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Single-Drop Solution Electrode Discharge-Induced Cold Vapor Generation Coupling to Matrix Solid-Phase Dispersion: A Robust Approach for Sensitive Quantification of Total Mercury Distribution in Fish

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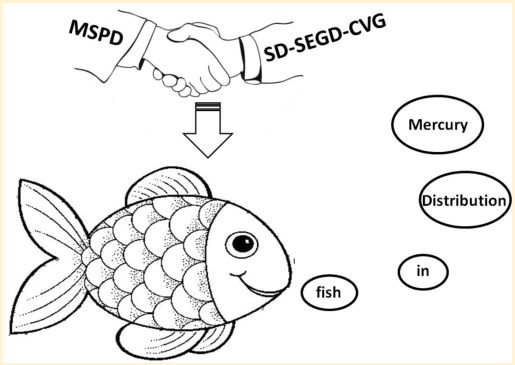
Supporting Information

ABSTRACT: Sensitive quantification of mercury distribution in fish is challenging because of insufficient sensitivities of conventional analytical methods, the limited mass of organs (tens of micrograms to several milligrams), and dilution of analyte concentration from sample digestion. In this work, a simple and robust approach coupling multiwall carbon nanotubes assisted matrix solid-phase dispersion (MWCNTs-MSPD) to single-drop solution electrode glow discharge-induced cold vapor generation (SD-SEGD-CVG) was developed for the sensitive determination of mercury in limited amount of sample. Mercury species contained in a limited amount of sample can be efficiently extracted into a 100 μL of eluent by MWCNTs-MSPD, which are conveniently converted to Hg^0 by SD-SEGD-CVG and further transported to atomic fluorescence spectrometry for their determination. Therefore, analyte dilution resulted from sample preparation is avoided and sensitivity is significantly improved. On the basis of consumption of 1 mg of sample, a limit of detection of 0.01 μg L^{-1} (0.2 pg) was obtained with relative standard deviations (RSDs) of 5.2% and 4.6% for 2 and 20 μg L^{-1}, respectively. The accuracy of the proposed method was validated by analysis of three Certified Reference Materials with satisfying results. To confirm that SD-SEGD-CVG-AFS coupling to MWCNTs-MSPD is a promising method to quantify mercury distribution in fish, this method was successfully applied for the sensitive determination of mercury in seven organs of common carps (muscle, gill, intestine, liver, gallbladder, brain, and eye) after dietary of mercury species. The proposed method provides advantages of minimum sample dilution, low blank, high sample introduction efficiency, high sensitivity, and minimum toxic chemicals and sample consumption.



It is well-known that the toxicity and bioavailability of mercury are strongly dependent on its chemical forms.[1,2] Previous works[1−3] revealed that about 95% methylmercury (MeHg) is accumulated in humans regardless of the route of exposure because it could readily cross biological barriers, whereas only 10% inorganic mercury (IHg) was remained. Recently, the mercury distribution in fish was found to be not only dependent on its chemical forms but also organ dependent.[4−6] MeHg is generally accumulated and permanently stored in muscle and brain, however, IHg is inclined to be accumulated in detoxification organs (liver and kidney). To date, the roles of organs for the uptake and distribution of Hg as well as the transportation and transformation of mercury species in organs are still not well understood. Consequently, quantification of mercury distribution in fish not only provides important information on which parts of fish are edible but also can provide insights of the mechanisms of bioaccumulation, transformation, and detoxification of mercury species.

Many efforts have been devoted to develop effective techniques for the quantification of mercury distribution.[7−12] Among these techniques, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is one of the most used techniques because of its excellent spatial resolution, high sensitivity, and selectively.[10−12] However, LA-ICP-MS is usually hampered by the lack of matrix matched standards for accurate quantification. To circumvent the requirement of matrix matched standards, a sample is usually digested into homogeneous solution prior to ICP-MS analysis. Unfortunately, conventional sample digestions (e.g., acid digestion and microwave assisted digestion) are tedious, often use concentrated mineral acid, and result in serious dilution of analyte,

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making them unsuitable for the determination of mercury in microamounts of sample. Matrix solid-phase dispersion (MSPD) is a simple and promising technique for the extraction of analyte from complex matrices without complete decomposition of sample.[13−15] Owing to its large surface area and excellent mechanical strength benefit for sufficient dispersion of sample matrix and diffusion of the eluent into the mixture of solid support and fish samples, multiwall carbon nanotubes (MWCNTs) was used as solid support for MSPD to successfully realize complete extraction of IHg and MeHg from 1 mg of fish tissues by using only 100 μL of eluent.[16] Compared to the conventional digestion techniques, MWCNTs-MSPD not only ensures the integrity of the original species of mercury but also minimizes analyte dilution, reduces consumption of toxic chemicals, and alleviates reagent blank.

