone permeation tube is consistent with the Hg solution formation in previously published findings using similar methods (Zheng et al., 2019). The maximum concentration observed after 2 months of equilibration was approximately 27 ppb, within the 25 to 40 ppb (0.13 to 0.20 μ M) concentration range observed by Zheng et al. (2019), though the Hg(II) formation was higher in the procedure used in our experiments. This allowed for the potential of a mixed Hg(0)-Hg(II) solution to be made by reducing the equilibration time, as almost all of the Hg(II) that would have formed in solution was present by 48 hours. The limit for the Hg(II) formation is expected to be from available oxidant being rapidly consumed before 48 hours. By limiting the equilibration time with the Hg bead, and thereby limiting the amount of Hg(0) that would diffuse into the solution, a mixed Hg(0)-Hg(II) solution was generated. However, as demonstrated by the time-zero ($t = 0 \min$) measurements of the solution composition used in the experiments (Fig. 2), there is variability in concentration, Hg(II) formation, and isotopic compositions between different solutions prepared in the same manner.

4.2. Equilibrium reaction of mixed Hg(II)-Hg(0) solutions with thiols and humic acids

In contrast to the observations of Zheng et al. (2019), the results in Figure 2 do not show oxidation of Hg(0) by MCA or MPA. Although the thiol experiments were performed with Hg(II) present in the initial solution, the oxidation expected from the

previously published equilibrium oxidation reactions were not observed in the experiments conducted here. The extent of oxidation that was observed by Zheng et al. (2013) found equilibrium oxidation of 85% and 91% with 25-30 nM MCA and MPA, respectively, at a 100:1 thiol: Hg molar ratio. Additionally, higher thiol: Hg ratios up to 1000:1 were also tested and showed that higher oxidation extent correlated with a higher thiol:Hg ratio (Zheng et al., 2013). Zheng et al. (2019) similarly used an 80:1 thiol:Hg molar ratio and observed up to 50% Hg(II) formation. In the experiments conducted here, the thiol:Hg ratio of MCA and MPA used was near 2000:1, but no net oxidation was definitively observed in either experiment (Fig. 2 a,b). This is observed particularly in the MCA experiment where the Hg(II) fraction accounted for 30% of THg, and the Hg(II)formation expected from thiol-driven Hg(0) oxidation is absent (Zheng et al., 2013). The results obtained do not indicate any net redox transformation of Hg, suggesting that the formation of Hg-thiol complexes between MCA or MPA and the initial Hg(II) in the mixed solutions was the only reaction. The oxidation of Hg(0) by thiol functional groups may be possible in these conditions as a potential mechanism of exchange between Hg pools of different oxidation states (Cohen-Atiya and Mandler, 2003), but net oxidation may not occur in the absence of an external electron transporter/accepter such as anthraquinone-2,6-disulfonate (AQDS) (Appendix 1).

In the humic acid experiment, the Hg(II) fraction remained constant throughout the experiment, similar to the thiol experiments. The S:Hg molar ratio in the experiment was approximately 6000:1, though neither reduced-sulfur or cysteine content of the substance have been confidently determined (Averett et al., 1994). Similar to the thiol experiments, the unchanged Hg redox composition differs from the net oxidation that was observed by Zheng et al., 2012, and Zheng et al., 2019. Although this may be similar to the trend that was observed between the thiol experiments, Zheng and co-workers had additionally chemically reduced the humic acids used in their experiments with Pd catalysts and H₂ at ambient temperatures (Zheng et al., 2012). As reduced sulfur functional groups have been determined to be important factors to humic acid interactions with Hg (Skyllberg et al., 2006; Xia et al., 1999), it is unclear if the results obtained from the humic acid experiments here were in agreement with the thiol experimental results or resulted from oxidized sulfur functional groups in the humic acids used.

 δ^{202} Hg values for the MCA experiments show Hg isotope exchange towards an equilibrium isotopic composition (Fig. 2 c,d). The MCA experiment was the one of the three experiments to exhibit a prominent shift in isotopic composition, shifting from a ε^{202} Hg value 2.07‰ of the initial solution to 1.34‰ at equilibrium (Fig. 4). This equilibrium ε^{202} Hg value is similar to the value found by Zheng et al. (Table 2), and highlights the potential of systems in which Hg is at chemical equilibrium to reach isotopic equilibrium. In addition, the similar ε^{202} Hg values for both Hg(II) formation (Zheng et al., 2019) and equilibration between existing Hg(II) and Hg(0) pools as presented here indicates that the process driving isotopic fractionation was likely the same in both situations, the equilibrium isotope exchange between thiol-bound Hg(II) and dissolved Hg(0). The Suwannee River humic acid equilibrium ε^{202} Hg was similar to those measured for Elliot soil humic acid by Zheng et al., but the MPA equilibrium ε^{202} Hg differed (Table 2). As the MCA experiment demonstrated that there is the capability of establishing an isotopic equilibrium with thiol reactions with Hg solutions, it appears that the equilibrium isotope fractionation factors for MPA and the humic acids were close to

the initial Hg solution isotope compositions, exhibiting no visible departure from the initial isotopic composition.

