Directly-thiolated graphene based organic solvent-free cloud point extraction-like method for enrichment and speciation of mercury by HPLC-ICP-MS

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ABSTRACT

Directly-thiolated graphene (G-SH) was prepared by substitution of the epoxy and hydroxyl groups with mercapto on the bulk and edge of the graphene oxide. Cloud point extraction-like method (CPEL) was established for enrichment of divalent mercury (IHg) and monomethylmercury (MMHg) in the water samples, based on the prepared G-SH, with good hydrophilicity, strong affinity towards mercury species and huge surface area. The large amount of active adsorption sites, thiol groups, on the bulk and edge of the surface of the G-SH play a key role in extraction of mercury species. Under the optimal conditions, the enrichment factors were 78 and 77 based on only 40 mL samples, the detection limits (3δ) by high-performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS) were 3.8 and 1.3 ng L^{-1} for IHg and MMHg with good recoveries, respectively. The proposed CPEL-HPLC-ICP-MS method was successfully applied for speciation of mercury in surface water, underground water, domestic sewage and even sea water; moreover, the results suggested that it is a simple, sensitive and environment-friendly technique for speciation of mercury at trace levels.

1. Introduction

Mercury is one of the most hazardous heavy metal pollutants which widely exist in the environment. Even at trace concentrations, mercury is high toxicity due to its liposolubility and biomagnification [1]. The toxicity of mercury is highly depended on its chemical form [2]. The main mercury species in natural water consist of inorganic mercury (IHg) and monomethylmercury (MMHg). MMHg is the most toxic mercury species, which cannot be metabolized from biological bodies [3]. Its ability to penetrate membranes in transport routes can break the blood-brain barrier and further destroy the central nervous system [4,5]. MMHg can be produced from other mercury species in the environment by biogeochemical transformation processes [6]. Inorganic mercury is considered as the proximate toxic agent, which can directly react with the amino acid cysteine in proteins [7]. Therefore, it is great significant for speciation of mercury to evaluate its toxicity, bioavailability, and mobilities in the natural environment [8].

In fact, extremely low concentrations and matrix interferences of mercury species, especially, MMHg, in natural environmental samples make them difficult to directly analyze with the existing detecting techniques [9]. Thus, a simple and sensitive method for enrichment of mercury species in the water is necessary. So far, various sample enrichment and preparation procedures have been employed for the enrichment of mercury species, such as solid-phase (micro-)extraction (SPE/SPME) [10,11], liquid–liquid extraction (LLE) [12,13], cloud point extraction (CPE) [14] etc. SPE has been widely used due to its simplicity, low labor cost, low solvent consumption, safe working environment, and low cost [15,16]. Compared with traditional LLE, CPE is advantageous due to its small amount of relatively nonflammable and nonvolatile surfactants during the extraction procedure [17]. However, organic solvent or surfactant was usually applied in most of the extraction methods mentioned above, which were not environment friendly. The cloud point extraction-like method is a recently proposed based on grapheme oxide (GO), with the advantages of both SPE and CPE including organic solvents-free, easy-to-handle, rapid phase separation and low cost. Moreover, it dealt with the separation difficulty of graphene material in traditional SPE tactfully and reaches its full potential in analytical chemistry [18].

Graphene (G) has large surface area, thermal and mechanical stability, offering enormous potential for the pre-concentration of heavy metals [19,20]. Graphene oxide (GO) has a large amount of oxygen-containing functional groups on its surface including epoxy, hydroxyl, and

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carboxyl groups, which can be the adsorption sites for heavy metal ions [21–24]. However, its non-selective adsorption limits its application in the speciation analysis in complex matrix [25–27]. The specific chemical functionalization of G/GO is necessarily for adsorbing the analytes [28, 29]. Obviously, the sulfur-containing groups have been generally considered as "effective reactive sites for mercury", based primarily on the high affinity [30]. Modification of GO with additional ligands or coupling agents had been successfully applied for adsorbing Hg^{2+}, such as sulfur nanoparticles decorated reduced graphene oxide [30], graphene oxide-magnetic chitosan grafted with mercapto [31], graphene oxide modified with L-cystine [32]. However, these modifications were carried out merely on the edge of the surface, which may limit their application in analysis. Recently, simple directly-thiolation methods for functionalization of graphene by substitution of the hydroxyl and epoxy groups on the bulk and edge of the GO surface were reported [33,34]. The directly-thiolated graphene (G-SH) can provide large amount of active sites [34,35], which could be applied in enrichment of mercury species.

In this work, a simple, sensitive and effective cloud point extraction-like method (CPEL) based on the directly thiolated graphene (G-SH), for speciation of mercury in environmental water samples was developed. Due to high dispersity of the G-SH, a stable and uniform solution like colloidal solution could be formed, which could aggregate immediately by changing pH or adding electrolyte solution, such as NaCl, similar to that conventional CPE method [36]. Moreover, the G-SH could be an excellent adsorbent for enrichment of mercury species due to its simple preparation, the abundant reactive sites, and the high hydrophilicity.

The proposed CPEL-HPLC-ICP-MS method with high sample throughput, high extraction efficiency, and easily-operated step had been applied in speciation of mercury in various environmental water samples, especially, high salinity water samples, such as sea water, which is very difficult to analyze by conventional analytical techniques [18]. To our knowledge, the directly-thiolation of G/GO composites was used, for the first time, as an effective adsorbent for the preconcentration of mercury species.

