

A new vapor generation system for mercury species based on the UV irradiation of mercaptoethanol used in the determination of total and methyl mercury in environmental and biological samples by atomic fluorescence spectrometry

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Abstract A new vapor generation system for mercury (Hg) species based on the irradiation of mercaptoethanol (ME) with UV was developed to provide an effective sample introduction unit for atomic fluorescence spectrometry (AFS). Preliminary investigations of the mechanism of this novel vapor generation system were based on GC–MS and FT–IR studies. Under optimum conditions, the limits of determination for inorganic divalence mercury and methyl mercury were 60 and 50 pg mL^{-1} , respectively. Certified reference materials (BCR 463 tuna fish and BCR 580 estuarine sediment) were used to validate this new method, and the results agreed well with certified values. This new system provides an attractive alternative method of chemical vapor generation (CVG) of mercury species compared to other developed CVG systems (for example, the traditional KBH_4/NaOH –acid system). To our knowledge, this is the first systematic report on UV/ME-based Hg species vapor generation and the determination of total and methyl Hg in environmental and biological samples using UV/ME–AFS.

Keywords UV-induced metal vapor generation · Mercaptoethanol · Mercury · Methyl mercury · Speciation · Trace elements · Atomic fluorescence spectrometry

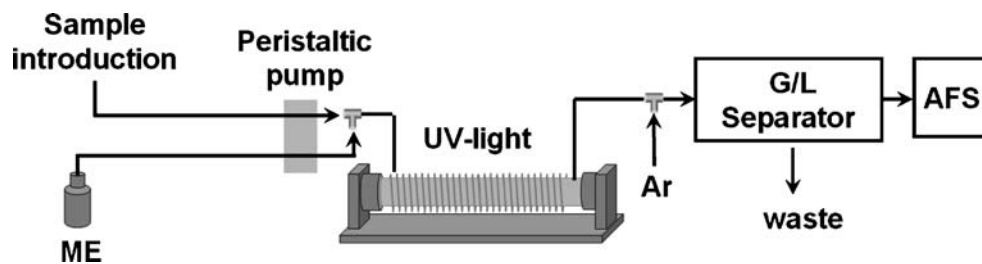
Introduction

Chemical vapor generation (CVG), including hydride generation, alkylation, halide generation, metal carbonyl and elemental vapor generation, is frequently employed as a method of introducing the sample analyzed in atomic fluorescence spectrometry [1–4]. CVG has several advantages over the conventional pneumatic nebulization system used for sample introduction, such as higher transport efficiency of target elemental species to the detector and efficient on-line matrix separation. Such merits are important not only for enhancing detection power and for eliminating possible physicochemical and spectral interferences from the matrix of a sample, but also for meeting the requirements of the interface linking hyphenated techniques used for elemental speciation, because only trace amounts of the target elemental species are usually present in the sample. Besides the most frequently used KBH_4/NaOH –Acid system, some new advances in CVG have recently been reported in terms of understanding the mechanism involved, developing new methodologies, and designing new vapor generation systems, including UV alkylation in the presence of low-molecular-weight organic compounds as well as photocatalytic reduction using the electrons in the conduction band of a nano TiO_2 semiconductor [5–11]. These approaches open up new routes to elemental determination and speciation analysis [12].

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Fig. 1 Schematic diagram of the (UV/ME)–AFS system



Mercury (Hg)—a well known and dangerous element—is a typical example of an element that is amenable to CVG, and Hg determination and speciation analyses have been widely performed and extensively reviewed [13–18]. In this study, we developed a new on-line vapor generation system for Hg species using the UV irradiation of mercaptoethanol (ME), used as a reductive radical precursor. The vapor generation mechanism associated with this approach was investigated based on the photochemistry of ME and evidence from capillary gas chromatography coupled with electron impact ionization mass spectrometry (GC–MS) and FT–IR studies. It was used as a sample introduction unit for atomic fluorescence spectrometry (AFS) and applied to total Hg and methyl Hg determinations in biological and environmental samples. To our knowledge, this is the first report of the systematic use of UV/ME–AFS for Hg species analysis.

