
Modified 3D-printed device for mercury determination in waters

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Abstract :

3D printing technology is increasingly used in flow analysis, to develop low cost and tailor-made devices. The possibility of grafting specific molecules onto 3D printed parts offers new perspectives for the development of flow systems. In this study, a MPFS system including a dicarboxylate 1,5-diphenyl-3-thiocarbazone grafted 3D-printed device has been developed for mercury determination. For this purpose, the surface of 3D-printed cuboids was first modified with amine functional groups and then grafted with dicarboxylate 1,5-diphenyl-3-thiocarbazone. This new grafted device resulted in selective mercury preconcentration with extraction and elution yields higher than 90% even at high sampling flow rates. The detection can then be carried out in two ways: a direct detection of mercury extracted onto 3D-printed grafted cuboids by atomic absorption spectrophotometry after amalgam on gold or a detection of mercury in solution after elution with L-cysteine by spectrophotometry or cold vapor atomic absorption spectrometry.

Highlights

► A grafting procedure of a ligand onto the surface of 3D printed supports is presented. ► Hg²⁺ can be selectively extracted from acidic medium even at high flow rate without clogging of the support. ► The LODs were between 2.6 ng/L and 0.3 µg/L, depending on the detection method used. ► Modification of 3D printed units opens new perspectives for flow systems.

Keywords : stereolithography, Poly(MethylMethacrylate) grafting, dicarboxylate 1,5- diphenyl-3-thiocarbazone, Multi Pumping Flow System, mercury

31 **Introduction**

32 Mercury is one of the most toxic metal, present in the environment in different chemical
33 forms. This post-transition metal has a high toxicity at low levels and may accumulate in
34 organisms [1] which increases its dangerousness. The exposition to mercury can cause
35 damages to organs [2] as respiratory and kidney diseases, dysfunctions of the nervous system
36 [3,4] with memory troubles and developmental delay for exposed children, or troubles in the
37 development of foetus. Mercury has the capacity to be exchanged between the different
38 reservoirs of the biosphere [5], whether in organic or in inorganic form. There are few natural
39 sources of mercury such as volcanism and geothermal sources [6], while anthropogenic
40 sources are very important: mining, combustion of fossil fuels [7,8], production of batteries
41 from mercury oxide, use in electrolytic processes for chemical or pesticides production. A
42 recent study [9] estimated anthropic mercury in the environment at 1 540 000 tonnes, of
43 which 73% was emitted between 1850 and 2010.

44 In the light of its toxicity and its numerous sources of pollution, the World Health
45 Organization has recommended a guideline value of $6 \mu\text{g L}^{-1}$ for inorganic mercury in
46 drinking water and European regulations have set a maximal allowable concentration (MAC)
47 in natural waters at $0.07 \mu\text{g L}^{-1}$ for mercury and its compounds. Accordingly, the development
48 of simple, rapid and sensitive methods for on-line Hg determination in natural waters has
49 attracted widespread attention in modern analytical chemistry. Flow analysis seems to be

50 appropriate to meet these specific requirements: this technology allows the decrease of
51 instrumentation size and of reagents and energy consumption, which are significant
52 advantages to simplify the manipulations required for the assays.

53 Many flow systems have been developed for quantification of mercury in waters, with a
54 detection step often performed by cold vapor atomic absorption spectrometry (CV-AAS) [10-
55 12]. Colorimetric detection has been also used for high concentrations of mercury [13,14].
56 Independently of the detection method, the quantification of low levels of mercury is usually
57 difficult because of sample storage problems [15]. A portable analytical system would avoid
58 these problems, but the coupling with complex laboratory detection techniques (atomic
59 absorption spectrometry (AAS), inductively coupled plasma mass spectrometry (ICP-MS) or
60 inductively coupled plasma atomic emission spectrometry (ICP-AES)) would remain difficult
61 to handle. A colorimetric detection step may be considered if a pre-concentration module is
62 used for low mercury concentrations.

63 3D printing eases the creation of three-dimensional custom fluidic modules as columns and
64 mixers and several flow systems based on such 3D-printed modules have been reported
65 previously for the determination of lead [16], cadmium [17] or chromium [18].

66 In this study, the 3D printing resin was used directly after the photopolymerization process as
67 a support for the grafting of a ligand for the selective extraction of mercury. Dicarboxylate
68 1,5-diphenyl-3-thiocarbazonate (DTZc) was chosen for this purpose because of its affinity for
69 mercury [19]. The grafting procedure is performed in two stages: first, amine functions are
70 integrated on the surface of the photopolymerized resin and then dicarboxylate 1,5-diphenyl-
71 3-thiocarbazonate is grafted by reaction between its carboxylic function and the amine function
72 added to the resin. Various detection methods have been considered and several applications
73 are discussed here as examples to illustrate the opportunity of using a 3D printed grafted
74 module.

75

76 2. Materials and methods

77 2.1. Reagents and solutions

78 All chemicals used were of analytical grade and used without further purification. Solutions
79 were prepared with ultra-pure water (Millipore, resistivity > 18 MΩ cm) and stocked in high
80 density polyethylene or Teflon flasks. Mercury standard solutions were prepared by dilution
81 of a commercial 1 g.L⁻¹ AAS mercury stock solution (Fisher Chemical, USA) and stabilized
82 with 1% v/v nitric acid trace metal grade (Fisher Chemical, USA).

83 A multi-metal solution (10 μmol.L⁻¹ for each metal) was prepared by diluting and mixing
84 eleven commercial AAS stock solutions of aluminium, cadmium, calcium, cobalt, copper,
85 iron, lead, magnesium, mercury, silver and zinc at 1 g.L⁻¹ (Fisher Chemical, USA) in ultra-
86 pure water.

87 Modification of 3D printed devices was carried out with hexane diamine (Fisher Chemical,
88 USA), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide (EDC, GenScript, USA) and 1-
89 Hydroxybenzotriazole (HOBt, Fluka, USA).

90 Detection was performed by two techniques: spectrophotometric detection with a reagent
91 composed of dithizone (Sigma-Aldrich, USA) and thiourea (Sigma-Aldrich, USA) prepared
92 in a glycine buffer solution (Sigma-Aldrich, USA) in 30% absolute ethanol v/v (Sigma-
93 Aldrich, USA), whereas hydrochloric trace metal grade (Fisher Chemical, USA) and sodium
94 borohydride (Sigma-Aldrich, USA) were prepared in hydroxide solution (Fisher Chemical,
95 USA) for CV-AAS detection.

96

97 2.2 Synthesis of DTZc

98 Dicarboxylate 1,5-diphenyl-3-thiocarbazone (DTZc) was synthesized following the
99 conditions defined by Shenashen et al. [19]. Briefly, 1.9 g of hydrazinobenzoic acid were

100 mixed with 0.6 mL of carbon disulfide, 20 mL of ethanol, 0.5 g of sodium hydroxide and 15
101 mL of distilled water. The mixture was heated to reflux for 5 hours; then DTZc was
102 precipitated as a white solid by addition of ethanol, filtered, washed and dried. The reaction
103 yield was 22% (0.95 g of dicarboxylate 1,5-diphenyl-3-thiocarbazone).

