SPECIATION OF ORGANOMERCURIALS BY CAPILLARY ELECTROPHORESIS WITH PRE-COLUMN UV DERIVATIZATION AND ON-LINE CONCENTRATION

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Abstract

Simultaneous separation and detection of methylmercury (MeHg), ethylmercury (EtHg) and phenylmercury (PhHg) was investigated by capillary electrophoresis with UV detection using thiosalicylic acid as derivatization reagent. The separation parameters such as buffer pH, ion strength were investigated. A baseline separation was obtained by using 30 mmol L^{-1} of borate as buffer at pH 9.2. Then an on-line concentration method was used to improve the detection sensitivity. Parameters such as concentration of NaCl in sample matrix and injection time were optimized. The limits of detection were 76.9, 83.0 and 76.4 μg L^{-1} for PhHg, EtHg and MeHg, respectively.

Introduction

Mercury is a kind of toxic heavy metal, and present in various environmental media all over the world [1]. Mercury exists in environmental media as different species which have different environmental behavior and toxicity. Methylmercury is one of the most toxic organometallic compounds and can be bioaccumulated and bioconcentrated through food chain. Ethylmercury and phenylmercury are used as bactericide and exist in environment as anthropogenic pollution [2, 3] or natural sources [4, 5]. Some microorganism and natural processes can transform mercury in environment from one form to another. Therefore, a simple and cost-effective method is necessary for determining mercury species and understanding the chemistry and risk assessment of mercury. In the past decades, chromatographic method such as GC and HPLC have been widely applied to separate species of mercury and various detectors were coupled with them, including UV, AAS, AFS, AES and ICP-MS [6]. CE was applied to the speciation of metal elements due to its advantages in speciation, such as high separation efficiency, low sample consumption, low operating cost and free of organic solvent. CE coupled with AAS [7], AFS [8], electrochemical [9], piezoelectric sensor [10], ICP-MS [11] and UV [12-15] detectors were used to the speciation of mercury recently. The use of element-specified detector such as AAS, AFS, ICP-MS can enhance the selectivity and sensitivity of detection. However, UV is still the most universal detector in commercial CE instrument. In recent years, various methods of CE with UV detection have been developed for mercury separation [12-15]. But some of these methods need non-aqueous solvent as electrophoresis media or commercially unavailable derivatization reagent, which limit their further application.

In the present study, a simple and cost-effective CE method coupled with UV detector using thiosalicylic acid as UV derivatization reagent for organomercurials speciation was developed. The pre-column derivatization procedure was simple and fast and three organomercurial derivatives could be well separated and detected. In order to improve the detection sensitivity, an on-line concentration method was further investigated. The developed method was verified by a certified reference material DORM-2 and applied to analyze

organomercurials in fish tissue sample exposed by ethylmercury. The results were accorded very well with the reference value and the HPLC-AFS results, which revealed that the developed method could be applied in the speciation of organomercurials in biological samples.

Materials and Methods

Instrumentation Capillary electrophoretic separations were performed on an HP^{3D}CE system (Hewlett Packard, Waldbronn, Germany) with a diode array detector (190-600 nm). Detection was carried out at 210 nm. A capillary of 75 μm id and 45 cm total length was used for the optimization of the separation. Capillaries of 75 μm id with 73.5 cm and 120 cm total length were used for on-line concentration. The capillary was thermo-stated at 20℃. The HPLC-AFS system used in the experiment was similar with the system developed in our lab before [16], except for a post-column UV digestion system.

Chemicals and reagents Stock solution of standard inorganic mercury (1 mg mL^{-1} as Hg) was prepared by dissolving appropriate amount of $HgCl_2$ (≥99.5%, Beijing Chemical factory, Beijing, China) in 5% (v/v) HNO_3. Stock solutions of standard organomercurials (1 mg mL^{-1} or 2 mg mL^{-1} as Hg) were prepared by dissolving appropriate amounts of methylmercury chloride (MeHg), ethylmercury chloride (EtMg) and phenylmercury chloride (PhHg) in methanol respectively. All the organomercurials were purchased from Merck (≥98%, Darmstadt, Germany). Thiosalicylic acid (TA, ≥99%, Beijing Hengye Zhongyuan Chemicals, Beijing, China) was dissolved in pure methanol to a concentration of 1mg mL^{-1} and was kept in refrigerator. All other chemicals were analytical grade. Buffer and standard solutions were prepared with de-ionized water from Barnstead ultra-pure water system.

Preparation of sample extracts An alkaline extraction method was used for the extraction of organomercurials from fish sample [16].

