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Research Article

Speciation analysis of mercury in water samples by dispersive liquid–liquid microextraction coupled to capillary electrophoresis

In this study, a method of pretreatment and speciation analysis of mercury by dispersive liquid–liquid microextraction along with CE was developed. The method was based on the fact that mercury species including methylmercury (MeHg), ethylmercury (EtHg), phenylmercury (PhHg), and Hg(II) were complexed with 1-(2-pyridylazo)-2-naphthol to form hydrophobic chelates and L-cysteine could displace 1-(2-pyridylazo)-2-naphthol to form hydrophilic chelates with the four mercury species. Factors affecting complex formation and extraction efficiency, such as pH value, type, and volume of extractive solvent and disperser solvent, concentration of the chelating agent, ultrasonic time, and buffer solution were investigated. Under the optimal conditions, the enrichment factors were 102, 118, 547, and 46, and the LODs were 1.79, 1.62, 0.23, and 1.50 μg/L for MeHg, EtHg, PhHg, and Hg(II), respectively. Method precisions (RSD, $n = 5$) were in the range of 0.29–0.54% for migration time, and 3.08–7.80% for peak area. Satisfactory recoveries ranging from 82.38 to 98.76% were obtained with seawater, lake, and tap water samples spiked at three concentration levels, respectively, with RSD ($n = 5$) of 1.98–7.18%. This method was demonstrated to be simple, convenient, rapid, cost-effective, and environmentally benign, and could be used as an ideal alternative to existing methods for analyzing trace residues of mercury species in water samples.

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1 Introduction

Mercury is considered as one of the most toxic elements impacting on human and ecosystem health with its persistent accumulation and large distribution [1–3]. A wide range of mercury species exist within our environment especially in some water samples and the common forms of mercury are inorganic mercury (Hg(II)) and organic mercury (methylmercury (MeHg), ethylmercury (EtHg), and phenylmercury (PhHg)) [3]. All mercury species are toxic, but, organic mercury compounds generally being more toxic than inorganic mercury [3–5]. Their toxicity depends strongly on their chemical forms, which control mercury transport, bioavailability, and persistence [3–5]. Therefore, speciation analysis of mercury ion and its organic compounds has become a crucial topic for pollution monitoring and remediation.

Many analytical techniques have been developed for the separation and determination of mercury species, such as GC [6], HPLC [7], ion chromatography [8], atomic absorption/fluorescence spectrometry (AAS/AFS) [9, 10], ICP-MS/optical emission spectrometry (ICP-MS/OES) [11, 12], as well as some combination techniques, such as GC-ICP-MS [13] and HPLC-ICP-MS [14]. Recently, CE has been widely used and become an extremely powerful technique in the analysis of mercury speciation since separation

in CE is mainly governed by difference in charge to size ratio of analytes [15]. In addition, CE has the advantages of small sample size, low solvent consumption, high separation efficiency, and short analysis time [16, 17]. However, because the injection volume is small, optical path length is narrow and UV–Vis absorbance of metal ions is weak, CE-UV faces a severe problem of poor sensitivity for use at trace and ultratrace analysis [16, 18]. Consequently, developing high-efficiency enrichment techniques has become more and more important to improve the analytical sensitivity in CE [19].

For mercury speciation, various pretreatment and preconcentration procedures have been well used to couple with some of the above-mentioned analytical techniques in previous studies, such as the conventional SPE [20, 21] and liquid–liquid extraction (LLE) [22] as well as some innovative concentration methods, including "homemade" C_{18} SPE microcolum [23], solid phase microextraction (SPME) [24], matrix solid-phase dispersion (MSPD) [25], hydrostatically modified EOF [26], single-drop microextraction (SDME) [27], cloud-point extraction (CPE) [28, 29], liquid–liquid–liquid microextraction (LLLME) [30, 31], and hollow fiber supported liquid–liquid–liquid membrane microextraction (HF-LLLMME) [32]. Dispersive liquid–liquid microextraction (DLLME) is a relatively novel miniaturized sample pretreatment technique that has the advantages of simplicity, rapidity, cost-effectiveness, and environmental benignity [33]. With the above features, it shows a wide application prospect in the analysis of organic compounds and metal ions, and even in the speciation analysis of trace elements [33–38]. Recently, in the aspect of mercury speciation analysis, the DLLME combined with HPLC-UV [37] and HPLC-ICP-MS [38] methods have been reported. Moreover, our group has determined Hg(II) in water samples using DLLME along with CE [36]. Inspired by these studies, we intend to develop a new method of DLLME coupled to CE for the identification and determination of mercury speciation.

