Possible alkylation of inorganic Hg(II) by photochemical processes in the environment

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ABSTRACT

The methylation of inorganic Hg by anaerobic bacteria in aquatic environments is considered to be the major pathway for methylmercury (MeHg) production. However, recent research has suggested that abiotic or chemical methylation by humic substances and other low-molecular-weight organic compounds in natural environments is also possible. Here, the aqueous photo-transformation of Hg²⁺ to organomercurials was investigated in the presence of ketones, aldehydes and low molecular weight organic acids under UV irradiation. MeHg and/or ethylmercury (EtHg) were identified as the main organomercurial products by multiple analytical techniques, including chromatography-atomic spectrometry and molecular mass spectrometry and further confirmed by stable isotope tracer experiments. The yield of organomercurials was markedly influenced by pH, NaCl concentration, alkylation donor concentration and the presence of chelating ligands in the aqueous solution. Electron paramagnetic resonance spectrometry demonstrated that the radical reaction was not the predominating alkylation pathway, although methyl radicals were detected in the photo-alkylation procedure. A mechanism based on intra-molecular alkyl transfer in the Hg²⁺-low-molecular-weight organic compound complex is proposed. The present work helps us better understand of MeHg and EtHg photo-generation in natural environments.

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1. Introduction

Methylmercury (MeHg), which is ubiquitous in various environmental compartments including water, sediment, and the atmosphere (Kieber et al., 2008), is of great concern because of its neurotoxicity and bio-accumulation in the food chain. Much effort has been made to explore possible sources of MeHg. Biological methylation by sulfate-reducing bacteria (Benoit et al., 2003) and iron-reducing bacteria (Fleming et al., 2006) is considered to be the main source of MeHg in aquatic environments. However, chemical methylation, another possible pathway for MeHg formation, has been studied only to a limited extent. Recent research has revealed that the abiotic production of MeHg by dissolved organic matter (DOM) under solar radiation is an important source of MeHg in the freshwaters of some lakes (Siciliano et al., 2005). Methylcobalamin (Imura et al., 1971), humic substances (Nagase et al., 1982; Weber et al., 1985) and methyltin compounds (Jewett et al., 1978; Cerrati et al., 1992) have been identified as possible methyl donors for MeHg production in the environment.

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Chemical and photochemical methylation play important roles in the transformation, transport and biogeochemical cycle of metals, metalloids and nonmetallic elements (Keppler et al., 2000; Hamilton et al., 2003; Moore, 2008). Photochemical alkylation of As (Guo et al., 2005; McSheehy et al., 2005), Se (Guo et al., 2003) and I (Grinberg et al., 2009) by low-molecular-weight organic acids was recently demonstrated. Under UV irradiation, acetic acid can transform As, Se and I to their corresponding methylated species. However, the photochemical methylation of mercury has been studied only to a limited extent (Akagi and Takabatake, 1973; Siciliano et al., 2005). In 1973, Akagi and coworkers observed that solar irradiation transformed Hg²⁺ into MeHg in the presence of acetic acid, methanol or ethanol (Akagi and Takabatake, 1973). Recently, Hammerschmidt et al. suggested that MeHg is possibly formed in wet deposition through the chemical methylation of labile Hg²⁺ by a methylating agent (especially acetate) (Hammerschmidt et al., 2007). A comprehensive study on the possible methylating donors and related mechanism for Hg methylation is needed. Low-molecular-weight organic compounds (LMWOCs) including ketones, aldehydes, and low-molecular-weight organic acids, are important metabolites of micro-organisms (Musa-Veloso et al., 2006). LMWOCs (Atkinson and Arey, 2003) are widely present in surface water (Hudson et al., 2007), soil (Jones et al., 2003), and

the atmosphere (Poschl et al., 2001) and are potential methyl donors for mercury methylation.

In the present study, the aqueous phase alkylation of inorganic mercury, Hg^{2+} , by ketones, aldehydes and low-molecular-weight organic acids under UV irradiation was investigated. Influencing factors such as pH, salinity, concentrations of Hg^{2+} and the methyl donor, chelating reagents and the reaction time of alkylation were considered. The mechanism of photochemical alkylation was also preliminarily studied. This work can help improve our understanding of the possible photochemical methylation process and the alkylation mechanism of mercury in natural environment.