Cold vapor generation (CVG) is frequently used to couple with atomic fluorescence spectrometry (AFS) or ICP-MS to improve their sensitivities and abilities of anti-interferences on the mercury analysis.[17−19] However, there are a number of serious impediments remaining to the determination of mercury in a microamount sample or individual organ even using CVG-AFS/ICP-MS, including serious dilution of analyte, high blank arising from digestants, and use of the relatively toxic and unstable chemicals.[20] Therefore, development of a new CVG technique which retains advantages of reduction of toxic chemicals consumption, alleviation of analyte dilution, and interferences will significantly advance the toxicological study of mercury. To generate volatile species of analyte from microamount of sample directly, we have successfully developed a single drop solution electrode glow discharge induced (SD-SEGD) chemical vapor generation technique to convert Zn(II) and Cd(II) to their volatile species from limited amounts of samples (5−20 μL) without using any chemicals.[21] This technique not only retains the advantages of conventional chemical vapor generation but also provides several unique advantages, including higher sensitivity, elimination of analyte dilution, and less consumption of toxic and unstable chemicals. Thus, we believe that SD-SEGD-CVG-AFS coupling to MWCNTs-MSPD would be a most promising technique for the determination of mercury in limited amount of samples.

The purpose of this work was to investigate the feasibility of SD-SEGD-CVG-AFS for the determination of mercury species in a microamount of sample and then further couple it to MWCNTs-MSPD for the quantification of mercury distribution in fish organs. The proposed system retains both the advantages of MWCNTs-MSPD and SD-SEGD-CVG and successfully used for the quantification of mercury distribution in fish organs.

EXPERIMENTAL SECTION

Reagents. All reagents used in this work were analytical reagent grade. 18.2 MΩ cm of deionized water (DIW) was produced from a water purification system (Chengdu Ultrapure Technology Co., Ltd., China). Formic acid and other chemicals were purchased from Kelong Factory (Chengdu, China). L-Cysteine was from Aladdin industrial Co. (Shanghai, China). A 60 Å of octadecyl-functionalized silica gel (DAISOGEL C_{18}) was purchased from DASIO Co., Ltd (Osaka, Japan). MWCNTs (purity, >95 wt %; 5−15 nm i.d.× 50 nm o.d. × 10−20 μm in length) were obtained from Chengdu Organic Chemicals Co. Ltd. (Chengdu, China). High purity Ar (99.999%) from Qiaoyuan Gas Co. (Chengdu, China) was used as both discharge gas and carrier gas. The stock solutions of IHg (1000 mg L^{-1}) and MeHg (1000 mg L^{-1}) were prepared by diluting mercury chloride ($HgCl_2$, 99%, Aladdin, Shanghai, China) and methylmercury chloride (CH_3HgCl, 99%, Aladdin) with methanol (HPLC grade, Aladdin), respectively. Standard stock solutions of IHg (GBW08617) and MeHg (GBW08675) from the National Research Center for Certified Reference Materials (Beijing, China) were used for the quality control of the stock solutions. Certified Reference Materials (CRMs) were obtained from National Research Council Canada (NRCC), including dogfish muscle (DORM-2 and DORM-4) and lobster hepatopancreas (TORT-3).

Instrumentation. The analytical system consisted of a MWCNTs-MSPD device, a SD-SEGD-CVG generator, and a commercial atomic fluorescence spectrometer (AFS-9600, Beijing Haiguang Instrument Co. Ltd., Beijing, China). A schematic diagram of this system is illustrated in Figure 1a. The

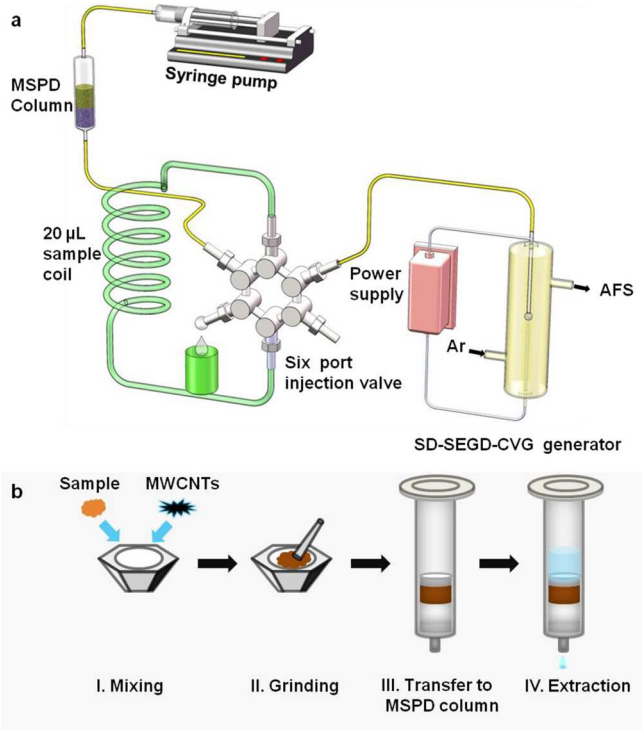

Figure 1. (a) Schematic of the experimental setup; (b) MWCNTs-MSPD procedure.