Herein, four mercury species including Hg(II), MeHg, EtHg and PhHg in water samples were simultaneously separated and detected using DLLME followed by CE. As far as we are aware, this is the first demonstration for mercury speciation analysis by virtue of DLLME-CE. Because mercury has no UV absorption, 1-(2-pyridylazo)-2-naphthol (PAN) and L-cysteine (L-Cys) were used as chelating reagents. The four species were complexed with PAN to form hydrophobic chelates, respectively, and thereafter were extracted into the fine drops of extractive solvent dispersed in the aqueous phase. In the back extraction process, L-Cys displaced the hydrophobic mercury–PAN complexes to form the hydrophilic mercury–L-Cys ones since the aqueous sample solution was naturally compatible with aqueous CE determination. Under the optimized DLLME-CE conditions, excellent analytical performances, including wide linear range, good reproducibility, low detection limit, and high enrichment factor (EF) were attained, and the developed method was also successfully applied to the mercury speciation of real water samples, such as tap water, lake water, and seawater.

2 Materials and methods

2.1 Reagents, instruments and water samples

The stock standard solution of inorganic mercury (Hg(II)) was prepared by dissolving mercury(II) chloride (purchased from Sinopharm Chemical Reagent Co., Shanghai, China.) in ultrapure water at a concentration of 1000 mg/L. The stock standard solutions of MeHg and PhHg at 1000 mg/L (as Hg) were obtained by dissolving appropriate amounts of MeHg chloride and PhHg chloride (all purchased from Aladdin, Shanghai, China) in methanol, respectively. EtHg standard solution at a concentration of 60 mg/L (as Hg) was purchased from National Institute of Metrology (Beijing, China). Working standard solutions were prepared daily by diluting the stock solutions to the required concentrations using ultrapure water.

PAN dissolved in ethanol solution and L-Cys dissolved in ultrapure water at a concentration of 0.1% (w/v) were both purchased from Sinopharm Chemical Reagent Co. Carbon tetrachloride (CCl_4), bromobenzene (C_6H_5Br), chlorobenzene (C_6H_5Cl), benzyl bromide (C_7H_7Br), boric acid, acetone, and chromatographic grade ethanol (EtOH) were all obtained from Tianjin Kermel Chemical Reagent Factory (Tianjin, China). Chromatographic grade methanol (MeOH) and ACN were purchased from J&K Chemical (Beijing, China).

A Beckman P/ACE^{TM} MDQ Capillary Electrophoresis System (Beckman Coulter, Fullerton, CA, USA) in conjunction with a DAD was used throughout the whole experiment. Bare fused-silica capillaries (Yongnian Photoconductive Fiber Factory, Hebei, China) were used as separation column for the separation of the four mercury–L-Cys complexes with 75 μm id, total length of 60.2 cm, and effective length of 50 cm. Ultrapure water used throughout the work was produced by a Milli-Q Ultrapure Water System (Millipore, Bedford, MA, USA). The pH of the solution was measured with a PHS-3C digital pH meter (Hangzhou Dongxing Instrument Factory, Hangzhou, China).

Tap water samples were collected when needed from our laboratory after flowing for about 5 min. Lake water samples were collected into a teflon bottle from an artificial lake located in Laishan District of Yantai City (China). Surface seawater samples were collected into a teflon bottle from the Fisheman's Wharf of the Yellow Sea located in the coastal zone area of Yantai City of China. And then they were all filtrated through a 0.45 μm PTFE syringe filter (Phenomenex, Los Angeles, CA, USA). The samples were directly analyzed or stored at 4°C for use.

2.2 CE separation

A new capillary for the first use was conditioned with water for 10 min, 0.5 mol/L of NaOH for 40 min, water for 10 min, and running buffer for 30 min. Every time before use, the capillary was conditioned by flushing with water (2 min), 0.1 mol/L NaOH (10 min), water (5 min), and

running buffer (5 min) sequentially. After the daily usage, the capillary needed to be rinsed sequentially with water (5 min), 0.1 mol/L NaOH (20 min), and water (10 min). Between two successive CE runs, the capillary should be rinsed with running buffer (2 min).

