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Mercury speciation in seawater by liquid chromatography-inductively coupled plasma-mass spectrometry following solid phase extraction pre-concentration by using an ionic imprinted polymer based on methyl-mercury–phenobarbital interaction

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ABSTRACT

Trace levels of inorganic mercury, methyl-mercury and ethyl-mercury have been assessed in seawater by high performance liquid chromatography (HPLC) hyphenated with inductively coupled plasma-mass spectrometry (ICP-MS) after solid phase extraction (SPE) pre-concentration with a novel synthesized ionic imprinted polymer. The adsorbent material was prepared by trapping a non-vinylated chelating ligand (phenobarbital) via imprinting of a ternary mixed ligand complex of the non-vinylated chelating agent, the template (methyl-mercury), and the vinyl ligand (metacrylic acid, MAA). Ethylene dimetacrylate (EDMA) and 2,2′-azobisisobutyronitrile (AIBN) were used as cross-linker and initiator reagents, respectively; and the precipitation polymerization technique was used in a porogen of acetonitrile/water (4:1). The best retention properties for methyl-mercury, inorganic mercury and ethyl-mercury species from seawater were obtained when loading 200 mL of sample adjusted to pH 8.0 and at a flow rate of 2.0 mL min^{-1} on a column-packed with 200 mg of the material. Quantitative mercury species recoveries were obtained using 4 mL of an eluting solution consisting of 0.8% (v/v) 2-mercaptoethanol and 20% (v/v) methanol (pH adjusted to 4.5) pumped at a flow rate of 2.0 mL min^{-1}. Mercury species separation was achieved on a Kinetex C18 column working under isocratic conditions (0.4% (v/v) 2-mercaptoethanol, 10% (v/v) methanol, pH 2.5, flow rate 0.7 mL min^{-1}). ICP-MS detection was performed by monitoring the mercury mass to charge ratio of 202. The limits of quantification of the method were 11, 6.7, and 12 ng L^{-1}, for inorganic mercury, methyl-mercury and ethyl-mercury, respectively (pre-concentration factor of 50); whereas, analytical recoveries ranged from 96 to 106%. The developed method was successfully applied to several seawater samples from unpolluted areas.

1. Introduction

Although the use of mercury (in the chloralkali industry and, previously, in the wood pulping industry) is decreasing, high mercury concentrations are still present in sediments associated with the industrial applications of this metal. In addition, the burning of fossil fuels and pollution of water by mine tailings can also be significant sources of mercury [1]. However, low levels of dissolved mercury are expected in the marine environment [2]. Concentrations range from 0.5 to 3.0 ng L^{-1} in open ocean; whereas, values within the 2–15 ng L^{-1} range can be found in coastal seawater [1]. Nevertheless, local variations from these values can be observed in coastal seawater and inland waters where near to anthropogenic sources, and where mercury associated with suspended material may also contribute to the total load [1]. Even at low concentrations, mercury species can drive bio-accumulation and bio-magnification to levels of this toxic metal in fish, posing human and ecological health risks [2–4]. Mercury is thus included in the Annex I (Environmental Quality Standards for Priority Substances and Certain Other Pollutants) of the Directive 2013/39/UE of the European Parliament and of the Council (12/08/2013), and a maximum allowable concentration (MAC) of mercury and its compounds of 70 ng L^{-1} in inland surface waters and other surface waters has been established [5].

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Despite the better performances (lower limit of detection and better separation) obtained when using gas chromatography-inductively coupled plasma-mass spectrometry (GC-ICP-MS), the need of derivatization of mercury species is drawback. HPLC-ICP-MS method, although less sensitive allows, however a direct liquid sample introduction. Pre-concentration procedures are therefore needed when using HPLC-ICP-MS because the expected mercury concentrations are within the ng per liter range, and complex samples, such as seawater, contain high salt levels. Solid phase extraction (SPE) has been demonstrated to be a suitable pre-concentration technique for extracting mercury species, allowing quantitative recoveries, efficient salt removal, and guarantying minimal/negligible mercury species changes [6]. Recent applications for mercury speciation include anion (quaternary amine type [7], and SAX [8]), and cation (SCX, benzenesulfonic acid-type) [9,10] exchange resins; and also polymeric resins, mainly reverse phase C18 [11–18]. The latter solid supports require mercury species complexation to enhance analyte interaction with the sorbent [11–13], or the immobilization of the complexing reagent on the C18 particles [14–18].

Current trends in SPE are mainly focused on the development of new sorbents such as nanometer-sized materials, egg-shell membranes, modified silica beads, molecularly/ion imprinted polymers, and mesoporous materials. These new solid supports are prepared mainly to improve selectivity [6]. As reported, molecularly imprinted polymers (MIPs) and ionic imprinted polymers (IIPs) have been proposed for achieving efficient pre-concentration of both organic and inorganic analytes, and also for clean-up purposes [19–22]. In contrast to organic molecules, the absence of functional groups in most ions difficults a template–monomer interaction, and the typical binary (template/vinylated monomer) pre-polymerization mixtures when synthesizing MIPs must be changed to ternary pre-polymerization mixtures in which an auxiliary non-vinylated reagent (a ligand exhibiting affinity for the metal ion and adequate functional groups) is mixed with the template and the monomer. After polymerization, the non-vinylated chelating ligand is therefore trapped via imprinting [22]. On other occasions, bifunctional reagents (a ligand exhibiting affinity for the metal ion and containing vinyl groups for polymerization after cross linking) can be used, and the complexing ligand is therefore chemically immobilized in the polymeric matrix. The main drawback of the latter approach is the fact that complexing ligands showing vinyl groups are scarce and are not commercially available, so the synthesis of these complexing monomers must be performed in the laboratory. Although some bifunctional monomers have been used for preparing IIPs for mercury recognition [23,24], most of the synthesized IIPs used non-vinylated ligands trapped in the polymeric matrix after ternary template–non-vinylated ligand–vinylated monomer interactions when using Hg(II) [25–30] and methyl-mercury (Me-Hg) [31] as templates.