Experimental

Instrumentation

A nondispersive atomic fluorescence spectrometer (ND-AFS 610A) (Beijing BRAIC Analytical Instrument Corporation, People's Republic of China) equipped with a high-performance Hg hollow cathode lamp (253.7 nm, Beijing Institute of Vacuum Electronics Research, People's Republic of China) and a sunlight-blinded photomultiplier tube (PMT) (Hamamatsu, Hamamatsu City, Japan) was used for Hg determination; signal acquisition and processing were carried out by HWH software Version 1.0 [19]. A LEAD-1 peristaltic pump (Baoding Longer Precision Pump Co., Ltd,

People's Republic of China) was used to introduce reagents during the experiments, and a 40-W low-pressure Hg lamp (Xinyuan Appliance Lighting Co., Ltd., People's Republic of China) surrounded by a PTFE tube (1.5 m in length \times 0.8 mm i.d. \times 1.6 mm o.d., Cole-Parmer, Vernon Hills, IL, USA) was employed as a light source. These were used as a sample introduction unit for AFS on-line photolysis and to generate the vapor of the Hg species. Each part of the hyphenated system was connected with PTFE tubes (0.8 mm i.d. and 1.6 mm o.d.), as shown schematically in Fig. 1, and the optimum operating conditions are listed in Table 1. A Shimadzu (Kyoto, Japan) QP 2010 GC-MS and a Nicolet (Thermo Fisher Scientific Inc., Waltham, MA, USA) Avatar 360 FT-IR spectrometer were also used when studying the UV/ME Hg species vapor generation mechanism.

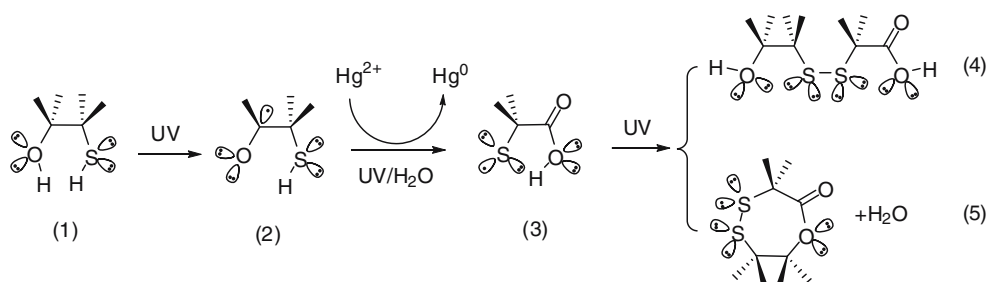
Chemical reagents

All chemicals used were of at least analytical-reagent grade, and ultrapure water (UPW) with a resistivity of 18 M Ω (Millipore, Bedford, MA, USA) was used throughout in this study. Methyl mercury chloride (methyl Hg) and mercury nitrate [Hg(NO₃)₂] were obtained, respectively, from Dr. Ehrenstorfer, Germany and Shanghai Chemicals, China. Methyl Hg and Hg(NO₃)₂ stock solutions were prepared using methanol (MeOH) (HPLC grade, Tedia, Fairfield, OH, USA) and UPW, respectively, and stored at 4 °C in the dark before use. Working solutions were prepared by further stepwise dilution with MeOH and UPW, respectively. Mercaptoethanol (ME) was purchased from Sigma (St. Louis, MO, USA). The Standard Reference materials BCR 463 (tuna fish) and BCR 580 (estuarine

Table 1 Optimum operating conditions

CVG parameters		AFS parameters	
UV light	40 W low pressure Hg Lamp	Resonance wavelength	253.7 nm
Reagent	0.1% ME	HCL current	40 mA
Flow rate	2 mL min ⁻¹	Argon flow rate	300 mL min ⁻¹
Concentration of mercury species (inorganic mercury as Hg ²⁺ and methyl mercury as CH ₃ Hg ⁺)	100 ng mL ⁻¹	Voltage of PMT	–310 V
Injection volume	50 μ L	Height of atomizer	7 mm
		Readout mode	Peak height

Scheme 1 Possible mechanism for the generation of Hg vapor via UV irradiation of mercaptoethanol



sediment) were obtained from the Community Bureau of Reference (BCR, Brussels, Belgium), and were subsequently used for method validation.