AFS was fitted with a coded high intensity mercury hollow cathode lamp (HCL), a quartz gas liquid separator (GLS), and a quartz atomizer. Optimizations of operation parameters for AFS were undertaken independently, as shown in Table S1 (see section 1 of the Supporting Information). The MWCNTs-MSPD device was constructed with a syringe pump (Harvard Apparatus, Inc. Massachusetts, USA), a MSPD column (4.6 mm i.d. × 50 mm length) with polyethylene frits, and a six-port injection valve (Genuine Rheodyne Co.) equipped with a 20 μL sample loop. The SD-SEGD-CVG generator offers both generation and gas−liquid separation functions and consists of a quartz tube (8 mm i.d. × 10 mm o.d. × 8.5 cm length), a tapered tungsten electrode, and a stainless steel tube (0.5 mm i.d. × 1.5 mm o.d. × 5 cm length). The liquid drop hung on the steel tube, and the tungsten electrode was used as the up and down electrodes, respectively. The plasma was ignited and sustained in the gap when a high voltage applied between the electrodes using a compact ac ozone generation power supply

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(YG.BP105P; 6 cm length × 4 cm width × 3 cm height; with a rated output of 4 kV, 20 kHz, and 12 W at 220 V, 50 Hz input (Electronic Equipment Factory of Guangzhou Salvage, Guangzhou, China).

A field-emission scanning electron microscopy (SEM) (JEOL, JSM-7500F) was used to characterize the fish organs ground with or without MWCNTs.

Sample Preparation. All sampling procedures were approved by the Aquitaine Ethics Committee for fish and birds. A total of 45 common carps (body weight = 10.6 ± 2.2 g wet weight (WW); standard length = 60 ± 5 mm) were obtained from a local supermarket. To make the fish adapt to the environmental change, 1 week was required before exposure of fish to mercury species. These common carps were separated into five groups and exposed to different dietary conditions during 14 days: (1) five of them used as control group, (2) 10 of them diet with 10 μg L^{-1} IHg, (3) 10 of them diet with 10 μg L^{-1} MeHg, (4) 10 of them diet with 1.0 μg L^{-1} IHg, and (5) another 10 fish diet with 1.0 μg L^{-1} MeHg. Five groups of fish were maintained in 10 L of tanks, separately, at a constant temperature of 25 °C. The stock density was 0.5 L of water per fish. The water containing mercury species was replaced daily to maintain water quality. After exposure to mercury species for 13 days followed by 1 day for depuration, fishes were harvested, and muscle, eye, brain, gill, liver, gallbladder, and intestine (Supporting Information, Figure S1) were separately collected, which were then freeze-dried for 24 h and stored in a refrigerator at 4 °C prior to analysis.

MWCNTs-MSPD Procedure. The MWCNTs-MSPD procedure is depicted in Figure 1b. Briefly, 1 mg of each fish organ (dry weight) or muscle were accurately weighed into an agate mortar and then blended with 0.5 mg of MWCNTs for 5 min using an agate pestle to obtain a homogeneous mixture. This mixture was quantitatively transferred to a 3 mL syringe wherein 0.15 g of C_{18} was placed between two polyethylene frits to prevent the sample mixture from being flushed into the liquid drop. Then a third polyethylene frit was placed at the top of the syringe and slightly compressed with a syringe plunger. Then 100 μL of eluent containing 0.5% L-cysteine and 4% HCOOH was injected into the syringe and reacted with the samples dispersed on MWCNTs for 3 min. Then the eluent was pushed out of the syringe and stored in a 0.5 mL of polypropylene tube. Finally, another 100 μL of the eluent was used to extract the sample again and mixed with the first extract prior to analysis.

Analytical Procedure. With the aid of the peristaltic pump, the eluent was initially directed to a 20 μL sample loop through the six-port valve. The valve was activated to pass Ar carrier gas to push the solution to form a drop, which was hung on the end of the steel tube. A 60 V voltage was supplied to generate and sustain microplasma for 10 s, and then the mercury species were converted to mercury cold vapor (Hg^0) and further swept to AFS for detection.

RESULTS AND DISCUSSION

Preliminary Studies and Optimization of Operation Parameters of SD-SEGD-CVG-AFS. Despite SD-SEGD-CVG having been successfully used to convert Zn(II) and Cd(II) to their volatile species,[21] this is the first attempt to utilize this technique for the generation of Hg^0 from IHg and MeHg. Therefore, initial experiments were conducted to prove the feasibility and practicability of SD-SEGD-CVG on the generation of Hg^0 from limited amounts of samples. When 20 μL of a standard solution containing 10 μg L^{-1} Hg(II) was pumped into the generator, intense mercury response was directly detected by AFS without using any atomization/ionization, confirming that the Hg^0 was indeed generated. As shown in Figure 2, a linear coefficient of a typical calibration

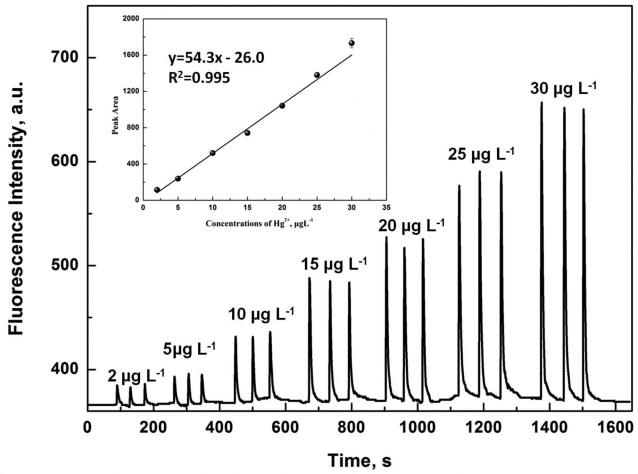

Figure 2. Calibration curves established using FI-SD-SEGD-CVG-AFS.

curve of the atomic fluorescence intensity versus the concentration of IHg better than 0.99 was obtained. These observations support the feasibility of SD-SEGD-CVG-AFS for the determination of mercury in limited amounts of samples.

