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Cold vapor generation interface for mercury speciation coupling capillary electrophoresis with electrothermal quartz tube furnace atomic absorption spectrometry: Determination of mercury and methylmercury

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ABSTRACT

A novel on-line coupled capillary electrophoresis (CE) cold vapor generation (CVG) with electrothermal quartz tube furnace atomic absorption spectrometry (EQTF-AAS) system for mercury speciation has been developed. The mercury species (inorganic mercury and methylmercury) were completely separated by CE in a 80 cm length × 100 μm i.d. fused-silica capillary at 20 kV and using a buffer of 100 mM boric acid and 10% (v/v) methanol (pH 8.30). The effects of the inner diameter of quartz tube, the acidity of HCl, the $NaBH_4$ concentration and N_2 flow rate on Hg signal intensity were investigated. Speciation of mercury was highlighted using CE-CVG-EQTF-AAS. The detection limits of methylmercury and mercury were 0.035 and 0.027 μg mL^{-1}, respectively. The precisions (RSDs) of peak height for six replicate injections of a mixture of 10 μg mL^{-1} (as Hg) were better than 4%. The interface was used for speciation analysis of mercury in dry goldfish muscle.

1. Introduction

Speciation analysis of trace elements may lead to better understanding of the chemical/biochemical processes, environmental availability, and toxicological risks associated with different species [1,2]. Mercury is a highly toxic element because of its accumulative and persistent nature in the environment and biota [3]. Organomercury compounds are generally more toxic than inorgnomercury compound. Therefore, the speciation of mercury has become one of the focuses of scientific research in the global environment [4–9]. Because vapor generation techniques (e.g., cold vapor generation and hydride generation) can decrease the detection limit by 1–3 orders of magnitude for those volatile-vapor forming elements when compared to conventional nebulization techniques, they are constantly used for improving sensitivity of elemental analysis [10]. The high-efficient capillary electrophoresis (CE) was developed rapidly in the eighties of the 20th century as a novel separation and analysis technique. It offers many advantages, such as high efficiency, quick analysis, different modes of analysis, little reagent consumption, wide application, and easy automation. Therefore, it exhibits many important applications to life sciences, biotechnology, clinical medicine, medicine analysis and environmental science, and thus becomes very impressive in the field of speciation analysis [11,12]. Currently, inductively coupled plasma optical emission spectrometry (ICP-OES) [13–20], inductively coupled plasma mass spectrometry (ICP-MS) [13,21–26] and other detectors [27–29] have been employed extensively as on-line element-specific detectors in CE for elemental speciation. However, the comparatively high instrumental and operational costs of these ICP-based techniques as well as their requirements for well-trained analysts might limit their wide acceptance for routine speciation analysis in general laboratories [27].

As an alternative to ICP, atomic absorption spectrometry offers some advantages in terms of simple structure, easy operation, lower equipment and operation cost, good selectivity and good precision, so it is still widely employed in the field of metallurgical, geological, chemical, medical, clinic investigation and food hygiene. Li et al. had developed a capillary electrophoresis (CE) directly interfaced to flame-heated furnace atomic absorption spectrometry (FHF-AAS) *via* a laboratory-made thermospray interface for nanoliter trace mercury speciation, and studied a novel interface of coupling capillary electrophoresis with electrothermal atomic absorption spectroscopy (ET-AAS) [28,29]. Vieira et al. reported a photochemical vapor generation technique [30]. Wu et al. introduced sample aerosol into flame AAS by hyphenating pneumatic nebulization (PN) with flame furnace-AAS (FF-AAS) [31,32]. Although widely used as a stand-alone technique, CVG-FF-AAS and CVG-ET-AAS were not reported as detector for CE. In this paper, a detailed

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description of the CE-CVG interface design is given. The analytical conditions of capillary electrophoresis cold vapor generation electrothermal quartz tube furnace atomic absorption spectrometry (CE-CVG-EQTF-AAS) have been investigated for optimum resolution and signal-to-noise ratio. The application of the developed technique for speciation analysis of mercury in dry goldfish muscle is demonstrated.

