Study on the simultaneous reduction of methylmercury by $SnCl_2$ when analyzing inorganic Hg in aqueous samples

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ABSTRACT

Mercury (Hg) is among the most concerned contaminants in the world. It has three major chemical forms in the environment, including Hg^0, Hg^{2+}, and methylmercury (MeHg). Due to their differences in toxicity, mobility, and bioavailability, speciation analysis is critical for understanding Hg cycling and fate in the environment. $SnCl_2$ reduction-atomic fluorescence spectrometry detection is the most commonly used method for analyzing inorganic Hg. However, it should be noted that MeHg may also be reduced by $SnCl_2$, which would result in the overestimation of inorganic Hg. In this study, the reduction of MeHg by $SnCl_2$ in both de-ionized (DI) water and four natural waters was investigated. The results showed that MeHg could be reduced by $SnCl_2$ in DI water whereas this reaction was hard to occur in tested natural waters. By investigating the effects of water chemical characteristics (dissolved organic matter, pH and common anions and cations) on this reaction, SO_4^{2-} was identified to be the dominant factor prohibiting $SnCl_2$ induced MeHg reduction in natural waters. SO_4^{2-} in natural waters was evidenced to be reduced to S^{2-} by $SnCl_2$ and the generated S^{2-} can complex with MeHg to form $MeHgS^-$ which is hard to be reduced by $SnCl_2$. Findings of this study indicate that the effect of MeHg reduction by $SnCl_2$ on inorganic Hg analysis is negligible in natural waters; however, at simulated experimental systems without SO_4^{2-}, SO_4^{2-} should be added as protecting agents to prevent MeHg reduction when analyzing inorganic Hg if it would not cause any other unwanted effects.

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Introduction

Mercury (Hg) is a global contaminant (Capelo et al., 2006) that can be released into the environment from both anthropogenic and natural sources. There are three major forms of mercury present in the environment, including elemental Hg (Hg^0), divalent inorganic mercury (Hg^{2+}), and methylmercury (MeHg). The cycling and fate of Hg in the environment largely depend on its speciation, due to their differences in toxicity, mobility, bioavailability, etc. (Schroeder and Munthe, 1998; Steffen et al., 2008) Hg^0 is the major specie of mercury in the atmosphere and it can be distributed worldwide via long-range atmospheric transport (Lindqvist and Rodhe, 1985; Mason et al., 1994). MeHg is the most toxic form of mercury and the major specie bioaccumulated via food web in the environment (Morel et al., 1998; Ullrich et al., 2001), while Hg^{2+}

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accounts for the largest portion of Hg in aquatic environments (Selin, 2009). In consideration of the importance of Hg speciation in Hg cycling and toxicology, it is necessary to determine both the total amount and the speciation of Hg in the environment.

As for Hg^{2+} determination in the environment, there are mainly two reported methods, $SnCl_2$ reduction of Hg^{2+} and subsequent detection of Hg^0 using AFS (US EPA, 2002; Guo et al., 1996; Jeremiason et al., 2015; Zheng et al., 2013) and sodium borohydride reduction (Oda and Ingle, 1981; Riisgård and Hansen, 1990). Between them, $SnCl_2$ reduction has become the most commonly used method (Dong et al., 2010; US EPA, 2002; Guo et al., 1996; Jeremiason et al., 2015; Johnson et al., 2015; Luo et al., 2017; Zheng et al., 2012, 2013) due to its virtues of high sensitivity and low detection limit when combining with gold trap pre-concentration. This method has been utilized in detecting Hg^{2+} and reactive Hg (RHg) in environmental water samples (Dalziel, 1995; Lindström, 2001; Mason and Fitzgerald, 1991; Seritti et al., 1980), as well as Hg^{2+} in filed and laboratory incubation samples for measuring the rates of Hg oxidation and reduction (Zheng et al., 2013) and the products of MeHg photodegradation (Inoko, 1981; Kim et al., 2016). However, it should be noted that MeHg usually coexists with Hg^{2+} when performing these measurements. As it is also a form of divalent Hg, MeHg has the potential of being simultaneously reduced by $SnCl_2$, which is expected to result in the overestimation of the determined Hg^{2+} if this reaction occurs.

Controversial results have been previously reported on the reduction of MeHg by $SnCl_2$ in aqueous phase (Zhang et al., 2018; Gill and Bruland, 1990; Rio-Segade and Bendicho, 1999). It has been previously found that $SnCl_2$ could reduce 20%–30% of methyl and ethyl mercury into Hg^0 in 0.5 mol/L HCl solution (Zhang et al., 2018). However, no significant reduction of MeHg by $SnCl_2$ was also observed in a surface fresh water (Gill and Bruland, 1990) and both de-ionized (DI) water and a river water (Rio-Segade and Bendicho, 1999). These inconsistent results indicate that the reduction of MeHg by $SnCl_2$ may depend on the chemical characteristics of water samples, *e.g.*, dissolved organic matter (DOM), pH, cations and anions.