As this is the first report of using SD-SEGD-CVG-AFS for the mercury analysis, it is necessary to undertake investigations of all influencing parameters on the generation of Hg^0 using SD-SEGD-CVG, including Ar carrier gas flow rate, discharge voltage, reaction medium, and discharge gap (see Figure S2 in section 3 of the Supporting Information). The effect of mercury species on generation efficiency of Hg^0 was also investigated, and the results indicate that both IHg and MeHg can be efficiently reduced to Hg^0.

Optimization of MWCNTs-MSPD. The previous work reported that mercury species in fish muscle could be extracted by the MWCNTs-MSPD using an eluent containing HCl and L-cysteine.[16] Unfortunately, many previous studies reported that Cl^- significantly depressed the CVG efficiencies for various mercury species.[17−19] Thus, HCOOH was used as an alternative to HCl for the MSPD. To improve the extraction efficiency of mercury, the effects of the concentrations of L-cysteine and HCOOH, the mass of MWCNTs, the eluent volume, and the extraction time on the extraction efficiency of mercury were investigated in detail, as shown in Supporting Information, Figure S3. DORM-2 (fish muscle) was used for these optimizations.

Similar to the previous work,[16] only 60% of mercury was extracted when 1 mg of DORM-2 was directly transported into the MSPD column without grinding with MWCNTs, and this extraction efficiency was increased to 98% with use of 0.4 mg MWCNTs, followed by decrease at the higher mass of MWCNTs, as shown in Supporting Information, Figure S3A. This is because lower MWCNTs mass cannot be dispersed in the fish tissues homogeneously, resulting in low extraction efficiencies whereas higher MWCNTs mass results in inadequate interaction between the tissues and the eluent due to the fact that only 100 μL of eluent was used. A MWCNTs mass of 0.5 mg was thus selected for all subsequent experiments. According to the previous studies,[22,23] both microplasma

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Table 1. Comparison of Performance with Other Methods

method	sample consumption (μL)	sensitivity (L μg $^{-1}$)	LOD (μg L^{-1})	absolute LOD (pg)	RSD (%)
FI-SD-SEGD-CVG-AFS[a]	20		0.01	0.2	5.2
FI-CVG using THB-AFS[b]	20				
FI-CVG using THB-AFS (ref25)	50	0.237	10	500	1.5−3.0
FI-PVG-AFS (ref26)	2000	590	0.005	10	2.2
FI−SI−CVG-AFS (ref26)	2000	522	0.01	20	3.1
FI-DBD-CVG-AFS (ref27)	300		0.0045	1.35	2.6
FI-CVG using $SnCl_2$-AAS (ref28)	5000		0.4	2000	5
FI-CVG using THB-AAS (ref29)	300		9	2700	5
FI-SCGD-CVG-OES (ref30)	100		1.2	1200	2.2
FI-CVG using THB-ICP-MS (ref31)	500		0.004	2	1.3
CVG using THB-AFS (ref32)	2400	580	0.03	72	2.2
UV LED-PVG-AFS (ref32)	2000	300	0.01	20	3.2
CVG using $SnCl_2$-AFS (ref33)	2400	544	0.01	24	2.2
UV-PVG-AFS (ref34)	2400	614	0.003	6.2	3.6
CVG using $SnCl_2$-AAS (ref35)			0.25		2.98
ICP-OES (ref22)	2500 μL min^{-1}		30		
PVG-ICP-OES (ref22)	2000 μL min^{-1}		0.1		
DBD-CVG-OES (ref23)	2000 μL min^{-1}		0.09		2.1
CVG using $SnCl_2$-ICP-MS (ref36)	1200 μL min^{-1}		0.08		

[a]The proposed work. [b]Obtained by the same AFS detector under optimum conditions. FI, flow injection; PVG, photochemical vapor generation; SI, sono-induced; DBD, dielectric barrier discharge; SCGD, solution cathode glow discharge; LED, light-emitting-diodes; ICP-OES, inductively coupled plasma optical emission spectrometry.