2. Experimental

2.1. Instrumentation

Important operating parameters for the CE-CVG-EQTF-AAS system are summarized in Table 1. An 80 cm length × 100 μm i.d. fused-silica capillary (Yongnian Optical Fiber, Hebei, China) was used for CE separation. The sample solution was hydrodynamically introduced into the separation capillary under a N_2 gas pressure of 0.05 MPa for 16 s. A new capillary was conditioned by flushing sequentially with methanol for 30 min, 1 M NaOH for 30 min, and double distilled water (DDW) for 5 min under N_2 gas pressurization. Prior to separation, the capillary was flushed with the running buffer solution for 5 min. The capillary was reconditioned daily by flushing with 0.1 M NaOH and DDW for 10 min.

2.2. CE-CVG-EQTF-AAS interface

The CE-CVG-EQTF-AAS interface used in this work is shown in Fig. 1. The separation capillary was inserted through a PTFE tubing, which HCl solution was pumped through. Another PTFE tubing, located underneath, was for $NaBH_4$ solution. To prevent excessive gaseous production during the vapor generation process which would otherwise produce a back pressure on the CE capillary and thus lead to loss of current during the electrophoresis analysis, the ends of two PTFE tubings are placed 2 mm apart. Also, to reduce dead volume and to avoid convection band broadening, the following measures were taken: first, the coating of the CE capillary was peeled off about 1 cm at the exit by burning; second, the make-up liquid flow and the electroosmotic flow from the CE capillary were combined inside a 0.5 mm long PTFE tubing at the exiting terminus before they react with the reduction reagents (cf. Fig. 1). The CE-CVG-EQTF-AAS interface was based on a connection design for introducing a makeup solution of 5% (m/v) HCl around the CE capillary and a Pt electrode, which provided an electrical connection for stable electrophoretic separations. The make-up solution of 5% (m/v) HCl was used to facilitate the CE effluent delivery and also served as the required medium for subsequent vapor generation. The mixture of the 5% (m/v) HCl solution and CE effluent merged with 2% (m/v) $NaBH_4$ solution for the generation of mercury vapor

Table 1
CE-HG-EQTF-AAS operating parameters.

Capillary electrophoresis parameters	
Fused-silica capillary	80 cm × 100 μm id
Electrolyte buffer	100 mM H_3BO_3–10% (v/v) methanol (pH 8.3)
Applied voltage	20 KV
Sample injection	0.05 MPa × 16 s
Hydride generation operating conditions	
HCl concentration	5% (v/v)
$NaBH_4$ concentration	2.0% (m/v, in 0.3% NaOH)
Flow rate of HCl	18 mL h^{-1}
Flow rate of $NaBH_4$	12 mL h^{-1}
AAS operating conditions	
Mercury hollow cathode lamp	3 mA
Spectrum bandwidth	0.4 nm
Carrier gas flow rate of N_2	0.2 L min^{-1}