Sample pretreatment

A microwave-assisted digestion method was used for total Hg determination. Appropriate amounts of samples dried at room temperature (0.1–0.2 g) were weighed and put into PTFE bombs, to each of which 5 mL of ultrapure HNO_3 was added. The samples were subjected to a cold predigestion step overnight in order to reduce any organic materials in the samples. The sealed bombs were then digested for 5 min (for biological samples) or 10 min (for the sediments) using a MK-III optical fiber pressure-controlled microwave decomposition system (Shinco, Shanghai, China) with a full power of 1200 W, placed inside a fume cupboard. After cooling the sealed bombs, the resulting transparent solutions were diluted with an appropriate amount of UPW to 10 mL and filtered through a 0.45- μm filter. An alkaline extraction procedure was applied to selectively extract the methyl Hg [20–22]. Briefly, 0.1 g of a biological sample or 1 g of a sediment sample and 25% (m/v) KOH (in methanol) were added to each 50-mL centrifuge tube. This mixture was sonicated

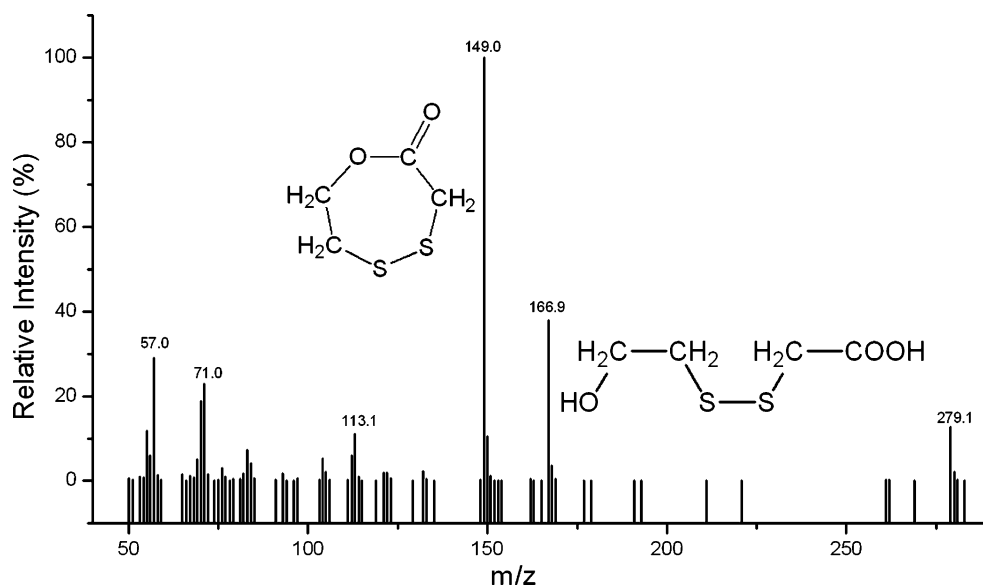
and shaken mechanically and then CH_2Cl_2 and concentrated HCl were added to the tube in sequence. Shaking extracted the methyl Hg into the CH_2Cl_2 phase, while the inorganic Hg remained in the water phase. After centrifuging at 3500 rpm for about 10 min, the CH_2Cl_2 phase was transferred into a 7-mL centrifuge tube, and 1 mL of a 10 mM sodium thiosulfate solution was added to strip the methyl Hg along with vigorous shaking for 45 min. After the water phase had been filtered through a 0.45- μm membrane, it was injected directly into the (UV/ME)–AFS system for determination.

