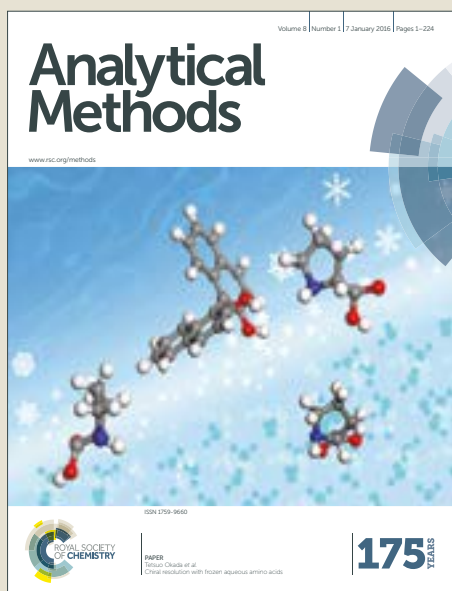


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3 1 **An easily prepared graphene oxide-ionic liquid hybrid nanomaterial**
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5 2 **for micro-solid phase extraction and preconcentration of Hg in water**
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8 3 **samples**
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12 5 **Annaly Cruz Sotolongo, Estefanía M. Martinis and Rodolfo G. Wuilloud***
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22 (R.G. Wuilloud)

Abstract

A preconcentration method based on the use of graphene oxide (GO) functionalized with an ionic liquid (IL) was developed for trace Hg determination in water samples. The IL-GO hybrid nanomaterial was prepared by a simple procedure to functionalize GO with the IL 1-butyl-3-dodecylimidazolium bromide ([C₄C₁₂im]Br) and its performance as a sorption material for Hg was evaluated. A microcolumn filled with the IL-GO nanomaterial was used for preconcentration and determination of Hg followed by electrothermal atomic absorption spectrometry (ETAAS) detection. Mercury was retained at pH 5.0 and 20% (v/v) HNO₃ was used for the elution of Hg from the microcolumn. The effects of different variables, including sample volume, extraction time, sample flow rate, type and concentration of eluent and eluent flow rate were carefully studied. High retention efficiency (100%) was achieved with the proposed IL-GO sorption nanomaterial without the need of additional chelating reagents or derivatization reagents, which is an important advantage compared with traditional preconcentration methods. A sensitivity enhancement factor of 100 and a low detection limit of 14 ng L⁻¹ were obtained under optimal experimental conditions. The proposed method can be considered as a simple, cost-effective and efficient alternative for Hg determination in water samples like river, rain, mineral and tap water.

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Keywords: *graphene oxide; 1-butyl-3-dodecylimidazolium bromide; mercury; solid*

phase microextraction; electrothermal atomic absorption spectrometry

47

1 Introduction

Mercury is one of the most well-known toxic elements and the World Health Organization (WHO) places it among the first ten chemicals that are of major public health concern. Although regulations and efforts are exercised at various levels, Hg pollution from both natural and anthropogenic sources have remained a major safety problem.¹ Thus, water contamination of Hg could be a problem worldwide and its control must be assured by appropriate analytical methods.²

Several detection techniques have been used for the determination of Hg including, electrothermal atomic absorption spectrometry (ETAAS),³ atomic fluorescence spectrometry (AFS)⁴ or inductively coupled plasma mass spectrometry (ICP-MS).⁵ ETAAS shows several advantages including, relative simplicity, reduced cost, and low sample volume requirements. However, since Hg concentrations are extremely low (ng L^{-1} levels) in natural waters, a preconcentration procedure must be applied. Therefore, to exploit the potential of ETAAS technique, this has to be combined with preconcentration systems involving efficient sorption materials.^{6, 7} In this way, the coupling of a microcolumn filled with a high performance sorption material to ETAA yields very low detection limits and allow trace Hg determination.

Graphene is a nanomaterial formed by layers having a thickness of a few atoms and have attracted great attention due to its applications in various fields: sensors, electronics, sorption material for remediation and analytical chemistry.⁸ Due to their unique physicochemical properties, this nanomaterial hold a great promise for its applications in chemical analysis.⁹ Graphene has a large specific surface area ($2630 \text{ m}^2 \text{ g}^{-1}$),¹⁰ suggesting a high sorption capacity. Furthermore, it can be easily modified to add different functional groups to its surface, e.g. by oxidation to obtain graphene oxide (GO) containing different groups (C-O, COO).⁹ The Hummer method have been

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3 74 generally employed to prepare GO due to its low cost and large scale production for
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5 75 analytical use.¹¹ However, the strength of interaction due to Van der Waals forces may
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7 76 inevitably result in an agglomeration of GO sheets, which compromises the overall
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9 77 yield of this carbon material.¹² Therefore, several types of functionalizations have been
10
11 78 used to obtain GO with greater dispersibility.^{8, 13} The functionalization approaches have
12
13 79 been classified as covalent functionalization, noncovalent functionalization,
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15 80 substitutional doping, and hybridization with nanoparticles, nanowires, and other
16
17 81 materials, considering the method and materials used.⁸ Recently, Ionic Liquids (ILs)
18
19 82 have been proposed to improve the analytical properties of GO, mainly due to the
20
21 83 modification of its surface properties and the increase of active sites.¹⁴ ILs are a class of
22
23 84 organic melt salts at room temperature which completely comprise cations and anions.
24
25 85 Due to their high conductivity, the wide potential window, the innate liquid state, low
26
27 86 melting points and negligible vapor pressures, ILs has been applied for GO
28
29 87 functionalization.¹⁵ Thus, the novel IL-GO nanomaterials are expected to possess the
30
31 88 advantages of the individual components, resulting in new, advanced adsorbents with
32
33 89 tunable extraction capabilities and improved performance.¹⁶ Moreover, implementation
34
35 90 of these hybrid nanomaterials in novel microextraction techniques can result into
36
37 91 efficient analytical methods for the determination of toxic trace elements such as Hg. In
38
39 92 this sense, micro-solid phase extraction (μ -SPE) is a widely used technique for
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41 93 environmental sample pretreatment due to its high recovery, high enrichment factor,
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43 94 low consumption of solvents and ease of automation.¹⁷ The sorption material
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45 95 determines the selectivity and sensitivity of the method and GO have demonstrated to
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47 96 be an effective sorbent for metal preconcentration.^{18, 19} Furthermore, on-line μ -SPE
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49 97 preconcentration systems are advantageous for metal determination because of their
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51 98 flexibility, simplicity, high sample throughput and versatility.²⁰ In this work, a novel
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99 preconcentration method based on the μ -SPE technique, using an IL-GO hybrid
100 nanomaterial as sorbent and ETAAS were combined for trace Hg determination. A
101 microcolumn filled with the new sorption material was used for preconcentration of the
102 analyte. The main factors influencing μ -SPE and the determination of Hg such as size
103 and shape of the microcolumn, sorption material load, sample pH, sample flow rate and
104 volume, type and concentration of eluent were examined in detail. The optimum
105 conditions were established and the determination of Hg in different types of water was
106 successfully performed reaching a high preconcentration factor and a low LOD.

107

108 **2 Material and methods**

109 **2.1 Instrumentation**

110 Measurements were performed with a PerkinElmer (Überlingen, Germany)
111 Model 5100 ZL atomic absorption spectrometer equipped with a transversely heated
112 graphite atomizer and a Zeeman-effect background correction system. A Hg hollow
113 cathode lamp (PerkinElmer) operated at a current of 170 mA and a wavelength of 228.8
114 nm with a spectral band width of 0.7 nm was used. All measurements were made based
115 on absorbance signals with an integration time of 5 s. The temperature vs. time program
116 for the atomizer is fully depicted in Table 1. A Horiba F-51 pH meter (Kyoto, Japan)
117 was used for pH determinations. A schematic of the microextraction system is shown in
118 Fig. 1, which consisted of: two Gilson (Villiers Le-Bell, France) Minipuls 3 peristaltic
119 pumps, two six-port two-position injection valves (Oak Harbor, WA, USA) and a home-
120 made microcolumn (8 mm length and 2 mm i.d.) filled with the retention material (an
121 homogenized mixture of 6 mg of IL-GO and 54 mg of milled glass). At the two ends of
122 the microcolumn, cotton plugs were used to avoid the loss or mixing of the sorption
123 material. Tygon-type pump tubes (Gilson) were employed for the delivery of all

124 aqueous streams. Solvent-resistant pump tubes (Gilson) were used for HNO₃ solutions.
125 The characterization of the hybrid material was performed by a Nicolete Protégé 460
126 Fourier transform infrared spectrometry (FT-IR) (Madison, WI, USA) and a Carl Zeiss
127 EM900 transmission electron microscope (TEM) (Jena, Germany).

128

129 2.2 Reagents

130 A commercially available 1000 mg L⁻¹ Hg²⁺ standard solution (Merck,
131 Darmstadt, Germany) was used. Lower concentrations were prepared by diluting the
132 standard solution with 0.1 mol L⁻¹ HNO₃. Stock methylmercury (MeHg), ethylmercury
133 (EtHg) and phenylmercury (PhHg) solutions (1000 mg L⁻¹) were prepared from
134 methylmercury chloride, ethylmercury chloride and phenylmercury chloride (Merck),
135 respectively, in methanol (Merck). Working standard solutions were prepared daily. The
136 organomercurial solutions were stored away from light at 4 °C to prevent
137 decomposition. Acetic acid–acetate buffer solution was prepared from a 2 mol L⁻¹ acetic
138 acid (Sigma-Aldrich, Milwaukee, WI, USA) solution adjusted to pH 5 with sodium
139 hydroxide (Merck). Ultrapure water (18 MΩ cm) was obtained from an Osmoion-U-0.5
140 ultrapure water equipment (APEMA, Buenos Aires, Argentina). For the synthesis of IL,
141 GO and IL-GO the following reagents were used: 1-butylimidazole (99%) (Fluka
142 Buchs, Switzerland), 1-bromododecane (97%) (Sigma-Aldrich), ethyl acetate (99.8%)
143 (Sigma-Aldrich), graphite powder (99.99%) (Sigma-Aldrich), H₂SO₄ (95-97%)
144 (Merck), KMnO₄ (99%) (Sigma-Aldrich), H₂O₂ (30%) (Sigma-Aldrich), HCl (37%)
145 (Merck), NaOH (98%) (Sigma-Aldrich), KOH (98%) (Sigma-Aldrich) and CH₂Cl₂
146 (99%) (Sigma-Aldrich), thiourea (99%) (Sigma-Aldrich) and EDTA (99%) (Sigma-
147 Aldrich) solutions were tested as eluents.

148

149 **2.3 Synthesis of 1-butyl-3-dodecylimidazolium bromide**

150 The synthesis of the IL [C₄C₁₂im]Br was performed as reported by Baltazar et al.
151 with a few modifications.²¹ Briefly, a 0.10 mol 1-butylimidazole (6.5 mL) solution was
152 mixed with 0.11 mol 1-bromododecane (13.2 mL) solution to obtain a homogeneous
153 mixture. The mixture was heated and refluxed (70 °C) under nitrogen for 48 h, with
154 constant stirring. The product was dissolved in 25 mL of ultrapure water and washed
155 with six aliquots of ethyl acetate (25 mL) to purify the product. Finally, water was
156 evaporated under vacuum at 80 °C and the IL was characterized by FT-IR technique.

158 **2.4 Synthesis of graphene oxide**

159 The GO was synthesized following a modified version of the Hummer's
160 method.^{11, 22} Briefly, commercial graphite powder (1.0 g) was placed into cold (10 °C)
161 concentrated H₂SO₄ (50 mL) and KMnO₄ (4.0 g) was added gradually under stirring.
162 The reaction was continued for 2 h at 10 °C. Afterwards, the mixture was stirred at 35
163 °C for 1 h. The solution was allowed to cool for 15 min and 100 mL of ultrapure water
164 were added gently in an ice bath. A color change from black to intense orange was
165 observed. Then, the mixture was stirred for 1 h water. After that, the mixture was
166 diluted to 300 mL with ultrapure water and 10 mL of 30% (v/v) H₂O₂ were added to
167 reduce the excess of KMnO₄ and it was allowed to stand for 19 h. The phase separation
168 was observed and the orange solid was settled on the bottom. The supernatant was
169 manually removed with a transfer pipette. In order to wash the product, 400 mL of 5%
170 (v/v) HCl were added to remove metal ions. The solid was finally washed with ultrapure
171 water until a pH of 5 was obtained. The supernatant was removed and GO was dried in
172 a stove at 60 °C for 5 h. The GO was characterized by FT-IR and TEM techniques.

173

174 **2.5 Functionalization of graphene oxide with the ionic liquid**

175 The functionalization of GO with the IL was performed following a procedure
176 described by Huang et al. with slight modifications.²³ An amount of 100 mg of GO was
177 diluted in 30 mL of ultrapure water. Then, 0.4 mL of 8 mol L⁻¹ KOH were added to
178 adjust the pH to 9. The solution was stirred for 5 h at room temperature. Subsequently,
179 the solution was mixed with 800 mg of [C₄C₁₂im]Br in 60 mL of chloroform followed
180 by stirring for 48 h. The organic phase was separated by centrifugation and washed with
181 CH₂Cl₂ and the functionalized nanomaterial was allowed to dry at room temperature.
182 Finally, the IL-GO was characterized by FT-IR and TEM techniques.

183

184 **2.6 Sample collection and conditioning**

185 Different water samples including tap water, river water, bottled mineral water
186 and rainwater collected in Mendoza province (Argentina) were analyzed in this work.
187 For the collection of tap water samples, domestic water was allowed to run for 20 min
188 and approximately a volume of 1000 mL was collected in a beaker. Mineral water was
189 purchased from local supermarkets. River water samples were collected in cleaned
190 bottles rinsed three times with water sample prior to sample collection. A sample
191 volume of 2000 mL was collected at a depth of 5 cm of the surface. Rainwater samples
192 were collected during a rain episode occurred in Mendoza city (Argentina) in acid-
193 cleaned bottles that were rinsed three times with the sample prior to collection. The
194 water samples were irradiated for 3 h with a 150 W UV lamp in order to photo-oxidize
195 organo-Hg compounds that could be present in water.²⁴ All the material used in this
196 work was previously washed with a 5% (v/v) HNO₃ solution followed by ultrapure
197 water before drying in a clean air hood.

198

199 2.7 General μ -SPE procedure for Hg preconcentration and determination

200 A schematic diagram of the preconcentration system is shown in Fig. 1. The
201 objectives of each step are summarized in Table 2. Before sample loading, the column
202 was conditioned with 20% (v/v) HNO₃ and then with an acetic acid–acetate buffer
203 solution to maintain the system at pH 5, driven by the peristaltic pump P₁ a flow rate of
204 2.5 mL min⁻¹ (3 minutes). After this procedure, 5 mL of sample (S) was propelled (Step
205 I) by pump P₁ at a flow rate of 0.3 mL min⁻¹. Simultaneously, pump P₂ supplied 20%
206 (v/v) HNO₃ to the loop (L) of valve V₂, until it was filled. Valve V₁ and V₂ were
207 switched to the loading position during these operations (Table 2). The pump P₂ was
208 then switched off.

209 In Step II, valves V₁ and V₂ were set at the injection position and Hg retained in
210 the microcolumn was eluted with 50 μ L of 20% (v/v) HNO₃. The eluent was supplied at
211 a flow rate of 0.3 mL min⁻¹. Subsequently, the eluate was diluted to 100 μ L with
212 ultrapure water. The concentration of Hg was determined by ETAAS, injecting 40 μ L of
213 the diluted eluate into the graphite furnace. Instrumental conditions for ETAAS
214 determination are mentioned in Table 1.

215 Calibration was performed with aqueous standards subjected to the same
216 preconcentration procedure. Blank solutions were analyzed in the same way as standard
217 and sample solutions.

218

219 3 Results and discussion

220 3.1 Morphology and structural characteristics of IL, GO and IL-GO

221 FT-IR technique was employed for the characterization of IL, GO and IL-GO.
222 Spectra were measured in the range from 4000 to 400 cm⁻¹ with a resolution of 4 cm⁻¹.
223 FT-IR spectra of IL-GO (red), GO (blue) and IL (light blue) are shown in Fig. 2. To

224 obtain the FT-IR spectra of GO, the KBr pellet method was used with a mass proportion
225 of 3% of the sample homogeneously dispersed in KBr. The GO spectrum was similar to
226 others reported in the literature.²⁵ This showed a significant shift of the stretching
227 vibration of the hydroxyl groups (O-H, C=O, C=C, C-OH, C-O), attributed to the
228 presence of these groups on the surface, with lower degree of association. A drop of
229 [C₄C₁₂im]Br was deposited on the KBr pellet to obtain the IL spectra. It exhibited a
230 typical strong peak centered at approximately 1494 cm⁻¹ corresponding to the draw
231 frequency of the functional group -C=N, while both peaks at 1168 and 1540 cm⁻¹ were
232 attributed to the stretching vibration of the imidazole ring.

233 The spectrum of IL-GO hybrid nanomaterial was also obtained by the KBr pellet
234 technique. From the comparison of IL and IL-GO spectra, a very coincident band
235 pattern was observed. The asymmetric ring stretching in the resulting plane of the
236 imidazolium ring resulted in a peak at 1164 cm⁻¹, which indicated that functionalization
237 of GO with the IL was successful. On the other hand, the characteristic band of GO for
238 C=O (1735-1720 cm⁻¹) bond was not observed in the spectrum of IL-GO so the
239 formation of a carboxylate (1595 cm⁻¹) was suggested. The characteristic bands
240 observed were: aromatic C=C (1615 cm⁻¹), epoxy C-OH (1225 cm⁻¹) and alkoxy C-O
241 (1050 cm⁻¹). The oxygen-containing functional groups and the imidazole were
242 confirmed in the FT-IR spectrum of the IL-GO hybrid nanomaterial. Moreover,
243 functionalization of GO with this type of IL could proceed thanks to ionic interactions,
244 electrostatic and Van der Waals forces, which are the most common interactions
245 occurring on the surface of GO with charged functional groups. Moreover, IL molecules
246 could be adsorbed on GO by interaction with carboxyl groups occurring on the surface
247 of this nanomaterial.²³

248 For TEM characterization, GO and IL-GO were dispersed by sonication into

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3 249 water at a concentration of 0.1 mg mL⁻¹. A drop of the dispersions were placed directly
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5 250 on two copper grids, dried at room temperature and TEM images were obtained
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7 251 working with the microscope at 80 kV, with a high-resolution Gatan SC1000-832 CCD
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9 252 camera. The Fig. 3a shows that GO formed sheets that were mostly dispersed in a single
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11 253 layer and were smooth and transparent. The TEM image of IL-GO (Fig. 3b) shows that
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13 254 functionalization of GO provided a layer of IL on the surface material. Although this
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15 255 process partially flattened the wrinkles on the GO sheets, the bound IL produced a
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17 256 corrugated surface. Therefore, it was proposed that the IL-GO hybrid nanomaterial
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19 257 could have excellent sorption performance due to the increase of the effective surface
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21 258 area and active sites.
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260 **3.2 Characteristics of the microcolumn and the filling material**

261 The preparation and dimensions of the microcolumn are key factors due to the
262 tendency of graphene-related materials to aggregate, which negatively affects the
263 hydrodynamic characteristics of the packed column, sample flow rate and ultimately,
264 analysis frequency.²⁶ For this reason, the preparation of the microcolumn was studied in
265 detail. A careful evaluation of the optimum dimensions of the microcolumn and special
266 characteristics of the filling material were performed. Thus, different materials with an
267 average particle size of 710 nm like feldspar, quartz and milled glass were tested to
268 avoid the compaction of IL-GO nanomaterial and allow solutions to flow through the
269 microcolumn. According to the results, milled glass was the best material because it
270 avoided the aggregation of the sorbent inside the microcolumn, preventing high back-
271 pressures in the system. Additionally, it has excellent chemical compatibility with the
272 mineral acids used in this work for the elution of Hg. An optimal weight proportion
273 consisting of ~90% of milled glass and ~10% of IL-GO hybrid nanomaterial was

274 applied. This proportion was suitable to prevent the compaction of IL-GO nanomaterial
275 inside the column.

276 Furthermore, the effects of microcolumn dimensions on the retention and elution
277 of the analyte were studied. The length of the microcolumn was assayed in the range of
278 5 - 15 mm, while the internal diameter (i.d.) in the range of 2 - 5 mm. The highest Hg
279 retention and efficient elution were achieved when the sorption material was packed in a
280 microcolumn of 2 mm i.d. and 8 mm length.

282 3.3 Evaluation of ETAAS conditions for Hg determination

283 A careful study of the conditions for ETAAS determination was necessary due
284 to the high volatility of Hg. Chemical modifiers were necessary to avoid deterioration of
285 Hg sensitivity by losses of the target metal during drying and pyrolysis steps. Different
286 amounts of $\text{Mg}(\text{NO}_3)_2$ and $\text{Pd}(\text{NO}_3)_2$ and their mixtures were assayed as chemical
287 modifiers. The stabilization of Hg in the atomizer by Pd (20 μg) was the most effective
288 approach as noble metals form stable amalgams with Hg.²⁷

289 The effect of pyrolysis temperature was studied in the range of 150 - 330 °C
290 (Fig. 1S in Supplementary Information). In this case, Hg was measured up to 250 °C
291 without significant losses and reduced background absorption during atomization.
292 Therefore, 250 °C was chosen as the pyrolysis temperature. Likewise, the effect of
293 atomization temperature on Hg absorption signal was studied within the range of 800 -
294 1500 °C (Fig. 2S in Supplementary Information). The highest absorbance signal was
295 achieved at 1300 °C. The ramp and hold times for pyrolysis step were studied, 10 and
296 20 seconds was chosen respectively. Final conditions for ETAAS detection are shown
297 in Table 1.

298

3.4 Optimization of the preconcentration procedure

Several parameters affecting Hg preconcentration including pH, sample volume, extraction time, sample flow rate, eluent type and concentration and eluent flow rate were carefully studied and optimized. The study of the preconcentration variables was performed by the univariate method. All parameters mentioned above were assayed with Hg aqueous standards prepared at known concentrations of the element.

3.4.1 Influence of pH on Hg retention

In order to evaluate the effect of pH on the process, a series of standard solutions at $0.5 \mu\text{g L}^{-1}$ Hg concentration were adjusted at different pH values (1.0 - 11.0) and the proposed preconcentration method was applied. The pH was adjusted by dropwise addition of 1 mol L^{-1} HCl or NaOH solutions. The main mechanism of Hg retention on the IL-GO hybrid material might proceed by electrostatic interactions between metal ions and oxygen functional groups ($-\text{COO}^-$ and $-\text{O}-$). Therefore, Hg retention could be mainly determined by the nature and the concentration of these groups on the surface of the IL-GO hybrid nanomaterial.²⁸ Likewise, a cation exchange mechanism between Hg^{+2} and the imidazolium cation of the IL could be considered. Previous works have proposed that the transfer rate of Hg^{+2} into the IL phase increases with the length of the alkyl chain of the imidazolium ring, meaning that when longer alkyl chains are present in the imidazolium ring, the extraction of Hg could be more efficient. Thus, the extraction of Hg was increased 20% compared to the extraction obtained with GO under the same experimental conditions.²⁹

The retention of Hg on the IL-GO nanomaterial was evaluated at different pHs (Fig. 3S in Supplementary Information). A constant 100% retention efficiency was obtained for Hg when the pH was increased up to 7 and after this value the retention

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3 324 started to diminish (60% for pH 11). The decrease in the retention of Hg can be
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5 325 attributed to the hydrolysis of the metal. According to these experiments, pH 5 was
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7 326 selected for further experiments because of the convenience of sample preparation using
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9 327 the acetic acid–acetate buffer solution. In order to avoid the use of additional reagents,
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11 328 the pH was adjusted using an acetic-acetate buffer solution.

329 **3.4.2 Type of eluent and concentration**

330 The desorption of Hg from the microcolumn was studied using different acids
331 solutions since decreasing the pH could lead to removal of Hg from the hybrid
332 nanomaterial into the aqueous phase. Moreover, a possible mechanism to explain the
333 elution of Hg from the sorption material could be attributed to a cation exchange
334 mechanism between Hg^{2+} and H^+ .²⁶ In order to determine the type and volume of eluent
335 required for Hg removal from the microcolumn, 500 μL of different inorganic acids
336 (HCl and HNO_3), EDTA and a mixture of thiourea and HCl were evaluated (Fig. 4). As
337 a result, a 100% elution of Hg from the microcolumn was observed with 20% (v/v)
338 HNO_3 . Thus, HNO_3 was selected as eluent to promote the desorption of Hg from the IL-
339 GO nanomaterial. Furthermore, the effect of the volume of eluent on Hg removal was
340 investigated (25-1000 μL). It could be determined that Hg was quantitatively eluted
341 from the microcolumn with only 50 μL of 20% (v/v) HNO_3 . In addition, the best results
342 were obtained when the elution of the analyte was developed in countercurrent mode
343 through the microcolumn, which contributed to obtain a more efficient elution by
344 minimizing the dispersion of the analyte.³⁰

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346 **3.4.3 Influence of sample volume and flow rate**

347 The sample volume is an important variable since it affects frequency of
348 analysis, enhancement factor and consumption index of preconcentration methods.

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3 349 Therefore, the effect of sample volume on analyte retention was evaluated in a range of
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5 350 1 – 10 mL. The results showed that a sample volume of 5 mL was optimum for analysis.
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7 351 When higher volumes were applied, the retention of the analyte in the microcolumn
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9 352 decreased significantly. Therefore, a volume of 5 mL of sample was selected (Fig. 5a).

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11 353 Also, sample flow rate through the microcolumn was crucial to be optimized in
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13 354 the preconcentration system as sensitivity of the method and enhancement factor were
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15 355 directly related to this variable. Sample flow rate influences the contact time between
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17 356 the analyte and the sorption material, consequently the dynamic sorption capacity of the
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19 357 microcolumn could be affected too. The effect of sample loading flow rate on Hg
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21 358 retention was studied in the range of 0.3 – 7 mL min⁻¹, while the elution flow rate was
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23 359 kept constant at 7 mL min⁻¹ and the sample volume was 5 mL. The experimental results
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25 360 showed that the analytical signal decreased upon an increase of the sample flow rate,
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27 361 with a maximum at 0.3 mL min⁻¹ (Fig. 5b). The almost linear decrease in absorbance
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29 362 signal within the tested range revealed the analyte followed a slow, but effective, mass
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31 363 transfer process from the IL-GO nanomaterial into the eluent, which led to quantitative
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33 364 removal from the microcolumn.

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35 365 The influence of the elution flow rate on the removal of Hg was also examined
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37 366 within the range of 0.3 – 7 ml min⁻¹. Mercury was less recovered upon increasing the
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39 367 elution flow rate (Fig. 5c). Therefore, a flow rate of 0.3 ml min⁻¹ was chosen for sample
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41 368 loading and elution of Hg from the microcolumn.

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46 370 **3.5 Microcolumn re-utilization and sorption capacity**

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48 371 Stability and regeneration of the IL-GO sorption material were also investigated.
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50 372 It was observed that the micro-column could be re-used after regeneration with 5 mL of
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52 373 20% (v/v) HNO₃, followed by a washing with ultrapure water. The microcolumn was
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3 374 stable without deterioration of Hg retention and capacity. This study comprised at least
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5 375 100 preconcentration-elution cycles.

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7 376 In addition, it was evaluated the dynamic sorption capacity of the microcolumn,
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9 377 which is defined as the maximum amount of analyte retained when the active sites of
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11 378 the IL-GO nanomaterial were saturated. In that experiment, a 0.1 mg L⁻¹ Hg solution
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13 379 was loaded into the microcolumn at 0.3 mL min⁻¹. Simultaneously, aliquots of 500 µL
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15 380 of effluent were collected every 2 min in centrifuge tubes and Hg was determined in
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17 381 each aliquot. Mercury was efficiently retained in the microcolumn up to 20 mL of the
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19 382 0.1 mg L⁻¹ Hg solution was loaded. Therefore, the calculated dynamic sorption capacity
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21 383 of the IL-GO nanomaterial was 0.033 mg of Hg per g of IL-GO nanomaterial.
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25 385 **3.6 Interference study**

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27 386 The ionic strength effect on Hg retention was evaluated within a range of 0 – 1
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29 387 mol L⁻¹ NaNO₃. It was observed that the ionic strength did not influence the
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31 388 performance of the preconcentration system within the studied range. Likewise, the
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33 389 effect of concomitant ions regularly found in natural water samples was evaluated. This
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35 390 experiment was performed by analyzing 3 mL of 0.5 mg L⁻¹ Hg solution containing
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37 391 concomitant ions at different concentrations. A concomitant ion was considered to
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39 392 interfere if it resulted in an analytical signal variation of ±5%. It was determined that
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41 393 Cu²⁺, Zn²⁺, Ni²⁺, Co²⁺, Mn²⁺ and Fe³⁺ could be tolerated up to at least 100 mg L⁻¹.
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43 394 Analytical signal of the blank was not modified in the presence of the concomitant ions
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45 395 assayed.
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50 396 Furthermore, the retention of organic Hg species on the IL-GO hybrid
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52 397 nanomaterial was evaluated and different yields were obtained: MeHg (57%), PhHg
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54 398 (89%) and EtHg (83%). Therefore, since the IL-GO nanomaterial was not selective to
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399 Hg species, and their retention was different depending on each species, a
400 photooxidation process was applied to ensure that all organic species were transformed
401 into inorganic Hg species before preconcentration.

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403 **3.7 Analytical performance and determination of Hg in real samples**

404 Several parameters characterizing the performance of the preconcentration
405 method were studied. A 100% retention efficiency was achieved for Hg under the
406 optimal experimental conditions (Table 1). The relative standard deviation (RSD)
407 resulting from the analysis of 10 replicates of 5 mL of a solution at 0.5 $\mu\text{g Hg L}^{-1}$ was
408 3.9%. Also, the sensitivity enhancement factor (EF) was obtained from the ratio of the
409 calibration curve slopes for Hg with and without application of the preconcentration
410 method. Calibration curve without preconcentration was obtained by direct injection of
411 20 μL of Hg standard solutions into ETAAS at different concentrations. The regression
412 equation obtained for the calibration curve obtained without preconcentration was $A =$
413 $0.00185C + 0.09$, where A is the absorbance and C is the concentration of Hg in $\mu\text{g L}^{-1}$.
414 Also, the calibration curve obtained after preconcentration was linear with a correlation
415 coefficient of 0.9987 from levels near the detection limits and up to at least 8 $\mu\text{g L}^{-1}$.
416 The regression equation was $A = 0.185C + 0.0011$. Thus, an EF = 100 was achieved in
417 this work when the two values of analytical sensitivity are compared. The limit of
418 detection (LOD) obtained after preconcentration was calculated based on the signal at
419 the intercept and three times the standard deviation about regression of the calibration
420 curve.³⁰ A LOD of 14 ng L^{-1} Hg was obtained and the analysis frequency was 3 samples
421 h^{-1} .

422 The accuracy of the proposed method was evaluated by analysis of a certified
423 reference material (CRM), NIST-SRM 1641e (total Hg in water) with a Hg

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3 424 concentration of $0.1016 \pm 0.0017 \text{ mg L}^{-1}$. The concentration of Hg found in this CRM
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5 425 after applying the proposed method was $0.1025 \pm 0.0019 \text{ mg L}^{-1}$ indicating an
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7 426 acceptable accuracy of the method ($p < 0.01$). Likewise, the method was applied for the
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9 427 determination of Hg in different water samples collected in Mendoza province
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11 428 (Argentina). Concentrations of Hg in water samples were in the range of <LOD for tap
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13 429 water, <LOD – $0.05 \text{ } \mu\text{g L}^{-1}$ for mineral water, $0.06 - 0.08 \text{ } \mu\text{g L}^{-1}$ for rain water and 0.09
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15 430 – $0.13 \text{ } \mu\text{g L}^{-1}$ for river water. A recovery study was also developed on samples spiked at
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17 431 known concentration of Hg. The results are shown in Table 3. Recoveries of Hg varied
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19 432 between 95.6% and 105%.

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22 433 Finally, a comparison with other methods reported in the literature for Hg
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24 434 determination is given in Table 4. In this work, an easily prepared sorbent nanomaterial
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26 435 implemented in a flow μ -SPE technique allowed to obtain a simple and effective
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28 436 microscale sample preparation technique. The high retention capacity shown by the
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30 437 sorbent material allowed trace Hg determination with a minimal amount of sorbent. The
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32 438 proposed method required a low volume of sample, which also diminished waste
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34 439 generation in the analytical laboratory. In addition, the proposed method showed a low
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36 440 LOD and a higher enhancement factor than other works. This method can be considered
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38 441 as a cost-effective and selective approach for trace Hg determination.
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443 **4 Conclusions**

444 The low concentrations of Hg occurring in water samples determine that
445 preconcentration must be applied when ETAAS detection is intended to be used for
446 trace element determination. A sensitive analytical methodology was developed in this
447 work for Hg preconcentration and determination in different water samples at trace
448 levels (ng L^{-1}) using a novel IL-GO sorption material. The prepared IL-GO hybrid

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3 449 nanomaterial showed excellent retention efficiency and can be used for several
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5 450 preconcentration-elution cycles without deterioration of the retention efficiency. The
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7 451 use of an IL containing long alkyl chain groups increased the retention efficiency of GO
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9 452 for the analyte, thus providing additional physicochemical properties that were useful
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11 453 for the extraction of Hg and its determination at trace levels. The mixing of IL-GO
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13 454 nanomaterial with milled glass prevented agglomerations and overpressures in the
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15 455 microcolumn. Furthermore, the μ -SPE preconcentration system can be proposed as an
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17 456 environmentally friendly approach as no volatile organic solvent was required during
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19 457 analysis and minimal amounts of reagents were used. The preconcentration method
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21 458 allowed Hg determination in river, rain, mineral and tap water samples with high
22
23 459 accuracy and reproducibility.

24 460 **Acknowledgements**

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26
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33 464 06/M099 and 06/M039) (Argentina).

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532 **Figure captions**

Fig. 1 Flow injection device and its operation sequence for μ -SPE preconcentration and determination of Hg. P₁ and P₂: peristaltic pumps; V₁ and V₂: injection valves; L: solvent loop; S: sample; E: eluent; A: air; W: waste.

Fig. 2 FT-IR spectra of IL (—), GO (....) and IL-GO (—).

Fig. 3 TEM images of a) GO and b) IL-GO.

Fig. 4 Influence of the type and concentration of eluent on Hg desorption from the column: 1) 15% (v/v) HCl; 2) 30% (v/v) HCl; 3) 0.25 mol L⁻¹ EDTA; 4) 20% (v/v) HCl / 5% (w/v) Thiourea; 5) 40% (v/v) HNO₃; 6) 20% (v/v) HNO₃. All experiments were performed in triplicate. Other conditions were as mentioned in Table 1.

Fig. 5 Effect of different variables on the analytical performance of the system: a) Sample volume, b) Sample flow rate, c) Eluent flow rate. Other conditions were as indicated in Table 1.

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Table 1
Instrumental and experimental conditions for Hg determination.

Wavelength	228.8 nm			
Spectral band width	0.7 nm			
Lamp (EDL) current	170 mA			
Injection volume	40 μ L			
Matrix modifier	20 μ g Pd [as Pd(NO ₃) ₂]			
Graphite furnace temperature program				
Step	T (°C)	Ramp time (s)	Hold time (s)	Argon flow (mL min ⁻¹)
Drying 1	110	1	30	250
Drying 2	130	15	15	250
Pyrolysis 1	250	10	20	250
Atomization	1300	0	5	0
Cleaning	2400	1	2	250
Optimal μ-SPE conditions				
Working pH	5.0			
Sample volume	5.0 mL			
Eluent solvent	20% (v/v) HNO ₃			
Eluent volume	50 μ L			
Loading flow rate	0.3 mL min ⁻¹			
Elution flow rate	0.3 mL min ⁻¹			

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Table 2
Operating parameters and sequence of the μ -SPE system.^a

Step	Flow rate (mL min ⁻¹)	Sample volume (mL)	Time (min)	Active pump	Valve positions V ₁ V ₂		Purpose
I	2.5	5	2	P ₁	L	L	Column conditioning
I	0.3	5	16.7	P ₁ -P ₂	L	L	Sample and eluent loading
II	0.3	0.1	0.3	P ₂	I	I	Analyte elution

^a Refer to Fig. 1

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Table 3
Determination of Hg in water samples and analyte recovery.

Sample	Hg added ($\mu\text{g L}^{-1}$)	Hg found ($\mu\text{g L}^{-1}$)	Recovery (%) ^a
Mineral water	-	<LOD	-
1	0.5	0.51±0.02	102
	1	0.99±0.03	99.0
Mineral water	-	0.05±0.02	-
2	0.5	0.54±0.02	98.0
	1	1.07±0.04	102
Tap water	-	<LOD	-
1	0.5	0.49±0.02	98.0
	1	1.03±0.03	103
Tap water	-	<LOD	-
2	0.5	0.50±0.02	100
	1	0.99±0.03	99.0
Rain water	-	0.06±0.01	-
1	0.5	0.58±0.02	104
	1	1.04±0.04	98.0
Rain water	-	0.08±0.01	-
2	0.5	0.56±0.02	96.0
	1	1.07±0.04	99.0
River water	-	0.09±0.01	-
1	0.5	0.57±0.02	96.0
	1	1.09±0.03	100
River water	-	0.13±0.01	-
2	0.5	0.64±0.02	102
	1	1.09±0.03	96.0

^a100 x [(Found-base) / added]

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Table 4
Comparison of the developed methodology with others reported for Hg determination in water.

Method	LOD (ng L ⁻¹)	RSD (%)	Sensitivity enhancement factor	Sample consumption (mL)	Ref.
HF- LPME/HPLC- ICP-MS ^a	110-230	-	27-48	-	5
EME ^b -ETAAS	500	6.2-7.1	102-108	-	3
Photo-CVG ^c - ETAAS	20	5	-	7	31
LIS-SH-SDME ^d - ETAAS	480	4.2	75	-	32
CPE ^e -ETAAS	1200	4.7	-	-	33
μ-SPE-ETAAS	14	3.9	100	5	Proposed method

^aEME: Hollow fiber-liquid phase microextraction /high performance liquid chromatography- inductively coupled plasma mass spectrometry

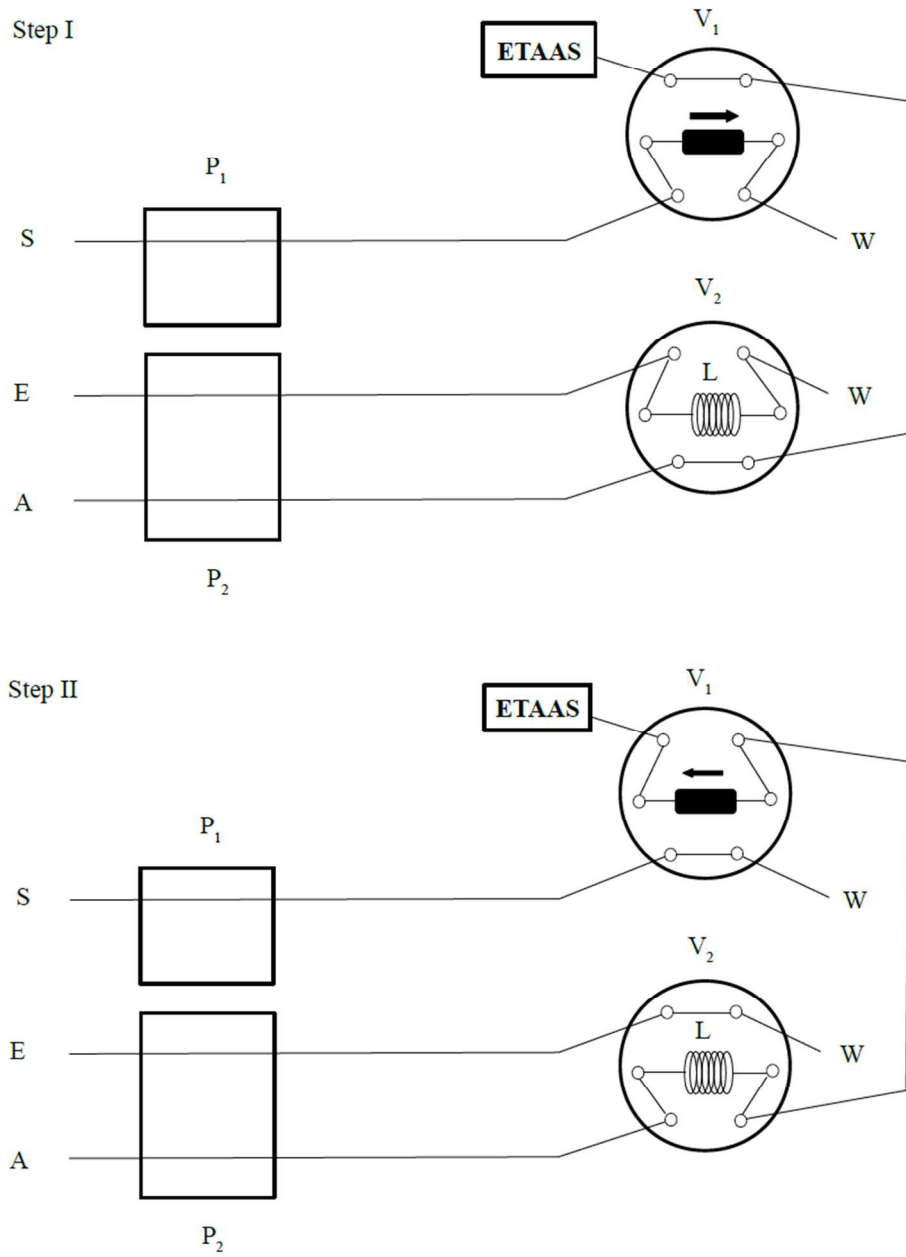
^bEME: Electromembrane extraction

^cPhoto-CVG: Photochemical vapor generation

^dLIS-SH-SDME: Automated headspace single drop microextraction

^eCPE: cloud point extraction

Fig. 1



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Fig. 2

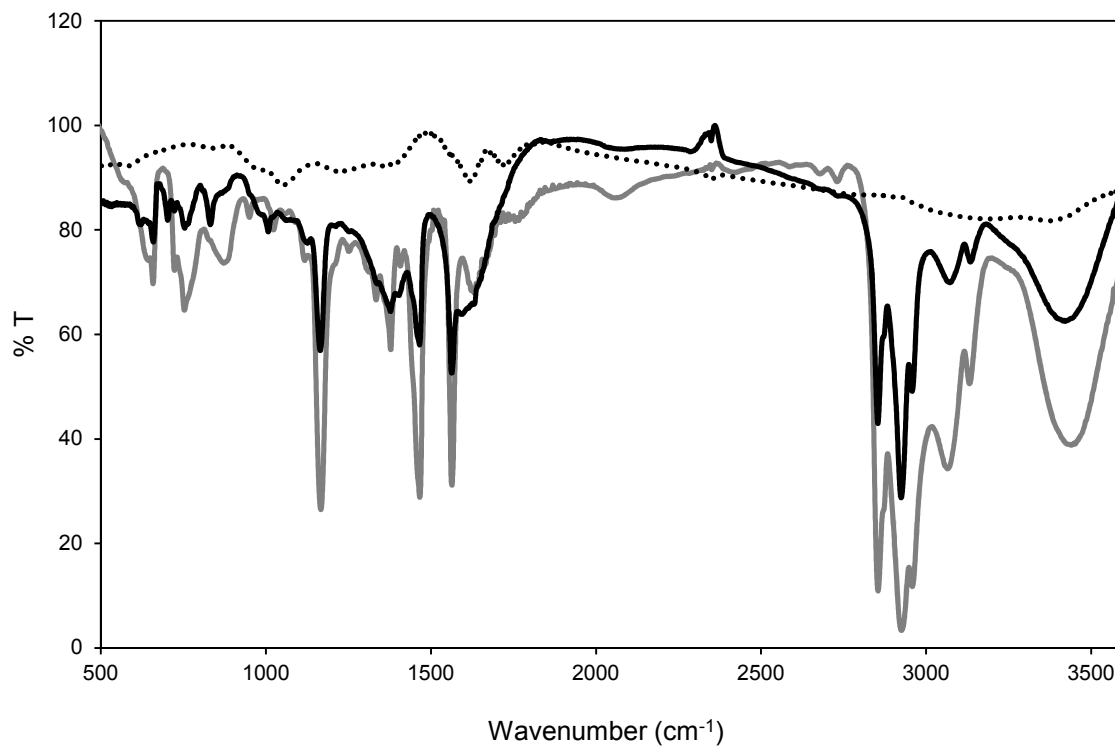
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Fig. 3a

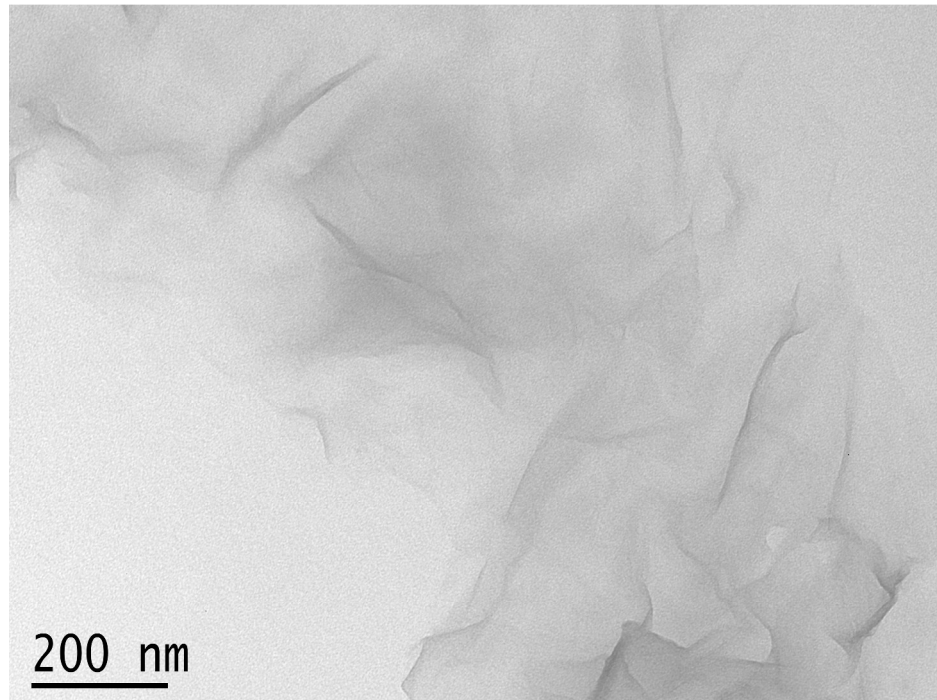
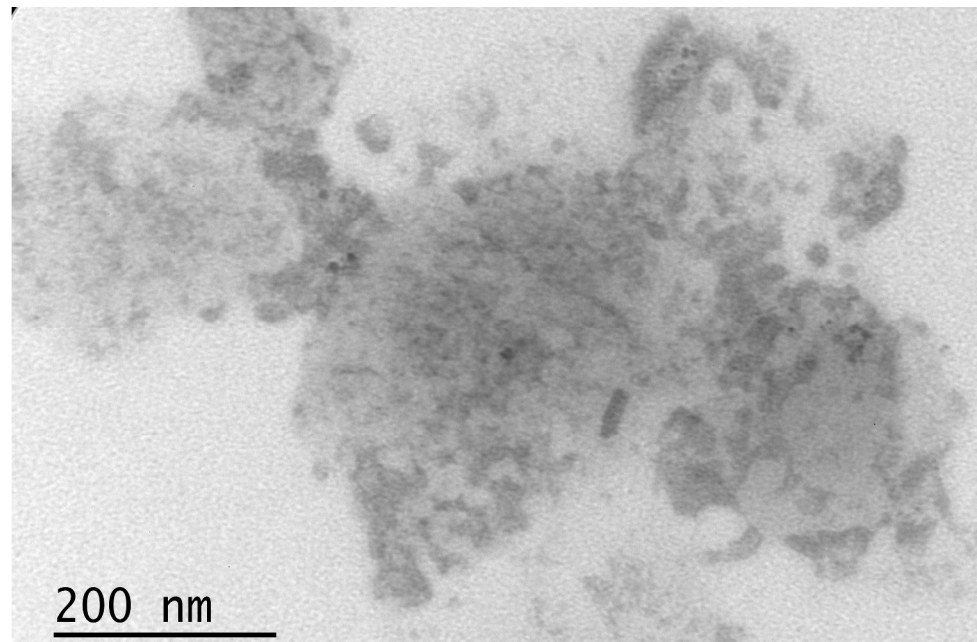


Fig. 3b



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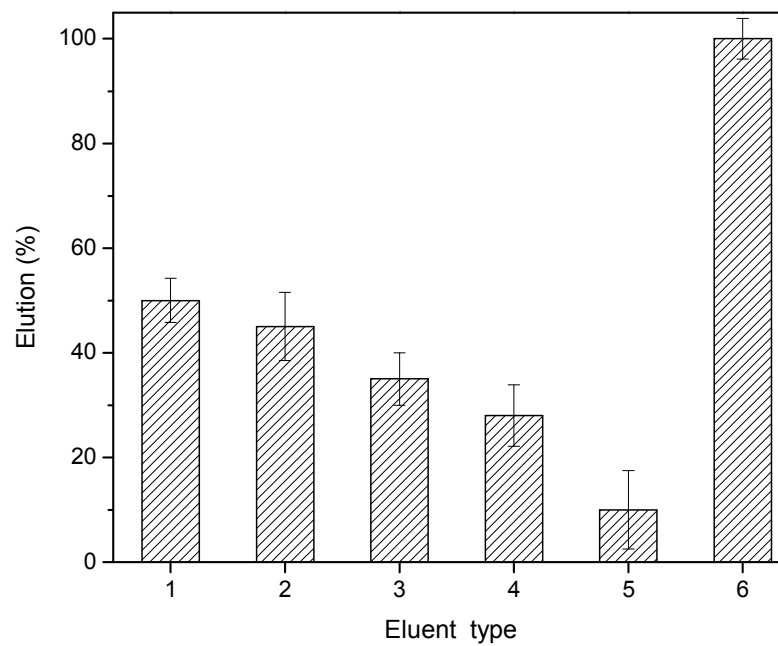
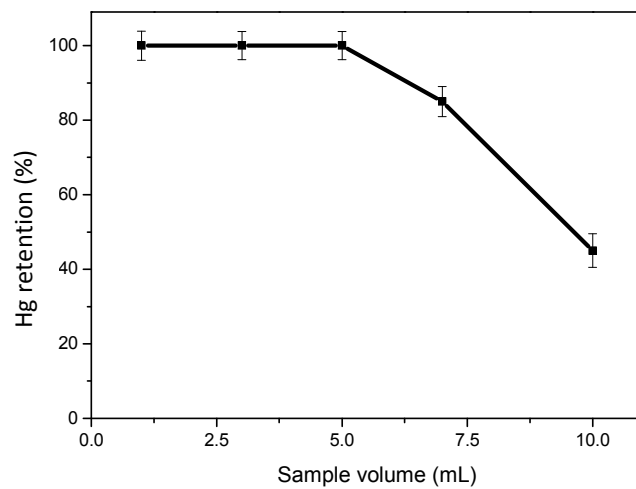
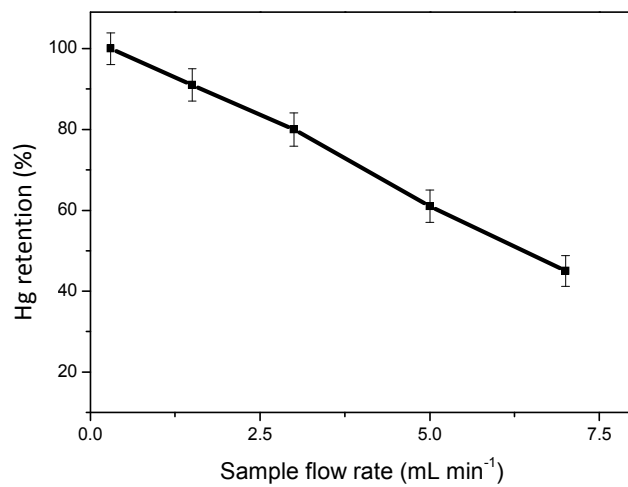
Fig. 41
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Fig. 5

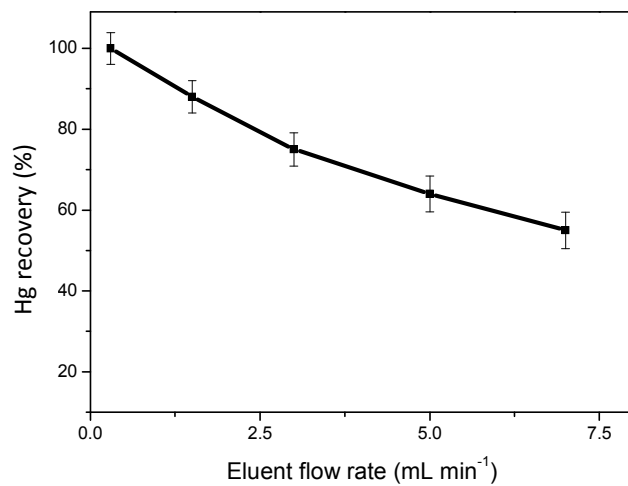
(a)

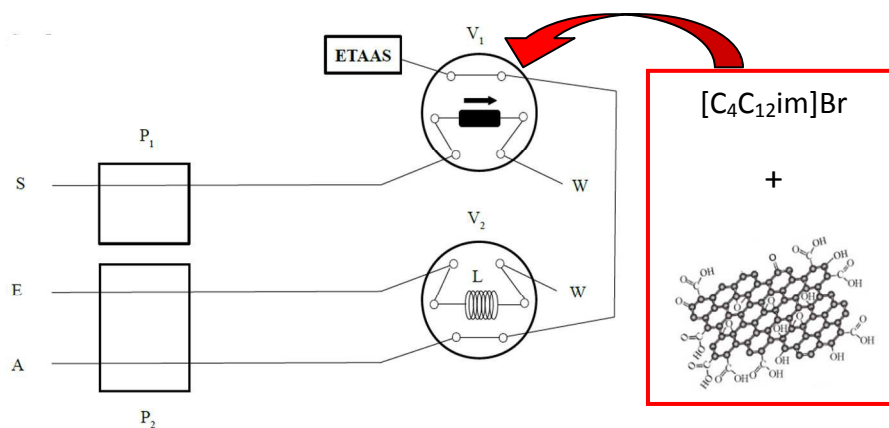


(b)



(c)



**Entry:**

High retention of Hg and sensitive determination with a graphene oxide-ionic liquid hybrid nanomaterial.