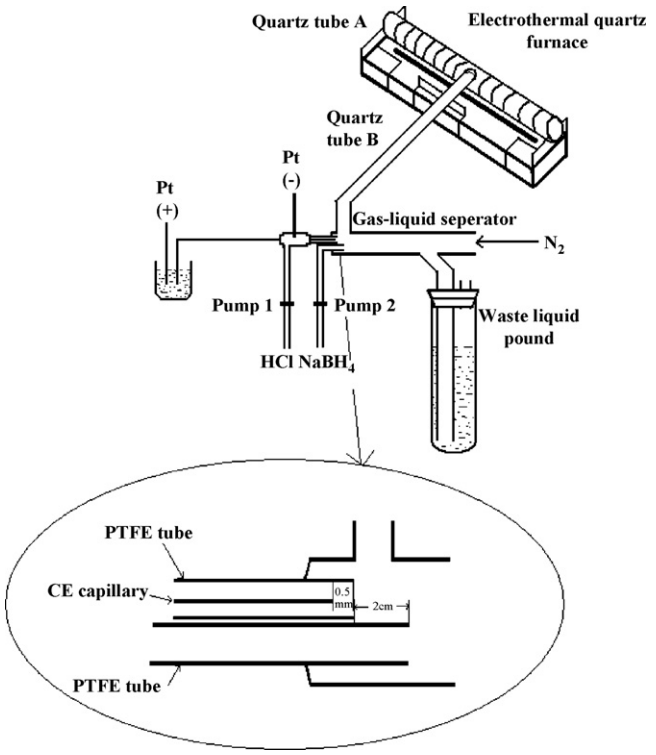

Fig. 1. Schematic diagram of the CE-CVG-EQTF-AAS.

(MV) and entered the hard glass tube together. The mixture of MV and hydrogen produced by VG reaction was transported directly into the quartz tube A with an argon gas flow and the waste liquid was flowed into the waste liquid collection pond, the waste gas was exhausted through the top outlet of atomic absorption spectrometry. Quartz tube A (4 mm i.d. × 13 cm length) was used as the electrothermal quartz tube furnace, into which the separated species was directly transported into a hole of 3 mm diameter in the center of the quartz tube wall. An atomic absorption spectrophotometer (Beijing Purkinje General Instrument, China) was used for data acquisition and data treatment. The advantage of the vapor generator mainly lies in good stability and high gas–liquid separate efficiency, washing easily, loading and unloading easily. In order to well separate analytes, the plus or minus electrode of the outlet end of capillary electrophoresis can be ascertained depending on the analytes that take different electric charges.

2.3. Reagents

All reagents were of the highest available purity and at least of analytical grade. The running electrolytes were a mixture of 100 mM boric acid (Beijing Chemicals, Beijing, China) and 10% (v/v) methanol (Shanghai Chemicals, Shanghai, China). The pH of the buffer solution was adjusted to 8.3 with 0.1 M NaOH (Beijing Chemicals). The buffer was filtered through a 0.45 μm filter prior to use. The stock solutions of methylmercury (MeHg) and mercury(II) chloride of 1000 mg L^{-1} (as Hg) were prepared, respectively, by dissolving suitable amounts of methylmercury chloride (Alfar Aesar) in methanol. Mercury(II) chloride was dissolved in 1% (v/v) HCl to prepare the stock solution of inorganic mercury (Hg (II)) of 1000 mg L^{-1}. All stock solutions were stocked at 277 K in the dark. Working standard solutions of mercury species were prepared by stepwise dilution of the stock solutions in 0.01% (m/v) aqueous cysteine solution just before use.

2.4. Sample preparation

Three of the goldfishes were used in the experiment with average weights of (5.60 ± 0.8) g. The muscle was freeze-dried (273 K) and used for later species analyses. Samples were prepared by using the well-known Westöö procedure [33,34]. 1.5 g of freeze-dried sample was extracted with a mixture of 10 mL DDW, 5 mL concentrated HCl and 10 mL toluene at 298 K for shaking 25 min, followed by centrifugation at 4000 rpm for 10 min. The organic phase in the supernatant was collected and the residue was extracted with another 5 mL of toluene as described above. The organic extracts were combined and back-extracted with 5 mL of 0.1% (m/v) L-cysteine solution. The final aqueous extracts were subjected to capillary electrophoresis separation [21].