Results and discussion

Possible mechanism of the generation of Hg species vapor by UV irradiation of ME

When the mixtures of $\text{Hg}(\text{NO}_3)_2$ and/or methyl Hg with ME were irradiated with UV light, a signal from Hg could be detected by AFS after sweeping with argon from a liquid–gas separator. Cowan and Drisko [23] noted that the electron of the ketyl radical generated by the UV light might undergo intermolecular migration to the sulfur atom of the mercaptan in the solution, and a hydrogen could be

Fig. 2 Mass spectrum of the solution analyzed using GC–MS. Column: DB-XLB 15 m \times 0.25 mm \times 0.25 μm ; injection temperature, 280 $^\circ\text{C}$; oven temperature program beginning at 120 $^\circ\text{C}$ and holding for 2 min, then rising to 200 $^\circ\text{C}$ at 20 $^\circ\text{C min}^{-1}$; carrier gas (He) flow rate: 1.2 mL min^{-1} ; temperature of interface between GC and MS: 280 $^\circ\text{C}$; electron impact (EI): 70 eV; ion source temperature: 200 $^\circ\text{C}$; detector voltage: 1.2 kV. The solution (3 mL) was prepared by mixing 0.1% ME in methanol and 10 ng Hg^{2+} irradiated with UV light for 30 s



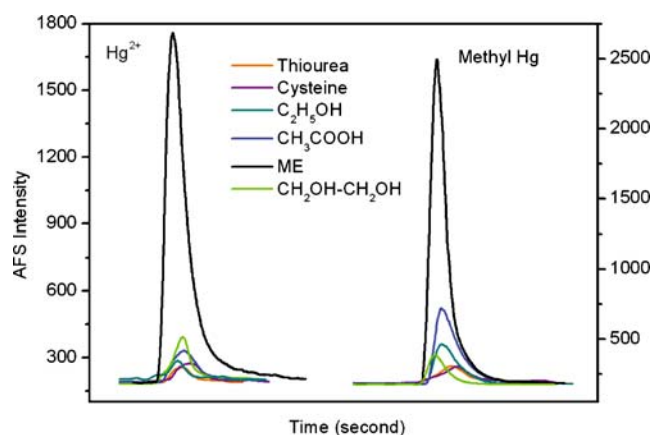


Fig. 3 Effectiveness of some radical precursors at generating the cold vapors of mercury species: 1% thiourea, 0.1% cysteine, 0.1% C_2H_5OH , 0.1% CH_3COOH , 0.1% ME and 0.1% CH_2OH-CH_2OH . The experimental conditions used are indicated in Table 1

transferred from the mercaptan to the ketyl radical. ME (1) contains both hydroxyl and mercapto in its molecule and, under UV irradiation, the ME radical ($HOCHCH_2SH$) (2) is generated. Then the electron on the carbon atom may transfer to the sulfur atom through intramolecular migration to form the $HOCH_2CH_2S$ radical, which is then further oxidized by the Hg^{2+} in the reaction medium to form the $HOOCH_2S$ radical (3), while Hg^{2+} is reduced to form Hg^0 , as detected by AFS. The resulting radicals (3) combine to form $HOCH_2CH_2S-SCH_2COOH$ (4) and a cyclic disulfide ester (5) by intramolecular esterification, as shown in Scheme 1. This mechanism was verified by analyzing the solution obtained after the photochemical reaction using GC-MS, as shown in Fig. 2, which shows that signals from $HOCH_2CH_2S-SCH_2COOH$ (m/z 167) and the cyclic disulfide ester (m/z 149) are present in the mass spectrum. Further evidence to support the proposed

