



Synthesis and application of ion-imprinted polymer for the determination of mercury II in water samples

Janaina E. Francisco¹ · Fernanda N. Feiteira¹ · Wanderson A. da Silva^{1,2} · Wagner F. Pacheco¹

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Abstract

In this study, an innovative analytical methodology capable of selectively identifying and quantifying mercury contamination by the association of solid-phase extraction using ion-imprinted polymers as a sorbent phase and differential pulse anodic stripping voltammetry is proposed. To this end, the ion-imprinted polymers were synthesized and characterized by infrared spectroscopy and atomic force microscopy. The sorption capacities and the selectivity of the ion-imprinted polymers were compared to the ones related to the non-imprinted ones. Next, the experimental parameters of this solid-phase extraction method (IIP-SPE) were evaluated univariately. The selectivity of this polymeric matrix against other cations (Cd II, Pb II, and Cu II) was also evaluated. Limits of detection (LOD) and quantification (LOQ) obtained for the here proposed methodology were $0.322 \mu\text{g L}^{-1}$ and $1.08 \mu\text{g L}^{-1}$, respectively. Also, the precision of 4.0% was achieved. The method was finally applied to three water samples from different sources: for the *Piratininga* and *Itaipu* Lagoon waters, Hg II concentrations were below the LOQ and for *Vargem* River waters a concentration equal to $1.35 \pm 0.07 \text{ mg L}^{-1}$ was determined. These results were confirmed by recovery tests, resulting in a recovery of $96.2 \pm 4.0\%$, and by comparison with flame atomic absorption spectrometry, resulting in statistical conformity between the two methods at 95% confidence level.

Keywords Ion-imprinted polymer · SPE · Mercury · Voltammetry

Introduction

Due to population growth and the development of the industrial sector, the supply and availability of potable water have become a global concern. Increased industrial activities lead to the production of contaminated effluents, rich for instance in toxic metals that are placed directly into the environment. In that sense, the toxicity, persistence, and high bioaccumulation potential of metals are the most dangerous points to deal with. Cadmium, lead, arsenic, and mercury are the metals that stand out based on their toxicity levels. Mercury is considered the most dangerous among them by the US Environmental Protection Agency (EPA - United States Environmental

Protection 1985). It is a natural element that can be found in the four subsystems of planet Earth (geosphere, hydrosphere, biosphere, and atmosphere) in several possible forms. The organic forms, such as methyl mercury and ethyl mercury, and the elemental form are neurotoxic, and the inorganic forms, such as mercury chloride, are mainly nephrotoxic (Oliveira et al. 2007; UNEP, 2013).

Many methodologies have been developed for the determination of mercury in aqueous matrices. The techniques employed are the most diverse such as UV-Vis molecular absorption (Babu and Reddy 2012), molecular fluorimetry (Liang et al. 2018), atomic fluorescence spectrometry (AFS) (Schlathauer et al. 2017), inductively coupled plasma optical emission spectrometry (ICP-OES) (Wuilloud et al. 2002), inductively coupled plasma mass spectrometry (ICP-MS) (Allibone et al. 1999), atomic absorption spectrometry (Sobhi et al. 2017), or voltammetry (Martín-Yerga et al. 2013). However, the instrumentation necessary to many of these methodologies is expensive, making them inaccessible to some research groups. Also, for most of these techniques, except for ICP-MS, the quantification of mercury species in effluents is strongly affected by concomitants present in the

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✉ Fernanda N. Feiteira
fnfeiteira@id.uff.br

¹ UFF - Universidade Federal Fluminense, Niterói, RJ, Brazil

² CEFET/RJ - Centro Federal de Educação Tecnológica Celso Suckow da Fonseca, Petrópolis, RJ, Brazil

matrix; thus, it is very common to make a previous step of sample preparation in order to promote the elimination of these interferences (Ghasemlou et al. 2013).

Solid-phase extraction (SPE) is a well-known method for isolating analytes from complex matrices. That approach makes use of the different interactions of a solid phase inside of a cartridge and the components of a liquid sample, allowing the separation of these components. It may offer the possibility of complex samples simplification, but many times the disadvantages of low selectivity and high cost of commercial cartridges are remarkable (He et al. 2007).

Obtaining new materials that might offer greater selectivity to the SPE sorbent phases when compared to the conventional commercial ones is a desired aspect in the development of new methodologies. In this context, many works in the field of molecularly and ion-imprinted polymers (MIP and IIP) have been developed by employing these ones as SPE sorbent phases (MIP-SPE/IIP-SPE), aiming the separation of interferences by the mechanism of analyte recognition (He et al. 2007; Moussa et al. 2017; Yusoff et al. 2017; Khoddami and Shemirani 2016).

Molecularly imprinted polymers (MIP) are synthesized in the presence of the template molecule, so that the growth of the polymer occurs around the substance of interest (Mazzotta et al. 2016). After polymerization, the template molecule is leached, leaving the binding sites available and resulting in a molecular memory by the polymer. This recognition mechanism is based on the selective mechanisms present in living beings such as enzyme-substrate and/or antigen-antibody (Han et al. 2016). When this model molecule is replaced by an ion, ion-imprinted polymers (IIP) are obtained (Shakerian et al. 2016; Luo et al. 2015).