3. Results and discussion

3.1. Optimization of CE separation conditions

The electrophoretic buffer is one of the key parameters for controlling CE separation. According to previous reports [28], the mixture of 100 mM boric acid and 10% (v/v) methanol (pH 8.3) was selected as the electrophoretic buffer in this work. This work utilized methanol as CE modifier to enhance the resolution by decreasing electroosmotic flow. The applied voltage was also examined in the range of 10–25 kV. The migration time decreased with the increasing of applied voltage. Lower voltage (less than 13 kV) resulted in poor peak shape and higher voltage produced severe noise. Therefore, a voltage of 20 kV was employed as the CE separation.

3.2. Effect of the electrothermal voltage

The optimization of analytical parameters was carried out using 10 μg mL^{-1} methylmercury. The effect of the electrothermal voltage on the signal intensity of CH_3Hg^+ was investigated. The rated power of furnace wire is 2125 W. The temperature of quartz tube furnace was controlled through changing the electrothermal voltage. When the voltage is over 100 V, the relative standard deviation is increasing. Therefore, 100 V was selected as the best electrothermal voltage in the next work.

3.3. Effect of the inner diameter of quartz tube

The optimization of the quartz tube inner diameters (QTID) consisted of determining the optimal absorbance of methylmercury without affecting the normal instrumental operating conditions. All the optimizations were performed by using 1 mm wall thickness and 13 cm length for quartz tube. The use of big QTID resulted in low absorbance due to leading to a dilution of the transient signals produced in the CE separation and reducing sensitivity. The use of less than 3 mm QTID in this work was found that the blank absorbance value of the instrument cannot be adjusted to zero. Because the light coming from instrument light source focuses on the central of quartz tube and both side ends have divergent phenomena, 3 mm QTID is too narrow for the light to completely go through the quartz tube. Therefore, 4 mm QTID was used for the remaining experiments.

3.4. Effect of the carrier gas flow rate

The effect of the carrier gas (N_2) flow rate on the signal of Hg was examined. With the set flow rate increases, the absorption reduced. However, the low flow rate will lead to time extension and the poor reproducibilities of results. Therefore, the carrier gas (N_2) flow rate was controlled in 0.2 L min^{-1}.

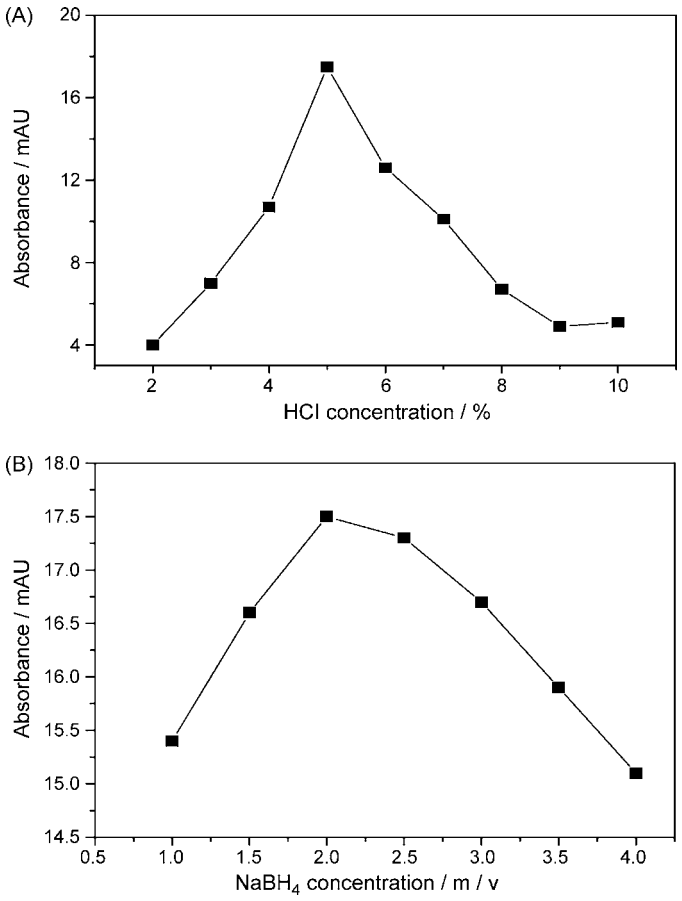

Fig. 2. The effect of acidity (A) and $NaBH_4$ concentration (B) on the signal intensity of 10 μg mL^{-1} CH_3Hg^+ (as Hg). All other conditions as in Table 1.