The major objective of this study was to elucidate whether MeHg can be reduced into Hg^0 by $SnCl_2$ in aqueous samples (both DI water and natural waters). To achieve this aim, reduction of MeHg by $SnCl_2$ in DI water and natural waters was examined. The effects of various factors, including $SnCl_2$ and MeHg concentrations, pH, DOM, and common cations and anions, on MeHg reduction by $SnCl_2$ were further examined.

1. Materials and methods

1.1. Reagents

MeHgCl and $HgCl_2$ standard were purchased from the National Institute of Metrology, China. $Me^{201}Hg$ was synthesized by methylcobalamin according to a previously reported method (Mizanur Rahman et al., 2003). Suwannee River natural organic matter (SRNOM) was purchased from the International Humic Substances Society. All reagents used were of reagent grade or higher. Ultrapure water (>18 MΩ/cm) was used to prepare all chemical solutions.

>3 kDa SRNOM solution was obtained by ultrafiltrating 10 mL of the SRNOM solution (~200 mg/L) using a 3 kDa centrifugal filter (Sartorius, Germany) at 6000 r/min for 60 min and then being cleaned 10 times using 10 mL of DI water. >1 kD NOMs were isolated from three natural waters, including Ink River, Laoshan Reservoir and Stone Old Beach of Yellow Sea using ultrafiltration method. Filtered (0.22 μm, PTFE) water samples were ultrafiltrated using a cross-flow ultrafiltration system with a 1 kDa tangential flow filter membrane (Pall, USA), and being cleaned ten times using DI water to remove the low molecular weight DOM and inorganic ions.

1.2. Reduction of MeHg by $SnCl_2$ in DI water and natural waters

Certain amounts of MeHg were added into 25 mL DI water to form a final concentration of 100 ng/L. After equilibrating at dark for 24 hr, 0, 50, 100, 125, 250, 500, 750, and 1000 μL of 20% (W/V) $SnCl_2$ (1.78–35.6 mmol/L) were added and Hg^0 in the solutions were determined using a MERX automated modular Hg system (Brooks Rand Labs, Seattle, WA, USA). Effects of MeHg concentrations (0, 10, 20, 50, 100 ng/L) on this reaction were also tested with the addition of 125 μL $SnCl_2$. In addition, reduction of MeHg (100 ng/L) in natural waters by $SnCl_2$ (125 μL) was examined. Four natural waters were tested, including a seawater from the Stone Old Beach of Yellow Sea (36°06′06″N, 120°28′52″E), and freshwaters from the Ink River (36°18′39″N, 120°21′15″E), Laoshan Reservoir (36°15′25″N, 120°30′57″E) and a rainwater collected on May 22, 2017 in Qingdao. Ancillary environmental parameters of these natural water samples, including dissolved organic carbon (DOC), Cl^-, NO_3^-, SO_4^{2-} and pH were measured and presented in Appendix A Table S1. Triplicates were employed for all treatments.

For quality assurance and quality control (QA/QC) purpose, total Hg in samples was measured following EPA method 1631E to avoid the unwanted loss of Hg by leaching or adsorbing on vessel walls. Acceptable recoveries, in the range of 93.5%–102.7%, were obtained. MeHg at treatments without significant production of Hg^0 was also measured using isotope dilution method to assure Hg^0 was not leached or adsorbed on particles or vessel walls. Detailed data are presented in the Appendix A Table S3. For each set of 20 samples, two method blanks and two 500 pg standard, were added. Triplicate analyses were employed for each sample. For Hg^0 and total Hg analyses, the method blanks ranged from 0.2 to 0.4 ng/L, within the acceptable value of EPA method (<0.5 ng/L). Recoveries of the standard additions were in the range of 93.5% to 102.7%. The relative standard deviations (RSD) of triplicate samples ranged from 0.8% to 9.9% for Hg^0 and total Hg and from 1.7% to 5.9% for MeHg, which were also within the acceptable ranges of the EPA method (<15%). The absolute method detection limits for Hg^0 and MeHg were 5 pg and 0.6 pg, respectively.

1.3. Effects of pH, DOM, common anions and cations on the reduction of MeHg by $SnCl_2$

To assess the effects of DOM, anions, cations and pH on the reduction of MeHg by $SnCl_2$, reductions of MeHg (100 ng/L) by $SnCl_2$ at various pH values (1–11, adjusted by adding HCl (2 mol/L)

or H_2SO_4 (2 mol/L) (pH 1–6) or NaOH (1 mol/L) (pH 8–11)) and various concentrations of DOM (0–10 mg/L), K^+ (0–200 mg/L), Ca^{2+} (0–500 mg/L), Na^+ (0–2000 mg/L), Mg^{2+} (0–500 mg/L), Cl^- (0–20,000 mg/L), NO_3^- (0–200 mg/L), and SO_4^{2-} (0–2000 mg/L) were further examined.