In CE separation, the running buffer that was consisted of 75 mmol/L boric acid and 10% (v/v) methanol (pH 9.0) was prepared daily prior to use. The pressure injection of the samples was performed using 0.5 psi for 5 s (1 psi = 6894.76 Pa) at the temperature of 25°C and the applied voltage of 20 kV. The detection wavelength was set at 210 nm. Before use, all electrolytes and samples were filtered through a 0.45 μm nylon membrane filter.

2.3 DLLME procedure

For the DLLME, 5 mL aqueous sample solution with the pH adjusted to 4.00 using H_3PO_4 and NaOH was placed into a 15 mL centrifuge tube with conical bottom consisting of EtHg, MeHg, PhHg and Hg(II), and PAN (chelating agent). A mixture of 600 μL EtOH (disperser solvent) and 50 μL benzyl bromide (extraction solvent) was rapidly (within approximate 3 s) injected into the sample solution using a 1 mL syringe. After the solution was gently shaken, fine droplets of the benzyl bromide were dispersed throughout the aqueous phase, thus, forming a so-called cloudy state [39] in the centrifuge tube and thereafter the four mercury–PAN complexes were extracted into the fine droplets of benzyl bromide. Then the cloudy solution was centrifuged for 5 min at 4000 rpm. The dispersed fine droplets of benzyl bromide were sedimented at the bottom of tube after centrifugation. Then the sedimentation was removed into a 0.5 mL conical centrifuge tube and 10 μL 0.1% (w/v) L-Cys was added into the tube subsequently. The mixture was then sonicated for 1 min in order to make L-Cys displace PAN to form the complexes of mercury-L-Cys more thoroughly. After centrifugation, the four mercury–L-Cys complexes were extracted into the upper aqueous phase, and then the supernatant aqueous phase was removed carefully with a pipette for CE analysis.

3 Results and discussion

3.1 CE separation of the four mercury species

In the procedure of DLLME, L-Cys displaced PAN to form the complexes of mercury–L-Cys for CE analysis. So, CE separation conditions of the four mercury–L-Cys complexes were first investigated. Many factors possibly affecting the separations, such as running buffer, organic modifier, detection wavelength, applied voltage, and the sample injection time, were investigated.

The boric acid and methanol system is mostly used in CE, and so herein it was adopted. The concentrations of boric acid and methanol, and the pH value of buffer system are required to optimize. So, different concentrations of boric acid

(25, 50, 75, and 100 mmol/L) and methanol (5, 10, 15, and 20% v/v) were studied, respectively. The results showed that the four mercury–L-Cys complexes obtained baseline separation within 8 min at the concentration of 75 mmol/L boric acid and 10% methanol. Because the buffer pH affected the ionization of analytes, resulting in the electrophoretic mobility of L-Cys and four complexes varying with different pH values, optimization experiments were performed in the alkaline conditions at the pH values of 8.0, 8.5, 9.0, 9.5, and 10.0, which were adjusted using NaOH and boric acid. When the buffer pH was 9.0, excellent resolution and peak-shape were displayed, so, it was selected as buffer pH for the further experiment. Therefore, the CE running buffer consisted of 75 mmol/L boric acid and 10% methanol, and its pH was adjusted to 9.0. To optimize the applied voltage, voltages of 18, 20, and 22 kV were investigated. A shorter separation time was obtained using 20 kV than 18 kV, and a better separation effect was obtained using 20 kV than 22 kV. Taking into account of the two factors, 20 kV was chosen as the applied voltage.

3.2 Optimization of DLLME conditions

In the DLLME procedure, to obtain the best extraction efficiency, several key parameters, such as the type and volume of extraction and disperser solvents, the concentration of chelating agent, sample pH, the volume of L-Cys, and ultrasonic time were investigated and optimized.