104

105 2.3 Modification of 3D printed device

106 3D printed devices were post-processed by cleaning in isopropyl alcohol for 10 minutes and
107 curing for 1 hour under UV light. Integration of amine functions was carried out by
108 immersion of 3D printed post-cured parts for 1 h in a 10% m/v solution of hexane diamine
109 prepared in a solution of borate buffer adjusted to pH 11.5. The aminated parts were carefully
110 cleaned by successive baths in water, in ethanol and in water afresh. The DTZc grafting was
111 then carried out by immersion in a mixture of 5 g.L⁻¹ solution of N-(3-Dimethylaminopropyl)-
112 N'-ethylcarbodiimide (EDC), 5 g.L⁻¹ solution of 1-Hydroxybenzotriazole (HOBt) and 1 g.L⁻¹
113 solution DTZc in absolute ethanol for 30 min at room temperature. Modified supports were
114 then cleaned by successive immersions in water, in sodium hydroxide 10 mM, in water, in
115 ethanol and in water again, and kept at 4 °C until use.

116

117 2.4 Detection

118 Elution of modified supports after mercury extraction was carried out with a 0.5% m/v
119 solution prepared from L-cysteine.

120 For CV-AAS detection, a 6 mol.L⁻¹ solution of hydrochloric acid (trace metal grade) was used
121 with a 0.135 mol.L⁻¹ solution of sodium borohydride prepared in 0.125 mol.L⁻¹ sodium
122 hydroxide solution.

123 Concerning the colorimetric detection, a post-elution photo-oxidation step was first performed
124 using hydrogen peroxide and hydrochloric acid in order to obtain a final concentration of 0.5

125 mol.L⁻¹ of each reagent in the eluent. At the end of the photo-oxidation step, a 1.1 mol.L⁻¹
126 solution of ascorbic acid was then added to eliminate the excess of hydrogen peroxide before
127 detection. The final colorimetric reagent was prepared according to the conditions described
128 by Theraulaz et al. [20] with a 0.01 mmol.L⁻¹ solution of dithizone prepared in a 0.01 mol.L⁻¹
129 glycine buffer solution with 30% ethanol v/v, and mixed with a 0.001 mol.L⁻¹ thiourea
130 solution (Sigma-Aldrich, USA).

131

132 2.5. Apparatus

133 2.5.1. Metal analysis

134 Cold vapour atomic absorption spectrometry (CV-AAS) was used to quantify mercury in
135 standard solutions and real samples. The measurements were carried out on a Thermo
136 Scientific ICE3500 (USA) atomic absorption spectrometer equipped with a mercury hollow-
137 cathode lamp operated at 6 mA (wavelength of 253.7 nm) and a VP 100 Vapour System, with
138 a peristaltic pump operated at 30 rpm. Determinations were carried out with the following
139 parameters: acid flow rate 0.7 mL.min⁻¹, reductant flow rate 1.6 mL.min⁻¹, sample flow rate
140 7.0 mL.min⁻¹, measuring time 6 seconds, argon flow rate 104 mL.min⁻¹.

141 Interferences of other metals on mercury extraction/elution were studied by inductively
142 coupled plasma-atomic emission spectrometry (ICP-AES) with a Jobin YVON JY2000
143 Ultratrace spectrometer, equipped with a CMA spray chamber and a Meinhard R50-C1 glass
144 nebuliser. Determinations were performed with the following settings: power 1000W, pump
145 speed 20 mL.min⁻¹, plasma flow rate 12 L.min⁻¹, coating gas flow rate 0.15 L.min⁻¹, nebuliser
146 flow rate 1.08 L.min⁻¹ and nebuliser pressure 2.6 bar.

147

148 2.5.2. 3D printing

149 The DTZc grafting procedure was optimized on 3D printed disks and cuboids. These pieces
150 were designed with Rhinoceros® 5.0 3D software (Robert McNeel & Associates Europe,
151 Spain) and then printed on the Form1+ stereolithographic printer (Formlabs, USA), using an
152 acrylate/methacrylate transparent resin (Clear, BV-002, Formlabs, USA). The integration of
153 amines functions and the DTZc grafting were first tested and optimized on 3D printed disks
154 (diameter 19 mm, thickness 2 mm) in batch experiments. Then the optimized parameters were
155 applied on a cuboids' column (14.1 x 54.1 mm, 284 cubes with a 1.5 mm diameter) which had
156 a grafted surface of 37.2 cm².

157

158 2.5.3. Flow systems

159 Sample was propelled using a peristaltic pump (Labcraft, Armtop, France). Reagents were
160 introduced by means of solenoid micro-pumps (Bio-ChemValve Inc., USA) with a stroke
161 volume of 20 µL and a highest frequency of 250 cycles/min and 3-way solenoid valves
162 (Takasago Electric Inc., Japan). The pumps and valves were computer-controlled by a
163 MCFIA/MPFS system (Sciware, Spain) with eight digital 12V output channels. Two flow
164 systems were developed according to the detection method used (CV-AAS or colorimetry),
165 their operating scheme is presented in Fig. 1 and Fig. 2.

166 In both systems, the first step of the analytical procedure was the mercury extraction from the
167 sample (PP and EV1 to waste) on the DTZc grafted 3D printed cuboid column. Then, a
168 solution of L-cysteine was pumped (P1) to elute the mercury extracted onto the grafted
169 column.

170 The first system included a CV-AAS detection system directly after the elution step,
171 previously described in section 2.5.1 (Fig. 1).

172 The second system offered a simple colorimetric detection (Fig. 2). This detection was based
173 on a photo-oxidation step to liberate the mercury from L-cysteine before a final colorimetric

174 detection with a dithizone reagent. A photo-oxidation module was used with a low-pressure
175 mercury lamp emitting at 254nm (90%) and 185 nm (10%), with a radiating part of 22.86 cm
176 in height and 0.95 cm in diameter, covered by a Suprasil quartz loop with an inner diameter of
177 2 mm (UVP PenRay, USA). Hydrogen peroxide oxidant solution (P2) was added to the eluate
178 to enhance photo-oxidation of L-cysteine and the mixture was exposed under UV-lamp for 30
179 minutes (P3 and EV2). An ascorbic acid solution (P4) was added at the end of this step to
180 eliminate excess of oxidant. The dithizone reagent (P5) was introduced in the system for the
181 detection of dithizone-mercury complex at 480 nm. Detection was carried out in a black 3D
182 printed spectroscopic flow-cell with a 5 cm optical pathlength. Two FC-UV600 optical fibers
183 (OF) (Ocean Optics, USA) were connected at the ends of optical pathlength, and isolated
184 from the reaction mixture with two tailor-made quartz discs, to guide the light from the source
185 to the spectrophotometric detector. The radiation of the halogen bulb of a DH-2000 UV-Vis
186 light source (Ocean Optics, USA) was transmitted to a USB2000 miniature spectrometer
187 detector (Ocean Optics, USA). The whole system was controlled by AutoAnalysis 5.0
188 software (Sciware, Spain).