The aim of the current work was the application of the precipitation polymerization approach for synthesizing an IIP for mercury species [Hg(II), Me-Hg, and ethyl-mercury (Et-Hg)] retention against major components in seawater (mainly sodium and chloride). A strategy based on using a ternary template/non-vinylated ligand/vinylated monomer (Me-Hg/phenobarbital/metacrylic acid (MAA) was adopted. Phenobarbital has not previously been proposed for IIP synthesis. However, phenobarbital was recently demonstrated to exhibit adequate functional groups for interacting with vinylated monomers such as MMA [32]. Mercury speciation (Hg(II), Me-Hg, and Et-Hg) was performed by high performance liquid chromatography (HPLC)-inductively coupled plasma-mass spectrometry (ICP-MS). The chromatographic separation was attained in 5 min, a shorter chromatographic time than those reported in other published applications (within the 10–25 min range) [33–36]). Because of the short analysis time (5 min) when comparing to those previously reported applications, and because of the high sensitivity inherent in the use IIP-SPE procedure, the developed method allows the assessment of mercury species in seawater at levels lower than the MAC established in the Directive 2013/39/UE for environmental quality standards (70 ng L^{-1}) [5].

2. Experimental

2.1. Apparatus

A Flexar LC HPLC (LC pump, column oven, and LC autosampler) from Perkin Elmer (Waltham, MA, USA) was used for mercury species separations. The HPLC system was coupled to a Perkin Elmer Nex-Ion 300X ICP-MS, instrument which was also used for total mercury determinations [in such case, a SeaFast SC2 DX autosampler from Elemental Scientific (Omaha, NB, USA) was used]. Reverse phase chromatographic separations were performed on a Kinetex C18 100 A (100 mm length × 2.10 mm i.d., 5.0 μm particle diameter) analytical column (Phenomenex, Torrance, CA, USA) connected to a C8 guard column (4 mm length × 3.0 mm i.d.) from Phenomenex. Other tested analytical columns were Kinetex C18 100 A (100 mm length × 4.60 mm i.d., 2.6 μm particle diameter) from Phenomenex, and Zorbax Eclipse XDB-C8 (4.6 × 150 mm) from Agilent (Santa Clara, CA, USA). Polymerization was performed by using a temperature-controlled incubation camera (Stuart Scientific, Surrey, UK) equipped with a low-profile roller (Stovall, Greensboro, NC, USA). IIPs were packed into 5 mL syringes (Braun, Wertheim, Germany) between replacement Teflon frits (Supelco, Bellefonte, PA, USA). SPE was performed by using a Miniplus 3 peristaltic pump equipped with a head for delivering low pulse flows of fluids in 8 channel standard flow rate (Gilson, Middleton, WI, USA), and PVC 2-stop tubing (3.175 mm i.d.) from SCP Sciences (Quebec, Canada). Seawater samples were filtered through Durapore 0.45 μm filters (Millipore, Billerica, MA, USA) by using a vacuum pump (Millipore). Synthesized IIP material was filtered through Durapore 0.22 μm filters (Millipore). IIP characterization was performed by using a scanning electron microscope EVO LS 15 from Zeiss (Oberkochem, Germany), and an energy dispersive X-ray fluorescence spectrometer (molybdenum-based anode) laboratory-made by RIAIDT (*Rede de Infraestruturas de Apoio á Investigación e ao Desenvolvemento Tecnolóxico*) at the University of Santiago de Compostela. Other laboratory devices were a Basic20 pH meter with a glass–calomel electrode (Crison, Barcelona, Spain) for pH measurements, a Reax 2000 (Heidoph, Niederbayern, Germany) for vortexing solutions, and an oven model 207 from Selecta (Barcelona, Spain) for IIP drying.

2.2. Reagents

Ultrapure water of resistivity 18 MΩ cm obtained from a Milli-Q purification device (Millipore) was used to prepare all the solutions. Hg(II) stock standard solution (1000 mg L^{-1}) was from Panreac (Barcelona, Spain). Methyl-mercury stock standard solution (1000 mg L^{-1}) was prepared from methyl-mercury chloride from Sigma (Steinhelm, Germany) by dissolving the reagent in a small volume of hot methanol, and further dilution with ultrapure water. Ethyl-mercury stock standard solution (1000 mg L^{-1}) was prepared from ethyl-mercury chloride from Alfa Aesar (Ward Hill, MA, USA) by reagent dissolution in a small volume of 5% (v/v) sulfuric acid in methanol before dilution with ultrapure water. Mercury (I) stock standard solution (1000 mg L^{-1}) was prepared by dissolving mercury (I) chloride from Sigma. Multi-element calibration standard 3 (10 mg L^{-1} of Al, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Sr, V, and Zn), and Y (10 mg L^{-1}) used as an internal