3.2.1 Effect of type and volume of the extraction solvent

In DLLME, the extraction solvent should have low solubility in water, excellent extraction ability, and high extraction selectivity for the target analytes. Also, it should have higher density than water in order to make the phase separation easily by centrifugation. Therefore, carbon tetrachloride, bromobenzene, chlorobenzene, and benzyl bromide were studied as the extraction solvents, respectively. The results showed that only benzyl bromide can extract the four mercury–L-Cys complexes simultaneously from the aqueous sample among the four extractions. At the same time, carbon tetrachloride had the extraction ability for MeHg-PAN, EtHg-PAN, and Hg-PAN, but not Ph-PAN; in addition, bromobenzene and chlorobenzene only had the extraction ability for PhHg-PAN and Hg-PAN. This may be because of the similar miscibility principle of functional groups. So, benzyl bromide was selected as the ideal extraction solvent.

To optimize the volume of benzyl bromide, experiments were performed using 600 μL ethanol containing different volumes of benzyl bromide (30, 40, 50, 60, and 70 μL). The extraction efficiency was increased with the increase of extracting volume from 30 to 50 μL, and then decreased with the volume of extractant continuing to increase, as is
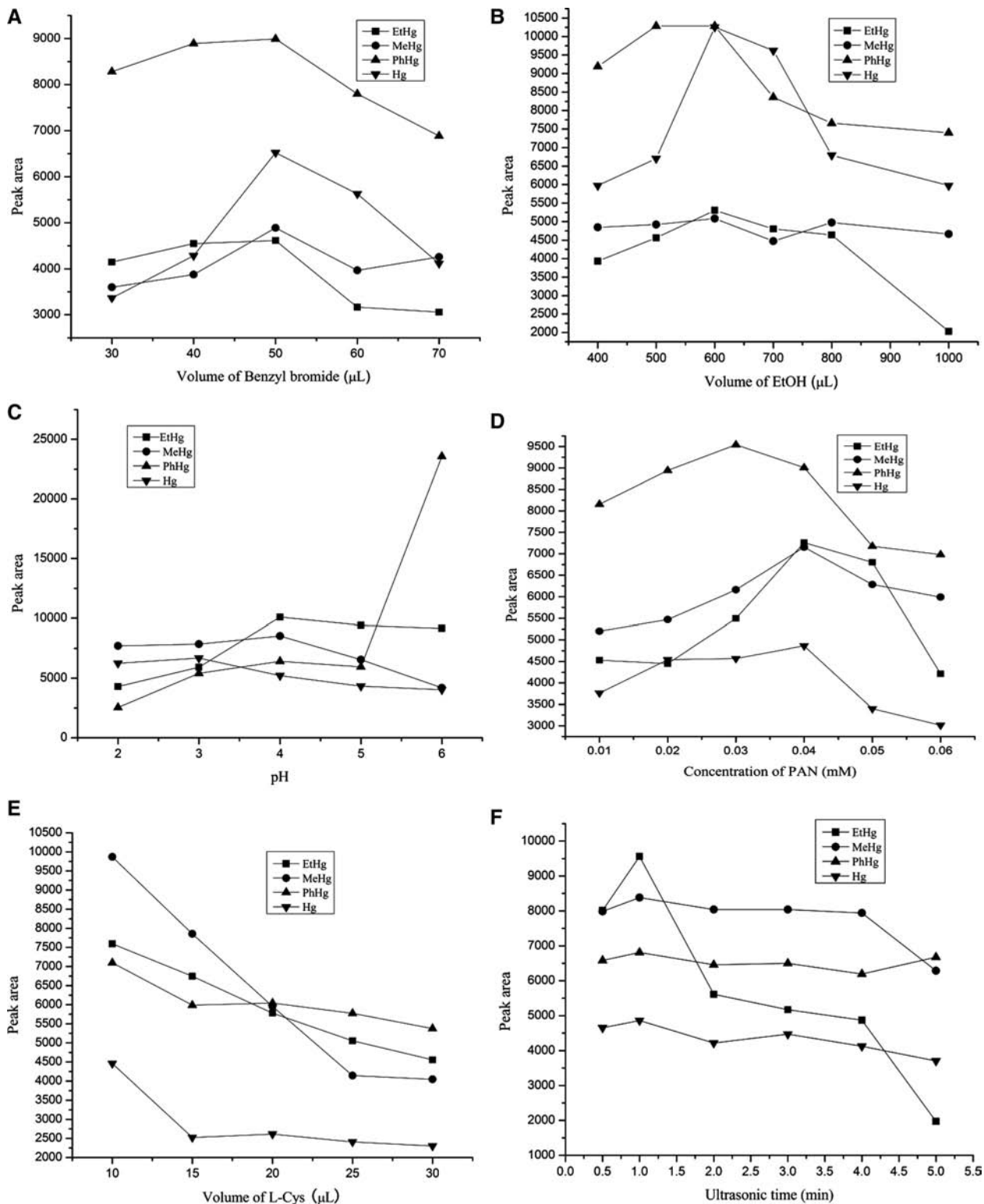
Figure 1. Effects of extracting solvent volume (A), dispersing solvent volume (B), pH (C), PAN concentration (D), L-Cys volume (E), and ultrasonic time (F) of DLLME for four mercury species. Extraction conditions: (A) 600 μL EtOH, pH 4, 0.04 mM PAN, 10 μL L-Cys, ultrasonic time of 1.0 min; (B) 50 μL benzyl bromide, pH 4, 0.04 mM PAN, 10 μL L-Cys, ultrasonic time of 1.0 min; (C) 50 μL benzyl bromide, 600 μL EtOH, 0.04 mM PAN, 10 μL L-Cys, ultrasonic time of 1.0 min; (D) 50 μL benzyl bromide, 600 μL EtOH, pH 4, 10 μL L-Cys, ultrasonic time of 1.0 min; (E) 50 μL benzyl bromide, 600 μL EtOH, pH 4, 0.04 mM PAN, ultrasonic time of 1.0 min; (F) 50 μL benzyl bromide, 600 μL EtOH, pH 4, 0.04 mM PAN, 10 μL L-Cys.