189

190 **3. Results and discussion**

191 3.1. Modification of 3D printed device

192 Stereolithography resins are mainly composed of methyl methacrylate, which is an ester
193 monomer. After photopolymerization and poly(methyl methacrylate) (PMMA) formation, this
194 ester group can be used to graft molecules onto the surface of 3D printed objects. This
195 grafting procedure requires two steps (global procedure is summarized in Fig. 3): first amine
196 functions were introduced onto 3D printed surface by amination reaction of the ester functions
197 of the PMMA with hexane diamine [21], followed by grafting of DTZc using an amidation
198 reaction with carboxylate function of DTZc. A pink coloration of the solid surface after

199 cleaning at the end of the procedure demonstrates efficiency of the DTZc grafting
200 (supplementary materials Fig. S1).

201 These two steps were optimized in batch experiments on 3D printed disks thanks to the results
202 of ADECA test (Amino Density Estimation by Colorimetric Assay). This test allows
203 quantification of the amine functions on a solid surface [22]. A chromogenic reagent, the
204 Coomassie brilliant blue, reacts with amine functions in acidic medium. Then the quantity of
205 Coomassie brilliant blue which has reacted with the amine functions is extracted in basic
206 medium and finally quantified by colorimetric detection in acidic medium at a wavelength of
207 611 nm.

208

209 *3.1.1. PMMA amination step*

210 The PMMA amination step was carried out according to the method previously described by
211 Fixe et al. [21] with a heated solution of hexane diamine in borate buffer (pH=11.5). 3D
212 printed disks were immersed in this solution with a reaction time between 30 min and 4 h.
213 Three parameters were studied by a one-factor-at-a-time method: temperature, percentage of
214 hexane diamine in solution, and time. The results of ADECA test obtained with the various
215 operating conditions are summarized in Table 1 (the higher the result of ADECA test, the
216 better the efficiency of the amination step). The results showed first the advantage of heating
217 the hexane diamine solution to at least 50°C. There was no significant difference between 50
218 and 70°C with 1.42 ± 0.04 and 1.48 ± 0.05 respectively. Moreover, the 3D printed disks have
219 shown signs of premature aging at 70°C with undesired cracking on the surface.
220 Consequently, a temperature of 50°C was chosen as the optimal condition. Concerning the
221 other parameters, a 10% m/v hexane diamine solution and a reaction time of 1 h were the best
222 conditions to obtain a maximum amination efficiency (1.75 ± 0.13).

223

224 3.1.2 DTZc grafting

225 The DTZc grafting was studied after amination of 3D printed disks according to the best
226 operating conditions described previously. Four parameters have been optimized by a one-
227 factor-at-a-time method: activating agents (EDC and HOBt) concentration, DTZc
228 concentration, temperature and grafting time. The influence of each parameter on the grafting
229 efficiency was evaluated based on the results of the ADECA test. In this case, a low value of
230 the ADECA test indicates a good efficiency of the grafting reaction, as it shows a decrease in
231 the number of free amine groups onto the PMMA surface.

232 The results are summarized in Table 2. Concerning activating agents EDC and HOBt, a
233 concentration of 5 g.L^{-1} for both reagents was enough to obtain the best grafting rate ($0.32 \pm$
234 0.01). For the DTZc concentration, we observed no significant difference between 1 and 2.5

235 g.L⁻¹ (with ADECA test results at 0.35 ± 0.02 and 0.33 ± 0.03 respectively). A concentration
236 of 1 g.L⁻¹ was thus selected for further experiments in order to limit the consumption of the
237 reagent. No effect of temperature has been observed and the grafting reaction could therefore
238 be performed at room temperature (25°C). Finally, a minimum reaction time of 30 min was
239 necessary to obtain a good grafting efficiency and no significant difference was observed
240 between 30 and 60 min.

241

242 3.2. Solid phase extraction of mercury on grafted 3D printed device

243 3.2.1 Extraction of metals

244 Shenashen et al. [19] have previously demonstrated that mercury was bonded to two
245 molecules of DTZc through S and one N atom, which is consistent with dithizone reactivity, a
246 well-known ligand which forms ML₂ complexes with various metals (including mercury)
247 through S and N bonding.

248 After the optimization of the grafting procedure, mercury extraction was studied following
249 various conditions. Raw resin, aminated resin, and grafted resin disks were immersed for 1 h
250 in a 100 µg.L⁻¹ mercury solution in PTFE tubes, at pH values between 1 and 9. The mercury
251 solution was analysed by CV-AAS before and after extraction. The results obtained
252 (extraction rate calculated from the concentrations before and after extraction) are
253 summarized in Fig. 4.

254 For pH value above 4, an average extraction of 38% of mercury by raw resin was observed
255 (min 24% at pH = 4; max 51% at pH = 9), which is characteristic of the adsorption of a metal
256 on a plastic material surface, as it was previously demonstrated for polyethylene [23]. In
257 acidic medium (pH=2), mercury was thus not extracted by the raw resin material (less than
258 1%): this result shows the importance to work at acidic pH to avoid the contamination of 3D
259 printed pieces during the flow procedure. Aminated resin disks also showed a partial

260 extraction of mercury in a pH range between 2 and 9, probably due to the complexation of
261 mercury by the amine functions (min 32% at pH = 2, max 55% at pH = 7).

262 Results showed complexation of mercury by DTZc onto the surface of disks, as shown by the
263 significant increase of extraction rate between the raw resin disks and the grafted resin disks
264 at acidic pH values. Concerning the grafted resin disks, the extraction in acidic medium was
265 very efficient, with a mercury extraction rate of 99%. The acidification of the samples at
266 pH=2 before extraction by the grafted resin was the best compromise to obtain good
267 extraction performance and to avoid adsorption phenomena on the plastic surfaces. At this pH
268 value, extraction kinetics was rapid since a mercury extraction rate of 90% was reached after
269 a contact time of 1 minute (supplementary materials Fig. S2).

270 The selectivity of the DTZc grafted resin was tested with a multi-metal solution (aluminium,
271 calcium, cadmium, cobalt, copper, iron, lead, magnesium, mercury, silver and zinc) at pH=2.
272 This solution was extracted on the aminated and the grafted resins, and solutions were
273 analysed by ICP-AES before and after extraction (Fig. 5). The results showed a good
274 extraction of silver, a partial extraction of aluminium and a low extraction of lead both for
275 aminated and grafted resins. Iron was also partially extracted on the aminated resin (20%), but
276 extraction rate on DTZc grafted resin was below 5%.

277 Three metals presented thus a noticeable affinity with the DTZc grafted resin: silver,
278 aluminium and lead. These interferences will be eliminated during the detection step: in the
279 first flow system the colorimetric dithizone reagent contains a masking agent (thiourea) to
280 limit foreign ions interferences, especially for silver, and in the second system the CV-AAS is
281 a selective analytical method for Hg.

282

283 *3.2.2 Elution*

284 After the extraction step, it was necessary to find a suitable eluent to recover the mercury
285 extracted by the DTZc grafted resin. Several reagents were studied, and the tests are
286 summarized in Fig. 6 (elution rates were calculated by the difference between the amount of
287 mercury extracted on the grafted supports and the amount of mercury in the eluate solutions).
288 Inorganic acids (hydrochloric, sulfuric and nitric acid), which are well known eluents for
289 metals, were not efficient to elute mercury, even with 1 mol.L⁻¹ concentration (higher
290 concentrations were not tested to avoid the 3D resin degradation). Dithizone, EDTA and
291 potassium iodide were also tested, but resulted respectively in 16%, 19% and 18% elution
292 rates.