induced CVG and PVG efficiencies of mercury were remarkably improved in the presence of formic acid. The formic acid not only plays the role of HCl in the reported MSPD but also provides reducing radicals to significantly improve the reduction of mercury species to Hg^0. Supporting Information, Figure S3B shows that the recoveries were increased significantly with increasing concentration of formic acid from 0 to 4% (v/v), followed by a plateau at higher concentrations. Because of the affinity of Hg^{2+} and sulfur-containing groups, L-cysteine can form stable complexes with mercury species and thus was frequently used to efficiently extract mercury species from complex matrices.[24] Supporting Information, Figure S3C demonstrates that the extraction efficiency was significantly increased over the range of 0−0.5% (m/v). However, the extraction efficiency unexpectedly and sharply decreased at higher concentrations, which is inconsistent with that reported in previous work.[16] It is speculated that the higher concentration of L-cysteine may suppress the SEGD-CVG efficiency of mercury by forming the complex of mercury and L-cysteine. Two approaches were used to validate this hypothesis: comparison of the SD-SEGD-CVG-AFS signal obtained from several standard solutions containing 10 μg L^{-1} of Hg^{2+} to which various concentrations of L-cysteine were added, respectively, direct analysis of the eluents obtained using mixture containing 4% HCOOH, and different concentration of L-cysteine by pneumatic nebulization ICP-MS. These results are presented in Supporting Information, Figure S4. It is evident that mercury species can be completely extracted when the concentration of L-cysteine is beyond 0.5%, and the CVG efficiency of mercury decreased to about 30% in the presence of 2% (m/v) of L-cysteine. Therefore, an eluent containing 4% (v/v) HCOOH and 0.5% (m/v) L-cysteine was selected for all subsequent experiments. The effects of the eluent volume and extraction time were also optimized and summarized in Supporting Information, Figure S3D,E, respectively. To avoid the drawbacks of conventional digestion techniques including dilution of analyte, a tedious procedure, low sample throughput, and the use of toxic chemicals, 100 μL of eluent and 3 min of extraction time were thus chosen for all subsequent experiments. The effects of concomitant ions on SD-SED-CVG of 10 μg L^{-1} Hg^{2+} were investigated and its detailed results summarized in section 5 of Supporting Information.

Analytical Figures of Merit. Table 1 summarizes analytical figures of merit achieved by SD-SEGD-CVG-AFS and comparisons of its performance with several other analytical methods. Under the optimum conditions, typical calibration curves (Supporting Information, Figure S6) obtained for IHg and MeHg can be characterized by the following calibration functions: $I_{IHg} = 69.0C_{IHg} - 10.9$ and $I_{MeHg} = 65.3C_{MeHg} - 16.9$ for IHg and MeHg, respectively, where C is the concentration (μg L^{-1}). Linear coefficients are better than 0.99 in both cases, and there is no significant difference in the generation efficiencies of IHg and MeHg. Therefore, a simple IHg standard can be used to construct a calibration curve for the determination of total mercury in samples. Precision of replicate measurements, expressed as a relative standard deviation (RSD, $n = 9$) is better than 5% regardless of the concentration of IHg. Supporting Information, Figure S7 shows the temporal profiles of repeat flow injection SD-SEGD-CVG-AFS analyses of 2 and 20 μg L^{-1} of IHg with the RSDs of 5.2% and 4.6%, respectively. The limit of detection (LOD), defined as the analyte concentration equivalent to 3 standard deviations of 11 measurements of a blank solution, is 0.01 μg L^{-1} (0.2 pg), comparable or better than those obtained by other similar techniques. More importantly, the absolute LOD obtained using the proposed method is 100-fold better than those using conventional CVG-AFS. Because only 20 μL of sample was used, it was suitable for the determination of trace mercury in limited amounts of sample. The severe memory effect encountered in CVG or pneumatic nebulization ICP-MS for the determination of Hg significantly limits further applications of CVG-AFS/ICP-MS for the determination of ultratrace mercury. Ten μg L^{-1} of Hg^{2+} was used to compare the memory effects obtained by SD-SEGD-CVG-AFS and CVG-AFS using

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the $NaBH_4$−HCl system, respectively. The results are summarized in Figure S8 and no significant memory effect was observed using the proposed system, whereas ∼10% of the initial signal still remained using the conventional technique even after three times washing. Three reasons may contribute to this significant alleviation of memory effect: (1) Only 20 μL of sample was used, whereas sample consumption was at least 1 mL in conventional CVG-AFS; (2) mercury adsorption on the surface of transport tube was inhibited because L-cysteine was used; (3) the length of the stainless steel transport tube was reduced to 5 cm and thus minimized the residual mercury in the transport tube. Although flow injection sampling technique can significantly alleviate the memory effect, the sensitivity and LOD of the conventional flow injection CVG-AFS were 80- and 100-fold worse, respectively, compared to the proposed technique. This is because analyte contained in 20 μL samples was remarkably diluted with the carrier solution when the conventional flow injection CVG-AFS was used. All above observations confirm that the proposed SD-SEGD-CVG-AFS method is the most ideal for the determination of trace mercury in limited amounts of samples because it not only retains the advantages of conventional continuous or flow injection CVG atomic spectrometry but also eliminates memory effect, analyte dilution, and consumption of toxic chemicals. In addition, it provides better sensitivity and LOD.

Validation of the Proposed Method. Several NRCC biological CRMs of DORM-2 (fish muscle), DORM-4 (fish muscle), and TORT-3 (lobster muscle) were analyzed to evaluate the accuracy of the proposed method. Analytical results are summarized in Table 2. The results of the *t* test show no significant differences between the obtained and certified values at the confidence level of 95%, demonstrating the accuracy of the proposed method, which offers a great potential for a simple, rapid, and sensitive determination of mercury in limited amounts of sample.