evidenced from Fig. 1A. Therefore, 50 μL of benzyl bromide was chosen in the following work.

3.2.2 Effect of type and volume of the disperser solvent

In DLLME, the disperser solvent has to be miscible with both the extraction solvent and aqueous sample so as to enable the dispersion of fine drops of the extractant into the aqueous phase containing the analytes. MeOH, EtOH, ACN, and acetone as the commonly used disperser solvents were investigated. Experimental results showed EtOH provided the highest extraction efficiency among these four disperser solvents. Moreover, EtOH has the characteristic of lower toxicity and lower cost. For these reasons, EtOH was selected as the optimum disperser solvent.

To investigate the effect of dispersant volume, different volumes (400, 500, 600, 700, 800, 900, and 1000 μL) of EtOH containing 50 μL benzyl bromide was studied. As the result shown in Fig. 1B, the peak area was gradually increased with the volume of EtOH, increasing from 400 to 600 μL and then decreased with the volume continuously increasing. When using a smaller volume of EtOH, benzyl bromide was not dispersed well, and the cloudy state was not formed well, yet. Nevertheless, when the volume of EtOH was excess, the solubility of the complexes in the aqueous phase was increased. The two patterns both caused the decrease of extraction efficiency; hence, 600 μL of EtOH was chosen in the subsequent experiments.

3.2.3 Effect of sample pH

Sample pH has an important effect on the pretreatment process since it affects the chelation reaction between mercury species and PAN as well as the subsequent extraction. Experimental results showed that the peak areas of the four mercury species complexes had reached maximum values with the pH value up to 4, but when the pH value continued to increase from 5 to 6 even to 7 and 8, the peak area of PhHg again increased, as seen in Fig. 1C. Considering the preconcentration efficiency for all the four species, a pH value of 4 was the most appropriate and was adopted.

3.2.4 Effect of the concentration of PAN

In this work, PAN was used as chelating agent to form stable complexes rapidly with mercury species which could be extracted into benzyl bromide. Therefore, studying the PAN concentration on the effect of extraction efficiency is very necessary. A series of PAN concentrations in the range of 0.01–0.06 mmol/L were examined. The peak area of PhHg increased with PAN concentration increasing from 0.01 to 0.03 mmol/L and then decreased, while the peak area of the other three ones increased with PAN concentration increas-

ing from 0.01 to 0.04 mmol/L and then decreased, as shown in Fig. 1D. In order to get higher extraction efficiency simultaneously for the four mercury complexes, 0.04 mmol/L PAN was chosen as the optimum concentration.