2. Experimental methods

2.1. Chemicals

All of the organomercurials were purchased from Merck ($\geq 98\%$, Darmstadt, Germany). Stock solutions of standard organomercury chloride (1 mg mL^{-1} as Hg) were prepared by dissolving the appropriate amounts of methylmercury chloride (MeHg) or ethylmercury chloride (EtHg) in methanol, respectively. A stock solution of standard inorganic mercury (1 mg mL^{-1} as Hg) was prepared by dissolving an appropriate amount of HgCl_2 ($\geq 99.5\%$, Beijing chemical factory, Beijing) in 10% (v/v) hydrochloric acid (HCl). Enriched ^{199}HgO (91.09%) was purchased from Oak Ridge National Laboratory (Oak Ridge, Tennessee) and dissolved in 10% (v/v) HCl. All of the ketones, aldehydes and low-molecular-weight organic acids were analytical grade (Beijing Chemical Reagent, China). All other chemicals were analytical grade or better. The mobile phase and standard solutions were prepared with de-ionized water obtained from a Barnstead ultra-pure water system (Barnstead International, Dubuque, IA, USA).

2.2. Photochemical experiments

Photo-reaction experiments were performed in a flow-through photo-reactor consisting of 4.5 m of PTFE tubing (0.8 mm i.d. and 1.5 mm o.d., Kaisheng insulation material company, Shenzhen, China) wrapped around a low-pressure Hg vapor UV lamp

(254 nm, 11 W, Beijing Research Institute for Light and Electric Instruments, Beijing, China). The flow-through photo-reactor was enclosed in a box for safety considerations. The reaction solution was pumped through the reactor with a peristaltic pump (D100C, Huxi Instrumental Company, Shanghai, China) at different flow rates, which resulted in varied irradiation times. The effluent from the reactor was analyzed directly by high-performance liquid chromatography coupled with atomic fluorescence spectroscopy (HPLC-AFS) or inductively coupled plasma mass spectrometry (ICP-MS) as well as gas chromatography hyphenated with mass spectrometry (GC-MS) after aqueous derivatization.

For the alkylation reaction of Hg^{2+} ($1 \mu\text{g L}^{-1}$) and propionic acid (1 mM), a bulk solution containing the reactants was irradiated with UV for 0.5 h instead of flowing through the photo-reactor. Briefly, the reaction solution was transferred to quartz reaction vessels ($12 \times 1 \text{ in.}$, 160 mL, Teflon screw top) and sealed before the photo-reaction. Irradiation in solution was performed using a Rayonet photochemical reactor (model RPR-100, Southern New England Ultraviolet) equipped with four 8 W low-pressure mercury lamps (wavelength 365 nm). The reaction products were analyzed by aqueous phenylation-purge-and-trap-GC coupled with ICP-MS.

2.3. Instrumentation and analytical methods

HPLC-AFS was used to qualitatively and quantitatively determine MeHg and EtHg. Moreover, HPLC-ICP-MS, aqueous ethylation-solid phase micro-extraction (SPME) coupled with GC-MS and aqueous phenylation-SPME coupled with GC-MS were all used to confirm the formation of MeHg and/or EtHg. For the reaction solution with $1 \mu\text{g L}^{-1}$ Hg^{2+} , only trace amounts MeHg or EtHg at the ng L^{-1} level were produced. Therefore, a highly sensitive aqueous a phenylation-purge-and-trap-GC coupled with ICP-MS method (Mao et al., 2008) was used to identify MeHg and EtHg. To further elucidate the mechanism of photochemical alkylation, UV-visible spectrometry, capillary electrophoresis (CE) and electron paramagnetic resonance spectrometry (EPR) were used to probe the formation of the Hg^{2+} -organic molecule complex, identify the photochemical products and determine the photo-induced

Fig. 1. HPLC-AFS chromatograms characterizing mercury species after UV irradiation in the presence of (a) acetone, (b) butanone, (c) 2-pentanone, (d) 3-pentanone, (e) propionaldehyde and (f) propionic acid. Peak identification: 1, unknown Hg species; 2, MeHg; 3, Hg(II) and 4, EtHg.

radicals, respectively. Details of the instrumentation and analytical methods are presented in the [Supplementary material](#).

