Photo-induced chemical vapour generation with formic acid: novel interface for high performance liquid chromatography-atomic fluorescence spectrometry hyphenated system and application in speciation of mercury†

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Based on a novel photo-induced chemical vapour generation (CVG) with formic acid instead of the conventional $K_2S_2O_8/KBH_4$ system, an on-line coupled HPLC and atomic fluorescence spectrometry procedure was developed for the speciation of inorganic mercury, methylmercury (MeHg), ethylmercury (EtHg) and phenylmecury (PhHg). Under UV irradiation, the decomposition of organic mercury species and the reduction of Hg^{2+} could be completed in one step with this proposed photo-induced CVG system. The novel CVG system used formic acid only, which simplified the flow system and avoided the possibility of contamination originating from additional chemicals. A number of operating parameters including organic acid species, concentration of formic acid, pH, flow rate of formic acid and flow rate of carrier gas were optimized, and comparable method sensitivities with the conventional $K_2S_2O_8/KBH_4$ system were obtained. The limits of detection at the optimized conditions were 0.81, 0.20 and 0.87 µg L^{-1} for MeHg, EtHg and PhHg, respectively. The developed method was validated by determination of certified reference material DORM-2 and was further applied in determination of seafood samples. The new CVG system is simple, environmentally benign, and inexpensive for the speciation analysis of mercury. It is expected to have similar applications in other analytical atomic spectrometric techniques as well as speciation of other organic metal compounds.

Introduction

Concentration of mercury has increased considerably during the industrial age.[1] Different species of mercury have been found in environmental media due to anthropogenic mercury release or alkylation in natural processes.[2,3] Speciation plays an important role in not only the toxicity and exposure of mercury to an organism, but also the transport of mercury between different environmental media. Therefore, the development of sensitive and specific methods for the speciation of mercury is of great importance for elucidation of the toxicity and geochemical behaviour of mercury.

HPLC and GC are often used to separate inorganic and organic mercury. HPLC on-line hyphenation with element-specific detection methods such as atomic absorption spectrometry (AAS),[4–6] inductively coupled plasma mass spectrometry (ICP-MS),[7,8] atomic fluorescence spectrometry (AFS),[9–11] and atomic emission spectrometry (AES)[12] have been developed for the speciation of mercury in recent years.

Direct introduction of eluents of organic mercury into the detector often results in low sensitivity and high interference. To obtain high sensitivity and selectivity, an on-line post-column oxidation and hydrogen generation system for the generation of cold mercury vapour was often used as the interface between HPLC and atomic spectrometry. For some years UV irradiation has been used to reduce metal ions to their lower oxidation states or zero-valence states.[13–16] This chemical vapour generation (CVG), in the presence of organic acid under UV irradiation, has also been coupled with atomic spectrometry for the analysis of selenium and mercury.[17,18] Compared with the traditional KBH_4 system, the UV CVG technique is green, simple and selective. More recently, mercury vapour generation by UV photo-reduction in acetic acid solution was coupled with AAS for mercury determination,[19] and on-line photo-assisted vapour generation was coupled with AAS for determination of Se.[20–22] Further, a UV–nano-TiO_2 vapour generation device with formic acid was coupled between HPLC and ICP-MS for the determination of inorganic Se(IV) and Se(VI).[23] Recently, Zheng *et al.*[24] found that UV irradiation with formic acid converts not only Hg^{2+} but also MeHg to Hg^0; thus a method was proposed for the speciation of Hg^{2+} and MeHg under room natural light or ultraviolet irradiation.

In the present work, a novel HPLC-AFS hyphenated system based on photo-induced chemical vapour generation with formic acid was developed for the speciation of mercury. In

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this proposed post-column digestion and vapour generation process, the decomposition of organic mercury and the reduction of mercury were completed in one step and only one cheap chemical (formic acid) was introduced into the flow system. Optimum conditions for the new photochemical vapour generation procedure were studied, and comparable method sensitivity to the $K_2S_2O_8/KBH_4$ procedure was obtained.

Experimental

Instrumentation

The schematic diagrams of the UV CVG system with formic acid and the conventional post-column UV $K_2S_2O_8/KBH_4$ CVG system are shown in Fig. 1. Samples were injected through a Rheodyne Model 7725i injection valve (Rheodyne, Cotati, CA, USA) and the mobile phase was delivered by a P680 HPLC pump (Dionex, Sunnyvale, USA).

In the UV CVG system with formic acid (Fig. 1B), chromatographic eluent was mixed with formic acid solutions and entered into a polytetrafluoroethylene (PTFE) tube. In the PTFE tube wrapped around an UV lamp, organomercury was digested to Hg^{2+} and further reduced to cold mercury vapour. By using argon as carrier gas, the mercury vapour generated in the reaction was separated in a gas–liquid separator and brought to the AFS (AF-610A, Beijing Raileigh Analytical Instrument Company) for determination.

Similar instrumentation was constructed in the traditional post-column UV $K_2S_2O_8/KBH_4$ CVG system (Fig. 1A). Briefly, the chromatographic eluent firstly mixed with 0.5% (w/v) $K_2S_2O_8$ (in 10% HCl (v/v)) at flow rate about 1.7 mL min^{-1}, and then into a PTFE tube (5 m $\times$ 0.5 mm id) wrapped around a UV lamp to digest organomercury to Hg^{2+}. The effluent then mixed with 0.5% (w/v) KBH_4 solution (in 0.2% KOH (w/v)) at flow rate about 1.7 mL min^{-1}. The mercury

vapour generated was separated in a gas–liquid separator and determined by AFS.

The experimental conditions for the proposed UV CVG system with formic acid are listed in Table 1.

Chemicals and reagents

Mercury chloride ($\geq$ 99.5%) was obtained from Beijing Chemicals. Methylmercury chloride (MeHg), ethylmercury chloride (EtMg) and phenylmercury chloride (PhHg) were purchased from Merck ($\geq$ 98%, Darmstadt, Germany). Potassium tetraborate ($>$95%) was purchased from the Institute of Geophysical and Geochemical Exploration (Langfang, Hebei). TiO_2 nano-particle (Degussa P25, Degussa) was obtained from Germany. All other chemicals were analytical grade or better.

Preparation of sample extracts

An alkaline extraction method was used for the extraction of organomercurials from biological samples. Briefly, 2 mL of 25% (m/v) KOH (in methanol) was added to about 0.2 g of freeze-dried sample in a 50 mL glass centrifuge tube and the tube was shaken overnight. Then 6 mL of CH_2Cl_2 were added, and the solution was neutralized by addition of 1.5 mL of concentrated HCl dropwise. The resulting solution was shaken for 15 min to extract organic mercury into the CH_2Cl_2 phase. After centrifuging at 3000 rpm for 15 min, the CH_2Cl_2 phase was transferred into a 10 mL glass centrifuge tube and 1 mL of 10 mmol L^{-1} sodium thiosulfate was added. The mixture was shaken for 45 min to extract the organomercurials into the water phase. After centrifuging at 3500 rpm for 15 min, the water phase was taken as the sample for the determination of organomercurials by HPLC-AFS under optimized conditions.

Results and discussion

Effect of organic acid species

Three kinds of low molecular weight organic acid, viz. formic acid, acetic acid and propionic acid, were tested in the range from 0–360 mmol L^{-1} for the proposed UV-CVG system. Only formic acid was found to enhance the fluorescence intensities of mercury compounds, although acetic acid at a concentration of 3.0 mol L^{-1} was used to determine total mercury by cold vapour atomic absorption spectrometry utilizing UV photoreduction.[19] This is probably because acetic acid and propionic acid are more stable than formic acid under UV irradiation, which results in low production of radical to decompose and reduce mercury species.

Effect of formic acid concentration

When no formic acid was added into the reaction solution all four mercury species could still be detected but with much low sensitivities. This perhaps can be ascribed to the degradation and reduction of mercury species by singlet oxygen and hydrogen radicals generated under UV irradiation.[25,26] The fluorescence intensities of mercury species sharply increased when formic acid was present in the reaction solution and the fluorescence intensities of mercury compounds were found to depend greatly on the concentration of formic acid, as is
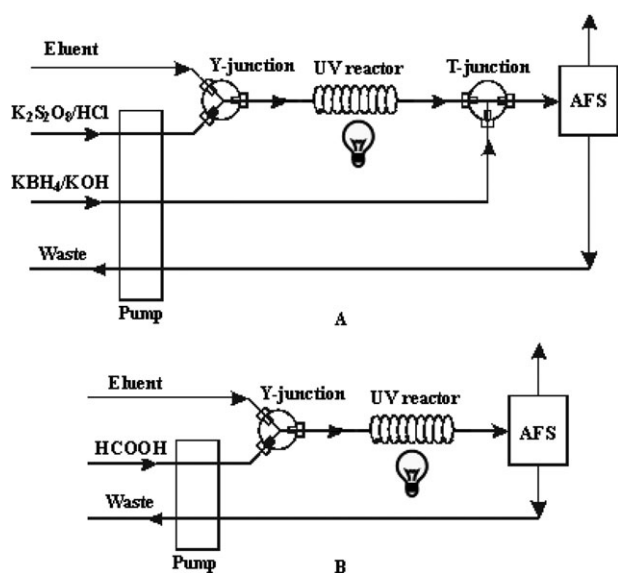

Fig. 1 Schematic illustration of conventional post-column UV $K_2S_2O_8/KBH_4$ CVG system (A) and the newly developed UV CVG system in the presence of formic acid (B).

Table 1 Operating conditions for the HPLC-AFS system

Parameter	Optimized value
HPLC	
Column	Shim-pack CLC-ODS (Shimadzu), 15 cm × 6.0 mm id × 5 μm
Mobile phase	A: 5% (v/v) CH_3CN, 10 mmol L^{-1} NH_4Ac, 0.01% 2-mercaptoethanol; B: 30% (v/v) CH_3CN, 10 mmol L^{-1} NH_4Ac, 0.01% 2-mercaptoethanol. 0–11 min, 100% A; 11–12 min, 100% A → 100% B, 12 min–30 min: 100% B, 30–31 min: 100% B → 100% A
Flow rate of mobile phase	1.2 mL min^{-1}
Sample injection	20 μL
UV digestion and vapour generation	
Power of UV lamp	11 W
Digestion coil	PTFE tube, 5 m × 0.5 mm id × 0.8 mm od
Formic acid reagent	240 mmol L^{-1}, pH 2.0
Flow rate of formic acid	50 rpm (about 3.8 mL min^{-1})
AFS	
Lamp	Mercury hollow cathode lamp, 253.7 nm
PMT voltage	270 V
Primary current	50 mA
Carrier gas	Ar, 500 mL min^{-1}

shown in Fig. 2. This should be attributed to the different efficiencies of photo-induced CVG in the present of different concentrations of formic acid.[24] Considering the signal intensity and the stability, a 240 mmol L^{-1} formic acid solution was used in the later research.

Effects of salt, hydrochloric acid and nano-TiO_2

The influence of Na_2SO_4, NaCl and NH_4Ac as additives was studied in the concentration range from 0 to 1.0 mol L^{-1}. No improvement of sensitivity was obtained by adding Na_2SO_4 or NaCl. Although a good baseline and the highest method sensitivity were obtained with 50 mmol L^{-1} NH_4Ac as additive, increased about 35% relative to that without NH_4Ac, no salt was added into the formic acid solution to preserve the simplicity of the CVG system.

In conventional CVG, such as hydride generation, hydrochloric acid solution is generally used as the reaction medium. Also, TiO_2 catalysis was used for photocatalytic reduction of

Hg^{2+} [14] and some other metal cations.[15] The signal intensity sharply decreased with the addition of hydrochloric acid and TiO_2 nano-particle in the formic acid solution. Therefore, no hydrochloric acid and TiO_2 nano-particle were added into the formic acid solution in the following studies.