3.2.5 Effect of the volume of L-Cys

A concentration of 0.1% (w/v) L-Cys was used as back extraction solvent to displace PAN and then formed more stable and hydrophilic complexes with the four mercury species. Different volumes (10, 15, 20, 25, and 30 μL) of L-Cys were investigated. It is evident from Fig. 1E that the peak areas of the four mercury complexes were all decreased when the volume of L-Cys increased. After the back extraction process and centrifugation, supernatant aqueous phase was removed for CE analyses. Given the fact that with the decrease of the volume of L-Cys, removal of the supernatant aqueous phase also became more difficult, the volume less than 10 μL was not studied. Therefore, 10 μL of L-Cys was used as the optimum volume.

3.2.6 Effect of the ultrasonic time

The ultrasonic process can accelerate the substitution reaction between mercury–L-Cys and mercury–PAN. To investigate the effect of ultrasonic time on the extraction efficiency, different ultrasonic time of 0.5, 1, 2, 3, 4, and 5 min was studied. As illustrated in Fig. 1F, the peak areas of the four mercury species complexes were all increased with the ultrasonic time increasing from 0.5 to 1 min and then decreased with the ultrasonic time continuously increasing. The decrease of peak areas probably was owing to that the produced thermo-energy in the ultrasonic process made mercury–PAN redissolve into aqueous phase and thereby the subsequently attained mercury–L-Cys decreased. Thus, the ultrasonic time of 1 min was selected for the work.

3.3 Interference study

To study the selectivity of the developed method for mercury speciation, some commonly found alkali and alkaline earth metal ions (Na^+, K^+, Ca^{2+}, and Mg^{2+}) and heavy metal ions (Fe^{3+}, Pb^{2+}, Cd^{2+}, Zn^{2+}, and Cu^{2+}) in environmental water samples were chosen for their possible interference investigation. Different amounts of ions were added to the tested solutions containing four mercury species at the concentration of 10 μg/L for $PhHg^+$ and 100 μg/L for the other three ones. The same DLLME procedure was performed. The results showed that a 500 times excess (50 mg/L) of Na^+, K^+, Ca^{2+}, and Mg^{2+}, and a 20 times excess (2 mg/L) of Fe^{3+}, Pb^{2+}, Cd^{2+}, Zn^{2+}, and Cu^{2+}, did not cause significant interferences with the recoveries of all the four mercury species ranging from 94.6 to 105.2%. Therefore, the present DLLME-CE showed high selectivity and reliability for mercury speciation.

Table 1. Parameters of analytical performance of the DLLME-CE for mercury speciation

Species	Linear equation	Correlation coefficient (R)	Linear range (μg/L)	RSD (%, $n = 5$)		LOD (μg/L)	LOQ (μg/L)[a]	EF
				Migration time	Peak area			
EtHg	$y = 40.296x + 184.93$	0.9983	10.0–200.0	0.29	3.08	1.62	5.40	118
MeHg	$y = 36.429x + 133.72$	0.9990	10.0–200.0	0.43	3.19	1.79	6.00	102
PhHg	$y = 394.45x + 917.69$	0.9978	1.0–20.0	0.48	3.23	0.23	0.77	547
Hg(II)	$y = 38.665x + 606.37$	0.9960	5.0–200.0	0.54	7.80	1.50	5.00	46

a) The LOQ is estimated using a criterion of ten times S/N.