From the experiments conducted, the process that is most likely occurring is isotope exchange involving redox transformation between thiol-bound Hg(II) initially available in the solution, and dissolved Hg(0) in solution. As the rapid bonding between Hg(II) and thiols is well established (Colombo et al., 2013; Wiederhold et al., 2010), the isotopic exchange observed would have involved exchange across oxidation states even in the absence of net redox transformation of the Hg.

4.3. Reduction of Hg(II) by siderite (FeCO₃)

The rate of Hg(II) reduction by siderite was very rapid, exceeding the reaction rate and extent that was found by Ha et al. (2017). The surface area of the siderite used here was not characterized, but the approximate mass-concentration of siderite that was used in solution corresponds to a surface area concentration of 12.36 m²/L (Ha et al., 2017). During the experiments of Ha et al., 60% of Hg(II) was reduced in approximately 30 minutes. The reduction measured here reached 88% Hg(II) loss and began to establish chemical equilibrium within the same time frame. Previously published results for reduction of Hg(II) by magnetite (Fe₃O₄), a mixed Fe(II)/Fe(III) mineral, resulted in a reduction extent that was similar to the results presented here, with approximately 80% loss after 2 hours (Wiatrowski et al., 2009). The Hg reduction by magnetite provides evidence that the reduction observed here by siderite can be reached by Fe-Hg redox interactions, even if previous characterizations of reduction by siderite do not initially agree with the reaction extent observed.

The cessation of reduction of Hg(II) at 30 minutes was also reflected in the δ^{202} Hg values. During the reduction, the increase in δ^{202} Hg of Hg(II) indicates that the remaining Hg in solution was depleted of light isotopes (Fig. 6a), while the Hg(0) formed was enriched in light isotopes (Fig. 6b). After the net reduction ended at 30 minutes, the δ^{202} Hg of Hg(0) remained constant while the δ^{202} Hg of Hg(II) abruptly began to be enriched in lighter isotopes to a constant δ^{202} Hg value, suggesting that the reduction reaction that had dominated the reaction had ended at 30 minutes and reached equilibrium. The δ^{202} Hg of Hg(II) after 30 minutes then approached isotopic equilibrium with the much larger Hg(0) pool, reaching isotopic equilibrium after an additional 30 minutes and indicating an underlying isotope exchange process still active in the solution.

The ε^{202} Hg value obtained from the closed-system model for the reduction reaction, -1.59‰ (Fig. 7a), is similar to the isotope enrichment factor found for the reduction of Hg(II) by tin (II) chloride (SnCl₂; -1.56‰) (Zheng and Hintelmann, 2010), though it is uncertain if there is a shared mechanism of Hg reduction between the Fe(II) in siderite and SnCl₂. The closed system model ε^{202} Hg for the observed reduction is also within the range of values observed for microbial Hg(II) reduction (-1.20‰ to -2.00‰). Again, it is uncertain if there is any similarity between reduction by Fe(II) in siderite and microbial reduction mechanisms (Kritee et al., 2013). The ε^{202} Hg value calculated from the Rayleigh distillation value, -1.07‰, is of smaller extent than those of previously characterized reduction ε^{202} Hg values presented above.

As equilibrium isotopic fractionation between Hg and reduced-iron minerals have not been measured, there are no directly comparative ϵ^{202} Hg values available. The equilibrium ϵ^{202} Hg for the reduction by siderite observed after final isotopic equilibrium is established, -0.67, is different from the available fractionation observed for equilibrium surface sorption to ferric mineral goethite [FeO(OH)] (-0.30‰ to -0.44‰) (Jiskra et al., 2012).

Upon closer inspection of the reaction progress, while it is clear that the reaction occurs in at least 2-phases (reduction before 30 minutes and equilibrium after 30 minutes), evidence of earlier phases of the reaction can also be seen occurring before 20 minutes of reaction time (Fig. 9). The fraction of Hg(II) in solution rapidly decreased to approximately 33% in the first 4 minutes, followed by a brief period with no change in Hg(II). This initial reduction reaction, indicating loss of 67% of Hg(II) from solution, is consistent with the Hg(II) loss that was found by Ha et al. (2017) with siderite. The following intermediate equilibrium that is observed between 4 and 20 minutes is reflected in the δ^{202} Hg of Hg(0) composition as the constant δ^{202} Hg values of -0.75‰ (Fig. 10b). Accepting this 20-minute initial reaction, a second reduction reaction can be detected, occurring between 20 and 30 minutes of reaction time, which dominates the isotopic fractionation observed for the Hg(II) reduction of the reaction with siderite (Fig 11). Unfortunately, the reaction phases are not reflected in the δ^{202} Hg of the remaining Hg(II) in solution, as is seen for the Hg(0) produced by the reaction. The time points used for the siderite experiment also do not provide for sufficient resolution to characterize the isotope fractionation from the reduction reaction that occurred in the first 4 minutes, or account for any effect it may have had on the isotopic enrichment factor determined from the overall reaction. It is also unknown if the reaction with siderite is responsible for the Hg(II) reduction observed over the course of the whole reaction, or if a Fe(II)/Fe(III) system may have developed and contributed to the second reduction reaction observed.



Figure 9. Reaction phases of reduction of Hg(II) by siderite (FeCO₃). Fraction of remaining Hg(II) of total in solution is represented by black squares. The vertical dotted line indicates 30 minutes of reaction time. Error bars in $f_{\text{Hg(II)}}$ represents an analytical uncertainty of 2 σ . Colored boxes indicate different identifiable concentration trends.



Figure 10. δ^{202} Hg of Hg(II) and Hg(0) during reaction phases of Hg(II) reduction by siderite. a) measured δ^{202} Hg of Hg(II) (black squares); b) calculated δ^{202} Hg of Hg(0) (white squares). δ^{202} Hg of HgCl₂ source is represented by a horizontal dashed black line in both graphs. 30 minutes of reaction time is indicated by a vertical dotted line in both graphs. Error bars in δ^{202} Hg represent analytical uncertainty from the double-spike method (0.06‰). Colored boxes in both graphs correspond to data points in the respectively colored and numbered boxes in Figure 9, indicating different concentration trends during reduction reaction.



Figure 11. Experimental δ^{202} Hg of Hg(0) product with modeled product formation from Hg(II) reduction by siderite, with respect to reaction phases. a) fractionation in a closed system model; b) fractionation in a Rayleigh distillation model. δ^{202} Hg of HgCl₂ source is represented by a horizontal dashed black line in both graphs. Cumulative Hg(0) product formation from fractionation models are shown as a grey lines. Error bars in $f_{Hg(0)}$ represents an analytical uncertainty of 2σ . Error bars in δ^{202} Hg represent analytical uncertainty from the double-spike method (0.06‰). Colored boxes in both graphs correspond to data points in the respectively colored and numbered boxes in Figure 9, indicating different concentration trends during reduction reaction.

4.4. Environmental Implications

The findings presented here both reinforce and challenge current understandings about mercury interactions in anoxic systems. In anoxic environments with high levels of organic matter, reduced-sulfur functional groups are still important for mercury complexation. Hg(II) in solution is known to be capable of binding to thiol groups, but the isotope exchange also indicates that equilibrium with dissolved Hg(0) across redox states is also possible. The equilibrium reaction of binding to thiols may even be a dominant stable isotope fractionation effect observed in systems with high dissolved organic matter. This means that thiols have a more important effect on mercury chemistry by being capable of complexation reactions with both dissolved Hg(II) and Hg(0). However, the results of this research indicate that thiol compounds do not readily oxidize Hg(0) which is in disagreement of the previously published work of Zheng et al (2013). The experiment results shown in Figures 2 and 3 show that MCA, MPA, and humic acids exhibit very little or no capacity for Hg(0) oxidation.

In anoxic environments with low levels of organic matter like in groundwater aquifers, Hg and potentially other redox-active metal contaminants can be reduced by minerals found in those conditions. The reduction of Hg(II) by at least two ferrous iron containing minerals is rapid (Ha et al., 2017; Wiatrowski et al., 2009) and the data presented in this thesis demonstrate that Hg stable isotopes can be used to track the reaction progress. The results displayed in Figure 6 indicate that Hg stable isotope fractionation occurs during the kinetic phase of the reaction where Hg(II) is reduced to Hg(0). However, the isotope results also show that at chemical equilibrium, isotopic exchange between Hg(II) and Hg(0) continues.