1.4. Effects of SO_4^{2-} and S^{2-} on the complexation of MeHg with DOM in the presence of $SnCl_2$

The influence of SO_4^{2-} and S^{2-} on MeHg-DOM complexation in the presence of $SnCl_2$ was tested using ultrafiltration method. SO_4^{2-} or S^{2-} was added into 10 mL DI water containing MeHg (100 ng/L) and DOM (10 mg/L (as C)) to form a final concentration of 2 g/L and 0.2 mg/L (for SO_4^{2-}) or 0.005 and 0.05 μg/L (for S^{2-}), respectively. 125 μL $SnCl_2$ were then added into the solution. Trials without adding $SnCl_2$, or SO_4^{2-} and S^{2-} were employed as controls. After equilibrating for 24 hr at dark, the solutions were ultrafiltrated using a 3 kDa centrifugal filter (Sartorius, Germany) at 6000 r/min for 25 min. MeHg concentrations in the two fractions (>3 kDa and <3 kDa) were analyzed using an isotope dilution method and the percentage of MeHg complexed with DOM was calculated according to Eq. (1) (Han et al., 2017). Triplicates were employed for each trial. p values with and without the addition of SO_4^{2-} or S^{2-} were compared to evaluate whether they can affect the complexation of MeHg with DOM

$$P = \frac{(C_1 - C_2) * V_1}{C_1 * V_1 + C_2 * V_2} \tag{1}$$

where C_1 and C_2 are the concentrations of MeHg in the residual and filtrate solutions, respectively. V_1 and V_2 are the volumes of residual and filtrates solutions in the centrifugal tubing.

1.5. Analysis of total Hg and methylmercury

Total Hg in water samples was analyzed following US EPA method 1631E. Twenty-five milliliters of water samples were digested by adding 125 μL of BrCl for 12 hr, and then 62.5 μL of $NH_2OH·HCl$ was added to consume the residual BrCl. After that, 125 μL of $SnCl_2$ was added to convert Hg^{2+} to volatile Hg^0, which was finally detected by a Model III atomic

fluorescence spectrometer (Brooks Rand Lab., Seattle, WA, USA).

For MeHg analysis, an isotope dilution method was adopted (Song et al., 2017). 1 mL water samples were pipetted into a distilling flask with 45 mL of DI water, and then $Me^{201}Hg$ was added into the samples to form a final concentration of 1 ng/L. After equilibration at dark for 12 hr, the samples were distilled at 125 ± 3°C on a heating block under 90 ± 10 mL/min N_2 for about 2.5–4 hr to get 35 mL water at the receiving tube. 65 mL of DI water was added into the samples and the mixture was reacted with 50 μL $NaBEt_4$ (1%) for 15 min and then trapped on a Tenax trap by purging with N_2 (200 mL/min) for 15 min. MeHg on the Tenax trap was thermally desorbed at 200°C, separated by a GC column, converted to Hg^0 by heating at 800°C, and finally detected by the ICP-MS (Thermofisher iCAP Q, Thermo Fisher Scientific, Germany).

1.6. Data analysis

The efficiency constant (k) of MeHg reduction by $SnCl_2$ was calculated according to Eq. (2):

$$k = (C_{Hg^0}/C_{TMeHg}) * 100\% \tag{2}$$

where C_{Hg}^0 (ng/L) is the concentration of Hg^0 generated from MeHg reduction by $SnCl_2$; C_{TMeHg} (ng/L) is the spiked MeHg concentration in water samples at time zero.

2. Results and discussion

2.1. Possible $SnCl_2$ induced reduction of MeHg in DI water and natural waters

As shown in Fig. 1a, approximate 40%–45% of MeHg was reduced to Hg^0 in DI water and the reduction efficiency (k) depended on the added concentrations of $SnCl_2$ (Fig. 1b). k increased from 23.4% to 92.7% with the added concentrations of $SnCl_2$ increasing from 1.78 to 35.6 mmol/L. With the increase in the concentrations of MeHg, the absolute amounts of Hg^0 generated increased accordingly; however, the reduction efficiency was relatively stable, approximately

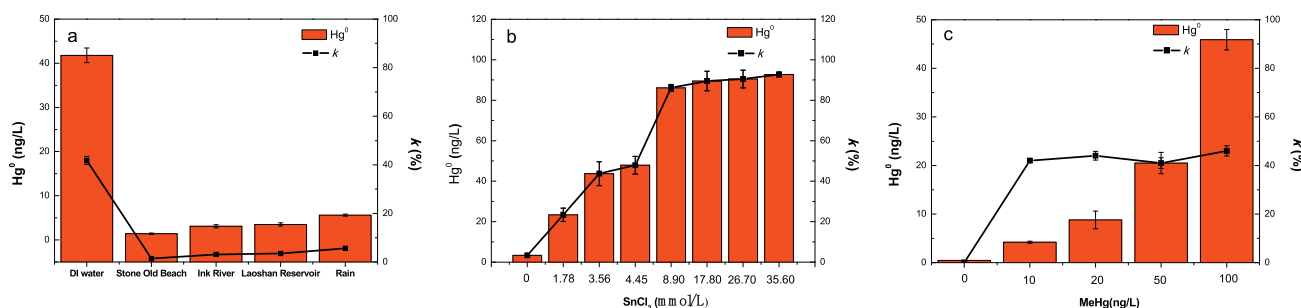**Fig. 1 – (a) $SnCl_2$ induced MeHg reduction efficiency constants (k) in de-ionized (DI) water and natural waters (Stone Old Beach, Ink River, Laoshan Reservoir and Rain water). (b) and (c) Effects of $SnCl_2$ (b) and MeHg (c) concentrations on the reduction of MeHg by $SnCl_2$.**