These materials called IIP have attracted much attention as a highly selective sorbent for SPE in order to pre-concentrate and clean-up samples prior to analyses. IIPs show outstanding characteristics such as high selectivity, low cost, high surface area, durability, and reusability (Rajabi et al. 2017; Shamsipur et al. 2014). A variety of IIP have been reported for separation of copper (Rajabi et al. 2017), selenium (Khajeh et al. 2007), uranyl (Shamsipur et al. 2007), mercury (Ghanei-Motlagh et al., 2016; Alizadeh et al. 2011; Rajabi et al. 2013), cesium (Shamsipur and Rajabi 2013), zinc (Shamsipur et al. 2014), nickel (Vatanpour et al. 2011), and many other ions.

Three steps are involved in the ion-imprinting process: (i) complexation of templates (metal ions) to a suitable ligand, (ii) polymerization of this complex, and (iii) removal of the template after polymerization. After ion-imprinting polymerization, the imprinted metal ion is removed from the polymeric material by leaching with a mineral acid which generates available binding sites. These polymeric imprinted particles are complementary

in shape and size to the metal ion and, for that reason, show an affinity for the template ion over other ones structurally related (Rajabi et al. 2017; Shamsipur et al. 2014).

In order to combine the sensitivity of the voltammetric instrumental technique and the selectivity of the IIPs as solid phases for IIP-SPE, a new analytical methodology capable of determining mercury in environmental water samples (river and lagoons waters) was proposed here. At first, an Hg^{2+} -IIP was synthesized and characterized, then the IIP-SPE procedure was optimized and quantifications were performed by differential pulse anodic stripping voltammetry using gold electrode.

Table 1 shows a comparison of different methodologies for mercury determination in environmental water samples, some of them using IIP-SPE as sample preparation step and others that employ different strategies.

The results presented in this work show that the here developed methodology offers limits of detection (LOD) and quantification (LOQ) and recoveries as good as or better than others suggested in literature, except for AFS, ICP-OES, and ICP-MS. Though, it should be highlighted that this work proposes a more affordable alternative, one that can be accessible to many more research groups. Also, the results here exposed confirm that this present method offers better limits of detection and quantification than other works that synthesized Hg^{2+} -IIP.

Experimental procedures

Reagents, solutions, and instrumentation

4-Vinylpyridine (4-VP), benzoyl peroxide (BPO), and ethylene glycol dimethacrylate (EGDMA) were purchased from Sigma-Aldrich (USA) and mercury II chloride from Qhemis (Brazil). All reagents were of the analytical grade used without further purification. Ultrapure water was obtained by Arium Pro Sartorius (Germany) purification system.

1.25 mL of a 1000 mg L⁻¹ Hg II standard solution was transferred to a 250.0 mL volumetric flask and the final volume was completed to the mark with deionized water, resulting in a 5.00 mg L⁻¹ intermediate solution. Working solutions were prepared daily by diluting the intermediate solution.

A 4.00 × 10⁻² mol L⁻¹ Britton Robinson buffer solution was prepared by dissolving 0.248 g boric acid, 1.433 g sodium phosphate, and 0.328 g sodium acetate in about 50.0 mL of deionized water, adjusting the pH with NaOH aqueous solution and completing the volume to the mark of 100.0 mL in a volumetric flask with deionized water.

The IR spectra were measured by a Spectrum Two FTIR Spectrometer (PerkinElmer, USA). The morphological

Table 1 Different methodologies present in literature for mercury determination in environmental water samples

Reference	Sample preparation	Detection	LOD/LOQ	Recovery (%)
Babu and Reddy 2012	Formation of colored complex	UV-Vis molecular absorption	240 $\mu\text{g L}^{-1}$ (LOQ)	96.7–101.1
Liang et al. 2018	Quenching of derived carbon nanodots	Molecular fluorimetry	151 $\mu\text{g L}^{-1}$ (LOD)	100.4–105.0
Sobhi et al. 2017	Magnetic dispersive SPE	CV-AAS	0.2 $\mu\text{g L}^{-1}$ (LOD)	87–114
Dal Borgo et al. 2013	–	Voltammetry	0.39 $\mu\text{g L}^{-1}$ (LOD)	100.1 \pm 3.9
Rajabi et al. 2013	Hg ⁺² -IIP	Voltammetry	1.0 $\mu\text{g L}^{-1}$ (LOD) / 1.8 $\mu\text{g L}^{-1}$ (LOQ)	92.5–99.5
Hande et al. 2017	Hg ⁺² -IIP	Molecular fluorescence	4.0 $\mu\text{g L}^{-1}$ (LOD)	95.0–98.0
Schlathauer et al. 2017	SPE with nanogold-coated silica	AFS	4 ng L^{-1} (LOD)	90.8–101.5
Wuilloud et al. 2002	Cloud point extraction	CV-ICP-OES	4 ng L^{-1} (LOD)	96–101
Allibone et al. 1999	–	ICP-MS	32 ng L^{-1} (LOD)	98.5–99.5
Dos Santos et al. 2009	Retention in polyurethane foam	HPLC-ICP-MS	4.6 ng L^{-1} (LOD)	93.4–94.0

structure and surface roughness of the polymers were investigated by a NanoSurf Atomic Force Microscope (Switzerland).