Results and Discussion

Selection of derivatization reagent

Organomercurials such as MeHg, EtHg have no chromosphere group, and pre- [13], in- [14], or post-column [17] derivatization using chromogenic compounds is often required. Selection of proper chromogenic compounds for the derivatization is very important for the CE separation and detection. The derivatization reagent should have good water solubility and the derivatization product could be separated and detected easily [18]. Three derivatization reagents dithizone, ammonia pyrrolidine dithiocarbamate (APDC) and TA were investigated using EtHg as a model compound in this experiment. Dithizone was often used for the UV derivatization and extraction in spectroscopic determination for mercury. In our study, although dithizone could form complex with EtHg, it is difficult for it to be used in aqueous CE due to its poor aqueous solubility. So some water-soluble derivatives of dithizone synthesized were applied in the derivatization of mercury in ion chromatography [17] and capillary electrophoresis [14, 15]. However, the synthesis procedure of derivatization reagent is labor-intensive and time-consuming. Therefore, two other water-soluble derivatization reagents, APDC [19] and TA [20] were further considered. 100 μL of 1 mg mL^{-1} APDC or TA was added to 900 μL of 40 mg L^{-1} EtHg respectively. Then the resulting solution was injected directly as sample without other pre-treatment. Although there are reports about the derivatization of organomercurials by APDC in chromatographic separation [19], the peak of derivatization

product of EtHg could not be detected in our experiment. This perhaps was ascribed to the adsorption of hydrophobic derivatization product on the capillary wall. TA could complex EtHg quickly and the derivatization product was stable in the analytical procedure, which could be detected at 210 nm by UV. The spectroscopy of the three derivatization products is shown in Figure 1. Therefore, the commercial available and low-cost reagent, TA was developed as a novel derivatization reagent for the speciation of mercury for the first time.

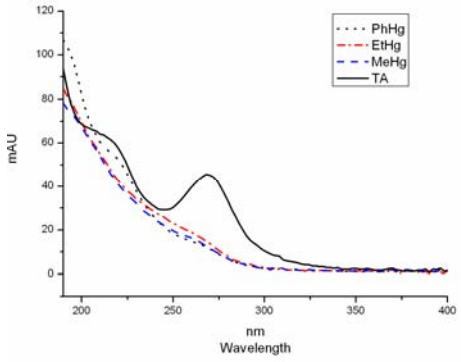

Figure 1. UV-visible spectroscopy of PhHg, EtHg, MeHg derivatization product and thiosalicylic acid.

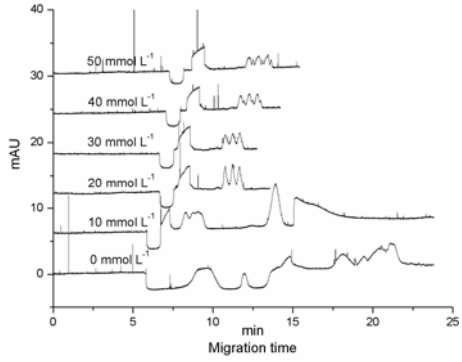

Figure 2. Effect of NaCl concentration in sample matrix on stacking.

Composition of buffer and buffer pH

To achieve efficient separations, two buffer systems including NH_4Ac-HAc and borate buffer were studied. Three organomercurials could be separated in baseline in both of the two buffer systems, but a shorter electrophoretic time was achieved in borate buffer system. Therefore, borate was adopted as the running buffer. The effects of running buffer pH were studied in a pH range from 7.2 to 10.2 using 30 mmol L^{-1} borate buffer as the background electrolyte. It was found that pH of the borate buffer has great influence on the separation and detection. Broaden peaks were observed at low buffer pH which resulted in poor resolution and sensitivity. With the increase of buffer pH, the peak area of organomercurials increased and high theoretical plates were acquired and the resolution of three organomercurials increased with the increase of buffer pH. However, when buffer pH is higher than 9.2, the peak area decreased sharply and the peak shape deteriorated. These maybe due to unstability of the derivatization products in high pH buffer and decomposition was happen in the separation procedure. At pH 9.2, highest peak area and baseline separation were observed. Therefore, borate buffer at pH 9.2 was selected in further research.

Effect of borate buffer concentration

The influence of the borate buffer concentration on separation and detection of organomercurials was also investigated at pH 9.2. Concentrations of borate buffer ranged from 5 to 40 mmol L^{-1} were tested. When the concentration of borate buffer was lower than 10 mmol L^{-1}, three organomercurials could not reach a baseline separation. With the increase of borate concentration, resolution and migration time of organomercurials increased. While when the concentration of borate buffer reached 40 mmol L^{-1}, the electrophoretic current is not stable and the peak area decreased because of excessive peak broadening induced by Joule heat effect. Therefore, a final concentration of borate buffer at 30 mmol L^{-1} was selected for further studies. In addition, SDS and organic modifiers including methanol and acetonitrile as buffer additive were also studied to improve resolution. However, it was found that addition of SDS and organic modifiers resulted in longer separation time and no

improvement of separation was observed. Therefore, no SDS and organic solvent were added in the background electrolyte.