40% at 10–100 ng/L MeHg concentrations (Fig. 1c). Minor reduction of MeHg by $SnCl_2$ was observed in all tested natural water samples (<5%, Fig. 1a) and they were in the order of Stone Old Beach (1.4%) < Ink River (3.1%) < Laoshan Reservoir (3.5%) < Rainwater (4.6%). These results suggest that the reduction of MeHg by $SnCl_2$ is tolerable for Hg^{2+} analysis in natural waters; however, for experimental simulated experiments performing in DI water or other solutions, the reduction of MeHg by $SnCl_2$ could result in the overestimation of Hg^{2+}.

Reduction of MeHg by $SnCl_2$ in 0.5 mol/L HCl has been reported in a previous study (Zhang et al., 2018), while some other studies proposed that $SnCl_2$ can't reduce organomercury in natural waters (Gill and Bruland, 1990; Oda and Ingle, 1981), in consistence with this study. However, there is still a lack of knowledge on which constituents in natural waters prohibit the reduction of MeHg by $SnCl_2$. Effects of various chemical characteristics of natural waters, including DOM, pH, common cations and anions, on the reduction of MeHg by $SnCl_2$, were then examined.

2.2. Effects of natural water chemical characteristics on MeHg reduction by $SnCl_2$

The effect of DOM on MeHg reduction by $SnCl_2$ was firstly investigated. As shown in Fig. 2a, MeHg reduction efficiency decreased from 40.6% to 12.8% and 11.8%, respectively, with the addition of 10 mg/L SRNOM and DOM (>3 kDa) isolated from Ink river, indicating that DOM can inhibit the reduction of MeHg by $SnCl_2$ in natural waters. However, it should be noted that more than 10% of MeHg can still be reduced by $SnCl_2$ in DOM solution as high as 10 mg/L, which is higher than DOM in most natural waters (Albinet et al., 2010; Muir et al., 1994), indicating that DOM may be not the dominant factor inhibiting MeHg reduction. This was further confirmed by testing the reduction of MeHg by $SnCl_2$ in DOM isolated from several natural waters. 24.9%–31.2% of added MeHg was reduced by $SnCl_2$ in these DOM solutions at their natural levels (3.35 mg/L for Stone Old Beach, 12.97 mg/L for Ink River and 5.12 mg/L for Laoshan Reservoir), much higher than that in the natural waters (1.4%–3.5%) and <3 kDa filtrates (1.9%–3.8%) (Fig. 2b). Cations or anions of natural waters were still

present in the <3 kDa filtrates. These results suggest that some cations or anions, rather than DOM, may play a crucial role in inhibiting MeHg reduction by $SnCl_2$.

Common cations and anions, including K^+, Ca^{2+}, Na^+, Mg^{2+}, and Cl^-, NO_3^-, SO_4^{2-} were then examined. As shown in Table 1, K^+, Ca^{2+}, Na^+, Mg^{2+} and NO_3^- had a negligible influence on MeHg reduction (one-way ANOVA, $p > 0.05$) at all tested concentrations, while MeHg reduction was decreased from 40.6% to 17.2% at 20 g/L Cl^-. Since Cl^- at natural water concentrations (0–200 mg/L for fresh water, 200–20,000 mg/L for saline water) showed a minor effect on MeHg reduction by $SnCl_2$ (k was expected to be 18.6%–21.8% in fresh water and 17.2%–19.2% in saline water), Cl^- could not be the major factor prohibiting $SnCl_2$ induced MeHg reduction in natural waters. SO_4^{2-} largely reduced MeHg reduction, <5% MeHg was reduced by $SnCl_2$ at 0.2 mg/L to 2 g/L SO_4^{2-}. SO_4^{2-} in the tested natural waters was in the range of 21.2–1925.3 mg/L, indicating that SO_4^{2-} in these waters may play a dominant role in prohibiting the reduction of MeHg by $SnCl_2$. The effects of SO_4^{2-} in the presence of >1 kD NOM (3.35 mg/L for Stone Old Beach, 12.97 mg/L for Ink River and 5.12 mg/L for Laoshan Reservoir) isolated from the three tested natural waters were also examined. Less than 5% of MeHg was reduced with the addition of SO_4^{2-} at natural levels (1925.3 mg/L for Stone Old Beach, 306.4 mg/L for Ink River and 62.8 mg/L for Laoshan Reservoir) (Fig. 2b), similar to the results obtained in SO_4^{2-} solutions.

