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Journal of Chromatography A, 1154 (2007) 437–443
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Dual-cloud point extraction as a preconcentration and clean-up technique for capillary electrophoresis speciation analysis of mercury

Xue-Bo Yin *

Research Center for Analytical Chemistry and Department of Chemistry, College of Chemistry, Nankai University, Tianjin 300071, China

Received 15 December 2006; received in revised form 27 March 2007; accepted 30 March 2007
Available online 4 April 2007

Abstract

A novel dual-cloud point extraction (dCPE) technique is proposed in this paper for the sample pretreatment of capillary electrophoresis (CE) speciation analysis of mercury. In dCPE, cloud point was carried out twice in a sample pretreatment. First, four mercury species, methylmercury (MeHg), ethylmercury (EtHg), phenylmercury (PhHg), and inorganic mercury (Hg(II)) formed hydrophobic complexes with 1-(2-pyridylazo)-2-naphthol (PAN). After heating and centrifuging, the complexes were extracted into the formed Triton X-114 surfactant-rich phase. Instead of the direct injection or analysis, the surfactant-rich phase containing the four Hg species was treated with 150 μL 0.1% (m/v) L-cysteine aqueous solution. The four Hg species were then transferred back into aqueous phase by forming hydrophilic Hg–L-cysteine complexes. After dCPE, the aqueous phase containing the Hg–L-cysteine complexes was subjected into electrophoretic capillary for mercury speciation analysis. Because the concentration of Triton X-114 in the extract after dCPE was only around critical micelle concentration, the adsorption of surfactant on the capillary wall and its possible influence on the sample injection and separation in traditional CPE were eliminated. Plus, the hydrophobic interfering species were removed thoroughly by using dCPE resulted in significant improvement in analysis selectivity. Using 10 mL sample, 17, 15, 45, and 52 of preconcentration factors for EtHg, MeHg, PhHg, and Hg(II) were obtained. With CE separation and on-line UV detection, the detection limits were 45.2, 47.5, 4.1, and 10.0 μg L^{-1} (as Hg) for EtHg, MeHg, PhHg, and Hg(II), respectively. As an analysis method, the present dCPE–CE with UV detection obtained similar detection limits as of some CE–inductively coupled plasma mass spectrometry (ICPMS) hyphenation technique, but with simple instrumental setup and obviously low costs. Its utilization for Hg speciation was validated by the analysis of the spiked natural water and tilapia muscle samples.

Keywords: Dual-cloud point extraction; Mercury; Preconcentration; Speciation analysis

1. Introduction

Cloud point extraction (CPE) as an environmentally benign separation approach, has been adopted as an alternative to traditional solvent extraction [1,2]. Compared with solvent extraction, CPE requires simple instrumentation setup with low cost and is carried out without the use of the dangerous and toxic organic solvents [1–4]. This makes CPE very popular in the preconcentration and clean-up procedures for atomic spectroscopic detection and chromatographic/electrophoretic separation. In CPE the solution is separated into two phases, aqueous phase and surfactant-rich phase, when the temperature of the solution is higher than the cloud point of the surfactant used. With almost 100% of surfactant in the surfactant-rich phase, the concentration of surfactant in the aqueous phase is at about the critical micelle concentration [1]. The hydrophobic analytes are transferred into the surfactant-rich phase and the preconcentration to the analytes is achieved [1,2]. The analytes-containing surfactant-rich phase is then introduced into the detector or separation column as sample.

But compared with the enhancement of surfactant to the atomic spectroscopic signals [5], the adsorption of surfactant in the surfactant-rich phase onto the chromatographic stationary phase or the inner wall of electrophoretic capillary may interfere with the chromatography/electrophoresis injection and separation resulting in poor reproducibility and efficiency [6–11]. To overcome the drawback, dilution to the surfactant-rich phase with organic solvent, such as acetonitrile–methanol containing

* Tel.: +86 22 23494777; fax: +86 22 23502458.
E-mail address: xbyin@nankai.edu.cn.

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doi:10.1016/j.chroma.2007.03.113

2 mM perchloric acid [8], methanol [4,9], tetrahydrofuran [10], and acetonitrile [11], was used. The dilution partly eliminated the effects of the surfactant on the injection and separation of chromatography/electrophoresis, but the content of surfactant in the diluted surfactant-rich phase was still about 50%. Organic solvent back-extraction [12–15] to the analytes from the surfactant-rich phase was applied to remove surfactant to some extents. Moreover, because CPE is primarily based on the hydrophobic interaction between the solutes and surfactant, other hydrophobic species can be extracted into the surfactant-rich phase and may interfere with the analysis of interested analytes. In addition, the use of volatile and flammable organic solvent for dilution and back-extraction were negated the advantage CPE held over solvent extraction.