Then, 30 mmol L^{-1} borate buffer at pH 9.2 was selected as the separation buffer in CE. Under the optimized conditions, the baseline separation of three organomercurials species could be finished in 4 min.

An on-column derivatization method [17] for the mercury speciation was also investigated, but the derivatization efficiency is not satisfactory. The poor derivatization efficiency was ascribe to the inadequately mix in the electrophoresis procedure.

The LODs of the method were about 1 mg L^{-1} for three organomercurials species, which was far from satisfactory for analysis of real environmental sample. Therefore, an on-line concentration method was studied further.

On-line concentration

The parameters of NaCl concentration in sample matrix and the injection time were optimized to realize an on-line concentration effect.

The influence of concentration of NaCl on the stacking of 400 μg L^{-1} of mercury species was studied. It is reported that the possible mechanism of stacking was ascribed to transient isotachophoresis [23]. In the electrophoresis procedure, chloride in the sample served as leading ion, while borate ion in the background electrolyte as the terminating one. The results are shown in Figure 2. It is found that without NaCl in sample matrix no stacking was observed. When the concentration of NaCl was higher than 30 mM, splitting peaks appeared for three mercury species. The splitting of peaks was perhaps caused by the existence of Kohlrausch regulating functions and electrolyte "memory" [21, 22]. High sensitivity and good separation can be obtained when the concentration of NaCl in sample matrix was 20 mmol L^{-1}. Therefore, 20 mmol L^{-1} NaCl was selected as additive in sample matrix.

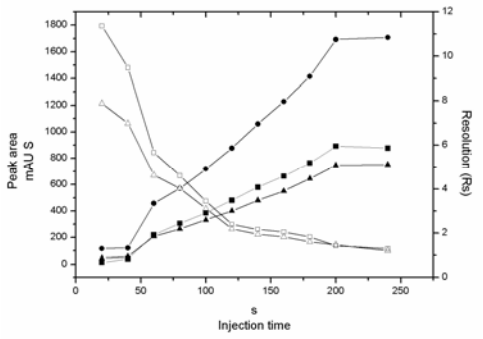

Figure 3. Effect of injection time on separation and sensitivity of PhHg (5 mg L^{-1}), EtHg (10 mg L^{-1}) and MeHg (5 mg L^{-1}). Injection pressure: 30 mbar. Capillary: 75 μm id with 120.0 cm total length; 20 mmol L^{-1} NaCl in sample matrix. ■, PhHg; ●, EtHg; ▲, MeHg; □, resolution of PhHg and EtHg; △, resolution of EtHg and MeHg.

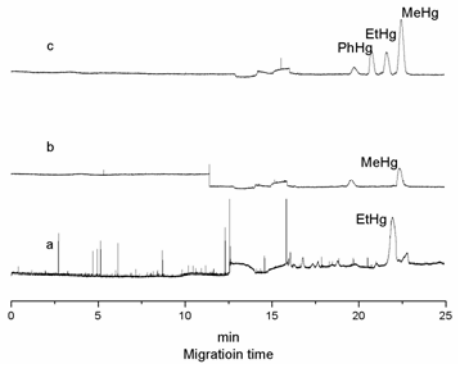

Figure 4. Electropherograms of (a) the extract of fish tissue exposed by ethylmercury (b) the extract of a certified reference material DORM-2 (dogfish muscle); (c) the extract of DORM-2 spiked with organomercury of 600 μg L^{-1} each (as Hg).

Injection time was also investigated from 20 s to 240 s at the injection pressure of 30 mbar. The results are as seen in Figure 3. The peak area increased with the increasing injection time, but when the injection time was longer than 180 s, three organomercurials could not be separated effectively. Considering of the resolution and

sensitivity, the injection time of 180 s is appropriate.

Effect of $Na_2S_2O_3$ in sample matrix

An alkaline extraction method was often used for the biological sample preparation, and the final sample for CE injection was dissolved in a 10 mmol L^{-1} $Na_2S_2O_3$ solution. Therefore, the influence of $Na_2S_2O_3$ in sample matrix on the stacking and separation was investigated. The results showed that 10 mmol L^{-1} $Na_2S_2O_3$ had no influence on the on-line stacking and separation of the three organomercurials.

Analytical performance

Typical electrohopherograms of PhHg, EtHg and MeHg in fish tissue and spiked sample extracts at optimized conditions is shown in Figure 4. As can be seen, three organomercurials species could be separated in 25 min. The linearity of mercury compounds was in the range from 400-2000 μg L^{-1} for PhHg, 200-2000 μg L^{-1} for EtHg and MeHg. The detection limits (S/N=3) were 76.4 μg L^{-1} for MeHg, 83.0 μg L^{-1} for EtHg and 76.9 μg L^{-1} for PhHg, respectively. As shown in Table 1, the detection limits obtained in this work are comparable with other CE-hyphenated methods [7, 8, 10, 11].