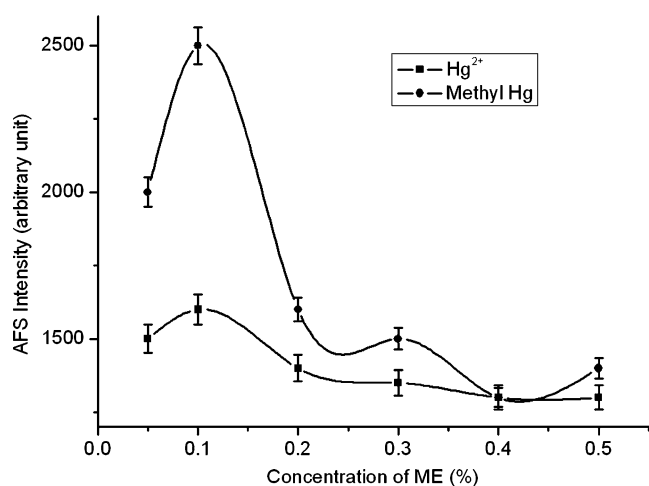


Fig. 4 Effect of ME concentration on the vapor generation of Hg species. Experimental conditions are indicated in Table 1. Uncertainties, shown by the error bars, are expressed as the standard deviation when $n=9$

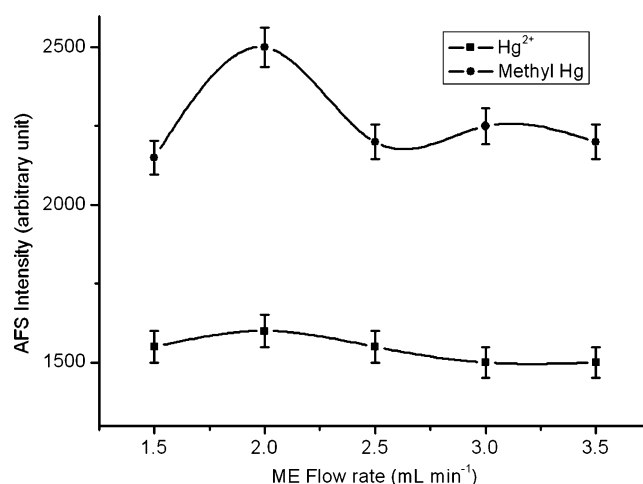


Fig. 5 Effect of ME flow rate on the vapor generation of Hg species. The other experimental conditions were the same as in Fig. 4. Uncertainties, shown by the error bars, are expressed as the standard deviation when $n=9$

mechanism is provided by the pH of the Hg^{2+} and ME mixture, which decreases from 5.3 to 4.5 after reduction under UV irradiation, as well as the presence of typical absorption peaks of $\nu_{C=O}$ 1709 cm^{-1} , ν_{C-O} 1048 cm^{-1} and ν_{OH} 3334 cm^{-1} as well as δ_{OH} 1292 cm^{-1} in the FT-IR spectrum of the solution.

Moreover, comparison of the use of CH_3COOH [9], CH_3CH_2OH [24], $HOCH_2CH_2OH$, cysteine and thiourea [25] with ME for Hg vapor generation indicated that the ME is the most effective for this purpose (Fig. 3). A possible reason for this is that only ME contains both hydroxyl and mercapto in its molecule.

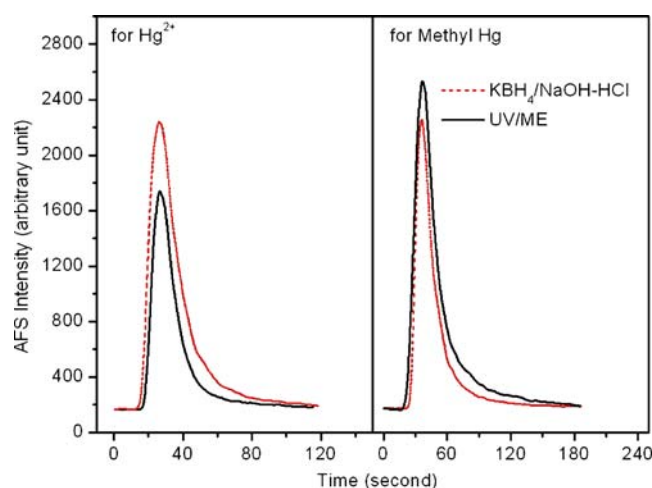


Fig. 6 Comparison of the $KBH_4/NaOH-HCl$ -AFS system and the UV/ME-AFS system. Experimental conditions: $KBH_4/NaOH$, 1.5% containing 0.5% NaOH; HCl, 2%; the flow rate of both $KBH_4/NaOH$ and HCl was 2.0 mL min^{-1} . The other experimental conditions are indicated in Table 1