3.5. Effect of acidity of solution and concentration of $NaBH_4$ solution

The effect of the concentration of the acid solution and of the $NaBH_4$ solution on the CE-CVG-EQTF-AAS signal intensity of methylmercury was studied. The optimization was performed by injecting the methylmercury standards solutions in the CE-CVG-EQTF-AAS system. Fig. 2A showed absorbances of methylmercury plotted versus HCl concentration with 10% (v/v) HCl concentration established as the optimum concentration. As illustrated in Fig. 2A, when the HCl concentration above 5%, signal intensities of CH_3Hg^+ of 10 μg mL^{-1} can be partially reduced by hydrogen. Therefore, 5% HCl was chosen for subsequent experiments.

The $NaBH_4$ concentration not only affects the efficiency of mercury vapor generation but also relates to the data stability. Low concentration of $NaBH_4$ probably gave incomplete reduction of the Hg^{2+} and CH_3Hg^+, leading to low signal intensity. Higher $NaBH_4$ concentration would produce a great deal of hydrogen and produce high noise in quartz tube. The influence of $NaBH_4$ concentration was investigated at a flow rate of 18 mL h^{-1} (Fig. 2B). Taking noise into consideration, therefore, 2.0% (m/v) $NaBH_4$ was chosen as the optimum concentration for subsequent experiments.

3.6. Mercury speciation

All separation conditions for mercury speciation were summarized in Table 1. Fig. 3A showed that the standard mixture of Hg^{2+} and CH_3Hg^+ could be well-separated, therefore, the CE-CVG-EQTF-AAS was feasible for mercury speciation. In order to identify the peaks that corresponds to Hg species in Fig. 3A, 10 μg mL^{-1} CH_3Hg^+

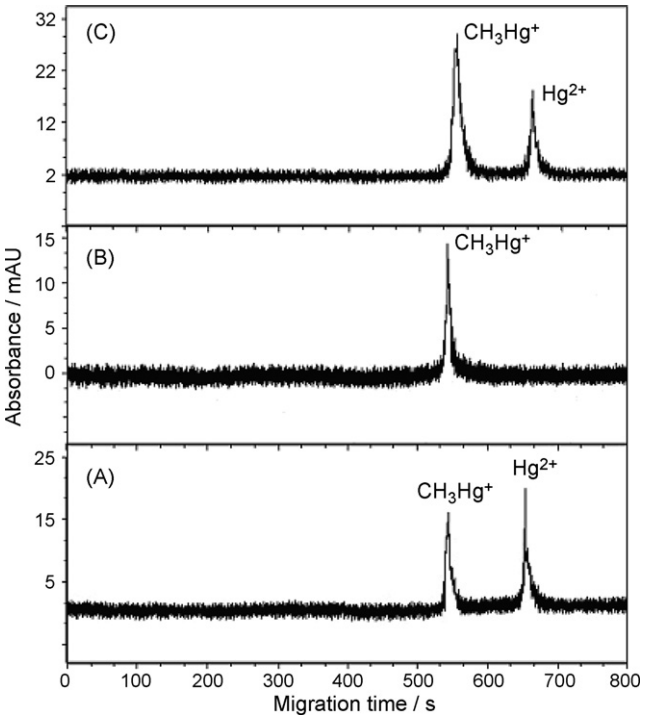

Fig. 3. Electropherogram of Hg speciation. A standard mixture containing 10 μg mL^{-1} of each individual Hg^{2+} and CH_3Hg^+ (A), 10 μg mL^{-1} CH_3Hg^+ (B), A spiked with 10 μg mL^{-1} CH_3Hg^+ (C). All other conditions as in Table 1.