Table 2. Analytical Results of Mercury in CRMs

sample	determined[a] (mg kg^{-1})	certified (mg kg^{-1})
TORT-3	0.319 ± 0.087	0.292 ± 0.022
DORM-4	0.407 ± 0.085	0.410 ± 0.055
DORM-2	4.32 ± 0.17	4.64 ± 0.26

[a]Mean and standard deviation of results (n = 3).

Quantification of Mercury Distribution in Common Carp. Although the accuracy of the proposed method for determination of mercury in 1 mg of fish muscle has been validated, the components of eye, brain, gill, liver, intestine, and gallbladder are quite different from those of muscle. Thus, the capability of MWCNTs-MPSD on complete extraction of mercury from various organs of fish was initially investigated prior to the use of the proposed method for the quantification of mercury distribution in common carp. Because the exact concentrations of mercury in the tested organs are not known, thus the extraction efficiency of each extraction (E_n) is thus defined as

$$E_n\% = \frac{C_n \cdot 100}{C_1 + C_2 + C_3 + C_4 + C_5}$$

where C_n is the concentration (μg L^{-1}) of mercury in eluent of the nth extraction (n = 1, 2, 3, 4 or 5). As shown in Figure 3, the extraction efficiencies of MeHg from the first extraction are higher than 80% in the case of dietary MeHg, whereas those of dietary IHg are below 75%. This agrees well with the previous work[16] wherein it was reported that the extraction of MeHg from fish tissue was easier compared to that of IHg. In general, more than 90% of mercury can be extracted via two times extraction regardless of dietary mercury species or organs. In consideration of achieving complete extraction of mercury, improvement of sample throughput, and elimination of analyte dilution, extraction two times was thus chosen for the quantification of mercury distribution.

It is interesting to know why this simple extraction method can efficiently extract mercury with using only 200 μL of eluent under such mild conditions without restriction of sample matrix. Parts a−d and i−k Figure 4 show the SEM images of the ground organs without using MWCNTs, respectively. It is clear that without MWCNTs the organ tissues still aggregate together and, even worse, liquid film densely coated on the surface of the organ tissue once the eluent flowed through the MSPD column, inhibiting sufficient penetration of the eluent into the tissues for the efficient extraction of mercury. On the contrary, the SEM images of the mixtures of organ ground with MWCNTs (Figure 4e−h and m−o) show that the organ tissues were evenly dispersed on MWCNTs which benefit the eluent easily diffused into the tissues, improving extraction of mercury species. This is attributed to the unique properties of MWCNTs such as its excellent mechanical strength, high surface area, flexibility, and dramatic hydrophobic surface, which means it not only cannot be ground into powder (Figure 4l,p) but also generate abundant carbon nanofibers, thus improving the dispersion of the fish tissues and preventing the aggregation of the mixture.

Above results confirm that complete extraction of mercury from various organs was achieved using the proposed method, thus it was applied for the quantification of mercury distribution in seven organs (muscle, gill, intestine, liver, gallbladder, brain, and eye) of common carp. The concentrations of mercury in the organs from the control group were below detection limit, indicating no significant Hg contamination arising from DIW and tank environment used for fish. Because of the high sensitivity of the proposed technique, the concentrations of mercury contained in all the tested organs can be detected regardless of the dietary mercury species and concentrations. The analytical results summarized in Figure 5 show that the concentration of mercury in intestines is the highest among those of other organs in all cases, possibly due to the intestine being the major site of digestion and ingested mercury species was not transported to other organs in such short dietary time (14 days). The concentrations of mercury in each tested organs exposed to MeHg are much higher than these exposed to IHg, particularly in brain and eye. These agree well with the fact that MeHg is more lipophilic than IHg and easily passes through the biological barriers such as placental, brain−blood, and retinal−blood barriers. According to previous works, the bioaccumulation factor (BAF) was calculated as the following function:

$$\mathrm{BAF} = \frac{C_o}{C_W}$$

where C_o and C_w are the concentrations of mercury in fish organs and water, respectively. The BAFs of MeHg and IHg of various organs are presented in Table 3; it is evident that the BAFs of MeHg in each organ is much higher than that of IHg. In addition, the BAFs obtained at low levels of mercury exposure are higher than those obtained at high levels. Clearly, IHg favored accumulation in detoxification organs (liver and