293 Several eluents with thiol or thiocarbonyl functions were also studied: L-cysteine, ethanethiol,
294 thioglycolic acid and thiourea. These four reagents were very efficient to elute mercury with
295 elution rates higher than 90 %, except thiourea with 83%. These results are consistent with the
296 high mercury affinity for thiolate compounds (R-SH) [24]. Recently Liem-Nguyen et al. [25]
297 determined a formation constant of Hg(L)₂ complexes with cysteine and thioglycolic acid of
298 41.5 and 37.5 respectively. The three thiol compounds tested showed almost identical elution
299 rates, and L-cysteine was chosen for further experiments as the least toxic and malodorous
300 sulfur compound.

301

302 3.3. Application

303 3.3.1. *Off-line sampling*

304

305 Given the difficulties of on-site analysis of mercury and real sample storage for further
306 laboratory analysis, a cuboid column was used to carry out on-site mercury extraction. For
307 this purpose, a column was developed to incorporate a removable rectangular assembly of
308 grafted cuboids (Fig. 7), these latter ones being able to be changed for each sample.

309 To optimize the efficiency of the extraction step, the mercury extraction flow rate was studied
310 between 1 and 10 mL.min⁻¹ by means of a peristaltic pump. The results are depicted in Fig. 8.
311 Flow rate showed no significant effect on extraction rate between 1 and 5 mL.min⁻¹ with an
312 average mercury extraction rate of 85%. Comparison of extraction efficiency versus higher
313 sample flow rates showed a 12% and 17% decrease respectively for sample flow rates of 7.5
314 mL.min⁻¹ (mercury extraction rate of 75%) and 10 mL.min⁻¹ (mercury extraction rate of 72%).
315 Nevertheless, despite the 17% loss, a sample flow rate of 10 mL.min⁻¹ could be a good
316 compromise between the extraction efficiency and the extraction time (or sample volume). A
317 sample flow rate of 10 mL.min⁻¹ enables in a given time a significant increase of the
318 preconcentration factor by percolating higher volumes of sample. For a 1 h sample
319 percolation, a preconcentration factor of 23 is thus obtained for a sample flow rate of 5
320 mL.min⁻¹ versus 38 for a sample flow rate of 10 mL.min⁻¹.
321 After the extraction step, the entire cuboids assembly can be removed from the column and
322 introduced in a nickel boat of a mercury analyzer Altec AMA 254 which is specifically
323 conceived for the fast, precise and simple determination of mercury traces in solids samples.
324 Note that with this technique, it was necessary to perform a blank on grafted cuboids before
325 extraction, because the analysis of the raw resin used for 3D printing has showed a mercury
326 concentration of $9.6 \pm 3.2 \text{ ng.g}^{-1}$.

327

328 3.3.2. Flow systems

329 Mercury can also be quantified in solution after elution by L-cysteine by CV-AAS (Fig. 1).
330 As the mercury concentration is usually low in environmental samples, it is necessary to work
331 with large sample volumes to achieve a large preconcentration factor. In this study, the
332 sample volume was set at 500 mL to limit the analysis time (mercury extraction was
333 successfully tested for sample volumes up to 1 L). Experiments were carried out to determine

334 the minimum volume of L-cysteine which has to be used to quantitatively elute the mercury
335 extracted on modified supports. Results showed that a minimum volume of 10 mL of 0.5%
336 m/v L-cysteine in borate buffer (pH=11) should be used to elute 92% of the mercury extracted
337 on the modified support (Supplementary materials, Figure S3).

338 The analytical features of this procedure were thus determined for a sample volume of 500
339 mL and an elution volume of 10 mL: a limit of detection (LOD, 3σ ; $n=10$) of 2.6 ng.L^{-1} and a
340 limit of quantification (LOQ, 10σ ; $n=10$) of 8.6 ng.L^{-1} were achieved, with a linear range from
341 8.6 to 200 ng.L^{-1} . The overall analysis time by this analytical procedure was 60 minutes. The
342 3D printed DTZc grafted supports can be reused for 3 successive extractions. The tests
343 showed a decrease in the extraction rate to 65% from the fourth extraction. This method was
344 validated on two samples of fresh water and drinking water: as the mercury concentration was
345 below the LOD, samples were spiked to 40 ng.L^{-1} . The results obtained were consistent with
346 $38.2 \pm 5.1 \text{ ng.L}^{-1}$ for fresh water and $44.1 \pm 4.2 \text{ ng.L}^{-1}$ for drinking water (recovery rates
347 ranged from 83% to 120% in sample replicates).

348 The analytical procedure of the second flow system (Fig. 2) was divided in four steps:
349 extraction of mercury, elution, photo-oxidation, and colorimetric detection. Dithizone reagent
350 was used for the mercury detection because of its sensibility to mercury and to avoid
351 interferences with other extracted metals (silver and aluminium). A linear domain was
352 observed between 1 and $200 \text{ } \mu\text{g.L}^{-1}$ for a 500 mL sample volume and a 10 mL elution volume.
353 The limit of detection was $0.3 \text{ } \mu\text{g.L}^{-1}$ ($n=10$), and the limit of quantification was $1.0 \text{ } \mu\text{g.L}^{-1}$.
354 Coefficient of variation obtained for a mercury concentration of $50 \text{ } \mu\text{g L}^{-1}$ was 2.2%. The
355 overall analysis time by this analytical procedure was 90 minutes.

356 Given these results, the flow system with colorimetric detection was more intended for
357 analysis of wastewater or industrial water, while the CV-AAS flow system was most adapted
358 for fresh water and seawater because of its lower LOD and LOQ.

359

360 Conclusion

361 In this study, a new possibility offered by 3D printing has been explored. The
362 acrylate/methacrylate resin can be used as an inert support to graft selective molecules. The
363 two steps of the grafting procedure were developed and optimized. The first step was the
364 integration of amine functions on PMMA, followed by reaction between these amine
365 functions and the carboxylate function of DTZc. A very good extraction of mercury was
366 observed, and the grafted resin can be eluted with thiol eluents, more particularly with L-
367 cysteine, which has a high affinity with mercury.

368 A 3D printed column of cuboids has been grafted and several applications of this module have
369 been presented to show the different opportunities of the grafting of 3D resin. Other
370 molecules with carboxylate functions could also be grafted by the same procedure for the
371 extraction of various metals. This type of column (cubes were spaced 1.2 mm from each
372 other) could lead to many advantages for solid phase extraction: no clogging, use of unfiltered
373 or prefiltered samples and compatibility with high flow rates.

374

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379

380 Figure captions

381 Figure 1: Flow system for mercury determination with CV-AAS detection [P1: solenoid
382 micropump; PP: peristaltic pump; EV: solenoid valve; MC: mixing coil; W: waste; OF:
383 optical fiber; Ar: argon].

384 Figure 2: Flow system for mercury determination with colorimetric detection [P1-P4: solenoid
385 micropumps; PP: peristaltic pump; EV: solenoid valve; MC: mixing coil; W: waste; OF:
386 optical fiber; DTZ: dithizone].

