Determination of Mercury in Wastewater Using a Molecularly Imprinted Polymer as Solid Phase Extraction Sorbent and CV-ICP-OES

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INTRODUCTION

More than 140 nations signed a binding treaty in 2013 to reduce the release of mercury which has accumulated in the environment as a result of industrial and mining activities (1). One of the most promising sorbents for wastewater treatment and monitoring of metals are the ionic imprinted polymers due to their selectivity, because they are made with specific cavities for target analytes (2, 3). The polymers for remediation of waters are synthesized using Hg(II) as a template and ligands containing sulfur or nitrogen donating atoms such as dithizone (4), vinylcarbazole (5), or aminothiophenol (6). Mercury(II) imprinted materials such as crop stalks (7), polyaminated chitosan derivatives (8), cellulosic cotton fibers (9), or chelating fibers based on poly(ethylene terephthalate) (10) have also been used for the removal of mercury from waters. Solid phase extraction (SPE) has been performed previous to the analysis of waters using diazoamino-benzene-vinylpyridine copolymers (11), and imprinted polymeric sorbents with 1-(2-thiazolylazo)-2-naphthol (12), 4-(2-thiazolylazo)resorcinol (13), aminothiol monomers (14, 15), tetrakis(3-hydroxyphenyl)porphyrin (16, 17), 2-vinylpyridine (18), 2,2'-di pyridyl amine (19), 8-hydroxyquinoline (20), and 1-(2-acetatoxyphenyl)-3-(4-methylphenyl) triazine (21). Yordanova et al. (22) also synthesized polymeric ion-imprinted nanoparticles for mercury speciation in sur-

ABSTRACT

In this study, a molecularly imprinted polymer based on the formation of the phenobarbital-methylmercury complex was used as a solid phase extraction sorbent for the preconcentration of mercury in waters and subsequent analysis by CV-ICP-OES. A number of important operating parameters were optimized: pH of the sample (pH = 8), sample flow rate, elution volume (5 mL of 1M thiourea/1M HCl), mass of polymer in the cartridge (300 mg), and volume of sample (200 mL). The analytical performance of the method was evaluated with good recovery (105%) at 0.05 $\mu\text{g L}^{-1}$ of mercury (environmental quality standard established by the European Union) and a preconcentration factor of 40. The method was also applied to the analysis of wastewater samples.

face waters with 1-pyrrolidinedithiocarboxylic acid as complexing agent.

Several authors have developed organic-inorganic hybrid materials using thiol-functionalized mesoporous sorbents and methanesulfonic acid (23, 24), an imprinted amino-functionalized silica gel sorbent (25), 3-isocyanatopropyltriethoxysilane bearing thymine bases (26), a double imprinting technique with cetyltrimethylammonium bromide (CTAB) as a second template to improve the efficiency of the polymer (27, 28), or maghemite nanoparticles with mercaptoethylamino as functional monomer (29). A dithizone-Hg²⁺ imprinted sorbent was synthesized by a sol-gel process and applied to

speciation (30). Electrochemical (31-38) and optical sensors (39, 40) for Hg(II) based on imprinted polymers as recognition elements have also recently been developed.

Few studies have used methylmercury as a template to synthesize the imprinted polymers with methylmercury-methacryloyl(L)-cysteine (41), 4-(ethenylphenyl)-4-formate-6-phenyl-2,2'-bipyridine (42) or 3-methyl-5-(methylthio)-4-vinylthiophene-2-carboxylic acid (VTCA) (43) as monomers. Mercury has been used in classical methods for the analysis of barbiturates in biological fluids (44-46). In a previous work (47), the present authors synthesized a methylmercury-imprinted polymer using phenobarbital as a ligand which was applied to the determination of methylmercury in two certified reference materials of marine products after an extraction step with toluene. The polymer is versatile and can also be applied to the speciation of organic forms of mercury by HPLC-ICP-MS (48). In this study, use of a polymer for the preconcentration of waters and analysis by CV-ICP-OES was evaluated and applied to the analysis of several waste-water samples.

EXPERIMENTAL

Instrumentation

Mercury was analyzed using an Optima™ 3300DV inductively coupled plasma optical emission spectrometer (ICP-OES) (PerkinElmer, Inc., Shelton, CT, USA). The Optima spectrometer system includes a three-channel, software-controlled peristaltic pump for mixing the sample solutions and the

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sodium borohydride reducing solution. A homemade gas-liquid separator with forced outlet was connected to the torch using a 60-cm PTFE tube, and the liquid was pumped to waste using a Minipuls™ 3 peristaltic pump (Gilson, Villiers-le-Bel, France). All connections in the vapor generation system were made of PTFE tubing of 0.5 mm i.d., including the 500- μ L reaction loop.

The concentrations determined after the analysis of the wastewater samples were also compared to the results obtained using a PerkinElmer® flow injection mercury system (FIMS).

The polymer was synthesized using a Movi-Rod roll and tilt mixer (Selecta, Barcelona, Spain) placed into the Boxcult temperature-controlled incubation chamber (Selecta). A pH meter model 720 (Thermo Orion, Waltham, MA, USA) was used to adjust the pH of the samples prior to the SPE procedure. The Minipuls™ 3 peristaltic pump (Gilson, Villiers, France) was also used in the SPE experiments in column mode.

The following instruments were used for the characterization of the synthesized polymer (47): FLASH 1112 elemental analyzer (Thermo Finnigan (Waltham, MA, USA), EVO LS 15 microscope (Zeiss, Oberkochen, Germany) to obtain the micrographs, and a labmade spectrometer (Servicios Generales USC (General Services, University of Santiago de Compostela, Spain) with an anode of Mo for energy dispersive x-ray dispersion fluorescence measurements.

Reagents

All solutions were prepared using ultra-pure water of 18 M Ω ·cm resistance obtained from a Milli-Q® purification system (Millipore Corporation, USA).

Methylmercury chloride, methacrylic acid (MMA), ethylene dimethacrylate (EDMA), and phenobarbital and thiourea (Sigma, Steinheim, Germany). The stock standard solution (1000 mg L⁻¹) of Hg(II) was from Merck (Darmstadt, Germany). For polymerization, 2,2'-azobisisobutyronitrile (AIBN) was used (purchased from Fluka, Steinheim, Germany). All other chemicals (ammonia for the preparation of the buffer, hydrochloric acid, acetonitrile, toluene) were purchased from Panreac and Scharlau (Barcelona, Spain).

All glass and plastic material was cleaned and kept in 10% (w/w) nitric acid for at least 48 hours. The material was then rinsed three times with ultra-pure water before use.

Synthesis of the Molecularly Imprinted Polymer

The molecularly imprinted polymer (MIP) was synthesized using a precipitation method previously reported (47). Briefly, the procedure consists of the following steps:

- Preparation of the pre-polymerization mixture (for approximately 1.5 g of polymer, an amount of 0.075 mmol of MeHgCl, 0.3 mmol of phenobarbital, 0.75 mmol of MAA, and 12 mL of the porogen acetonitrile:water [4:1] was mixed and kept in darkness overnight);
- polymerization at 60 °C for 24 hours after addition of 4.5 mmol of the cross-linker EDMA and 0.25 mmol of AIBN (initiator);
- after removal of the template, portions of the dry polymer (150 mg) were packed in 5-mL syringes between Teflon® frits and treated with 200 mL of 1M thiourea in 1M HCl at a flow rate of 1 mL·min⁻¹.

Solid Phase Extraction Procedure

Portions of the MIP (300 mg) were packed in 5-mL syringes used as SPE cartridges. The cartridges were conditioned with 0.1/0.1M NH₃/NH₄⁺ buffer solution at pH 8.0

and the Hg(II) buffered standards or samples (typically 200 mL) were loaded at a flow rate of 0.5 mL min⁻¹. The cartridges were washed with the buffer solution, and elution was performed using 5 mL of 1M thiourea in 1M HCl at the same flow rate. The cartridges were kept in buffer solution after thiourea. Afterwards, the acidic thiourea extracts were analyzed by CV-ICP-OES.

Determination of Mercury by CV-ICP-OES

The determination of mercury was performed by ICP-OES after generation of the elemental cold vapor using a continuous-flow system. The samples were mixed with the reducing solution of 0.2% (w/v) NaBH₄ stabilized with 0.05% (w/v) NaOH. The reagents were transferred to the gas-liquid separator through a 500- μ L reaction loop and an 8-way peristaltic pump (Gilson, Villiers, France) equipped with a 3.18 mm i.d. Tygon tube used for extracting the waste from the gas-liquid separator. The Hg vapor was separated from the liquid mixture and swept to the torch using a 0.8 L min⁻¹ Ar flow rate. The operating conditions used for cold vapor generation and detection of mercury by ICP-OES are summarized in Figure 1 and Table I, respectively.

RESULTS AND DISCUSSIONS

Optimization of Working Conditions

The use of the MIP in column mode avoids losing mass of the polymer as well as time-consuming steps in solid phase extraction, such as weighing the polymer or filtering after extraction. In this study, the MIP was used in column mode as reported in previous studies (11,18, 23), and some of the operating parameters were optimized such as pH of the sample, sample loading flow rate, elution volume and mass of polymer.

Influence of pH on MIP Retention

Standards of 50 µg L⁻¹ of Hg²⁺ (25 mL) buffered at pH 6.0, 7.0, 8.0, and 9.0 were loaded in duplicate at 0.5 mL min⁻¹ into cartridges containing 150-mg portions of the polymer. Afterwards, mercury was eluted with 10 mL of 1M thiourea/1M HCl. Similar experiments were performed using 50 µg L⁻¹ of MeHg⁺. It was observed that the pH affects the recovery of MeHg⁺, whereas the recovery of Hg(II) is almost constant in the interval of the pH studied with a small decrease at pH 7.0 (Figure 2). As a compromise solution, pH 8.0 was selected to perform all further experiments.

The pH used for sample loading depends on the type of ligand selected to complex mercury. The pH in the present study is higher than was used with other methylmercury imprinted polymers with (4-ethenylphenyl)-4-formate-6-phenyl-2,2'-bipyridine (pH 5.0) (11), or methacryloyl-(L)-cysteine methylester (pH 7.0) as ligands (41), or Hg(II) imprinted polymer (pH 6) with vinylcarbazole (5). However, in the case of the polymer with 2,2'-di pyridyl amine, the pH used to load the sample was 9.5 (19).

Selection of Elution Volume

An experiment was performed to reduce the elution volume and

increase the sensitivity in the determinations by CV-ICP-OES. A standard of 0.9 µg L⁻¹ Hg(II) (25 mL) buffered at pH 8.0 was loaded at a flow of 0.5 mL min⁻¹ into a 150-mg cartridge. Mercury was eluted from the polymer using portions of 2.5 mL of 1M thiourea/1M HCl. Only 50% of the analyzed mercury was eluted in the first portion and another 2.5 mL portion was needed to elute the remaining mercury. Therefore, a volume of 5 mL of acidic thiourea was used in further experiments to elute the element from the cartridge.

Influence of Sample Loading Flow Rate

The loading flow rate was studied at four different values (0.5, 1.0, 2.5, and 5.0 mL min⁻¹) using 0.9 µg L⁻¹ Hg(II) standards (25 mL) buffered at pH 8.0. The results indicated that a low flow rate must be used to increase recovery (Figure 3); therefore, a flow of 0.5 mL min⁻¹ was selected for further extractions. An elution flow rate of 0.5 mL min⁻¹, previously selected (47), was used for all further experiments.

Fig. 1. Operating conditions of the cold vapor generation system.

Fig. 2. Influence of pH on the retention of Hg(II) and MeHg⁺.

TABLE I Instrumental Operating Conditions for CV-ICP-OES Determination of Hg	
Hg Wavelength	253.652 nm
ICP RF Power	1300 W
Argon Flow:	
Plasma	15 L min ⁻¹
Nebulizer	0.8 L min ⁻¹
Auxiliary	0.5 L min ⁻¹
Viewing Height	15 mm
Measurement Mode	Axial
Purge Gas Flow	Normal
Aperture Size	Normal
Read Delay	40 s
Replicates	5
Integration Time	Auto

Selection of the Mass of Polymer Used for SPE

Hg(II) aqueous standards were loaded at different concentrations (ranging from 50 ng L⁻¹ to 25 µg L⁻¹) and sample volumes of 10 mL to 200 mL using syringes containing 150 or 300 mg packaged MIP. The average recovery increased successfully to 100 ± 15% using 300 mg of polymer due to the larger breakthrough volume and Hg retention capacity. Therefore, 300 mg of polymer was introduced into the cartridges prior to the analysis of the wastewater samples. The mass of mercury expected in the water samples is lower than the capacity of the MIP sorbent which decreases starting from 20 µg g⁻¹ polymer onwards.

ANALYTICAL PERFORMANCE

Calibration Graphs

The standards were prepared with concentrations of 0, 2.5, 5.0, 7.5, and 10.0 µg L⁻¹ of Hg(II) in 1.0M thiourea/1M HCl and analyzed by CV-ICP-OES using the conditions shown in Table I and Figure 1. The equation of the obtained calibration graph was: $A = 98.291[\text{Hg}] - 1.092$, with a regression coefficient value of 0.999 (Figure 4).

Limit of Detection and Quantification

The limit of detection (LOD) is defined as the element concentration corresponding to three times the standard deviation of the measurement of a blank (n = 11). The limit of quantification (LOQ) is calculated as 10 times the standard deviation of the measurement of a blank. An instrumental LOD of 0.8 µg L⁻¹ was obtained when a solution of 1M thiourea/1M HCl was used as the blank. The instrumental LOQ was 2.7 µg L⁻¹. The LOD and LOQ of the sample was 0.02 and 0.07 µg L⁻¹, respectively, for a preconcentration factor of 40 and 200 mL of sample. The LOQ value of the present study was found to be lower than the environmental quality standards established by the European Union for mercury and its compounds in surface waters of 0.05 µg L⁻¹ (annual average) and 0.07 µg L⁻¹ (maximum allowable concentration) (49).

Accuracy

To evaluate accuracy, the recovery was studied after applying the solid phase extraction procedure and analysis by CV-ICP-OES to different samples containing mercury at different concentration levels (Table II). First, buffered standards of 0.36 µg L⁻¹ Hg(II) (25 mL) were

loaded into 300-mg MIP cartridges in duplicate, and the recovery obtained was 100 ± 16%, with a preconcentration factor of 5. The preconcentration procedure was applied to a standard containing 0.05 µg L⁻¹ of mercury (environmental quality standards established by the European Union, annual average). A volume of 200 mL of sample was loaded into the cartridge in duplicate and eluted with 5 mL of 1M thiourea/1M HCl (preconcentration factor: 40). The eluate was analyzed by CV-ICP-OES and the calculated concentration in the sample was 0.0525 µg L⁻¹, corresponding to a recovery of 105 ± 24%. The analytical recovery was also calculated after the addition of 0.3 µg L⁻¹ Hg(II) to a diluted wastewater sample. The average value

TABLE II
Accuracy in the Determination of Hg

Conc. (µg L ⁻¹)	Experimental Conc. (µg L ⁻¹)	Recovery (%)
0.05	0.0525 ± 0.012	105 ± 24
0.36	0.36 ± 0.06	100 ± 16
0.30 (addition to diluted wastewater)	0.31 ± 0.02	104 ± 2

Fig. 3. Influence of Sample Loading Flow Rate.

Fig. 4. Calibration graph obtained for the determination of Hg.

obtained with the standard deviation was 104 ± 2 % (Table II).

Reproducibility

The reproducibility of the whole procedure (solid phase extraction and determination of mercury by CV-ICP-OES) was evaluated after applying the procedure to five replicates of a diluted wastewater (1:100). The following concentrations were obtained for the replicates: 4.97, 3.74, 4.71, 4.87, and 4.63 $\mu\text{g L}^{-1}$. The mean concentration $\pm$ standard deviation measured in the sample was 4.58 ± 0.49 $\mu\text{g L}^{-1}$, with a standard relative deviation of 10.7%. The cartridges containing 300 mg of the imprinted polymer were reused more than 20 times before being discarded.

Applications

The method was applied to the analysis of 5 wastewater samples that had been previously analyzed using a flow injection mercury system (FIMS), a PerkinElmer CV-AAS instrument. The samples were acidified to pH <1 and kept at 4 °C before analysis.

The analyzed samples were diluted (1:100), undiluted concentrations ranging from 5 to 49 $\mu\text{g L}^{-1}$. The samples were diluted 1:100, 200 mL were loaded in duplicate into the SPE cartridges, and the acidic thiourea extracts were analyzed by CV-ICP-OES. The concentration values provided by the supplier, the concentration values calculated, and the recoveries are listed in Table III.

CONCLUSION

Solid phase extraction using an imprinted polymer is a good choice for the determination of mercury in waters. Preconcentration factors of 40 were obtained with the methylmercury imprinted polymer using phenobarbital as a ligand, thus allowing the determination of Hg by CV-ICP-OES in samples with con-

centrations lower or similar to the environmental quality standards established by the European Union for mercury ($0.05 - 0.07$ $\mu\text{g L}^{-1}$). The developed method was also applied successfully to the analysis of diluted wastewaters.

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TABLE III
Hg(II) Concentration in Analyzed Samples

Sample	Provided Concentration ($\mu\text{g L}^{-1}$)	Calculated Concentration ($\mu\text{g L}^{-1}$)	Recovery (%)
1	5.0 ± 0.1	5.6 ± 0.3	112 ± 6
2	49.0 ± 1.0	53.5 ± 0.1	109 ± 1
3	11.6 ± 0.4	12.1 ± 0.7	104 ± 6
4	29.5 ± 0.7	23.7 ± 6.8	80 ± 23
5	3.9 ± 0.8	3.4 ± 0.2	88 ± 6

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