3. Results and discussions

3.1. Identification of MeHg and EtHg

HPLC–AFS and HPLC–ICP–MS were applied to identify MeHg and EtHg by their retention times. The reaction products were further confirmed by GC–MS based on their structural information (mass-to-charge ratio). Typical HPLC–AFS chromatograms are presented in [Fig. 1](#). By matching their retention times and spiking with the corresponding mercury standards, MeHg and/or EtHg

were identified as the alkylation products of inorganic mercury. These results were validated using HPLC–ICP–MS by simultaneously monitoring the ^{199}Hg and ^{202}Hg isotopes. A ^{199}Hg -enriched Hg^{2+} solution was allowed to proceed under UV irradiation in the presence of ketones and HPLC–ICP–MS analyses revealed that ^{199}Hg was the main isotope in the detected MeHg and EtHg (as shown in [Fig. S1](#)), suggesting that the detected MeHg and EtHg were not from contamination in the analytical process but were photochemical products.

Chromatography coupled with atomic spectrometry (AFS) and elemental mass spectrometry (ICP–MS) can identify organomercurials only by matching retention times with standards, but cannot provide structural information. Therefore, the complementary use

Fig. 2. Mass spectra of (a) MeHgPh and (b) EtHgPh with the corresponding standards, and the mass spectra of (c) MeHgPh and (d) EtHgPh from the acetone and 3-pentanone photo-reaction system obtained with phenylation–SPME–GC–MS: $m/z = 308$, EtHgPh^+ ; 279, PhHg^+ ; 231, EtHg^+ ; 294, MeHgPh^+ ; and 217, MeHg^+ .

Fig. 2 (continued)

of molecular mass spectrometry is beneficial to confirming the structures of organomercurials. GC–MS has been widely used as a powerful technique for the identification of organic compounds because it can provide molecular and structural information. Ethylation–SPME–GC–MS was applied for MeHg identification (as shown in Fig. S2) but could not distinguish EtHg from Hg^{2+} because their ethylation products are both diethylmercury. Therefore, a phenylation–SPME–GC–MS procedure was further used to simultaneously identify MeHg and EtHg in the photo-reaction solution (Fig. 2). In both ethylation- and phenylation–SPME–GC–MS, the isotope patterns and mass spectra of the Hg species in the photo-reaction solution were essentially the same as those of MeHg

and/or EtHg standards, strongly suggesting that the photochemical products of inorganic Hg^{2+} are MeHg and/or EtHg.

The HPLC–AFS and/or ICP–MS analysis also revealed that some unknown mercury species were produced in the photo-reaction solution (Fig. 1c, d and f). Their retention times on the hydrophobic C18 column were shorter than those of MeHg and EtHg, indicating that these unknown mercury species are more hydrophilic than MeHg and EtHg. In a previous study on the photochemical alkylation of As^{3+} by acetate, anionic organoarsenic species containing carboxyl- or hydroxyl-containing side chains were identified using LC–MS (McSheehy et al., 2005). It is also possible that organomercurials containing hydrophilic groups were produced during the

photo-reaction of Hg^{2+} . However, their exact chemical structures need to be further determined.

3.2. Photo-alkylation by various alkylation donors

Some potential alkylation donors including ketones, aldehydes, low-molecular-weight organic acids, alcohols, acetonitrile and N, N-dimethylformamide (DMF) were compared for their capabilities to methylate Hg^{2+} . Their corresponding alkylation products are shown in Table S1. Formaldehyde, formic acid and oxalic acid, which do not contain any methyl or ethyl groups, cannot lead to the formation of organomercurials. MeHg was detected when other organic compounds were used as the alkyl donor. EtHg was generated when acetone, butanone, pentanone, propionaldehyde, propionic acid, and malonic acid were used as alkylation donors (butanone, pentanone, propionaldehyde, and propionic acid contain ethyl groups). Although acetone and malonic acid do not contain an ethyl group, other organic molecules containing an ethyl or ethylene group, which might have been produced by alkyl re-arrangement under UV irradiation (Szalai et al., 1998; Guo et al., 2005; Si and Ariya, 2008), may have functioned as ethyl donors.

The MeHg yields obtained with different alkyl donors were compared (Fig. S3). The results indicated that ketones had the highest MeHg yield, followed by aldehydes, low-molecular-weight organic acids, and alcohols. Molecules containing carbonyl groups, such as ketones, DMF, aldehydes, and low-molecular-weight organic acids, exhibited relatively higher methylation capabilities.