387 Figure 3: Grafting pattern of DTZc onto PMMA.

388 Figure 4: Batch extraction of a $100 \mu\text{g.L}^{-1}$ mercury solution ($V=50 \text{ mL}$) on raw resin,
389 aminated resin and DTZc grafted resin disks versus pH ($n=3$).

390 Figure 5: Batch extraction of a multi-metal solution at $10 \mu\text{mol.L}^{-1}$ (aluminium, calcium,
391 cadmium, cobalt, copper, iron, lead, magnesium, mercury, silver, and zinc; $V=50 \text{ mL}$) at
392 $\text{pH}=2$ on the aminated resin disks and the grafted resin disks.

393 Figure 6: Batch elution of mercury extracted on DTZc grafted resin with different eluents
394 ($V=50 \text{ mL}$; $n=3$).

395 Figure 7: Scheme of removable column of grafted cuboids.

396 Figure 8: Influence of flow rate on the extraction of a $100 \mu\text{g.L}^{-1}$ mercury solution in a grafted
397 cuboids column ($n=3$).

398

399 **References**

400 [1] E. Pelletier, Mercury-selenium interactions in aquatic organisms: A review, Marine
401 Environmental Research. 18 (1986) 111–132. doi:10.1016/0141-1136(86)90003-6.

402 [2] Cancer du poumon et exposition professionnelle aux métaux : une revue des études
403 épidémiologiques - Article de revue - INRS, (2018).
404 <http://www.inrs.fr/media.html?refINRS=TC%20120> (accessed April 16, 2018).

405 [3] C. Freire, R. Ramos, M.-J. Lopez-Espinosa, S. Díez, J. Vioque, F. Ballester, M.-F.
406 Fernández, Hair mercury levels, fish consumption, and cognitive development in preschool
407 children from Granada, Spain , Environmental Research. 110 (2010) 96–104.
408 doi:10.1016/j.envres.2009.10.005.

- 409 [4] T. Yorifuji, T. Tsuda, S. Inoue, S. Takao, M. Harada, Long-term exposure to
410 methylmercury and psychiatric symptoms in residents of Minamata, Japan, *Environment*
411 *International*. 37 (2011) 907–913. doi:10.1016/j.envint.2011.03.008.
- 412 [5] Prévention du risque chimique - Mercure, cycle et toxicité, (2018).
413 <http://www.prc.cnrs.fr/spip.php?rubrique42> (accessed April 25, 2018).
- 414 [6] J.C. Varekamp, P.R. Buseck, Global mercury flux from volcanic and geothermal
415 sources, *Applied Geochemistry*. 1 (1986) 65–73. doi:10.1016/0883-2927(86)90038-7.
- 416 [7] S. Tang, X. Feng, J. Qiu, G. Yin, Z. Yang, Mercury speciation and emissions from
417 coal combustion in Guiyang, Southwest China., *Environ Res.* 105 (2007) 175–182.
418 doi:10.1016/j.envres.2007.03.008.
- 419 [8] D.G. Streets, Z. Lu, L. Levin, A.F.H. ter Schure, E.M. Sunderland, Historical releases
420 of mercury to air, land, and water from coal combustion, *Science of The Total Environment*.
421 615 (2018) 131–140. doi:10.1016/j.scitotenv.2017.09.207.
- 422 [9] D.G. Streets, H.M. Horowitz, D.J. Jacob, Z. Lu, L. Levin, A.F.H. Ter Schure, E.M.
423 Sunderland, Total Mercury Released to the Environment by Human Activities, *Environ. Sci.*
424 *Technol.* 51 (2017) 5969–5977. doi:10.1021/acs.est.7b00451.
- 425 [10] A.N. Anthemidis, G.A. Zachariadis, J.A. Stratis, Development of a sequential
426 injection system for trace mercury determination by cold vapour atomic absorption
427 spectrometry utilizing an integrated gas–liquid separator/reactor, *Talanta*. 64 (2004) 1053–
428 1057. doi:10.1016/j.talanta.2004.05.003.
- 429 [11] H. Erxleben, J. Ruzicka, Atomic Absorption Spectroscopy for Mercury, Automated by
430 Sequential Injection and Miniaturized in Lab-on-Valve System, *Analytical Chemistry*. 77
431 (2005) 5124–5128. doi:10.1021/ac058007s.

- 432 [12] L.O. Leal, O. Elsholz, R. Forteza, V. Cerdà, Determination of mercury by
433 multisyringe flow injection system with cold-vapor atomic absorption spectrometry,
434 *Analytica Chimica Acta*. 573–574 (2006) 399–405. doi:10.1016/j.aca.2006.04.078.
- 435 [13] M. Garrido, M.S. Di Nezio, A.G. Lista, M. Palomeque, B.S. Fernández Band, Cloud-
436 point extraction/preconcentration on-line flow injection method for mercury determination,
437 *Analytica Chimica Acta*. 502 (2004) 173–177. doi:10.1016/j.aca.2003.09.070.
- 438 [14] J.F. van Staden, R.E. Taljaard, Determination of Lead(II), Copper(II), Zinc(II),
439 Cobalt(II), Cadmium(II), Iron(III), Mercury(II) using sequential injection extractions, *Talanta*.
440 64 (2004) 1203–1212. doi:10.1016/j.talanta.2004.06.020.
- 441 [15] C. R. Hammerschmidt, K. L. Bowman, M.D. Tabatchnick, C. H. Lamborg, Storage
442 bottle material and cleaning for determination of total mercury in seawater, *Limnol.*
443 *Oceanogr.: Methods* 9 (2011) 426–431. doi:10.4319/lom.2011.9.426
- 444 [16] E. Mattio, F. Robert-Peillard, C. Branger, K. Puzio, A. Margailan, C. Brach-Papa, J.
445 Knoery, J.-L. Boudenne, B. Coulomb, 3D-printed flow system for determination of lead in
446 natural waters, *Talanta*. 168 (2017) 298–302. doi:10.1016/j.talanta.2017.03.059.
- 447 [17] E. Mattio, F. Robert-Peillard, L. Vassalo, C. Branger, A. Margailan, C. Brach-Papa, J.
448 Knoery, J.-L. Boudenne, B. Coulomb, 3D-printed lab-on-valve for fluorescent determination
449 of cadmium and lead in water, *Talanta*. 183 (2018) 201–208.
450 doi:10.1016/j.talanta.2018.02.051.
- 451 [18] C. Calderilla, F. Maya, V. Cerdà, L.O. Leal, 3D printed device for the automated
452 preconcentration and determination of chromium (VI), *Talanta*. 184 (2018) 15–22.
453 doi:10.1016/j.talanta.2018.02.065.
- 454 [19] M.A. Shenashen, S.A. El-Safty, E.A. Elshehy, Architecture of optical sensor for
455 recognition of multiple toxic metal ions from water, *Journal of Hazardous Materials*. 260
456 (2013) 833–843. doi:10.1016/j.jhazmat.2013.06.025.