Effect of pH

The effect of the pH of the formic acid solution on the signal intensity of mercury was studied (Fig. S1, ESI†). No significant change in the signal intensity was observed in the pH range from 2.0 to 4.0, whereas a decreasing trend in the signal intensity was found as the pH increased from 4.0 to 7.0. Therefore, pH 2.0 (the inherent pH of 240 mmol L^{-1} aqueous formic acid) was selected for the following experiment to obtain a high sensitivity.

Effect of formic acid flow rate

The flow rate of formic acid has great influence on the fluorescence intensities of mercury compounds. The influence of the flow rate of formic acid was studied by changing the rotation speed of the peristaltic pump from 10 to 80 rpm (Fig. S2, ESI†). When the rotation speed of the peristaltic pump was lower than 40 rpm, broader peaks and low fluorescence intensity were observed. With the increase of pump speed, the signal intensity increased significantly at first and then decreased owing to the short reaction time induced by the high flow rate. The optimum pump speed depended on the mercury species. While the highest signal intensity was obtained at 70 rpm for EtHg and PhHg, it was obtained at 40 rpm for MeHg. This suggests that EtHg and PhHg can be degraded in a shorter time than MeHg. This result agreed with the different stabilities of organomercurials.[27] As a compromise for the sensitivities of all mercury species and the consumption of formic acid, 50 rpm pump speed (corresponding to about 3.8 mL min^{-1} formic acid solution) was selected for further studies. At this flow rate, the irradiation time was about 40 s, as calculated from the flow rate and the inner volume of the reaction coil.
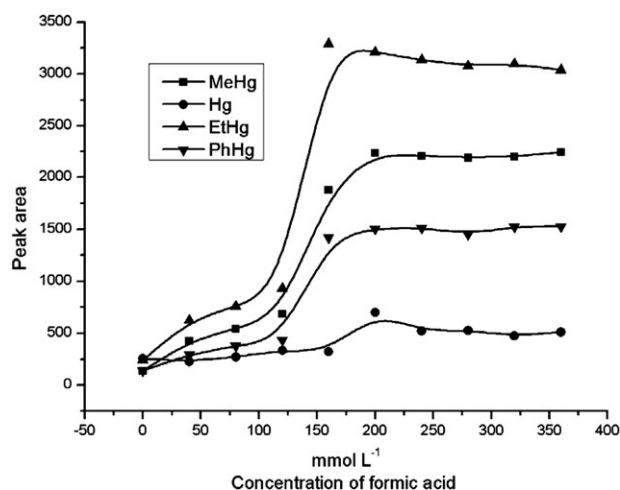

Fig. 2 Effect of formic acid concentration on the fluorescence intensities of mercury compounds. Concentration of mercury species: 100 μg L^{-1}. Flow rate of formic acid: 3.1 mL min^{-1}. Other conditions were given in Table 1.

Effect of carrier gas flow rate

The influence of flow rate of argon gas was also investigated in the range 200 to 800 mL min^{-1} (Fig. S3, ESI†). At flow rate of 500 mL min^{-1}, higher signal intensities of mercury species and low baseline noise were obtained. Therefore, a final flow rate of argon carrier gas of 500 mL min^{-1} was selected as the optimum condition.

Analytical performance

The signal intensities obtained by use of the proposed UV CVG system with formic acid and the traditional $K_2S_2O_8$/KBH_4 system were compared under respective optimum conditions (Fig. S4, ESI†). The proposed method possesses about 74% peak areas for the organomercurials and 35% peak area for inorganic mercury relative to that of the $K_2S_2O_8$/KBH_4 procedure, indicating that these two methods have comparable method sensitivity. In the proposed UV CVG system with formic acid, a significantly lower sensitivity was observed for inorganic mercury. A similar phenomenon was observed in a UV CVG system with mercaptoethanol.[28] The reason is not known, but this phenomenon is very interesting and needs further investigation.