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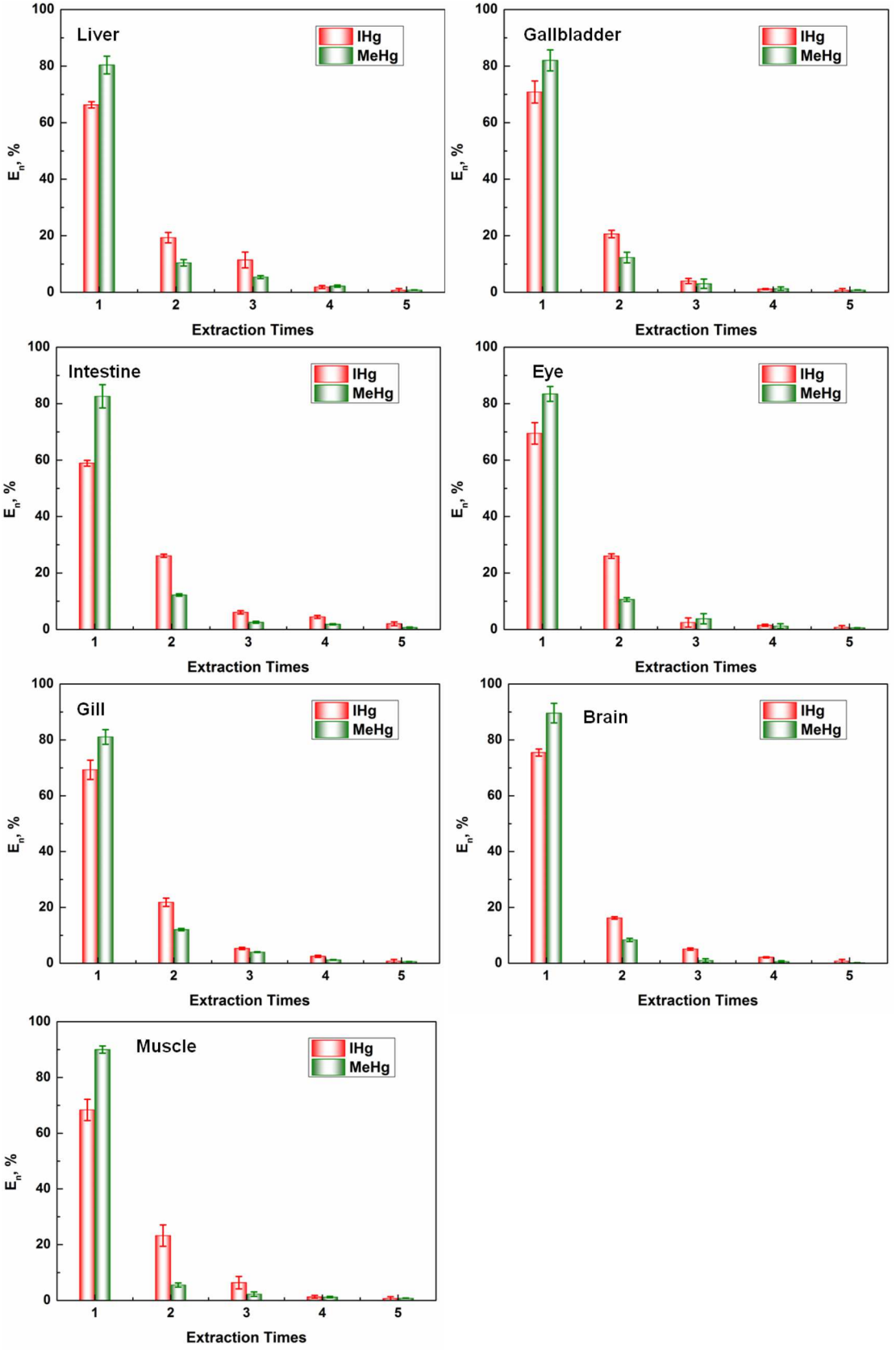

Figure 3. Extraction of mercury from different organs after dietary of different concentrations of MeHg or IHg.

kidney), in agreement with observations in previous studies.[4−6] It should be noted that when the dry masses of the gallbladder and liver are only several milligrams, the concentration of analyte would be diluted about 10000-fold if a conventional digestion method is used and thus making the conventional analytical methods unsuitable for such measurements.

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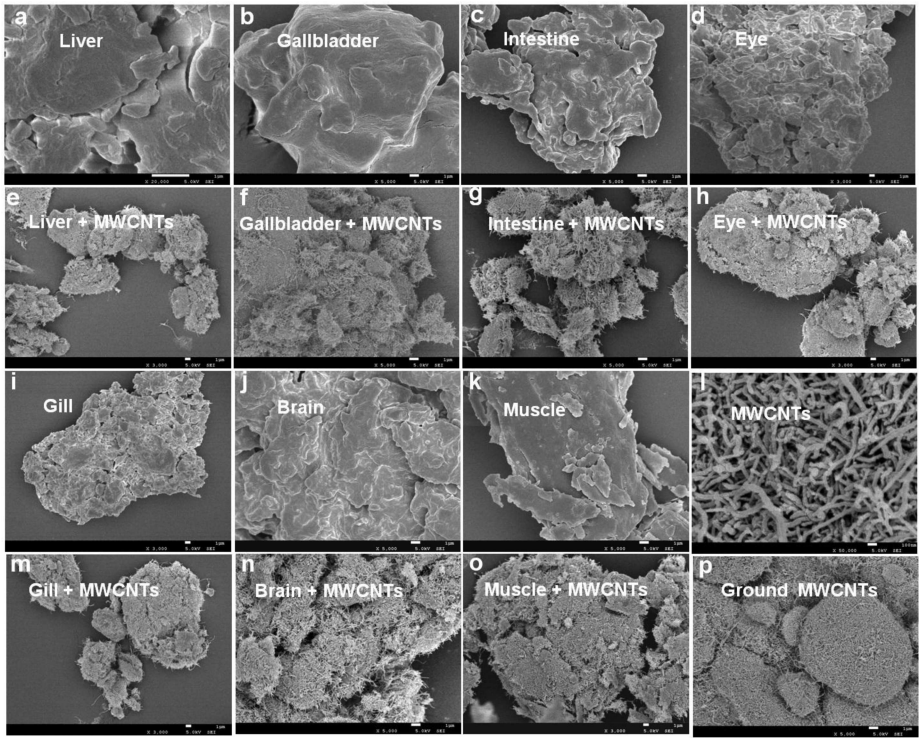

Figure 4. SEM images of organs ground without ((a) liver, (b) gallbladder, (c) intestine, (d) eye, (i) gill, (j) brain, (k) muscle) or with ((e) liver, (f) gallbladder, (g) intestine, (h) eye, (m) gill, (n) brain, (o) muscle) MWCNTs before (l) and after (p) grinding.