- 457 [20] F. Théraulaz, O.P. Thomas, Complexometric determination of mercury(II) in waters
458 by spectrophotometry of its dithizone complex, *Microchimica Acta*. 113 (1994) 53–59.
459 doi:10.1007/BF01243137.
- 460 [21] F. Fixe, M. Dufva, P. Telleman, C.B.V. Christensen, Functionalization of poly(methyl
461 methacrylate) (PMMA) as a substrate for DNA microarrays, *Nucleic Acids Res.* 32 (2004) e9.
462 doi:10.1093/nar/gng157.
- 463 [22] G. Coussot, C. Perrin, T. Moreau, M. Dobrijevic, A. Le Postollec, O. Vandenabeele-
464 Trambouze, A rapid and reversible colorimetric assay for the characterization of aminated
465 solid surfaces, *Anal Bioanal Chem.* 399 (2011) 1061–1069. doi:10.1007/s00216-010-4363-7.
- 466 [23] A. Turner, L.A. Holmes, Adsorption of trace metals by microplastic pellets in fresh
467 water, *Environ. Chem.* 12 (2015) 600–610. doi:10.1071/EN14143.
- 468 [24] P. Pohl, B. Prusisz, Preconcentration of Mercury Using Duolite GT-73 in the Analysis
469 of Water Samples by Inductively Coupled Plasma Atomic Emission Spectrometry, *Analytical*
470 *Sciences.* 20 (2004) 1367–1370. doi:10.2116/analsci.20.1367.
- 471 [25] V. Liem-Nguyen, U. Skyllberg, K. Nam, E. Björn., Thermodynamic stability of
472 mercury(II) complexes formed with environmentally relevant low-molecular-mass thiols
473 studied by competing ligand exchange and density functional theory, *Environmental*
474 *Chemistry* 14 (2017) 243-253doi:10.1071/EN17062.

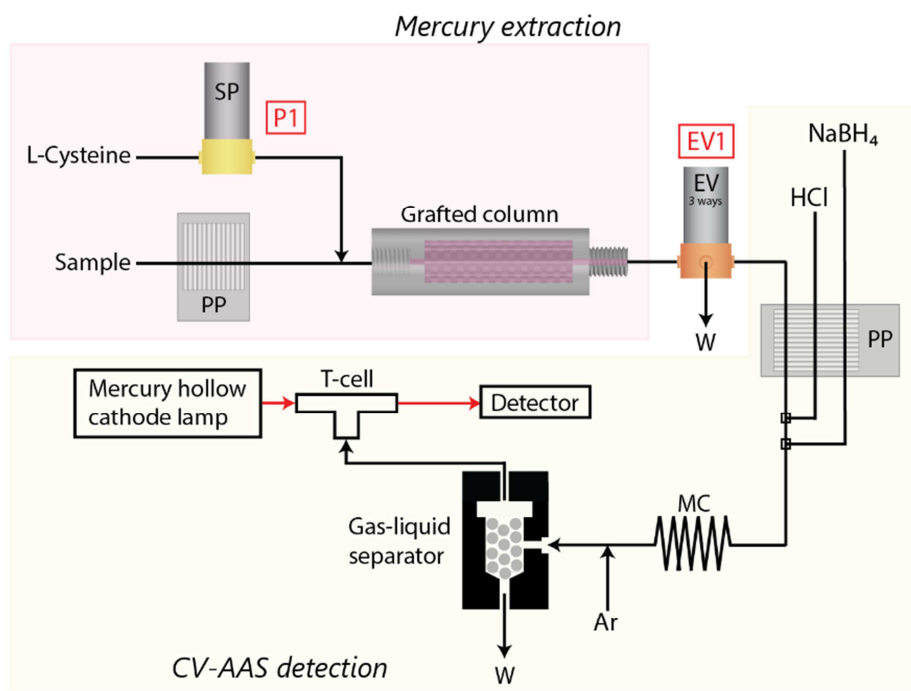
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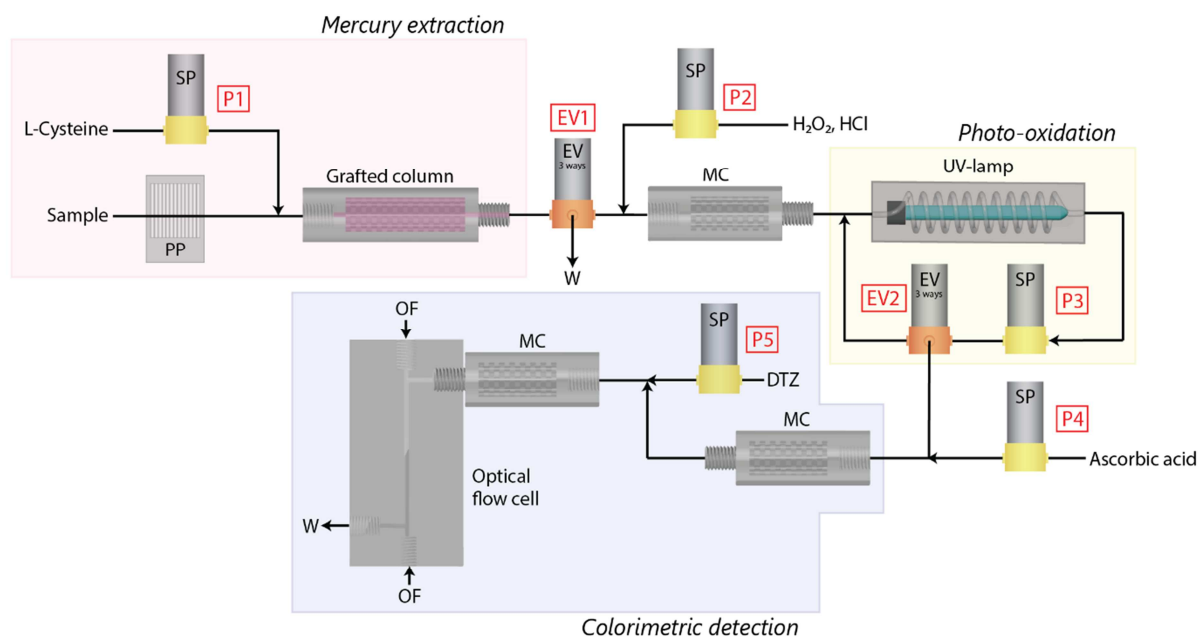
Table 1. Optimization of the PMMA amination reaction according to the ADECA test (n=3)