Hg II was detected by differential pulse anodic stripping voltammetry, using Metrohm 747 VA Stand (Switzerland) and a gold electrode as a working electrode, stainless steel as counter electrode and Ag/AgCl as a reference electrode. The supporting electrolyte solutions were prepared daily by dissolving 0.147 g of sodium chloride (VETEC, Brazil) and 2.70 mL of sulfuric acid 95% (Merck KGaA, Germany) in deionized water to the final volume of 200.0 mL in a volumetric flask.

The Ashcroft (Brazil) SPE manifold to perform IIP-SPE was coupled to a TBV5 Tander (Brazil) vacuum pump. The comparative AAS method for Hg II quantification was performed by using AA-6200 Flame Atomic Absorption Spectrometer (Shimadzu, Japan).

Electrochemical measurements

In order to improve the electrochemical signal of measurements, a conditioning procedure was performed daily on the gold electrode. It consisted of six voltammetric cycles from 0.100 to 0.550 V in NH_4OH 2.5% m v^{-1} solution (METROHM 2018).

The electrochemical measurements were realized by transferring 25.00 mL of the supporting electrolyte to a 50.00 mL borosilicate cell, then adding 100 μL of the sample solution, followed by successive additions of 10 μL of a standard solution of Hg II.

Differential pulse anodic stripping voltammetry (DPASV) was performed in the range from 0.200 to 0.750 V, using 5 s for resting time, 0.005 V for potential step, 0.200 V for time step, 0.050 V for pulse amplitude, 0.300 V for deposition potential, and 60 s for deposition time. Each analytical measurement was taken in three replicates. It is worth to mention that square wave anodic stripping (SWASV) voltammetry was also studied. Though SWASV is a faster technique, it showed

less peak resolution and more noise; thus, the experiments were conducted in DPASV.

Hg⁺²-IIP synthesis

The imprinted material synthesis was an adaptation of the reaction described by Mergola and coworkers (Mergola et al. 2015), in which the concept of bulk polymerization is employed. 0.050 mmol of HgCl_2 and 0.20 mmol of 4-VP were initially dissolved in 3.00 mL of a mixture containing acetonitrile and water (4:1, v/v^{-1}). That solution was table stirred for 10 min to guarantee complete complexation.

Thereafter, 0.90 mmol EGDMA and 0.040 mmol benzoyl peroxide were added. After fully dispersing and deoxygenating the mixture by nitrogen gas purge, it was heated to 60 °C in a water bath for 24 h under stirring. The resulting polymers were crushed, ground, and sieved with a 0.20-mm sieve. Subsequently, the synthesized polymers were treated with 20.0 mL EDTA 0.100 mol L^{-1} for 2 h under stirring, followed by washing steps to remove the Hg II ion, firstly with 10.0 mL ethanol, then with 15.0 mL HNO_3 (10% v/v^{-1}) and finally with water enough to adjust the pH to neutral condition. The Hg²⁺-IIP was finally dried at 60 °C in a laboratorial oven. Non-imprinted polymers (NIP) were synthesized under the same conditions, including the washing steps, although not containing the ion template, in order to later evaluate the sorption characteristics of the imprinted material.

IIP-SPE

Forty milligrams of the polymer were transferred to empty commercial cartridges. The solid-phase column was conditioned with 5.00 mL of 4.00×10^{-2} mol L^{-1} Britton Robinson buffer at pH 6.0. Then, 20.0 mL of sample or standard solution, buffered at the same concentration and pH were

loaded. The cartridge was later washed with 5.00 mL of deionized water, and finally, the adsorbed analyte was eluted with 10.0 mL HNO_3 25% v/v^{-1} .

These parameters were studied and optimized univariately. All extracts were analyzed in three replicates by differential pulse anodic stripping voltammetry.

Sample preparation

About 500 mL of water samples from the *Vargem* River, the *Itaipu* Lagoon, and the *Piratininga* Lagoon, all located in the State of Rio de Janeiro, Brazil, were superficially sampled in a sterilized glass bottle and stored under refrigeration at 5 °C. Before the IIP-SPE procedure, the samples were subjected to a sequence of filtration steps, using decreasing porosities of filters to eliminate the particles that were suspended in the samples. After this treatment, the samples' pH was adjusted to pH 4.0 with a small volume of HCl 6.0 mol L^{-1} (Ghanei-Motlagh et al., 2016; Alizadeh et al. 2011; Rajabi et al. 2013).

Results and discussion

Hg^{+2} -IIP preparation

As shown in Fig. 1, the IIP active sites were formed by the reaction between 4-VP and Hg II. This bifunctional ligand is capable of coordinating to the analyte by forming a stable complex and further has a polymerizable vinyl group which, through the action of the binding reagent (EDGMA) and the radical initiator (BPO), forms the polymer matrix (Shakerian et al. 2016).

For the complete template ion removal, three washing protocols were studied. First, 1.00 g of the synthesized IIP was put in contact with 20.0 mL of EDTA 0.100 mol L^{-1} for 2 h in a polypropylene tube and agitated on a horizontal stirring

table, followed by washing and filtrating with 100.0 mL of deionized water. The second method was the same as the first in terms of EDTA complexation, though the washing step was performed with 100.0 mL of HNO_3 10% v/v^{-1} . The third method was identical to the first one, except for the contact time with EDTA, changed for 72 h.

To evaluate the efficacy of template ion removal by each of the three methodologies, aliquots of 5.00 mL of the washing solutions were collected after the filtration process. The analyte concentration in these aliquots was determined by anodic stripping voltammetry.

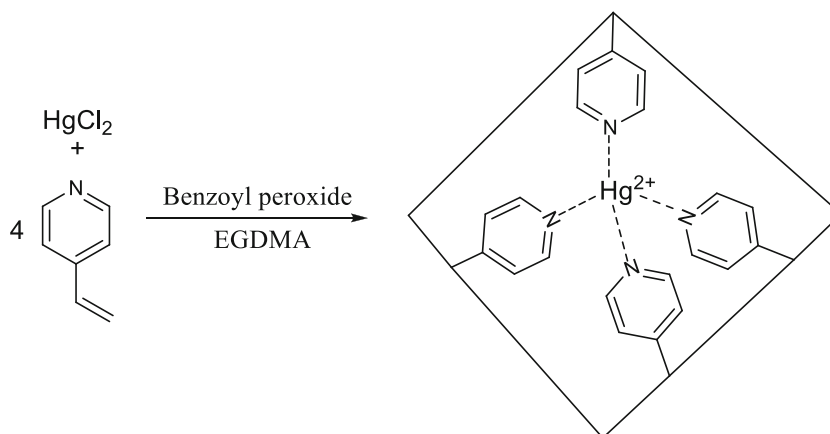
Both second and third procedures successfully corresponded to the same result of totally leaching the analyte. The one based on EDTA together with HNO_3 was chosen by considering the best throughput for the entire method.

EDTA is a chelating agent widely used to complex with metal ions. Because of the presence of acetate and amino groups on its structure, the best EDTA performance is generally in basic pH. However, the Hg II ions present the tendency to precipitate in hydroxide form; therefore, the best solution, in this case, is to use a strongly acid medium (Skoog et al. 2006). This assesses the mercury ion removal efficiency performed by the second procedure.

Hg^{+2} -IIP Characterization

The IR spectra of NIP, Hg^{+2} -IIP, and IIP (after Hg II extraction) are shown in Fig. 2. When analyzing the spectra corresponding to NIP and Hg^{+2} -IIP, it is possible to observe great similarity, as expected, since the same reagents were used in both syntheses, being distinguished only by the template ion. Around 2990 cm^{-1} region, there is a doublet in Hg^{+2} -IIP spectrum, that is assigned to quaternized nitrogen atoms belonging to 4-VP. In this same spectral region, NIP shows a single band that can be attributed to the axial deformation of the N-H bond of aromatic amines. These observations demonstrate the formation of the complex with mercury

Fig. 1 Scheme for the Hg^{+2} -IIP formation



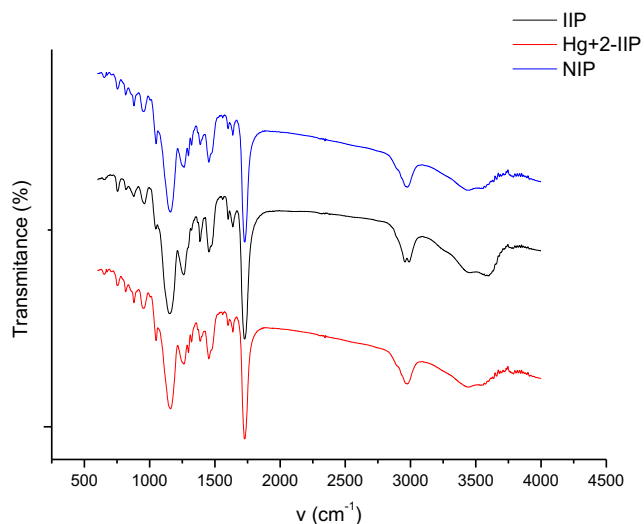


Fig. 2 IR spectra for the polymers

ion (Valle et al. 2011). When comparing the IR spectra of the washed and complexed IIP, it can be noticed that

the doublet absent for the first one, attesting that the washing process is efficient.

Atomic force microscopy (AFM) was used to analyze morphological differences between the Hg^{+2} -IIP and NIP particles. The images by AFM are shown in Fig. 3. On the one hand, the Hg^{+2} -IIP analysis (a) made it possible to observe a more uniform distribution of particles and a higher average roughness ($R_{ms} = 51,45 \text{ nm}$), which is of extreme importance in sorption studies, since it facilitates mass transfer from a liquid phase, containing the analyte, to the synthesized solid phase (Herrmann et al. 1997). It was also possible to conclude that the Hg^{+2} -IIP particles are products of smaller particles aggregation, thus conferring on them greater porosity. On the other hand, NIP (b) showed larger particles, lower average roughness ($R_{ms} = 33.43 \text{ nm}$) and a heterogeneous surface. This behavior suggests advantages of inserting the printing practice in the synthesis, to promote the formation of selective cavities on the solid material (Huang et al. 2019; Liu et al. 2018; Liu et al. 2016).

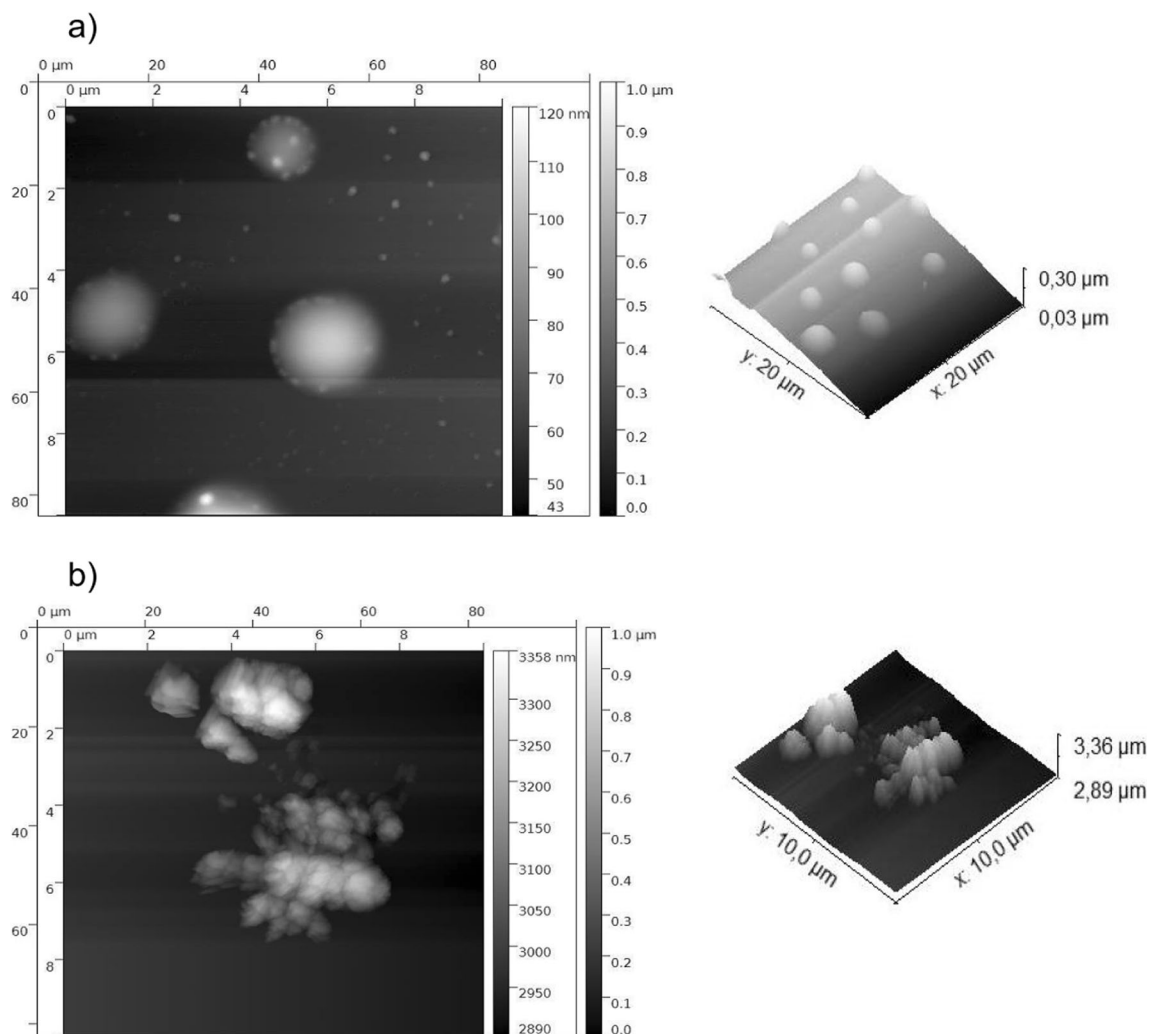


Fig. 3 Atomic force microscopy images for **a** Hg^{+2} -IIP and **b** NIP

Optimization of IIP-SPE

Study of loading pH

The effect of loading pH over the percentage of Hg II removal using IIP as sorbent phase was evaluated in the range from 3.0 to 8.0. The experiments were conducted by conditioning the cartridges with 5.00 mL of $4.00 \times 10^{-2} \text{ mol L}^{-1}$ Britton Robinson buffer at the same pH that 10.0 mL of 15.0 mg L^{-1} Hg II solution prepared in this buffer were loaded. Forty milligrams of IIP were employed as solid phase, using 5.00 mL of deionized water at the washing step and 10.0 mL of HNO_3 25% v/v^{-1} for elution.

This study was restricted to the pH range from 3.0 to 8.0, since the polymer is unstable in extremely acid environments and in very basic medium mercury ions tend to form hydroxides (Mergola et al. 2015).

It is possible to observe in Fig. 4 that the analytical signal increases with increasing pH to 6.0, after decreasing in more alkaline medium. The IIP sorption process depends both of electrostatic interactions between the species of opposite charges available in the systems and of the availability for entrapment in the cavities (Rao et al. 2004). Since 4-VP presents $\text{pK}_a = 5.2$, below pH 5.2, the nitrogen atoms present in the major portion of these molecules are protonated, that is, unavailable for extracting Hg II ions. This fact justifies the lower recovery of mercury ions when the loading medium is strongly acid (Batlokwa et al. 2012).

At pH 5.5, 4-VP pK_a region, about 50% of the total amine groups remain protonated and theoretically unavailable to form complexes with mercury ions, in agreement with the experimental result shown in Fig. 4.

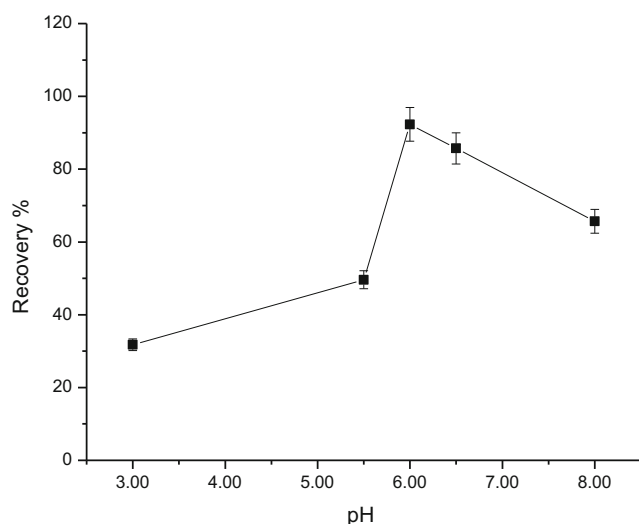


Fig. 4 Study of loading pH over the IIP-SPE method. Forty milligrams of IIP solid phase, conditioned with 5.00 mL of $4.00 \times 10^{-2} \text{ mol L}^{-1}$ Britton Robinson buffer, loaded with 10.0 mL of 15.0 mg L^{-1} Hg II solution in the same buffer, washed with 5.00 mL of deionized water, eluted with 10 mL of HNO_3 25% v/v^{-1} ($n = 3$)

As expected, it was experimentally observed that the increase of loading pH reduced the percentage of protonated 4-VP nitrogen atoms, then increasing the interaction between them and Hg II ions and consequently the recovery efficiency. At loading pH 6.0 and 6.5, the recovery percentages were between 85 and 97%.

In more alkaline conditions, however, the recovery percentages tend to lower, since mercury hydroxides are formed, decreasing the amount of free Hg II ions to be adsorbed on the polymer cavities (Batlokwa et al. 2012).

Analyzing this study, higher percentages of analyte retention were achieved at pH 6.0, so that this was the sample loading pH employed in further experiments.

Study of IIP and NIP sorption capacities

The maximum sorption capacity for IIP and NIP cartridges were calculated by building breakthrough curves, shown in Fig. 5. This study consisted in monitoring the analyte concentration after passing through the polymeric solid phase, and then plotting the relation between the sorbed analyte mass per one gram of solid material as a function of the volume of analyte solution loaded. Naturally, the effects of analyte dispersion and mass transfer until the columns achieve total saturation have to be considered (Knox et al. 2016; El-Sayed et al. 2016).

As shown in Fig. 5, the sorption capacities of both polymers initially increased when increasing the volume of loaded analyte solution (Hg II 15 mg L^{-1} buffered at pH 6.0). After loading more than 100.0 mL of the analyte solution, NIP reached exhaustion, so that its sorption capacity remained constant in $19.8 \pm 0.2 \text{ mg g}^{-1}$. IIP reached exhaustion after loading a greater volume of the same solution (more than

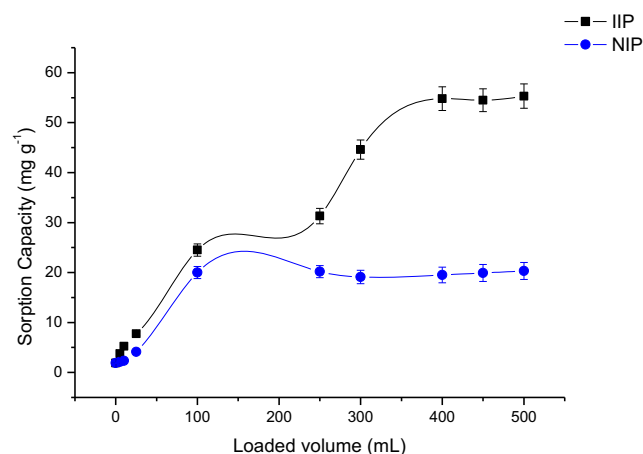


Fig. 5 Sorption capacity for IIP and NIP. Forty milligrams of each polymer, conditioned with 5.00 mL of $4.00 \times 10^{-2} \text{ mol L}^{-1}$ Britton Robinson buffer at pH 6.0, loaded with 15.0 mg L^{-1} Hg II solution in the same buffer ($n = 3$)

400 mL); thus, its sorption capacity was calculated as $54.9 \pm 0.6 \text{ mg g}^{-1}$, being greater than the NIP sorption capacity.

These differences in behavior between IIP and NIP suggest that the complexation between Hg II ions and 4-VP has played an important role in terms of sorption capacity of the analyte. The IIP morphological characteristics significantly favor this parameter, since there are more active sites available to interact with the analyte.

Study of the elution conditions

Choosing the appropriate eluent for the IIP-SPE process was considered fundamental for achieving satisfactory recovery percentages of the analyte. Thus, 10.0 mL of different solutions were evaluated: ethanol, EDTA 0.100 mol L^{-1} , the supporting electrolyte (H_2SO_4 0.270 mol L^{-1} , NaCl $0.0126 \text{ mol L}^{-1}$) and HNO_3 $10\% \text{ v/v}^{-1}$. Table 2 shows the analyte recovery obtained when each eluent was employed.

The best results were obtained in the strongly acid medium, in agreement to the observations discussed in item 3.1. Even though the supporting electrolyte is also composed by sulfuric acid, this solution presents a higher pH than the HNO_3 one, so that the recoveries are not satisfactory.

In order to improve the recovery percentages, different concentrations of HNO_3 (10, 25 and $50\% \text{ v/v}^{-1}$) were studied and these results are shown in Fig. 6. The same volume (10.0 mL) of eluent was loaded in all cases.

It was expected that increasing HNO_3 concentration the analyte recoveries would also increase, since the pH of eluent would be lower. However, in the case of $50\% \text{ v/v}^{-1}$ HNO_3 solution, this was not observed. This behavior can be attributed to the fact that the polymeric matrix is unstable in such acidic environments, and part of it may be leached or oxidized by the eluent. That decreases the analyte recoveries and interferes in the voltammetric quantification, contributing to the instability of the analytical signal, resulting in a greater standard deviation in measurements. In regard to that study, HNO_3 $25\% \text{ v/v}^{-1}$ was used as eluent in further experiments.

To evaluate the pre-concentration capacity of the IIP-SPE column, the elution step was performed by adding different volumes of HNO_3 $25\% \text{ v/v}^{-1}$. The results of this study are shown in Fig. 7.

There was no gain in terms of analyte recovery when volumes greater than 10.0 mL were used. In order to achieve a higher pre-concentration factor for the methodology, and, as a

Table 2 Effect of different solutions used as eluents for Hg II recovery ($n = 3$)

Eluent	Recovery (%)
Ethanol	0.521 ± 0.702
Supporting electrolyte	3.71 ± 0.92
HNO_3 $10\% \text{ v/v}^{-1}$	60.4 ± 4.2
EDTA 0.100 mol L^{-1}	32.3 ± 6.1

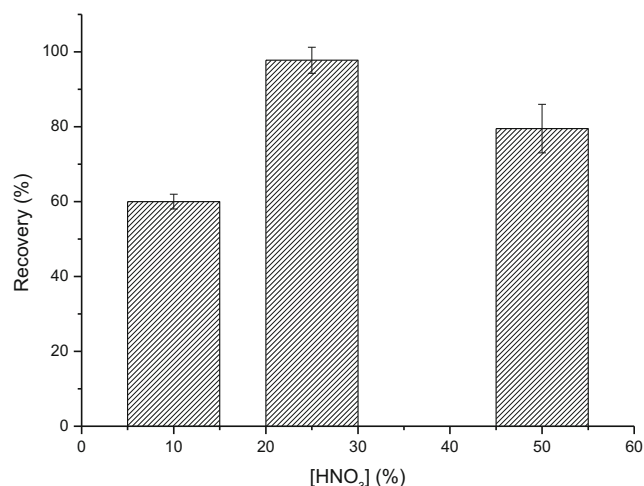


Fig. 6 Effect of HNO_3 concentration over Hg II recovery ($n = 3$)

consequence, lower limits of detection and quantification, 10.0 mL HNO_3 $25\% \text{ v/v}^{-1}$ was employed as eluent in the IIP-SPE protocol.

When analyzing samples, 20.0 mL of them were loaded in the cartridges and submitted to the optimized IIP-SPE protocol, assigning to the method a pre-concentration factor of two times.

Validation of methodology

After optimizing the IIP-SPE steps, in order to ensure the reliability of the method, several parameters were evaluated as follows.

Linear range, typical analytical curve, and coefficient of determination

The typical analytical curve for the voltammetric method was $Y = 1.969 \times 10^{-9} X + 8788 \times 10^{-9}$, in the linear range from 0.400 to $25.3 \mu\text{g L}^{-1}$. The coefficient of determination was 0.9977 .

Repeatability

The repeatability of the method was evaluated by submitting ten different Hg II 1.00 mg L^{-1} standard solutions to the optimized IIP-SPE protocol and determining the final concentration by differential pulse anodic stripping voltammetry. The coefficient of variation of these measurements was 4.0% , value employed to represent the repeatability of the proposed method.

Limits of detection and quantification (LOD and LOQ)

The limits of detection and quantification for the proposed method were calculated based on $\frac{3\delta}{S}$ and $\frac{10\delta}{S}$ criterion,

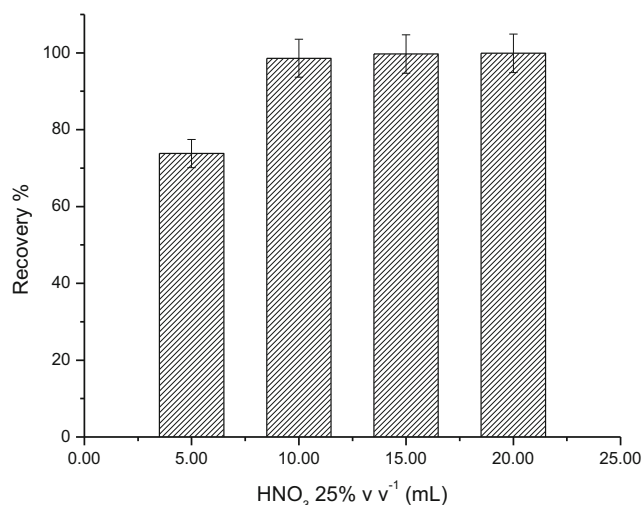


Fig. 7 Effect of volume of HNO₃ 25% v/v⁻¹ over Hg II recovery (n = 3)

respectively. Instrumental LOD was 0.644 μg L⁻¹ and LOQ 2.15 μg L⁻¹. These parameters were recalculated for the proposed method by dividing these values by the pre concentration factor obtained after the IIP-SPE optimization. Then, the LOD and LOQ for the method were equal to 0.322 μg L⁻¹ and 1.08 μg L⁻¹, respectively.

It is emphasized that these parameters for the proposed IIP-SPE voltammetric method were better than some others shown in Table 1, particularly ones that involve the synthesis of Hg⁺²-IIP. As expected, AFS, ICP-OES, and ICP-MS methods generate lower LOD and LOQ, but this work proposes a more affordable alternative.

Accuracy

Application to laboratorial sample To test the accuracy of the method three aliquots of Hg II 1.00 mg L⁻¹ standard solution were submitted to the proposed IIP-SPE protocol, assessing the accuracy of 98.6 ± 1.5%.

Application to environmental samples The IIP-SPE methodology was applied to three different environmental water samples, Piratininga and Itaipu Lagoons, located in Niterói-RJ/Brazil, and Vargem River, located in Itaboraí, RJ/Brazil. These waters were sampled superficially and filtered as mentioned in item 2.5.

Table 3 Quantifications of Hg II in lagoon and river water samples by the proposed and FAAS methods, both after IIP-SPE treatment. Results expressed as mean ± standard deviation (n = 3)

Method	Vargem River (mg L ⁻¹)	Piratininga Lagoon	Itaipu Lagoon
Voltammetric	1.35 ± 0.07	Below LOQ	Below LOQ
FAAS	1.54 ± 0.41	Below LOQ	Below LOQ

Initially, the three samples were directly analyzed by the voltammetric method, without employing the proposed IIP-SPE pretreatment, in order to investigate whether this procedure would be fundamental to the quantification or not. By doing so, no characteristic peak of mercury ions was obtained, even when spiking these samples with Hg II 12.5 μg L⁻¹. That observation made it possible to affirm that other matrix components were interfering in the voltammetric analysis.

When the IIP-SPE procedure was applied to the lagoon samples also no analytical signal was observed, but when spiking these samples with Hg II 12.5 μg L⁻¹ it was. In the case of spiking, a recovery of 90.0 ± 1.3% was achieved. These results show that the Hg II concentration in the lagoon samples is below the LOQ of the proposed methodology.

In opposition to the lagoon water samples, the Hg II concentration in Vargem River was high and determined 1.35 ± 0.07 mg L⁻¹ after IIP-SPE, resulting in a recovery of 96.2 ± 4.0% after spiking with Hg II 0.250 mg L⁻¹.

The accuracy of the method was also evaluated by comparing these results to a flame atomic absorption spectrometry (FAAS) reference method (Table 3). The IIP-SPE pretreatment was also necessary to accomplish the FAAS quantification, as other matrix components interfered in the analysis by strongly increasing the background signal.

By the application of Student’s t test at 95% confidence level to the results of the quantifications in Vargem River, it was possible to affirm that there was no statistical difference between the results obtained by the two techniques.

Study of possible interferents

In order to study the influence of other cations over the Hg II analytical signal for the proposed methodology, a laboratorial sample was simulated by considering the limits permitted by the Brazilian legislation CONAMA/357, which deals with river and lagoon waters quality (CONAMA, Conselho Nacional do Meio Ambiente 2005).

Thus, an aqueous solution containing Hg II 2.00 μg L⁻¹, Cd II 10.0 μg L⁻¹, Pb II 100 μg L⁻¹, and Cu II 90.0 μg L⁻¹ was submitted to the IIP-SPE method followed by differential pulse anodic stripping voltammetry quantification. It was possible to assess that in these conditions the studied cations did not influence the Hg II analytical signal, as the concentration of the analyte was determined 2.36 ± 0.42 μg L⁻¹.

Conclusions

Both imprinted and non-imprinted polymers were successfully synthesized by the methodology here proposed. These two polymers were differentiated in terms of sorption capacity, as IIP presented a higher capacity (31.0 mg g⁻¹) than NIP (20.0 mg g⁻¹). This differentiation was corroborated by

physical characterizations, by means of infrared spectrometry and atomic force microscopy.

After the optimum condition for IIP-SPE was settled, the imprinted polymer presented sufficient selectivity to Hg II against other cations (Cd II, Pb II, and Cu II), considering the expected concentration of those in river and lagoon waters according to the Brazilian legislation.

The extraction procedure using the ion-imprinted polymer as the solid phase was essential to perform mercury II quantification in the evaluated water samples, as it was responsible for eliminating the influence of other matrix components that caused interferences. The accuracy of the proposed methodology was $96.2 \pm 4.0\%$ calculated by means of recovery tests using river water samples. Also, the results of Hg II quantification in these samples using the IIP-SPE procedure and differential pulse anodic stripping voltammetry were compared to those obtained by using flame atomic absorption spectrometry for the analyte detection. Comparing these results by Student's *t* test at 95% confidence level, no statistical difference was observed.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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