Table 1 Comparison of detection limits for organomercury species using different CE-hyphenated methods

CE-hyphenated methods	LODs of organomercury species (μg L^{-1})			Reference
	PhHg	EtHg	MeHg	
CE-UV	76.9	83.0	76.4	This work
CE-FHF[a)]-AAS	50	-	48	7
CE-AFS	13.3	15.9	16.5	8
CE-PES[b)]	19	-	46	10
CE-MCN[c)]-MS		100	80	11

a) FTF, flame-heated furnace; b) PES, Piezoelectric sensor; c) MCN, microconcentric nebulization

Validation of the method and application

In order to validate the developed method, MeHg content in certified reference material DORM-2 (dogfish muscle) was determined. The determined result was in good agreement with the certified value. Also, this method was applied to determine concentration of EtHg in fish tissues (Chinese rare minnow) exposed to EtHg. The results agreed well with that obtained by HPLC-AFS.

In the present work, a novel capillary electrophoresis method was developed based on direct UV detection and on-line concentration for the speciation of three organomercurials by using thiosalicylic acid as pre-column derivatization reagent. NaCl in the sample matrix could facilitate on-line concentration in the electrophoretic separation procedure and improve detective sensitivity significantly. The proposed method was applied to determine MeHg and EtHg in certified reference material and exposed fish sample, which suggested that the method is simple and cost-effective for the speciation of organomercurials in biological samples.

Acknowledgements

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References

[1] UNEP Chemicals, *Global Mercury Assessment*, UNEP, Geneva 2002.

[2] Hintelmann H., Hempel M. and Wilken R.D. *Environ Sci Technol* 1995; 29: 1845.

[3] Yamanaka S. and Ueda K. *Bull Environ Contam Toxicol* 1975; 14: 409.

[4] Cai Y., Jaffe R. and Jones R. *Environ Sci Technol* 1997; 31: 302.

[5] Siciliano S.D., Sangster A., Daughney C.J., Loseto L., Germida J.J., Rencz A.N., O'Driscoll N.J. and Lean D.R.S. *J Environ Qual* 2003; 32: 2085.

[6] Leermakers M., Baeyens W., Quevauviller P. and Horvat M. *Trends Anal Chem* 2005; 24: 383.

[7] Li Y., Jiang Y. and Yan X.P. *Electrophoresis* 2005; 26: 661.

[8] Yan X.P., Yin X.B., Jiang D.Q. and He X.W. *Anal Chem* 2003; 75: 1726.

[9] Lai E.P.C., Zhang W., Trier X., Georgi A., Kowalski S., Kennedy S., MdMuslim T. and Dabek-Zlotorzynska E. *Anal Chim Acta* 1998; 364: 63.

[10] Manganiello L., Arce L., Ríos A. and Valcárcel M. *J Sep Sci* 2002; 25: 319.

[11] Lee T.H. and Jiang S.J. *Anal Chim Acta* 2000; 413: 197.

[12] Carro-Diaz A.M., Lorenzo-Ferreira R.A. and Cela-Torrijos R. *J Chromatogr A* 1996; 730: 345.

[13] Peng Z.L., Qu F., Song G.Q. and Lin J.M. *Electrophoresis* 2005; 26: 3333.

[14] Jones P. and Hardy S. *J Chromatogr A* 1997; 765: 345.

[15] Hardy S. and Jones P. *J Chromatogr A* 1997; 791: 333.

[16] Liang L.N., Jiang G.B., Liu J.F. and Hu J.T. *Anal Chim Acta* 2003; 477: 131.

[17] Shaw M.J., Jones P. and Haddad P.R. *Analyst* 2003; 128: 1209.

[18] Ptolemy A.S. and Britz-McKibbin P. *J Chromatogr A* 2006; 1106: 7.

[19] Dong L.M., Yan X.P., Li Y., Jiang Y., Wang S.W. and Jiang D.Q. *J Chromatogr A* 2004; 1036: 119.

[20] Bermudo E., Nunez O., Puignou L. and Galceran M.T., *J Chromatogr A* 2006; 1120: 199.

[21] Ermakov S.V., Zhukov M.Y., Capelli L. and Righetti P.G. *Anal Chem* 1994; 66: 4034.

[22] Ermakov S.V., Zhukov M.Y., Capelli L. and Righetti P.G. *Anal Chem* 1995; 67: 2957.

[23] Tu C. and Lee H.K. *J Chromatogr A* 2002; 966: 205.