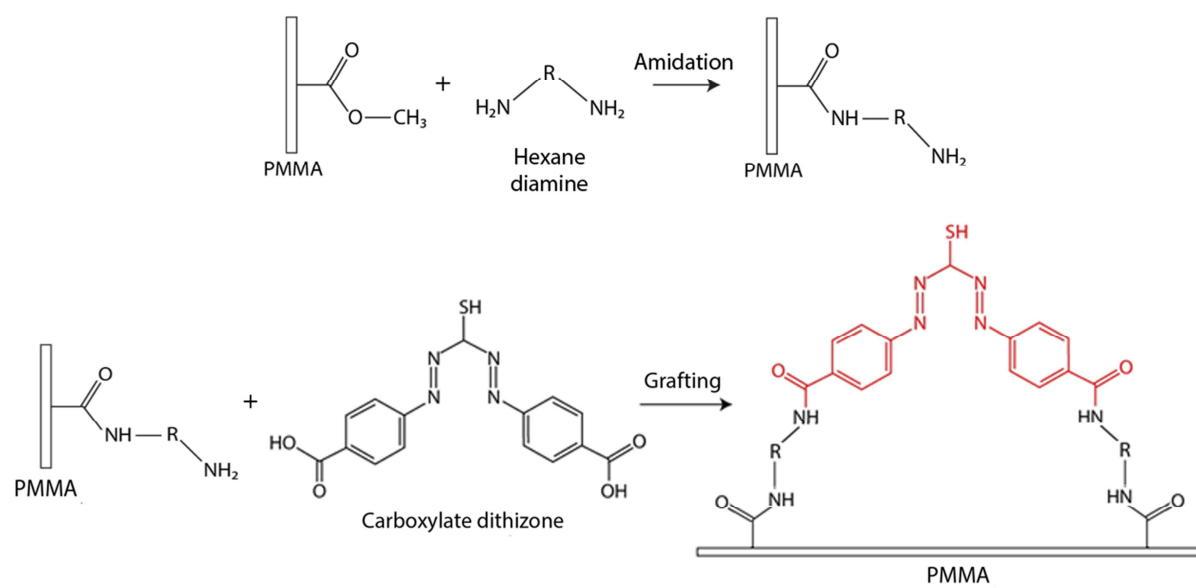
Operating conditions		Quantity of NH₂ functions ADECA test (a.u.)
Temperature (°C)	25	1.26 ± 0.15
	50	1.42 ± 0.04
	70	1.48 ± 0.05
Hexane diamine concentration (% m/v)	10	1.75 ± 0.13
	15	1.43 ± 0.04
	20	1.41 ± 0.06
	25	1.24 ± 0.06
Amination time (h)	0.5	1.02 ± 0.06
	1	1.75 ± 0.13
	2	1.27 ± 0.02
	4	1.11 ± 0.05

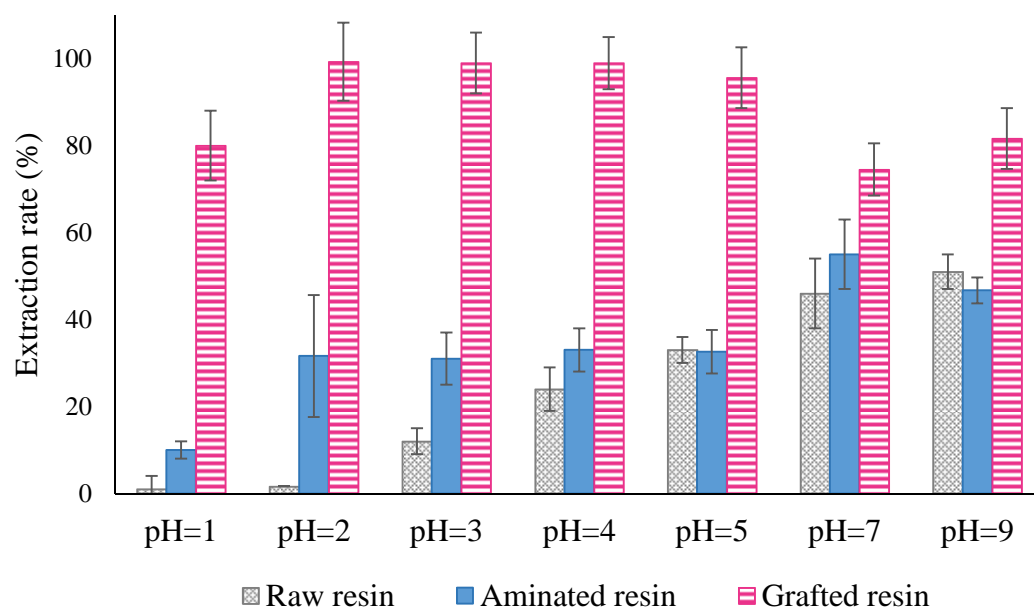
Table 2. Optimization of the PMMA grafting reaction [EDC: N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide; HOBt = 1-Hydroxybenzotriazole; DTZc = dicarboxylate 1,5-diphenyl-3-thiocarbazone] (n=3)

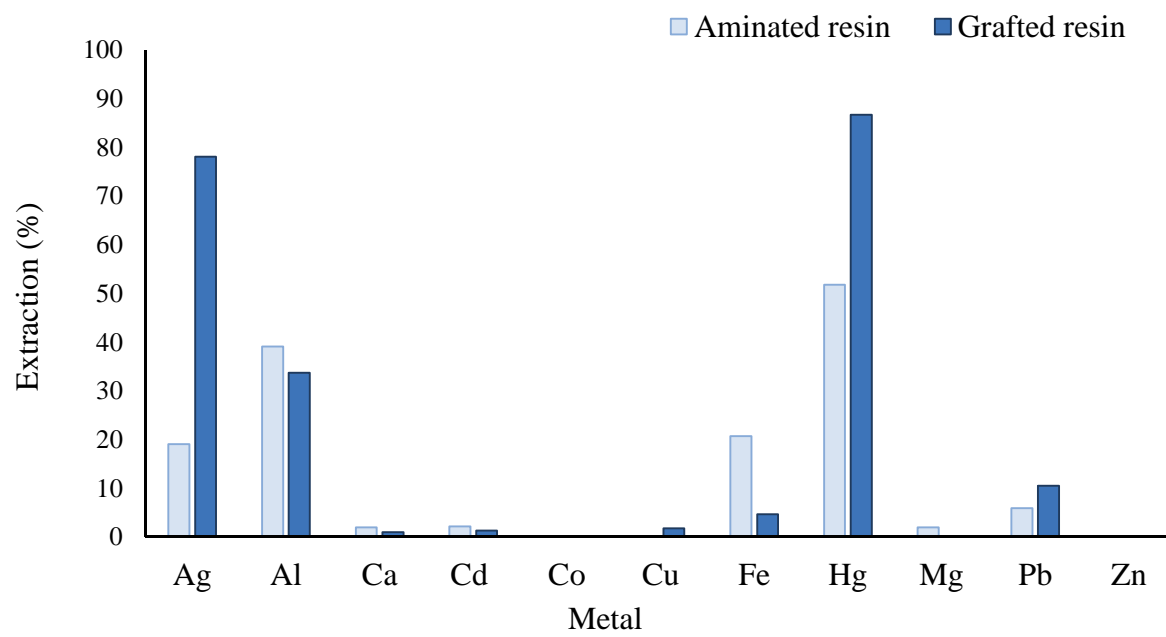
Operating conditions	Quantity of NH₂ functions ADECA test (a.u.)	
EDC/HOBt concentration (g.L ⁻¹)	5	0.32 ± 0.01
	10	0.37 ± 0.03
	20	0.37 ± 0.05
	40	0.38 ± 0.03
DTZc concentration (g.L ⁻¹)	1	0.35 ± 0.02
	2.5	0.33 ± 0.03
	5	0.43 ± 0.02
	10	0.77 ± 0.04
Temperature (°C)	25	0.35 ± 0.02
	40	0.35 ± 0.02
	60	0.37 ± 0.02
Grafting time (min)	10	0.80 ± 0.11
	30	0.31 ± 0.02
	60	0.31 ± 0.02