The alkylation of Hg^{2+} by ketones was studied both with and without UV irradiation to investigate the effect of UV irradiation on the alkylation reaction. MeHg or EtHg was also detected without irradiation, although the concentration of produced organomercurials was relatively lower. Trace levels of MeHg ($\sim 1 \mu\text{g L}^{-1}$) could be detected several minutes after $500 \mu\text{g L}^{-1} \text{Hg}^{2+}$ and 1 mM acetone were mixed under dark conditions, and the yield of MeHg did not increase significantly with the reaction time, in agreement with a previous study (Gardfeldt et al., 2003). Under UV irradiation, the yield of MeHg and EtHg increased significantly, demonstrating that photo-irradiation can lead to the production of organomercurials. Interestingly, our results contradicted those from another study showing that UV irradiation could inhibit the methylation of inorganic mercury by acetone (Gardfeldt et al., 2003). The lower yield of MeHg under illumination might have been due to the faster photo-degradation of MeHg than its photo-production under intensive irradiation (450 W Xenon lamp).

3.3. Photochemical processes of Hg

In addition to photo-alkylation of Hg^{2+} (methylation and ethylation), other photo-reactions including photo-reduction of Hg^{2+} , photo-oxidation of Hg^0 , photo-degradation of MeHg and EtHg, and photo-transformation of organic molecules also occurred under UV irradiation.

3.3.1. Photo-reduction of Hg^{2+}

The photo-reduction of Hg^{2+} to Hg^0 in the presence LMWOCs, including aldehydes, alcohols and low-molecular-weight organic acids, has been widely studied in recent years because of its significance for environmental (Pehkonen and Lin, 1998) and analytical (Han et al., 2007) purposes. The reduction of Hg^{2+} to Hg^0 was reported to be significantly promoted by UV irradiation and to be mediated by the Hg^{2+} -LMWOC complexes (Si and Ariya, 2008). In this study, we found that a fraction of Hg^{2+} in solution was reduced to Hg^0 and that photo-reduction by low molecular weight organic acids was much more significant than that by aldehydes and ketones. The solubility of Hg^0 in aqueous solution is very low and most of it was easily vaporized into the air phase (Andersson

et al., 2008). Therefore, the photo-reduction of Hg^{2+} to Hg^0 competes with photo-alkylation of Hg^{2+} .

3.3.2. Photo-oxidation of Hg^0

A fraction of the generated Hg^0 in the reaction solution can be re-oxidized into Hg^{2+} by $\cdot\text{OH}$, H_2O_2 or O_3 produced under UV irradiation (Zhang, 2006). The oxidation of Hg^0 was markedly accelerated by chloride (Yamamoto, 1996). The re-oxidation of Hg^0 to Hg^{2+} is favorable for photo-alkylation of Hg^{2+} .

3.3.3. Photo-degradation of MeHg and EtHg

MeHg and EtHg produced by photo-alkylation of inorganic mercury can also be photo-degraded under UV irradiation (Fig. S4), in agreement with previous studies (Sellers et al., 1996; Gardfeldt et al., 2001). Inorganic Hg^{2+} was found to be the photo-degradation product of MeHg and EtHg. Due to the instability of EtHg (Suda et al., 1993), the photo-degradation of EtHg was relatively faster than that of MeHg (as shown in Fig. S4).

3.3.4. Photo-transformation of LMWOCs

After UV irradiation, the pH of the solution containing the ketones and aldehydes was significantly decreased. Capillary electrophoresis with indirect UV detection was used to analyze the degradation product of the acetone solution (as shown in Fig. S5). Acetic acid and formic acid were identified as the main photo-degradation products of acetone. It is understandable that the generated low-molecular-weight organic acids decreased the pH of solution. The photolysis products of ketones, aldehydes, and low-molecular-weight organic acids are summarized in Table S2. The photo-decomposition of ketones, aldehydes and low-molecular-weight organic acids mainly produces shorter carbon-chain aldehydes and low molecular weight organic acids or CO_2 .