The effect of pH on the reduction of MeHg by $SnCl_2$ was further tested and the results are shown in Fig. 3a and b. MeHg reduction rate was highest at pH 4–6, and decreased to 21.2% at pH 1 (adjusted with HCl) and 22.1% at pH 11. As the reduction rate was over 20% at all tested pH, pH could not be the major factor of prohibiting MeHg reduction in natural waters. Inconsistent results were observed when pH was adjusted using H_2SO_4 (Fig. 3b). MeHg reduction rates were decreased gradually from 43.3% to 3.4% when pH decreased from 6 to 2; however, MeHg reduction rate increased back to 37.3% at pH 1. In consideration of the much weaker effect of adjusting using HCl, the greater effect of adding H_2SO_4 could not be due to the change in pH, but the increase in SO_4^{2-} (0.048 and 0.48 g/L at pH 3 and 2). The abnormal high reduction of MeHg at pH 1 could be due to the minor effect of SO_4^{2-} at high H^+ concentrations. This was validated by examining the
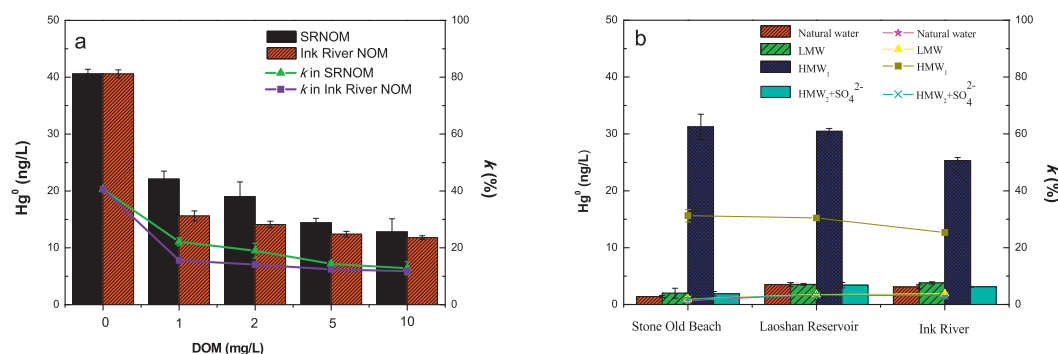
Fig. 2 – Effects of dissolved organic matter (DOM) on MeHg reduction by $SnCl_2$. (a) DOM concentration effects, (b) DOM fraction effects. High molecular weight (HMW) is >1 kD DOM isolated from natural waters. Low molecular weight (LMW) is the filtrates composed of low molecular weight DOM and ions.

Table 1 – Reduction of MeHg by SnCl$_2$ in the presence of common cations and anions.

| Concentration (mg/L)	Generated Hg^0 (ng/L)						
	Na$^+$	Ca^{2+}	Mg^{2+}	K$^+$	NO$_3^-$	SO$_4^{2-}$	Cl$^-$
0	40.6 ± 1.6	40.6 ± 1.6	40.6 ± 1.6	40.6 ± 1.6	40.6 ± 1.6	40.6 ± 1.6	40.6 ± 1.6
0.01	/	/	/	/	39.2 ± 0.9	/	/
0.05	/	/	33.2 ± 6.2	/	37.9 ± 2.3	/	/
0.1	30.8 ± 0.2	43.9 ± 0.2	37.0 ± 0.8	37.6 ± 5.1	/	/	/
0.2	/	/	/	/	/	3.1 ± 0.3	/
0.5	/	/	/	39.1 ± 3.0	/	/	19.2 ± 1.0
1	35.2 ± 0.6	44.9 ± 6.2	39.3 ± 2.5	40.2 ± 0.3	41.3 ± 1.5	2.7 ± 0.3	18.6 ± 0.7
5	/	/	/	/	/	/	/
10	32.1 ± 2.3	49.0 ± 3.1	36.9 ± 2.4	45.8 ± 4.5	43.9 ± 0.6	3.8 ± 0.3	/
20	/	45.5 ± 2.4	/	/	/	/	/
50	31.8 ± 0.5	/	/	/	/	/	21.8 ± 0.7
100	/	47.2 ± 8.3	37.1 ± 0.5	43.9 ± 3.6	39.2 ± 5.2	3.7 ± 0.3	/
200	/	/	/	42.3 ± 4.2	43.6 ± 1.2	/	/
500	/	48.2 ± 0.5	37.6 ± 0.8	/	/	/	19.2 ± 1.4
1000	48.4 ± 0.7	/	/	/	/	3.8 ± 0.5	17.2 ± 0.3
2000	42.1 ± 0.2	/	/	/	/	3.2 ± 0.3	/
20,000	/	/	/	/	/	/	18.2 ± 3.2

influence of pH (adjusted using HCl) on the inhibition effect of SO$_4^{2-}$ (4.8 g/L) (Fig. 3c). k was lower than 5% at pH 2–7, while it was increased to 18.7% at pH 1, in consistence with the results of adding H$_2$SO$_4$.