Herein, we report a dual-cloud point extraction (dCPE) technique which can overcome the drawbacks of traditional CPE. In our dCPE, cloud point procedure is carried out twice during a single sample pretreatment process. The first part of dCPE procedure is done just as traditional CPE. The surfactant is added into the solution containing the analytes that are hydrophobic or can form hydrophobic complexes with suitable ligands. Following the thermostatic bath and centrifugation, the interested analytes and other hydrophobic interfering species are extracted into the surfactant-rich phase. But, instead of the direct analysis, we perform another round of cloud point procedure, in which, surfactant-rich phase is treated with another aqueous solution containing a special ligand which can form new hydrophilic complexes with the interested analytes. After the thermostatic bath and certification, the interested analytes are back-extracted into aqueous phase. The obtained aqueous extract is injected in electrophoretic capillary as sample. Due to the introduction of the second cloud point procedure, the effects of the surfactant on the electrophoretic injection and separation are eliminated. The removal of interfering species through the dCPE procedure improves the analysis method selectivity extensively, and besides the aqueous sample solution is naturally compatible with the electrophoresis condition. The parameters influencing the dCPE and electrophoresis speciation analysis of mercury were investigated in detail.

2. Materials and methods

2.1. Instrumentation

A homemade capillary electrophoresis (CE) setup with an UV detector was used in this work. The high-voltage power supply was obtained from Tianjin Dongwen High Voltage Power Supply Plant (Tianjin, China) and operated in a positive voltage-controlled mode. UV detection was carried out with model 757 UV detector (Shanghai Huixing Apparatus Co. Ltd., Shanghai, China) designed originally for high performance liquid chromatography (HPLC). The flow-through cell for HPLC was machined so that 375 μm OD fused-silica capillary could be mounted in the optical path. The optical window facilitating the CE detection was replaced with a slice with 150 μm pinhole as slit to cut off the stray light. A chromatographic workstation (Shanghai Junrui Software Co. Ltd., Shanghai, China) was used for data acquisition and treatment. Detection was performed at 210 nm. Uncoated fused-silica capillaries (Yongnian Optical Fiber Co. Ltd., Hebei, China) with 60 cm total length, 40 cm effective length, 75 μm ID 375 μm OD were used to separate the four mercury–cysteine complexes. Sample was introduced into capillary by a hydrostatic pressure with a height-difference of 20 cm between the inlet and outlet of capillary for 20 s.

2.2. Reagents

The stock solutions of methylmercury (MeHg), ethylmercury (EtHg), and phenylmercury (PhHg) of 500 mg L^{-1} (as Hg) were prepared by dissolving suitable amounts of methylmercury chloride, ethylmercury chloride, and phenylmercury chloride (all from Alfar Aesar) in methanol. Mercury(II) nitrate (Beijing Chemical factory, Beijing, China) was dissolved in doubly deionized water directly to obtain the solution of inorganic mercury (Hg(II)) of 500 mg L^{-1} (as Hg). Working standard solutions containing mercury species were prepared by stepwise diluting the stock solutions as described above.

Triton X-114 and X-100 (both from Sigma) were used for the cloud point procedure. 1-(2-Pyridylazo)-2-naphthol (PAN), thionine, and 8-hydroxy quinoline (8-OX) (all from Tianjin Chemicals Co., Tianjin, China) were used to investigate the effect of complex agents on the dCPE. 0.1% (m/v) L-cysteine (Sigma) was used to back-extract mercury species into aqueous phase at the second cloud point procedure. A mixture of 100 mmol L^{-1} of boric acid and 10% methanol (Tianjin Taixing Chemicals Co., Tianjin, China) at pH 8.5 was used as buffer solution to separate the four mercury–L-cysteine complexes.

2.3. Samples and sample pretreatments

The accuracy of the present dCPE–CE technique for mercury speciation was checked by analyzing three mercury-spiked natural water samples and a tilapia muscle sample. Lake water and river water samples were collected locally and filtered through 0.45 μm filter after collection. Tilapia fish was obtained from local markets.

Aqueous sample pretreatment with the present dCPE is shown in Fig. 1. Briefly, the concentrated PAN (150 μL 5% (w/v) alcohol solution) and Triton X-114 (200 μL 4% (w/v) aqueous solution) were added into aliquots of 10 mL of the sample solution. After holding in a thermostatic bath at 40 °C for 10 min, the solution was separated into two phases by centrifugation for 5 min at 3500 rpm. The supernatant aqueous phase was removed carefully. 150 μL 0.1% L-cysteine solution was added to the surfactant-rich phase (about 100 μL). Subsequently, the mixture of surfactant-rich phase and L-cysteine was shaken acutely followed by thermostatic bath and centrifugation treatment the same as of the first procedure. The aqueous phase obtained finally was injected into electrophoretic capillary as sample.

The pretreatment for biological sample with dCPE was carried out as following. 0.500 g of homogenized tilapia muscle powder was added into 10 ml 0.075% (m/v) PAN aqueous solution containing 0.08% Triton X-114 at 4 °C in refrigerator. Under