In aqueous solution, some compounds containing longer carbon chains can also be formed through free radical combinations. For example, propionic acid and acetic acid were found as the main products of malonic acid (Szalai et al., 1998). In addition, a small quantity of propionic acid was reported to be formed in the photolysis of acetic acid (Guo et al., 2005). The re-arrangement and combination of the carbon chain could partly contribute to the production of MeHg and EtHg in malonic acid and acetone solution, respectively.

3.4. The effect of different environmental factors

Different environmental factors including pH, concentration of Cl^- , reaction time, and chelating ligands were investigated for their influence on photochemical alkylation.

3.4.1. Effect of pH

Fig. S6 shows that the pH of the reaction solution significantly influenced the yield of MeHg and EtHg. Moreover, the pH dependence of alkylation varies with the alkylation donor. For acetone and butanone, the highest yield of MeHg or EtHg was achieved between pH 4.0 and 5.0. For acetic and propionic acid, the optimized pH favoring alkylation of Hg^{2+} was at 4.0. A similar phenomenon was also observed in the methylation of Hg^{2+} by acetone under dark conditions (Gardfeldt et al., 2003) and in the photo-methylation of Hg^{2+} by acetic acid (Zuo and Pang, 1985). However, the low pH of the reaction solution of pyruvic acid, acetaldehyde and propionaldehyde promoted a favorable yield of MeHg or EtHg, similar to the photo-methylation of Sn^{2+} by ethanol (Hamasaki et al., 1991).

An equilibrium exists between Hg^{2+} and $\text{Hg}(\text{OH})_2$ as a function of pH in the aqueous solution. Free Hg^{2+} is considered to have a higher methylation reactivity than that of $\text{Hg}(\text{OH})_2$ (Gardfeldt et al., 2003; Chen et al., 2007). The increased pH of the reaction

solution decreases the availability of Hg^{2+} , which in turn causes a decrease in the yield of MeHg. The pH of the reaction solution of acetone, butanone, acetic and propionic acid influences not only the availability of Hg^{2+} , but also the photolysis of LMWOCs and the interaction between Hg^{2+} and LMWOCs.

3.4.2. Effect of concentration of alkylation donors and Hg^{2+}

For most investigated LMWOCs including ketones and low molecular weight organic acids, the alkylation efficiency of Hg^{2+} is initially enhanced with an increased concentration of alkylation donor but then decreases. At low concentrations, most alkylation donors are responsible for the formation of Hg^{2+} -organic molecule complexes, facilitating photo-alkylation of Hg^{2+} . However, an increased concentration of the alkylation donor suppresses Hg^{2+} alkylation, which could be attributed to the reduction of penetration depth of the UV irradiation into the bulk solution. For propionaldehyde, the alkylation efficiency kept increasing within the range of investigated concentrations from 1 mM to 1000 mM.

The yield of MeHg or EtHg was enhanced by increasing the Hg^{2+} in solution for all of the investigated alkylation donors except for pyruvic acid. For pyruvic acid, the yield of MeHg peaked at $500 \mu\text{g L}^{-1}$ of Hg^{2+} and then decreased with increasing Hg^{2+} concentration.

3.4.3. Effect of concentration of Cl^-

Figs. S7 and S8 shows the effect of Cl^- concentration ($[\text{Cl}^-]$) on the alkylation of Hg^{2+} , which was surprisingly found to depend on the concentration of the alkylation donor. With an acetone concentration of 1 M, Cl^- significantly increased the methylation of Hg^{2+} . However, with an acetone concentration of 1 mM, the MeHg yield initially increased and then dropped significantly with increasing $[\text{Cl}^-]$. Therefore, Cl^- potentially exerts two different effects on the photochemical reaction. On the one hand, Cl^- could form a complex with Hg^{2+} and decrease the availability of Hg^{2+} for alkylation. On the other hand, Cl^- could be converted to Cl_2^- under UV irradiation (Liu et al., 2009), facilitating the photolysis of LMWOCs and consequently improving the yield of MeHg or EtHg. In addition, the presence of Cl^- can also promote the re-oxidation of Hg^0 and inhibit the photo-reduction of Hg^{2+} (Si and Ariya, 2008), thereby increasing the availability of Hg^{2+} .