Chapter 5. Conclusions

The results of this work demonstrate that stable isotope fractionation occurs in mercury during anoxic reactions with both organic compounds containing reduced sulfur functional groups and inorganic ferrous minerals. In systems with thiols, there is evidence of isotope exchange between oxidation states of mercury when interacting with a solution of both dissolved Hg(0) and Hg(II), despite no net redox transformation. Reaction of a mixed Hg(0)-Hg(II) solution with MCA resulted in the convergence of the isotope compositions of Hg(0) and Hg(II) fractions to an equilibrium ε^{202} Hg of 1.34‰, which is similar to the fractionation observed in published findings of Hg(0) oxidation reactions with the same compound. The equilibrium ε^{202} Hg observed for MPA and Suwannee River humic acids were 2.03‰ and 1.50‰, respectively. These experiments showed that isotope equilibrium is reached in less than one hour. The mechanisms of isotope exchange between Hg(II) and Hg(0) also warrant future investigation to constrain dissolved-phase interactions, and the role of Hg binding to organic matter functional groups.

In reactions with siderite, there was rapid reduction of introduced Hg(II) and the change in processes that occurred during the reaction was clearly reflected in the isotope composition. The reaction reached chemical equilibrium at 30 minutes, with up to 87% of the mercury reduced. The reduction reaction enriched the Hg(0) product with light isotopes with an observed kinetic ε^{202} Hg of -1.59‰. The remaining Hg(II) later was observed to undergo isotopic exchange with the Hg(0) product, reaching an equilibrium ε^{202} Hg of -0.67‰. An initial reduction reaction was observed in the first 4 minutes of reaction, but was unable to be characterized due to insufficient resolution. Chemistry of interest may be occurring in the first 4 minutes of reaction that merits further study.

The findings from these experiments suggest that mercury may behave differently than previously thought and more research is needed to further refine the conclusions reached. An interesting future experiment is to examine mercury transformations in ternary Hg(II)-siderite-humic systems with applications to variable compositions of anoxic sediments. Based on the findings presented above, Hg(II) is likely be bound with thiol functional groups in humic substances, with reduction of available Hg(II) in solution to Hg(0) by siderite. At high concentrations of humic acid, the majority of available Hg(II) is expected to be bound to thiol groups in the organic matter with minimal available in solution for reduction. Low humic conditions are expected to have minimal Hg(II) bound to organic matter, with rapid reduction of unbound Hg(II) to Hg(0) by the siderite or other ferrous iron forms (Amirbahman et al., 2013; Mishra et al., 2011). These more complex experiments would test our predictive understanding of mercury transformations and provide a bridge to modeling the behavior of Hg in natural anoxic sediments.

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Appendix 1. Reaction of Hg solutions with MCA, with and without 1 mM AQDS. a) Hg reacted with MCA; Hg(II) and THg concentrations represented by solid squares and white squares, respectively; b) Hg reacted with MCA with AQDS; Hg(II) and THg concentrations represented by solid triangles and white triangles, respectively; c) δ^{202} Hg of Hg(II) during Hg reaction with MCA; d) δ^{202} Hg of Hg(II) during Hg reaction with MCA; d) δ^{202} Hg of Hg(II) during Hg reaction with MCA; d) δ^{202} Hg of Hg(II) during Hg reaction with MCA; d) δ^{202} Hg of Hg(II) during Hg reaction with MCA; d) δ^{202} Hg of Hg(II) during Hg reaction with MCA and AQDS. Measured δ^{202} Hg of THg in (c) and (d) are represented by horizontal

dashed black lines for the respective reactions. (a) and (c) are from Figs. 2a and 2c, respectively. MCA with AQDS experiment (b) was conducted using identical method as MCA experiment (a) with addition of AQDS. Error bars in Hg concentration represent 1σ of triplicate samples. Error bars presented for δ^{202} Hg represent analytical uncertainty from the double-spike method.



Appendix 2. Δ of ¹⁹⁹Hg, ²⁰⁰Hg, ²⁰¹Hg (MIF) as a function of δ²⁰²Hg (MDF). For a) MCA;
b) MPA; c) humic acid experiments. Δ^XHg values were calculated as described in
Bergquist & Blum (2007). In (c), δ²⁰²Hg of THg measurements are between -1.24‰ and -1.60‰



Appendix 3. Δ of ¹⁹⁹Hg, ²⁰⁰Hg, ²⁰¹Hg (MIF) as a function of δ^{202} Hg (MDF) for reduction by siderite (FeCO₃). Δ^{X} Hg values were calculated as described in Bergquist & Blum (2007). The values for the HgCl₂ source correspond to δ^{202} Hg of -0.37‰, the farthest set of points to the left.