Table 2 Total Hg, Hg²⁺ and methyl Hg in the Certified Reference Materials and real samples ($\mu\text{g g}^{-1}$, $n=4$)

Sample	Methyl Hg		Hg ²⁺		Total Hg		Recovery of methyl Hg (%)
	Certified value	Found	Certified value	Found	Certified value	Found	
CRM 463	3.04	3.00±0.06	–	–	2.85	3.13±0.2	96.7
CRM 580	75±3.7 ^a	68.2±2 ^a	–	127±5	132±3	131±2	92.7
<i>Ostrea</i>	–	290±13 ^a	–	261±14 ^a	–	682±15 ^a	
Lake sediment	–	120±3 ^a	–	9.00±0.3	–	9.20±0.4	

^a: ng g^{-1}

Performance of the UV/ME–AFS system

Both the ME concentration and the flow rate employed strongly influence the efficiency of Hg species vapor generation. These parameters were optimized using AFS in flow injection mode, and the results indicated that 0.1% ME at a flow rate of 2.0 mL min⁻¹ was optimum for Hg species vapor generation, as shown in Figs. 4 and 5. Although the chemical bond between the carbon atom and Hg in methyl Hg can be effectively broken under UV irradiation [24, 26, 27], it should be noted that the AFS signal intensity of methyl Hg is much higher than that of Hg²⁺. Obviously, this phenomenon needs further investigation. Under optimum operating conditions, the detection limits (3 σ) for Hg²⁺ and methyl Hg are 60 and 50 pg mL⁻¹, respectively. The signals for both Hg²⁺ ($R^2=0.9954$) and methyl Hg (0.9992) were linear in the range 1.0–100 ng mL⁻¹, and the RSDs ($n=9$; the concentrations of both Hg²⁺ and methyl Hg were 100 ng mL⁻¹) were less than 3.2%. A comparison of the UV/ME–AFS system with the conventional (KBH₄/NaOH–HCl)–AFS system indicated that UV/ME offered an attractive alternative for the generation of Hg species vapor (Fig. 6). On the other hand, a comparison of the detection limits obtained using our UV/ME–AFS system with previously published limits obtained using CV–AAS [28, 29] also indicated that the DL of the UV/ME–AFS system is lower than that of the CV–AAS system. Moreover, because ME is usually used as a component in the mobile phase during the HPLC separation of Hg species [30, 31], UV/ME is more convenient since no additional reagent is needed when it is used as an interface between HPLC and an element-specific detector for Hg speciation analysis.

Method validation and sample analysis

Two Certified Reference Materials—CRM 463 (tuna fish) and CRM 580 (estuarine sediment)—were used for method validation. The total Hg and methyl Hg concentrations determined by (UV/ME)–AFS agreed well with the certified values (as shown in Table 2), and the recoveries of methyl Hg ranged from 92.7 to 96.7%. This method was

applied also to a shellfish (*Ostrea*) and a lake sediment, which were collected, respectively, off the coast of and in Xiamen Island in September 2006. Both methyl Hg and inorganic Hg were found in the *Ostrea* and lake sediment samples (Table 2), indicating Hg pollution, probably from local thermoelectric power plants.

Conclusions

A new system based on the UV irradiation of mercaptoethanol was developed in order to generate Hg species vapor. Compared with the conventional KBH₄/NaOH–HCl system, UV/ME offers an attractive alternative for the generation of Hg species vapor, and it can be used as an effective sample introduction unit when determining Hg species by atomic spectrometry. Furthermore, UV/ME can be expected to be utilized in the near future, after appropriate modification, as an on-line interface between HPLC and various element-specific detectors used for Hg speciation analysis.

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