3.4 Method performance

In order to evaluate the analytical performance of the DLLME-CE for mercury speciation, linearity, correlation coefficient, RSD of the migration time and peak area, LODs, LOQ and EF were determined under the optimized conditions. The analytical data were summarized in Table 1. Good linear relations between peak areas and concentrations were obtained and the correlation coefficients were all over 0.9960. The RSD values of the migration time and peak area were in the rage of 0.29–0.54% and 3.08–7.80%, respectively, based on five replicate experiments at the concentration of 20 μg/L of PhHg and 200 μg/L of the other three mercury species. The LOD was estimated using a criterion of three times S/N and favorable LODs were achieved within 0.23–1.79 μg/L. The detection limit is lower than the maximum concentration level for Hg(II) in drinking water, namely 2.0 μg/L formulated by United States Environmental Protection Agency (EPA). The maximum concentration level for Hg(II) in drinking water is 1.0 μg/L stipulated by European Union (EU) and China, for MeHg is 0.001 μg/L and for EtHg is 0.1 μg/L required by China. Nevertheless, the presented method sensitivity can contribute to mercury speciation detection in water samples especially industrial and sanitary wastewater. The EF, which was obtained by calculating the ratio of the final analyte concentration after extraction and the initial aqueous sample concentration, were 102, 118, 547, and 46 for MeHg, EtHg, PhHg, and Hg(II), respectively. It should be noted that the EF for Hg(II) is significantly lower than that reported in our previous work [36] (EF = 625). Although this work relied on the same principle as [36], the actual extraction systems were different after condition optimization, especially that benzyl bromide was selected as the optimal extraction solvent for the simultaneous extraction of four mercury species herein, while chlorobenzene was chosen for Hg(II) only in [36]. Nevertheless, the developed method was demonstrated to be highly sensitive and highly reliable for determination of mercury speciation.

In addition, comparisons of the LODs and EFs with that of other reported methods for the analysis of the four mercury species simultaneously are made in Table 2. As seen from the table, lower or comparable LODs and higher EFs were obtained compared with the HPLC-hyphenated techniques [23, 27]. Moreover, the four mercury species complexes obtained baseline separation within 24 min with the method of SDME-HPLC-DAD [27] and 30 min with the method of SPE-HPLC-cold vapor AFS (CVAFS) [23]. In contrast, four mercury species complexes only need 8 min to reach the baseline separation with our developed method of DLLME-CE. Also, the obtained LODs are lower than that reported using CE-UV with dual-CPE [29] and CE-VSG (volatile species generation)-AFS with hydrostatically modified EOF [26], and the obtained EFs are higher than that reported using the method of dual CPE-CE [29]. On the other hand, the present LODs are slightly higher and EFs are lower than that attained using HF-LLLMME coupled with CE-UV [32]. Quite low LODs and high EFs that got with the method of PT/MS-LLLME-LVSS (large volume sample stacking)-CE-UV [31] probably resulted from the LVSS, since similar LODs were obtained by our method compared with that of the PT/MS-LLLME-CE-UV [31] without stacking, but our method was more simple and more easy to operate. Besides the four mercury species analysis, three and two mercury species related studies are also included for comparison. As seen from Table 2, we obtained much better LOD and EF for PhHg than that using the same DLLME method followed by HPLC-UV [37], while the DLLME coupled with HPLC-ICP-MS provided significantly low LODs for MeHg and Hg(II) [38]. Hence, overall, the superiority of our developed DLLME-CE method is obvious on account of the relatively low LODs and high EFs, and moreover, it is simple and easy to use as well as the running time is short.

3.5 Analysis of real water samples

To evaluate the practical applicability of the developed method, three kinds of real water samples of tap water, lake water, and seawater were analyzed. LODs related to the real samples were obtained for EtHg, MeHg, PhHg, and Hg(II), respectively, namely 1.62, 1.79, 0.23, and 1.50 μg/L in tap water, 1.89, 2.04, 0.30, and 1.71 μg/L in lake water, and 1.95, 2.10, 0.35, and 1.80 μg/L in seawater. As seen in Fig. 2, no endogenous mercury species were detected in all three water samples (A, B and C); on the other hand, for the spiked seawater sample, four resolved peaks corresponding to EtHg, MeHg, PhHg, and Hg(II), respectively, were found within 8 min (D), which was consistent with that of the standard solution with DLLME. The recovery experiment was implemented by spiking different levels of mercury species

Table 2. Comparisons of LOD and EF for mercury speciation analyses using CE-based and HPLC-based methods