A typical chromatogram of MeHg, Hg, EtHg and PhHg standards at the 100 μg L^{-1} level was obtained under the optimized conditions (as shown in Fig. S5, ESI†). As can be seen, the four mercury compounds can be well separated in 35 min. Table 2 shows the analytical features of the proposed method under optimized conditions.

Mechanism of photo-induced chemical vapour generation

UV irradiation can induce radical reactions in water to produce H, OH or singlet oxygen, which result in the decomposition of organo-mercurials[25] and reduction of Hg^{2+}.[26] Therefore, four mercury species can still be detected even without formic acid in the post-column reaction solution. Literature reveals that OH, hydrogen and carbon monoxide radicals are generated in the photo-induced decomposition of formic acid.[29] The photo-induced OH fills the role of decomposing organomercurials to Hg^{2+}, while hydrogen and carbon monoxide radicals reduce Hg^{2+} to elemental mercury vapour.

Validation of the method and application

An alkaline extraction method was used for biological sample preparation, and the final sample for injection contained 10 mmol L^{-1} $Na_2S_2O_3$. Therefore, the influence of $Na_2S_2O_3$ in the sample matrix on separation and detection was investigated. The results showed that 10 mmol L^{-1} $Na_2S_2O_3$ in the sample had no influence on the separation and detection of

Table 2 Analytical performance of the developed HPLC-AFS system

Compound	Calibration curve	Relation coefficient	Detection limit/μg L^{-1}	RSD (%)[a]
MeHg	$Y = 23.17\,X - 129.1$	0.9987	0.81	2.8
Hg	$Y = 4.394\,X + 169.2$	0.9919	1.01	13
EtHg	$Y = 27.10\,X + 1061$	0.9978	0.20	6.3
PhHg	$Y = 16.40\,X + 10.95$	0.9965	0.87	5.5

[a] Standard concentration, 20 μg L^{-1}, $n = 3$.

Table 3 Results of MeHg contents in DORM-2 and seafood samples

Sample	MeHg concentration (mean[a] ± SD[b]/ ng g^{-1})	Certified value (mean[a] ± SD[b]/ ng g^{-1})
DORM-2	4657 ± 177	4470 ± 320
Rapana venosa (Dalian)	38.40 ± 8.35	—
Rapana venosa (Qingdao)	27.96 ± 1.62	—
Rapana venosa (Yingkou)	41.90 ± 1.86	—
Venerupis variegate (Huludao)	17.47 ± 0.32	—
Amusium japonica (Penglai)	ND	—

[a] $n = 3$. [b] Standard deviation.

mercury species. The feasibility of the developed method was examined by the determination of mercury species in certified reference material Dogfish Muscle (DORM-2) and seafood samples. As is shown in Table 3, good agreement between the certified value and analytical result was obtained. These results indicate that the developed method can be used to determine organic mercury species in biological samples.

Conclusions

A novel photo-induced chemical vapour generation with formic acid method was developed as an interface for the HPLC-AFS hyphenated system, and a new method based on this hyphenated system was proposed for the speciation analysis of four mercury compounds. The developed CVG systems used only one chemical reagent (formic acid), and the digestion of organomercurials and reduction to cold mercury vapour could be realized in one step. Compared with the traditional on-line UV $K_2S_2O_8$ digestion/KBH_4 reduction CVG system, this new CVG system has many advantages such as minimizing the possibility of contamination, simplifying instrumentation, decreasing analytical cost and eliminating interference and, moreover, this procedure is friendly to the environment. The proposed procedure possesses comparable method sensitivity with the conventional $K_2S_2O_8$/KBH_4 procedure, and was successfully applied to determining MeHg in standard reference material DORM-2 and seafood samples. The new HPLC-AFS hyphenated system using photo-induced CVG with formic acid as interface was expected to be applicable to the speciation of other organic metal compounds such as As, Se, Pb and Sn. Moreover, the new, powerful, CVG system could be applied as an interface for HPLC with other analytical atomic spectrometric techniques such as AAS, ICP-AES and ICP-MS.

Acknowledgements

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