was examined at the same condition (Fig. 3B). Comparing Fig. 3A and B, the first peak in Fig. 3A had the same migration time (~550 s) as in Fig. 3B. It is highly plausible that the first peak in Fig. 3A corresponds to CH_3Hg^+. In order to further identify the peak in Fig. 3A, 10 μg mL^{-1} $HgCH_3^+$ spiked into the solution prior to CE separation (Fig. 3C). Comparing Fig. 3A and C, the peak height at migration time ~550 s was increased substantially in the spiked samples whereas another peak in the electropherogram was almost not changed. Since the migration time of the first peak in Fig. 3A had nearly identical migration time (550 s) as the peak in Fig. 3B (552 s), it is further plausible that the first peak in Fig. 3A corresponds to CH_3Hg^+. Therefore, the second peak in Fig. 3A was Hg^{2+}.

3.7. Analytical performance of the CE-CVG-EQTF-AAS

As conditions showed in Table 1, 10 μg mL^{-1} for CH_3Hg^+ and Hg^{2+} each was detected by injecting 6 times, the relative standard deviations (RSDs) of CH_3Hg^+ and Hg^{2+} were 2.5% and 3.6%, respectively. The calibration curve for Hg^{2+} was described by the equation $A = 1.59 + 3.65C$ (C concentration unit in μg mL^{-1}, A unit in mAU), and that for methylmercury by $A = 1.44 + 2.88C$ (C concentration unit in μg mL^{-1}, A unit in mAU). The correlation coefficients of calibration curve for inorganic mercury and methylmercury were 0.9935 and 0.9952, respectively. The limits of detection (LOD, 3σ) for inorganic mercury and methylmercury were found to be 0.027 and 0.035 μg mL^{-1}, respectively.

3.8. Application

Introduction of the application of the CE-CVG-EQTF-AAS interface was demonstrated through the analysis of extract from exposure goldfish. The goldfish was grown in Little Dong River (Guilin, China). The extract procedures were introduced in Section 2. The electropherogram of extract from dry goldfish muscle was shown in Fig. 4. The peak of CH_3Hg^+ was achieved, but none of the

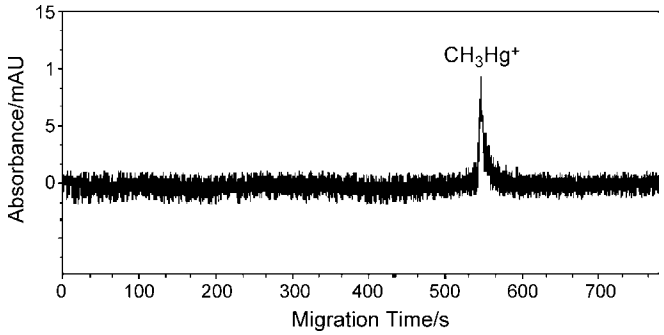

Fig. 4. Electropherogram of extract from dry goldfish muscle. All other conditions as in Table 1.

samples analyzed detected Hg^{2+}. According to absorbance from the calibration curve, the content of methylmercury was 8.17 μg g^{-1} for the dry goldfish muscle. The recoveries of CH_3Hg^+ spiked with 8.0 μg g^{-1} in the sample were 98.6%. The RSD ($n = 5$) of absorbance was less than 5%.

4. Conclusions

The results in the present work have demonstrated the feasibility of the proposed CE-CVG-EQTF-AAS technique for mercury speciation. Electrophoretic separation of the two mercury species was accomplished in less than 12 min with detection limits less than 0.04 μg mL^{-1} for each species. The low cost, simple structure, easy operation, less dead volume, good conductivity, good gas–liquid separation efficiency and good selectivity of AAS make it attractive as an on-line element-specific detector of CE for speciation of hydride-forming elements.

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