Detection technique	Pretreatment technique	LOD (µg/L)[a]	EF[a]	Ref.
CE-UV	DLLME	1.79, 1.62, 0.23, 1.50	102, 118, 547, 46	This work
CE-UV	dCPE[b]	47.5, 45.2, 4.1, 10	15, 17, 45, 52	[29]
CE-VSG[c]-AFS[d]	HSMEOF[e]	16.5, 15.9, 13.3, 6.8	–	[26]
CE-UV	PT/MS-LLLME[f]	2.21, 2.38, 1.40, 5.21	478, 316, 319, 160	[31]
CE-UV	HF-LLLMME[g]	1.0, 0.7, 0.07, 0.8	511, 265, 683, 103	[32]
LVSS[h]-CE-UV	PT/MS-LLLME	0.087, 0.12, 0.042, 0.37	12138, 6271, 10595, 2250	[31]
HPLC-UV	SDME[i]	1.0, 1.6, 7.1, 22.8	27, 31, 11, 3	[27]
HPLC-CVAFS[j]	SPE	4.3, 1.4, 0.8, 0.8	–	[23]
HPLC-UV	DLLME	0.96 (MeHg), 1.91 (PhHg), 0.32 (Hg(II))	114, 106, 107	[37]
HPLC-ICP-MS	DLLME	0.0076 (MeHg), 0.0014 (Hg)	138, 350	[38]

a) Sequence of the four species: MeHg, EtHg, PhHg, Hg(II).
b) Dual-CPE.
c) Volatile species generation.
d) Atomic fluorescence spectrometry.
e) Hydrostatically modified EOF.
f) Phase transfer/membrane supported liquid–liquid–liquid microextraction.
g) Hollow fiber supported liquid–liquid–liquid membrane microextraction.
h) Large volume sample stacking.
i) Single-drop microextraction.
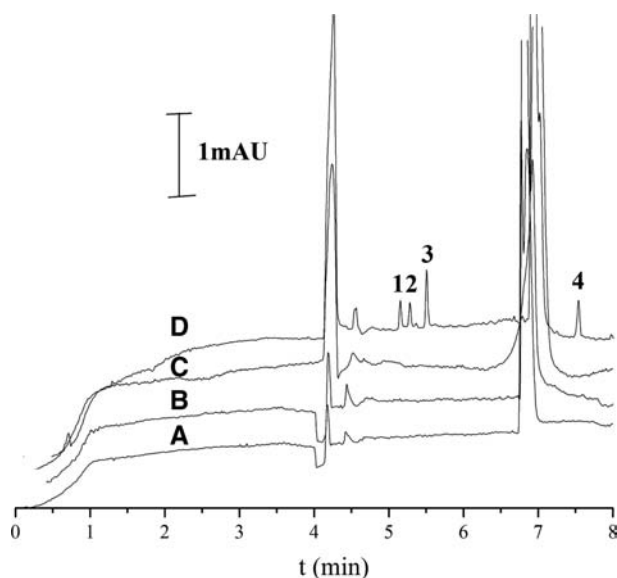j) Cold vapor atomic fluorescence spectrometry.

Figure 2. Electropherograms of tap water (A), lake water (B), and seawater (C) without spiking mercury species and seawater (D) spiked with 10 µg/L MeHg, 10 µg/L EtHg, 10 µg/L Hg(II), and 2.0 µg/L PhHg after DLLME. (1) EtHg–L-Cys, (2) MeHg–L-Cys, (3) PhHg–L-Cys, and (4) Hg–L-Cys. CE separation conditions: buffer, 75 mmol/L boric acid and 10% v/v methanol (pH 9.0); injection pressure and time, 0.5 psi and 5 s; injection voltage, 20 kV; detection wavelength, 210 nm.

standards in the three water samples, respectively, with PhHg at 5, 10, and 20 µg/L and others at 50, 100, and 200 µg/L. Recoveries were obtained between 82.38 and 98.76%, with the RSD lower than 7.18%, listed in Supporting Information Table 1. The developed DLLME-CE method proved greatly applicable for the determination of mercury speciation in water analysis.

4 Concluding remarks

In conclusion, a DLLME procedure coupled to CE was successfully developed for the separation and determination of MeHg, EtHg, PhHg, and Hg(II) in water samples simultaneously. High EFs in the rage of 46–547 and low detection limits within 0.23–1.79 µg/L were attained, indicating a strong preconcentration power of the DLLME. The DLLME-CE method with simple UV detection provided good quantitative ability, high precision, and wide linear range, and it was proved to be a simple, rapid, cost-effective, and eco-friendly option for the speciation analysis of mercury in water samples. Given the advantages, further explorations on the various combinations of versatile off/online enrichment techniques will be promising and are currently on the way in our laboratory, for speciation analysis of heavy metals in complicated matrices by using CE, potentially offering higher sensitivity.

The authors have declared no conflict of interest.

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