2. Material and methods

2.1. Chemicals and materials

All chemicals used in the experiment were of analytical grade or better. Natural graphite was obtained from Bailingwei Technology co., Ltd. (Beijing, China). Sodium nitrate, hydrogen peroxide (30%), thiourea, hydrobromic acid, and sodium chloride were purchased from Kermel Chemical Reagent Company (Tianjin, China). Sulfuric acid (95–98%), sodium hydroxide, potassium permanganate (99.5%), ethanol (99.7%), and ammonium acetate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). L-Cysteine was obtained from Beijing Solarbio Science & technology Co., Ltd. (Beijing, China). Methanol was obtained from Tedia Company Inc. (Fairfield, USA). High-purity hydrochloric acid (BV-III) was obtained from Beijing Institute of Chemical Reagents (Beijing, China). Deionized water produced by Milli-Q water purification system (Bedford, Ma, USA), 18.2 MΩ, was used for the preparation of regents and standards.

Stock solutions of inorganic mercury (GBW(E) 080124, 100 ± 0.8 μg mL^{-1}) and methylmercury in methanol (GBW 08675, 76.6 ± 2.9 mg kg^{-1}) were obtained from the National Institute of Metrology, China. All stock solutions were stored in the dark at 4 °C and used in stepwise dilution. The mobile phase of high performance liquid chromatography (HPLC) for the separation of IHg and MMHg is 1.0 g L^{-1} L-cysteine solution with 60 mmol L^{-1} ammonium acetate. It was prepared daily by dissolving the 1.0 g L-cysteine in 1 L deionized water with the addition of 20 mL ammonium acetate solution (3 mol L^{-1}).

All glassware in the experiment were soaked in bromine monochloride (BrCl) solution (3–5%, V/V) for 24 h and rinsed several times with deionized water immediately before use. The BrCl solution was prepared by dissolving 2.70 g of reagent grade KBr in 250 mL of high-purity hydrochloric acid with stirring for 1 h and adding 3.80 g reagent grade KBrO$_3$ with reaction time of 1 h [37].

2.2. Apparatus

The synthesis of material was carried out with motor stirrer (100 W, 3000 rpm, Zhongda instrument plant, Yuntai, China) and electro-thermostatic water bath (DZKW-D-2, Guangming Medical Equipment Company, Beijing, China). The aggregate of adsorbent and analyte ions was easily deposited to the bottom of the centrifuge tube by a high speed centrifuge (HC-3018, Anhui USTC Zokia Scientific Instruments Co., Ltd., China). A benchtop ultrasonic cleaner (KQ-600KDE, Kunshan Ultrasonic Instruments Co., Ltd., China) was applied to disperse equally in solution. The dry GO or G-SH were obtained by the vacuum freeze dryer (FD-1, Detanyou Technology and Development Co., Ltd., Beijing, China). The pH values were measured with a PHS-3C pH-meter (Shanghai Precision & Scientific Instrument Co., Ltd., Shanghai, China).

The size and morphology of the GO or the G-SH was observed by transmission electron microscopy (TEM, JEM-1011, Japanese Electronics Co., Ltd., JEOL), and the chemical composition was confirmed by X-ray photoelectron spectroscopy (XPS, ESCALAB 250, ThermoFisher SCIENTIFIC). Scanning electron microscopy (SEM) images were recorded using on a SUPPATM 55 (Zeiss, Germany) equipped with an energy dispersive X-ray spectroscopy (EDS) analyzer (INCAx-act, US Phillip). Besides, Fourier transform infrared spectrometer (FT-IR, Nicolet710, Thermo Nicolet, USA) and a UV–vis absorption spectrometer (UV–vis, UV-2550, Shimadzu, Japan) were respectively applied in materials characterization, respectively.

The separation of mercury species was performed on the HPLC system, which consist of Shim-pack CLC-ODS C18 column (15.0 mm × 6.0 mm × 5.0 μm, Shimadzu, Japan), Flexar LC pump (PerkinElmer, USA) and Rheodyne model 7725i injector (Rheodyne, Cotati, CA, USA) equipped with a 100 μL sample loop. After different mercury species separated, they were injected in NexION 300X ICP-MS (PerkinElmer, USA) for elemental determinations by a PEEK (0.1 mm I.D. × 1/16 mm O.D.). The optimum conditions of the HPLC-ICP-MS are given in Table 1.

2.3. Synthesis of adsorbents

2.3.1. Synthesis of GO

Graphite oxide (GO) was synthesized according to the modified Hummers method [38,39]. Briefly, 0.50 g graphite, 23.0 mL H$_2$SO$_4$ and 0.50 g NaNO$_3$ were mixed in a 250 mL round-bottom flask at 0 °C, and the mixture was stirred for 1 h. KMnO$_4$ (3.0 g) was then added in portions at 0 °C. Subsequently, the mixture solution was heated up to 35 °C with continuous stirring for 1 h. With deionized water (40 mL) added, the temperature of the mixture rise to 90 °C. After stirring at

Table 1
Optimized conditions of the HPLC-ICP-MS for mercury speciation.

Parameters	Optimized value
HPLC	
Column	Shim-pack CLC-ODS C18 column (15.0 cm × 6.0 mm I.D. × 5.0 μm)
Mobile phase	1.0 g L^{-1} L-cysteine, 60 mmol L^{-1} ammonium acetate
Flow rate of mobile phase	1.0 mL min^{-1}
Injection volume	100 μL
ICP-MS	
ICP-MS	PerkinElmer NexION 300X
Nebulizer	Meinhard Nebulizer
Isotope	202
RF power	1500
Plasma gas flow rate	15.0 L min^{-1}
Auxiliary gas flow rate	1.25 L min^{-1}
Nebulizer gas flow rate	0.80 L min^{-1}
Dwell time	20 ms