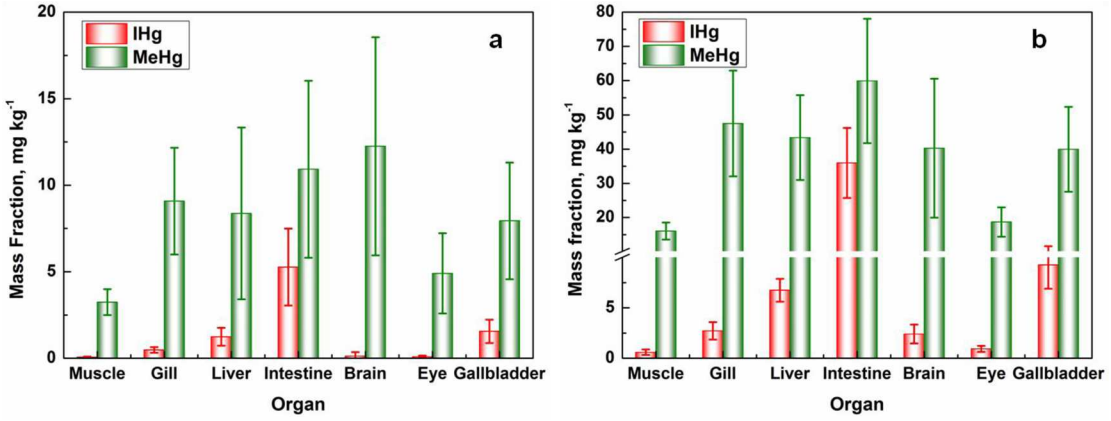

Figure 5. Distribution of mercury in meat, gill, liver, intestine, brain, eye, and gallbladder of fish after 13 days of exposure to mercury species and 1 day of depuration (n = 5). (a) Dietary of 1 μg L^{-1} MeHg or IHg; (b) dietary of 10 μg L^{-1} MeHg or IHg.

Table 3. Bioaccumulation Factors of Mercury Species in Various Organs[a]

	1 μg L^{-1} of IHg	10 μg L^{-1} of IHg	1 μg L^{-1} of MeHg	10 μg L^{-1} of MeHg
muscle	65 ± 34	59 ± 28	3200 ± 7450	1600 ± 240
gill	480 ± 170	270 ± 86	9100 ± 3100	4700 ± 1500
liver	1200 ± 510	670 ± 110	8400 ± 5000	4300 ± 1200
intestine	5300 ± 2200	3600 ± 1000	11000 ± 5200	6000 ± 1800
brain	122 ± 104	240 ± 94	12000 ± 6300	4000 ± 2000
eye	90 ± 60	93 ± 30	4900 ± 2300	1900 ± 430
gallbladder	1600 ± 670	930 ± 240	7900 ± 3400	4000 ± 1200

[a]Mean and standard deviation of results (n = 5).

CONCLUSIONS

Highly efficient generation of Hg^0 from IHg and MeHg contained in a limited amount of samples was achieved in the presence of formic acid by SD-SEGD-CVG. This method was further coupled with MWCNTs-MSPD to demonstrate its successful application to the accurate quantification of mercury distribution in fish organs. Compared to the conventional method, the proposed method retains both advantages of SD-

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SEGD-CVG and MWCNTs-MSPD, including higher sensitivity, elimination of analyte dilution, alleviation of reagent blank, and less consumption of toxic and unstable chemicals. Thus, this method is helpful for the toxicological study of mercury. Meanwhile, it remains to explore the potential of the proposed method for the quantification of other elements in fish because the determination of Zn and Cd in microamounts of sample by SD-SEGD-CVG-AFS has been accomplished.[21] In addition, when the proposed system coupled with a capillary electrophoresis or high performance chromatography, it retains a great potential for the quantification of elemental species distribution in fish or other animals, advancing the toxicological studies of elements.

■ ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org/. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.6b04753.

Optimum parameters for AFS; photographs of the organs of common carp; optimization of SD-SEGD-CVG; effect of the Ar discharge gas; effect of discharge voltage; effect of discharge gap; effect of formic acid; optimization of MWCNTs assisted matrix solid phase dispersion; effect of L-cysteine concentration of CVG efficiency of mercury by FI-SD-SEGD-CVG and extraction efficiency of mercury from DORM-2; interference of coexisting ions; typical calibration curves for IHg and MeHg; repeatability of the proposed method; comparison of the memory effects obtained by conventional CVG-AFS and FI-SD-SEGD-CVG-AFS (PDF)

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Notes

The authors declare no competing financial interest.

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