3.4.4. Effect of reaction time

The effect of UV irradiation time on the alkylation reaction was also investigated, as shown in Fig. 3. The concentration of MeHg or EtHg initially increased with reaction time and then decreased with prolonged reaction times. Reduced yields of MeHg or EtHg were ascribed to their photo-degradation under UV irradiation. Moreover, the reaction times for the maximal yield were approximately 2 and 4 min for EtHg and MeHg, respectively. The shorter reaction time for the maximal yield of EtHg was attributed to the faster photo-degradation kinetics of EtHg than that of MeHg (Suda et al., 1993).

3.4.5. Effect of chelating ligand

Considering the prevalence of chelating ligands for Hg^{2+} in the aquatic environment, the effect of the chelating ligand on alkylation was investigated. Organic (ethylenediaminetetraacetic acid (EDTA) and 2-mercaptoethanol (ME)) and inorganic chelating ligands ($\text{Na}_2\text{S}_2\text{O}_3$ and K_2S) were used in this study and the results are presented in Fig. S9. Interestingly, both EDTA and ME can methylate Hg^{2+} under UV irradiation without an alkylation donor. A methyl group can be produced by the transfer of a hydrogen atom to the methene group of EDTA and ME under UV irradiation. Generally, chelating ligands significantly decreased the yield of MeHg. The chelating ligands compete with the alkylation donor for Hg^{2+} , which in turn decreases the formation of the Hg^{2+} -alkylation donor

Fig. 3. Time evolution of organomercurials with different alkylation donors. Conditions: $500 \mu\text{g L}^{-1}$ Hg^{2+} and 1 mM alkylation donor. (Note: The product was MeHg with the exception of EtHg as the product for propionaldehyde and propionic acid.).

Fig. 4. EPR spectra of radicals trapped by DMPO under irradiation in (a) water and with (b) pyruvic acid, (c) acetone, (d) acetic acid and (e) ethanol. Conditions: 1 mg L^{-1} (as Hg) HgCl_2 , 1% NaCl (w/v), 0.2 M alkylation donor (except the concentration of acetic acid was 0.1 M) and 50 mM DMPO.

complex and consequently reduces the alkylation yield. The relatively higher MeHg yield in the presence of EDTA or ME can be attributed to the methylation of Hg^{2+} by the EDTA or ME ligand and not by the alkylation donors. These results suggested that the methylation capability of the alkylation donor decreases in aqueous environments in the presence of chelating ligands of Hg^{2+} .

3.5. Alkylation of Hg^{2+} by propionic acid at environmentally relevant concentrations

The concentration of Hg^{2+} in environmental compartments such as water is relatively low. Therefore, a low concentration of Hg^{2+} of $1 \mu\text{g L}^{-1}$ was used to mimic the methylation of Hg^{2+} by propionic acid in the aqueous environment. Considering the low concentration of the organomercurial product, a sensitive phenylation-purge-and-trap-GC coupled with ICP-MS method (Mao et al., 2008) was applied in this experiment to identify the

Fig. 5. Schematic diagram of the proposed mechanism for the (a) photo-methylation and (b) photo-reaction between Hg^{2+} and the LMWOCs.

organomercurial species. The results are given in Fig. S10. After UV irradiation (365 nm), approximately 1 ng L^{-1} of ^{199}Hg -enriched EtHg was generated and trace MeHg was also detected. The calculated ethylation efficiency is approximately 0.1%, indicating the significance of photo-chemical alkylation of Hg^{2+} in the environment.

3.6. Mechanism of photo-alkylation of inorganic Hg

Two different mechanisms have been proposed for the photo-methylation of Hg^{2+} in previous studies: free radical pathways (Oldekop et al., 1960; Hayashi et al., 1977) and an intra-molecular methyl transfer of the Hg^{2+} -organic molecule complex (Akagi et al., 1974). However, experimental evidence is needed to confirm these hypotheses. UV-visible spectrometry and electron spin resonance were applied to explore the possible intermediates or radicals, and further a mechanism for the photo-alkylation of Hg^{2+} was proposed.

3.6.1. Hg^{2+} -organic molecule complex

As a strong Lewis acid, Hg^{2+} can form complexes with Lewis bases such as ketones, aldehydes and organic acids (Banerjee and Singh, 1955; King et al., 2002). The interaction of Hg^{2+} with LMWOCs including acetone, pyruvic acid, acetic acid, and ethanol was studied using UV-visible spectrometry. The results showed that the addition of Hg^{2+} into a solution of organic molecules slightly enhanced the absorption of the organic molecules. Moreover, the absorption peak was shifted to the red region, which is consistent with the results of Hg^{2+} -oxalic acid (Gardfeldt and Jonsson, 2003). These data strongly suggested the formation of a complex of Hg^{2+} and LMWOCs. These complexes may contribute

to further photochemical alkylation. In another study (Akagi et al., 1974), the replacement of HgCl_2 by $\text{Hg}(\text{OCOCH}_3)_2$ increased the methylation of Hg^{2+} by acetate, which also suggested that the $\text{Hg}(\text{OCOCH}_3)_2$ complex is essential for the photo-methylation of Hg^{2+} .

3.6.2. Methyl radical in photochemical methylation

Electron spin resonance (ESR) with 5,5-dimethyl-1-pyrroline N-oxide (DMPO) as the free radical trapping agent was applied to probe the free radicals in the aqueous solution under UV irradiation (Fig. 4). The results indicated that methyl radicals were produced in a solution containing ethanol, pyruvic acid, acetic acid, and acetone under UV irradiation. Previous studies have demonstrated that acetic acid, acetaldehyde, ethanol and methanol can generate methyl radicals under UV irradiation (300–400 nm) (Kaise et al., 1994) or chemical oxidation (Nakao et al., 1999). Notably, ESR analyses revealed that the concentration of methyl radicals is uncorrelated with the yield of MeHg. Acetone, which exhibited a higher methylation yield, produced fewer methyl radicals than ethanol and acetic acid did. Although methyl radicals are generated in the photo-alkylation process, these results indicated that recombination of the methyl radicals with Hg^{2+} is not likely the dominant pathway of Hg^{2+} methylation.

3.6.3. Methylation and the bond energy of $\text{C}(\text{H}_3)\text{-O}$ and $\text{C}(\text{H}_3)\text{-C}$

Experimental results of the alkylation of Hg^{2+} exhibited that the alkylation efficiency of Hg^{2+} by LMWOCs follows the order: ketones > aldehydes > low-molecular-weight organic acids > alcohols. While, the energies of the chemical bond connected to the

methyl group follow a reversed order (Table S3), indicating that the alkylation efficiency is related to the bond energies of the methyl group. Weaker bond energies of the methyl group lead to higher methylation efficiencies of Hg^{2+} . In addition, the alkylation efficiency possibly was negatively correlated with the photo-reduction ability of Hg^{2+} by small organic molecules, which can be regarded as side reactions of alkylation.

We propose a mechanism for the photochemical alkylation of Hg^{2+} (Fig. 5). Firstly, the Hg^{2+} –LMWOC complex is formed and absorbs energy from UV irradiation. Then, the intra-complex transfer of an alkyl group (i.e., methyl or ethyl) to Hg^{2+} results in the formation of MeHg, EtHg, low-molecular-weight organic acid, CO_2 , or other products (Fig. 5a). In the photochemical reaction, in addition to the methylation of Hg^{2+} , other photochemical processes also occur simultaneously, including photo-degradation of MeHg or EtHg, photo-reduction of Hg^{2+} , and photo-oxidation of Hg^0 (Fig. 5b).

4. Conclusions

This study demonstrates the possibility of alkylation of inorganic Hg^{2+} to MeHg and/or EtHg by ketones, aldehydes and low-molecular-weight organic acids in aqueous solution under UV irradiation. The effect of UV irradiation, pH, concentration of Cl^- and chelating ligands on alkylation of Hg^{2+} was investigated and a tentative photo-alkylation mechanism of Hg^{2+} in aqueous phase is proposed. Alkylated mercury is the main photochemical products for ketones. However, for aldehydes and low-molecular-weight organic acids, volatile elemental mercury is the main product and the alkylation efficiency is much lower than ketones. The results from this study will contribute to a better understanding of the photo-generation of MeHg and EtHg in natural environments. However, the UV irradiation used throughout the photo-alkylation experiments is different from natural solar irradiation. Further studies are still needed to validate the possibility of photochemical alkylation of Hg^{2+} under natural sunlight. Mass-independent isotope fractionation fingerprint of MeHg in environmental samples is a promising tool to further probe the importance of this photochemical process in real environment (Bergquist and Blum, 2009; Malinovsky and Vanhaecke, 2011).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2012.01.006.

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