Cl$^-$ is an important binding ligand for Hg in natural waters (Morel et al., 1998). The increase of Cl$^-$ in water can increase the proportion of MeHg complexed with Cl$^-$. Since high energy is needed to break down the C-Hg of MeHg when bonding with Cl$^-$ (Tossell, 1998), it is reasonable to observe the low reduction of MeHg at higher Cl$^-$ concentrations. The low MeHg reduction efficiency in the presence of DOM may also be due to the formation of MeHg-DOM complexes (Aiken et al., 2011; Qian et al., 2014), which may further inhibit the reduction of MeHg by SnCl$_2$ at some extent.

SO$_4^{2-}$ was identified to be the dominant factor protecting MeHg from reduction by SnCl$_2$ in natural waters even at a concentration of as low as 0.2 mg/L. Since most natural waters have a SO$_4^{2-}$ of higher than this concentration, the effect of MeHg reduction by SnCl$_2$ could be negligible when analyzing natural water samples. However, at some simulated experimental systems without any SO$_4^{2-}$, e.g., the laboratory or filed incubation studies on Hg reduction/oxidation/demethylation (Zheng et al., 2013; Monperrus et al., 2007) or the products of MeHg photodegradation (Inoko, 1981; Kim et al., 2016; Sun et al., 2012), SO$_4^{2-}$ should be added as protecting agents when determining Hg^{2+} in these samples. Otherwise, the determined Hg^{2+} could be greatly overestimated. However, in some previous laboratory studies performed in DI water or solutions without SO$_4^{2-}$, for example the determination of MeHg photodegradation products (Inoko, 1981; Kim et al., 2016; Sun et al., 2012), Hg^{2+} was determined using the SnCl$_2$ reduction without the consideration of MeHg reduction by SnCl$_2$. The determined Hg^{2+} in these studies could be significantly overestimated and should be re-evaluated.

The dramatic decrease in MeHg reduction in the presence of SO$_4^{2-}$ could be caused by several pathways, including (1) affecting the reduction capability of SnCl$_2$, (2) forming complexes with MeHg that are difficult to be reduced, (3) affecting the determination of Hg^0, or (4) generating some products that can inhibit MeHg reduction. The complexation constant of SO$_4^{2-}$ with MeHg (log$K$) was 0.94, much lower than Cl$^-$ (log$K$ = 5.25), OH$^-$ (log$K$ = 9.37) (Rabenstein, 1978) and DOM (log$K$ = 13.00–15.00) (Amirbahman et al., 2002; Hintelmann et al., 1997; Karlsson and Skyllberg, 2003). This indicates that the inhibition effect of SO$_4^{2-}$ may not be via forming complexes with MeHg. It has been previously reported that SO$_4^{2-}$ can be reduced to S^{2-} in the presence of SnCl$_2$ (Kiba et al., 1955). S^{2-} has a high affinity with MeHg (log$K$ = 21.50) (Morel et al., 1998). The formation of
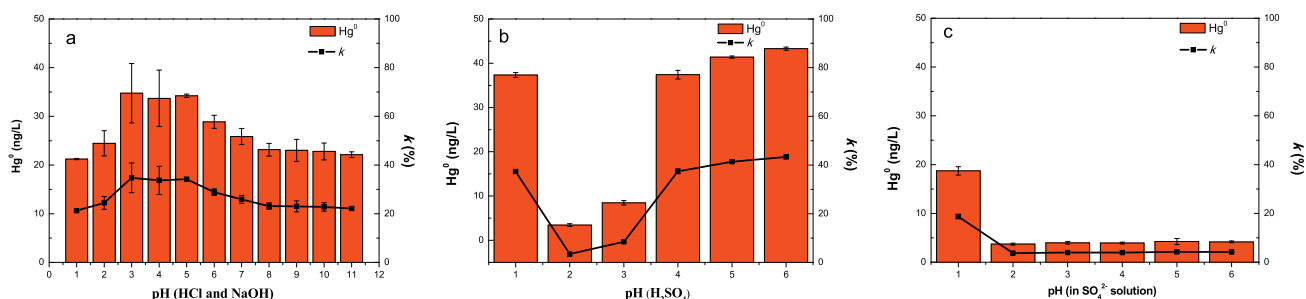
Fig. 3 – Effects of pH on MeHg reduction by SnCl$_2$. (a) adjusting pH using HCl and NaOH, (b) adjusting pH (1–6) using H$_2$SO$_4$, (c) SO$_4^{2-}$ solution at various pH values (1–6) adjusted using HCl.

MeHgS⁻ may prohibit the reduction of MeHg by SnCl₂. These possible pathways were further tested in the next section to elucidate the mechanism of SO₄²⁻ inhibition on MeHg reduction.

2.3. Possible mechanisms of inhibition of SO₄²⁻ on SnCl₂ induced MeHg reduction

The effects of SO₄²⁻ on the reducing capability of SnCl₂ and determination of Hg⁰ were firstly tested. As shown in Table 2, good recoveries of Hg²⁺ and Hg⁰, 94.9%–96.6% for Hg²⁺, and 94.7%–96.9% for Hg⁰ were observed with the addition of 2 g/L SO₄²⁻. These results indicate that the inhibition effects of SO₄²⁻ are neither due to its influence on the reducing capability of SnCl₂, nor the determination of Hg⁰. The effects of SO₄²⁻ on MeHg complexation were further examined. As shown in Fig. 4b, the complexation of MeHg with DOM was only slightly affected by the added SO₄²⁻. The percentage of MeHg complexed with DOM was determined to be 92.3% without adding any SO₄²⁻, while this ratio was 89.7% and 85.1% at 0.2 mg/L and 2 g/L SO₄²⁻, respectively. This finding ruled out the possible influence of SO₄²⁻ on MeHg reduction by direct complexation with MeHg.

S²⁻ in SO₄²⁻ solutions and natural waters after the addition of SnCl₂ were analyzed using the Methylene Blue Method (Reese et al., 2011). 0.005–0.050 μg/L of S²⁻ were detected at 0.5 mg/L-2 g/L SO₄²⁻ while the concentrations were determined to be 0.025–0.053 μg/L in natural waters with the addition of SnCl₂ (Appendix A Table S2). In addition, less than 5% of MeHg was observed to be reduced by SnCl₂ at 0.005–0.05 μg/L S²⁻ (Fig. 4a). These results suggest that the inhibition effect of SO₄²⁻ on MeHg reduction by SnCl₂ may be due to the generation of S²⁻ in the presence of SnCl₂. The produced S²⁻ may subsequently complex with MeHg, and then prohibits its reduction. This was further validated by testing the influence of SO₄²⁻ and S²⁻ on MeHg-DOM complexation in the coexistence of SnCl₂. With the addition of SnCl₂ (4.5 mmol/L), the percentage of MeHg complexed with SRNOM (10 mg/L) was decreased from 92.3% to 59.5% due to the complexation of MeHg with Cl⁻. The addition of 2 g/L SO₄²⁻ and 0.05 μg/L S²⁻ significantly decreased this percentage to 9.8% and 10.9%, respectively. These findings suggest that S²⁻, produced from the reduction of SO₄²⁻ by SnCl₂, can form stable MeHgS⁻ and then prohibit SnCl₂ induced MeHg reduction in natural waters.

Thermodynamic calculations were performed to validate this hypothesis. The major species of MeHg in natural waters in the presence of SnCl₂ and S²⁻ were calculated. MeHg⁺,

MeHgS⁻, MeHgCl, MeHg-DOM complexes were considered to be the major species of MeHg in aqueous phase (Morel et al., 1998). Since the pH was in the range of 1–2 after adding acidic SnCl₂, MeHgOH could be omitted. MeHg-DOM complexes were described as MeHgSR. The complex stability constants of MeHg with S²⁻, Cl⁻ and DOM were 21.50, 5.75 and 13.00–15.00, respectively (Appendix A Table S4). By calculating with Appendix A Eq. (S7), the percentage of MeHg complexed with S²⁻ was >99.7% at 0.05 μg/L S²⁻, suggesting that SO₄²⁻ in natural waters could significantly affect the complexation of MeHg with ligands in the presence of SnCl₂. The abnormal increase of MeHg reduction in the presence of SO₄²⁻ at pH 1 can also be explained by the mechanism proposed. At pH 1, most S²⁻ is expected to be present in the form of H₂S (>99.5%), as calculated according to Eqs. (3) and (4). This can inhibit the complexation of S²⁻ with MeHg and subsequently weaken the inhibition effect of SO₄²⁻ on MeHg reduction by SnCl₂.

$$\delta_{H_2S} = \frac{[H_2S]}{[H_2S] + [HS^-] + [S^{2-}]} = \frac{\left[H^+\right]^2}{\left[H^+\right]^2 + K_{a1}\left[H^+\right] + K_{a1}K_{a2}} \qquad (3)$$

$$\delta_{S^{2-}} = \frac{[S^{2-}]}{[H_2S] + [HS^-] + [S^{2-}]} = \frac{K_{a1}K_{a2}}{\left[H^+\right]^2 + K_{a1}\left[H^+\right] + K_{a1}K_{a2}} \qquad (4)$$
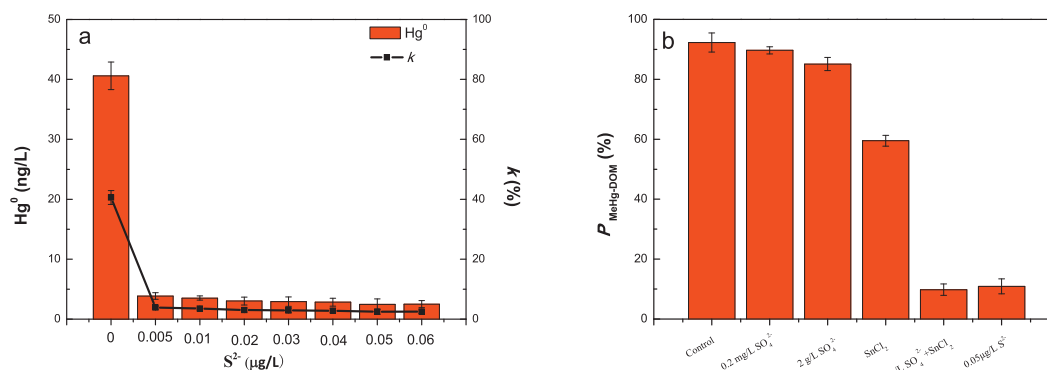
3. Conclusions

Speciation analysis is critical for understanding Hg cycling and fate in the environment. SnCl₂ reduction-purge and trap-atomic fluorescence spectrometry detection is the most commonly used method to determine Hg²⁺ or RHg. However, MeHg may also be reduced by SnCl₂, which could result in the overestimation of inorganic Hg analyzed. In this study, the reduction of MeHg by SnCl₂ in both de-ionized (DI) water and four natural waters were investigated. However, it should be noted that MeHg may also be reduced by SnCl₂, which would result in the overestimation of inorganic Hg. In this study, reduction of MeHg by SnCl₂ in DI water and natural waters was examined. The effects of various factors, including SnCl₂ and MeHg concentrations, pH, DOM, and common cations and anions, on MeHg reduction by SnCl₂ were further examined.

Approximate 40%–45% of MeHg was reduced to Hg⁰ in DI water, whereas <5% of MeHg was reduced in tested four

Table 2 – Effects of SO₄²⁻ and S²⁻ on the reducibility of SnCl₂ and the determination of Hg⁰. $P\left(\frac{Hg^{2+} \text{ in } SO_4^{2-}}{Hg^{2+} \text{ in DI water}}\right)$ (%) is the ratio of Hg²⁺ detected in SO₄²⁻ solution to that detected in DI water. $P\left(\frac{Hg^0 \text{ in } SO_4^{2-}}{Hg^0 \text{ in DI water}}\right)$ (%) is the ratio of Hg⁰ detected in SO₄²⁻ solution to that detected in DI water.

		Hg²⁺ (pg)	$P\left(\frac{Hg^{2+} \text{ in } SO_4^{2-}}{Hg^{2+} \text{ in DI water}}\right)$ (%)	Hg⁰ (pg)	$P\left(\frac{Hg^0 \text{ in } SO_4^{2-}}{Hg^0 \text{ in DI water}}\right)$ (%)
SO₄²⁻ (g/L)	2	500	96.6 ± 0.2	500	96.2 ± 1.0
	2	1250	94.9 ± 0.2	1250	95.6 ± 1.3
	2	2500	95.8 ± 0.6	2500	94.7 ± 1.5
S²⁻ (μg/L)	0.05	/	/	500	95.3 ± 2.1
	0.05	/	/	1250	96.7 ± 2.4
	0.05	/	/	2500	96.2 ± 4.2

Fig. 4 – Reduction of MeHg by $SnCl_2$ in 0–0.06 μg/L S^{2-} solutions (a) and the effects of SO_4^{2-} and S^{2-} on the complexation of with DOM (%) in the presence of $SnCl_2$ (b).

natural waters. These results suggest that the reduction of MeHg by $SnCl_2$ is tolerable for Hg^{2+} analysis in natural waters while significant reduction of MeHg was observed in DI water. K^+, Ca^{2+}, Na^+, Mg^{2+} and NO_3^- had a negligible influence on MeHg reduction. Although MeHg reduction was significantly decreased with the addition of DOM and Cl^-, they should not be the dominant factors. SO_4^{2-} was observed to largely inhibit MeHg reduction (<5% MeHg was reduced by $SnCl_2$), indicating that it may be the factor prohibiting MeHg reduction by $SnCl_2$ in natural waters.

Theoretically, the dramatic decrease in MeHg reduction in the presence of SO_4^{2-} could be caused by four pathways, including (1) affecting the reduction capability of $SnCl_2$, (2) forming complexes with MeHg that are difficult to be reduced, (3) affecting the determination of Hg^0, or (4) generating some products that can inhibit MeHg reduction. Good reducing capability of $SnCl_2$ and recoveries of Hg^0 were obtained in the presence of SO_4^{2-}. In addition, SO_4^{2-} had a minor influence on the complexation of MeHg with DOM. These experimental evidences ruled out pathways 1–3. S^{2-} was found to be produced via the reduction of SO_4^{2-} by $SnCl_2$. Further experiments showed that S^{2-} significantly prohibited $SnCl_2$ induced MeHg reduction by forming $MeHgS^-$ which are hard to be reduced by $SnCl_2$. These results suggest that SO_4^{2-} can inhibit the reduction of MeHg by $SnCl_2$ through the generation of S^{2-}.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2018.02.017.

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