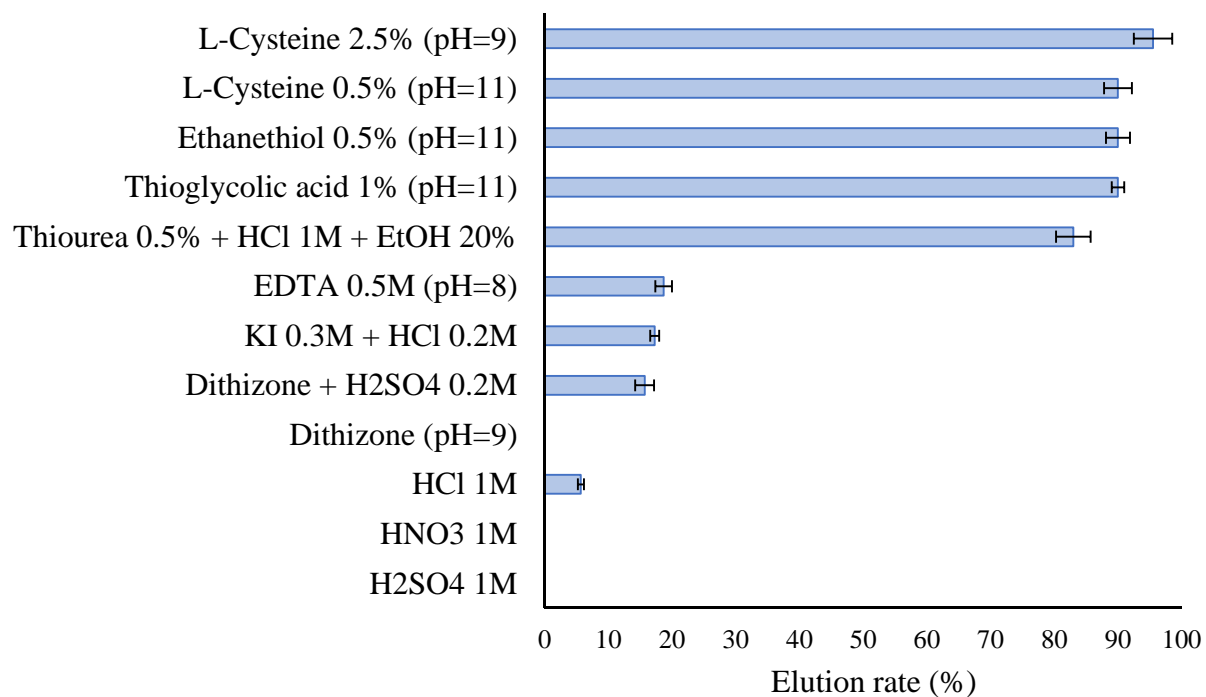


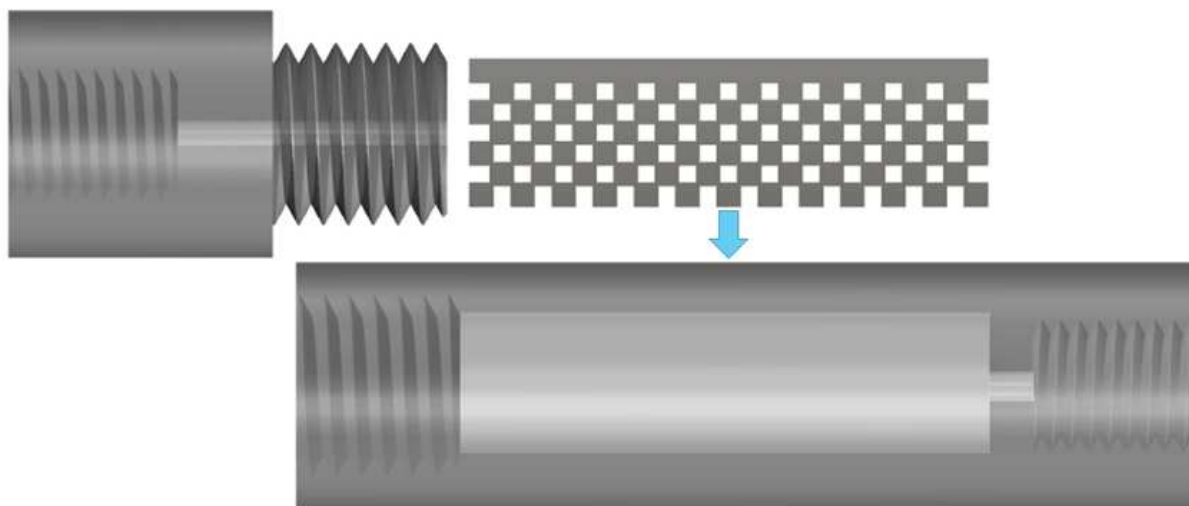




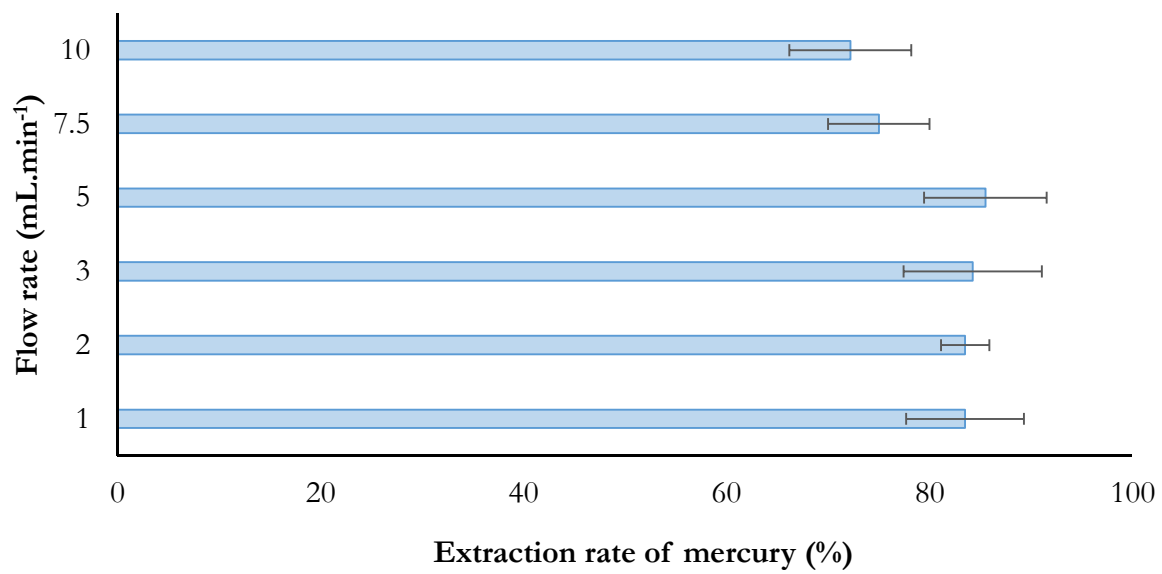








ACCEPTED MANUSCRIPT



Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: