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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.

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

Introduction

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

In the light of its toxicity and its numerous sources of pollution, the World Health Organization has recommended a guideline value of 6 µg L^{-1} for inorganic mercury in drinking water and European regulations have set a maximal allowable concentration (MAC) in natural waters at 0.07 µg L^{-1} for mercury and its compounds. Accordingly, the development of simple, rapid and sensitive methods for on-line Hg determination in natural waters has attracted widespread attention in modern analytical chemistry. Flow analysis seems to be appropriate to meet these specific requirements: this technology allows the decrease of instrumentation size and of reagents and energy consumption, which are significant advantages to simplify the manipulations required for the assays.

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

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

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

2. Materials and methods

2.1. Reagents and solutions

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

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

Modification of 3D printed devices was carried out with hexane diamine (Fisher Chemical, USA), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide (EDC, GenScript, USA) and 1-Hydroxybenzotriazole (HOBt, Fluka, USA).
Detection was performed by two techniques: spectrophotometric detection with a reagent composed of dithizone (Sigma-Aldrich, USA) and thiourea (Sigma-Aldrich, USA) prepared in a glycine buffer solution (Sigma-Aldrich, USA) in 30% absolute ethanol v/v (Sigma-Aldrich, USA), whereas hydrochloric trace metal grade (Fisher Chemical, USA) and sodium borohydride (Sigma-Aldrich, USA) were prepared in hydroxide solution (Fisher Chemical, USA) for CV-AAS detection.

2.2 Synthesis of DTZc

Dicarboxylate 1,5-diphenyl-3-thiocarbazone (DTZc) was synthesized following the conditions defined by Shenashen et al. [19]. Briefly, 1.9 g of hydrazinobenzoic acid were mixed with 0.6 mL of carbon disulfide, 20 mL of ethanol, 0.5 g of sodium hydroxide and 15 mL of distilled water. The mixture was heated to reflux for 5 hours; then DTZc was precipitated as a white solid by addition of ethanol, filtered, washed and dried. The reaction yield was 22% (0.95 g of dicarboxylate 1,5-diphenyl-3-thiocarbazone).

2.3 Modification of 3D printed device

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

2.4 Detection

Elution of modified supports after mercury extraction was carried out with a 0.5% m/v solution prepared from L-cysteine.
For CV-AAS detection, a 6 $mol.L^{-1}$ solution of hydrochloric acid (trace metal grade) was used with a 0.135 $mol.L^{-1}$ solution of sodium borohydride prepared in 0.125 $mol.L^{-1}$ sodium hydroxide solution.
Concerning the colorimetric detection, a post-elution photo-oxidation step was first performed using hydrogen peroxide and hydrochloric acid in order to obtain a final concentration of 0.5 $mol.L^{-1}$ of each reagent in the eluent. At the end of the photo-oxidation step, a 1.1 $mol.L^{-1}$ solution of ascorbic acid was then added to eliminate the excess of hydrogen peroxide before detection. The final colorimetric reagent was prepared according to the conditions described by Theraulaz et al. [20] with a 0.01 $mmol.L^{-1}$ solution of dithizone prepared in a 0.01 $mol.L^{-1}$ glycine buffer solution with 30% ethanol v/v, and mixed with a 0.001 $mol.L^{-1}$ thiourea solution (Sigma-Aldrich, USA).

2.5. Apparatus

2.5.1. Metal analysis

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

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

2.5.2. 3D printing

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

2.5.3. Flow systems

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

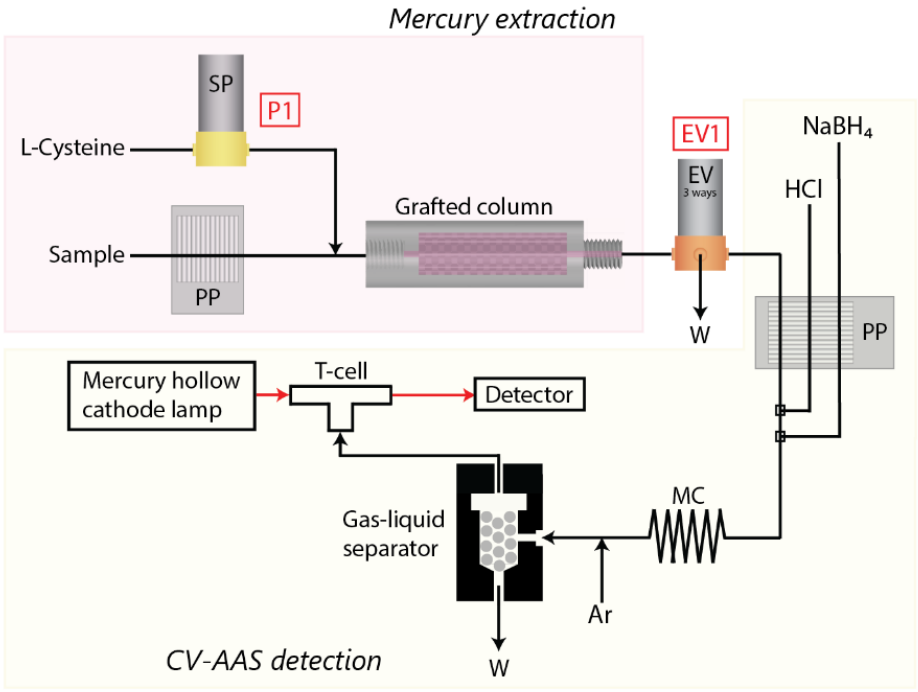

Figure 1: Flow system for mercury determination with CV-AAS detection [P1: solenoid micropump; PP: peristaltic pump; EV: solenoid valve; MC: mixing coil; W: waste; OF: optical fiber; Ar: argon].

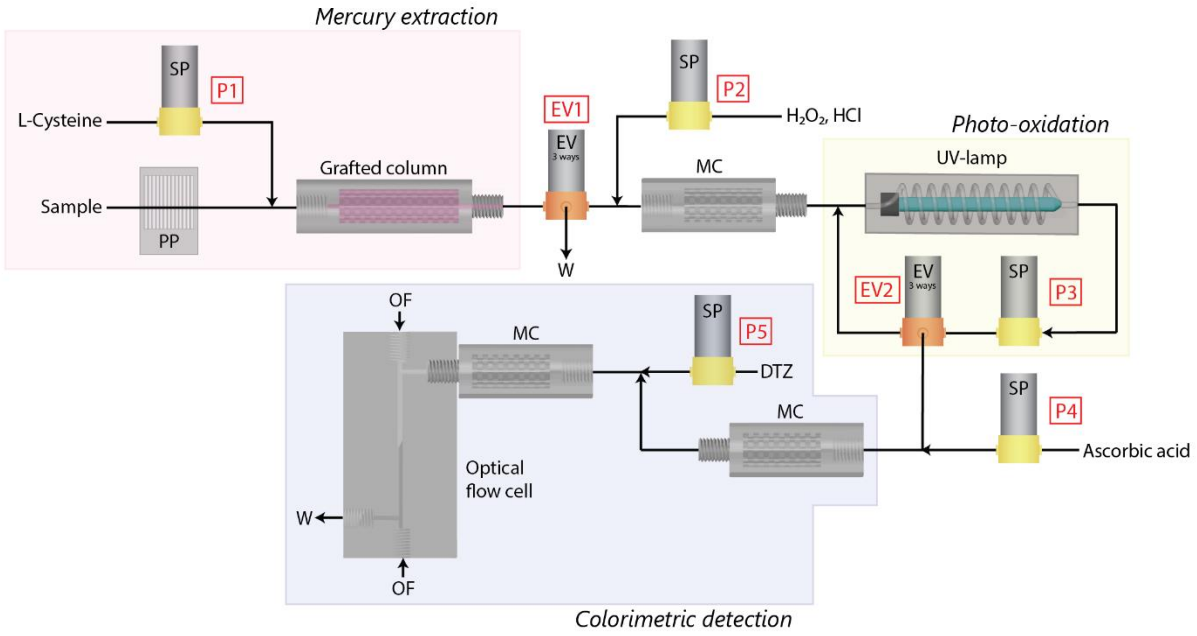

Figure 2: Flow system for mercury determination with colorimetric detection [P1-P4: solenoid micropumps; PP: peristaltic pump; EV: solenoid valve; MC: mixing coil; W: waste; OF: optical fiber; DTZ: dithizone].

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

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

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

3. Results and discussion

3.1. Modification of 3D printed device

Stereolithography resins are mainly composed of methyl methacrylate, which is an ester monomer. After photopolymerization and poly(methyl methacrylate) (PMMA) formation, this ester group can be used to graft molecules onto the surface of 3D printed objects. This grafting procedure requires two steps (global procedure is summarized in Fig. 3): first amine functions were introduced onto 3D printed surface by amination reaction of the ester functions of the PMMA with hexane diamine [21], followed by grafting of DTZc using an amidation reaction with carboxylate function of DTZc. A pink coloration of the solid surface after cleaning at the end of the procedure demonstrates efficiency of the DTZc grafting (supplementary materials Fig. S1).

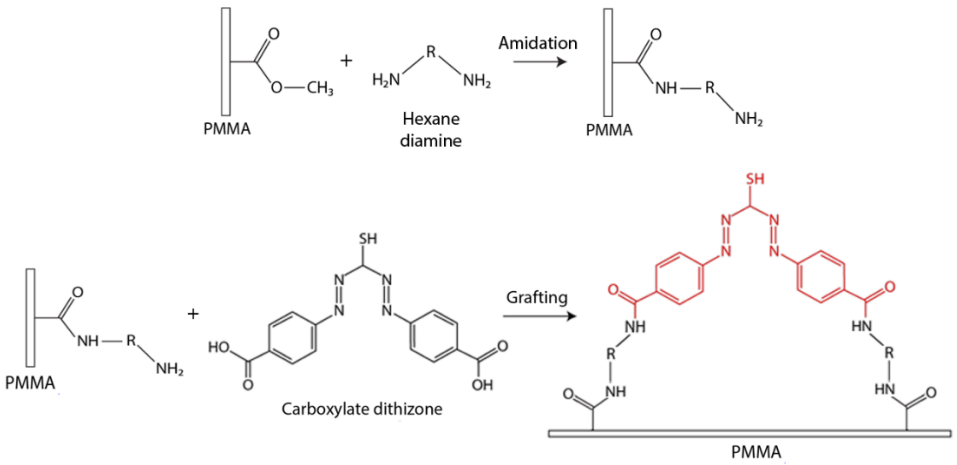

Figure 3: Grafting pattern of DTZc onto PMMA.

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

3.1.1. PMMA amination step

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

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

Operating conditions		**Quantity of NH_2 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

3.1.2 DTZc grafting

The DTZc grafting was studied after amination of 3D printed disks according to the best operating conditions described previously. Four parameters have been optimized by a one-

factor-at-a-time method: activating agents (EDC and HOBt) concentration, DTZc concentration, temperature and grafting time. The influence of each parameter on the grafting efficiency was evaluated based on the results of the ADECA test. In this case, a low value of the ADECA test indicates a good efficiency of the grafting reaction, as it shows a decrease in the number of free amine groups onto the PMMA surface.

The results are summarized in Table 2. Concerning activating agents EDC and HOBt, a concentration of 5 $g.L^{-1}$ for both reagents was enough to obtain the best grafting rate (0.32 ± 0.01). For the DTZc concentration, we observed no significant difference between 1 and 2.5 $g.L^{-1}$ (with ADECA test results at 0.35 ± 0.02 and 0.33 ± 0.03 respectively). A concentration of 1 $g.L^{-1}$ was thus selected for further experiments in order to limit the consumption of the reagent. No effect of temperature has been observed and the grafting reaction could therefore be performed at room temperature (25°C). Finally, a minimum reaction time of 30 min was necessary to obtain a good grafting efficiency and no significant difference was observed between 30 and 60 min.

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 NH_2 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

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

3.2.1 Extraction of metals

Shenashen et al. [19] have previously demonstrated that mercury was bonded to two molecules of DTZc through S and one N atom, which is consistent with dithizone reactivity, a well-known ligand which forms ML_2 complexes with various metals (including mercury) through S and N bonding.

After the optimization of the grafting procedure, mercury extraction was studied following various conditions. Raw resin, aminated resin, and grafted resin disks were immersed for 1 h in a 100 µg.L^{-1} mercury solution in PTFE tubes, at pH values between 1 and 9. The mercury solution was analysed by CV-AAS before and after extraction. The results obtained (extraction rate calculated from the concentrations before and after extraction) are summarized in Fig. 4.
For pH value above 4, an average extraction of 38% of mercury by raw resin was observed (min 24% at pH = 4; max 51% at pH = 9), which is characteristic of the adsorption of a metal on a plastic material surface, as it was previously demonstrated for polyethylene [23]. In acidic medium (pH=2), mercury was thus not extracted by the raw resin material (less than 1%): this result shows the importance to work at acidic pH to avoid the contamination of 3D printed pieces during the flow procedure. Aminated resin disks also showed a partial extraction of mercury in a pH range between 2 and 9, probably due to the complexation of mercury by the amine functions (min 32% at pH = 2, max 55% at pH = 7).

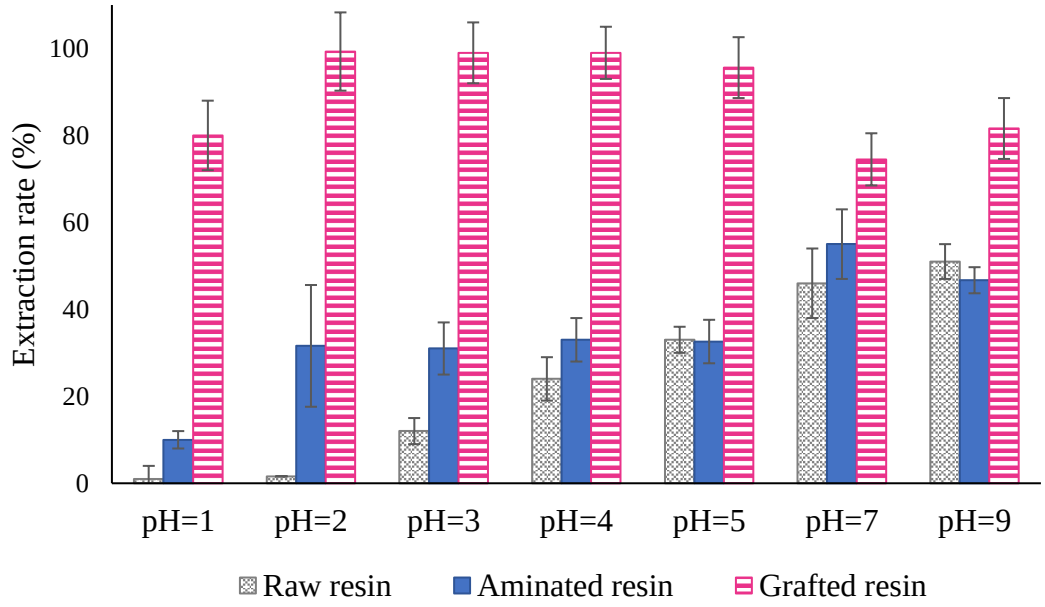

Figure 4: Batch extraction of a 100 µg.L^{-1} mercury solution (V=50 mL) on raw resin, aminated resin and DTZc grafted resin disks versus pH (n=3).

Results showed complexation of mercury by DTZc onto the surface of disks, as shown by the significant increase of extraction rate between the raw resin disks and the grafted resin disks at acidic pH values. Concerning the grafted resin disks, the extraction in acidic medium was very efficient, with a mercury extraction rate of 99%. The acidification of the samples at pH=2 before extraction by the grafted resin was the best compromise to obtain good extraction performance and to avoid adsorption phenomena on the plastic surfaces. At this pH value, extraction kinetics was rapid since a mercury extraction rate of 90% was reached after a contact time of 1 minute (supplementary materials Fig. S2).
The selectivity of the DTZc grafted resin was tested with a multi-metal solution (aluminium, calcium, cadmium, cobalt, copper, iron, lead, magnesium, mercury, silver and zinc) at pH=2. This solution was extracted on the aminated and the grafted resins, and solutions were analysed

by ICP-AES before and after extraction (Fig. 5). The results showed a good extraction of silver, a partial extraction of aluminium and a low extraction of lead both for aminated and grafted resins. Iron was also partially extracted on the aminated resin (20%), but extraction rate on DTZc grafted resin was below 5%.

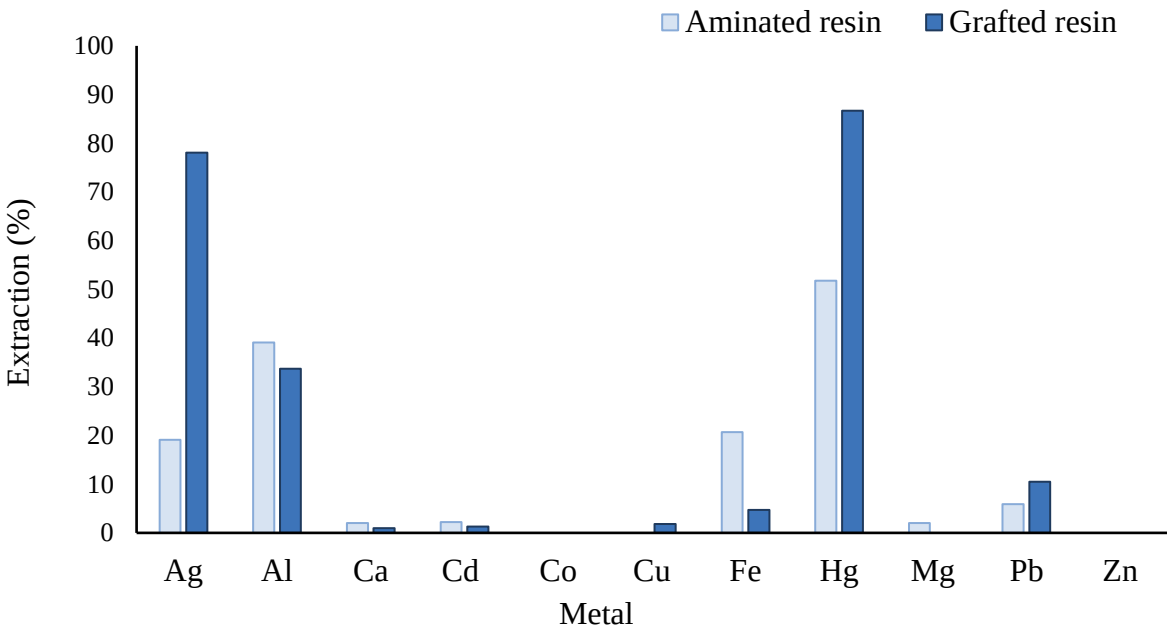

Figure 5: Batch extraction of a multi-metal solution at 10 µmol.L^{-1} (aluminium, calcium, cadmium, cobalt, copper, iron, lead, magnesium, mercury, silver, and zinc; V=50 mL) at pH=2 on the aminated resin disks and the grafted resin disks.

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

3.2.2 Elution

After the extraction step, it was necessary to find a suitable eluent to recover the mercury extracted by the DTZc grafted resin. Several reagents were studied, and the tests are summarized in Fig. 6 (elution rates were calculated by the difference between the amount of mercury extracted on the grafted supports and the amount of mercury in the eluate solutions). Inorganic acids (hydrochloric, sulfuric and nitric acid), which are well known eluents for metals, were not efficient to elute mercury, even with 1 mol.L^{-1} concentration (higher concentrations were not tested to avoid the 3D resin degradation). Dithizone, EDTA and potassium iodide were also tested, but resulted respectively in 16%, 19% and 18% elution rates. Several eluents with thiol or thiocarbonyl functions were also studied: L-cysteine, ethanethiol, thioglycolic acid and thiourea. These four reagents were very efficient to elute mercury with elution rates higher than 90 %, except thiourea with 83%. These results are consistent with the high mercury affinity for thiolate compounds (R-SH) [24]. Recently Liem-Nguyen et al. [25] determined a formation constant of $Hg(L)_2$ complexes with cysteine and thioglycolic acid of

41.5 and 37.5 respectively. The three thiol compounds tested showed almost identical elution rates, and L-cysteine was chosen for further experiments as the least toxic and malodorous sulfur compound.

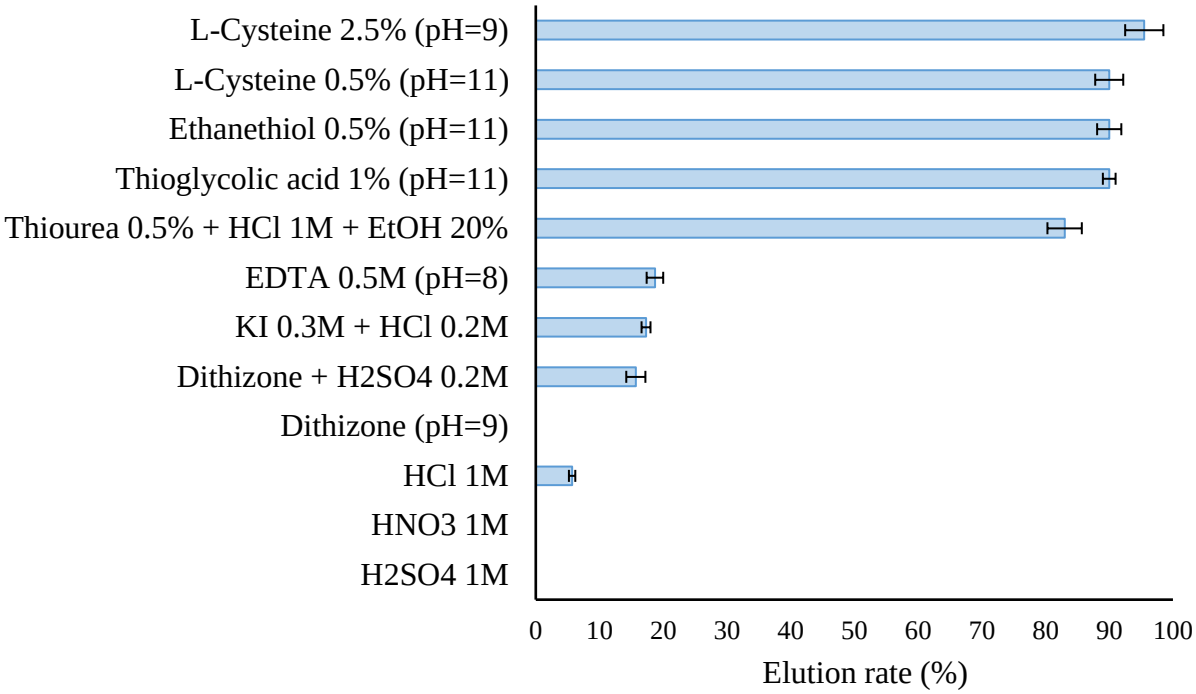

Figure 6: Batch elution of mercury extracted on DTZc grafted resin with different eluents (V=50 mL; n=3).

3.3. Application

3.3.1. Off-line sampling

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

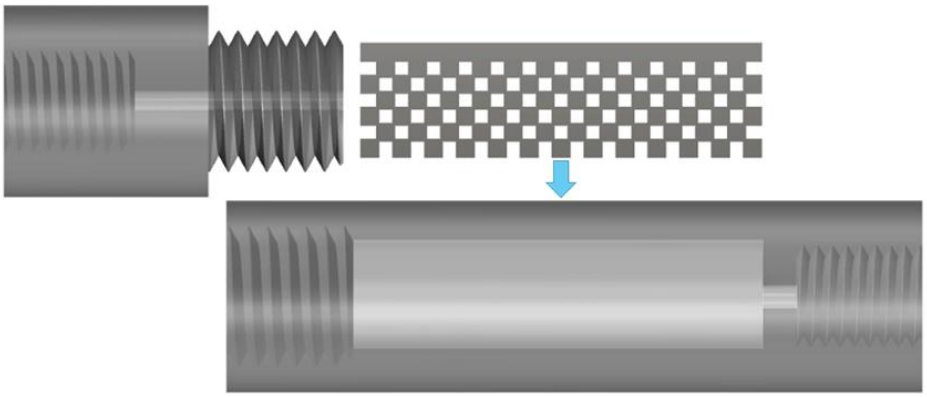

Figure 7: Scheme of removable column of grafted cuboids.

To optimize the efficiency of the extraction step, the mercury extraction flow rate was studied between 1 and 10 mL.min^{-1} by means of a peristaltic pump. The results are depicted in Fig. 8. Flow rate showed no significant effect on extraction rate between 1 and 5 mL.min^{-1} with an average mercury extraction rate of 85%. Comparison of extraction efficiency versus higher sample flow rates showed a 12% and 17% decrease respectively for sample flow rates of 7.5 mL.min^{-1} (mercury extraction rate of 75%) and 10 mL.min^{-1} (mercury extraction rate of 72%). Nevertheless, despite the 17% loss, a sample flow rate of 10 mL.min^{-1} could be a good compromise between the extraction efficiency and the extraction time (or sample volume). A sample flow rate of 10 mL.min^{-1} enables in a given time a significant increase of the preconcentration factor by percolating higher volumes of sample. For a 1 h sample percolation, a preconcentration factor of 23 is thus obtained for a sample flow rate of 5 mL.min^{-1} versus 38 for a sample flow rate of 10 mL.min^{-1}.

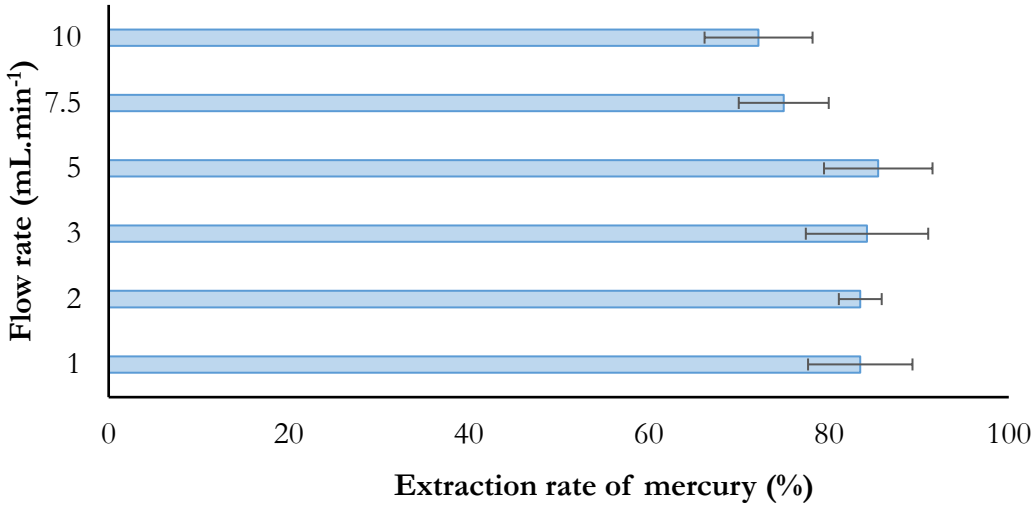

Figure 8: Influence of flow rate on the extraction of a 100 µg.L^{-1} mercury solution in a grafted cuboids column (n=3).

After the extraction step, the entire cuboids assembly can be removed from the column and introduced in a nickel boat of a mercury analyzer Altec AMA 254 which is specifically conceived for the fast, precise and simple determination of mercury traces in solids samples. Note that with this technique, it was necessary to perform a blank on grafted cuboids before extraction, because the analysis of the raw resin used for 3D printing has showed a mercury concentration of 9.6 ± 3.2 ng.g^{-1}.

3.3.2. Flow systems

Mercury can also be quantified in solution after elution by L-cysteine by CV-AAS (Fig. 1). As the mercury concentration is usually low in environmental samples, it is necessary to work with large sample volumes to achieve a large preconcentration factor. In this study, the sample volume was set at 500 mL to limit the analysis time (mercury extraction was successfully tested for sample volumes up to 1 L). Experiments were carried out to determine the minimum volume of L-cysteine which has to be used to quantitatively elute the mercury extracted on modified supports. Results showed that a minimum volume of 10 mL of 0.5% m/v L-cysteine in borate

buffer (pH=11) should be used to elute 92% of the mercury extracted on the modified support (Supplementary materials, Figure S3).

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

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

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

Conclusion

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

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

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Modified 3D-printed device for mercury determination in waters

Supplementary Materials

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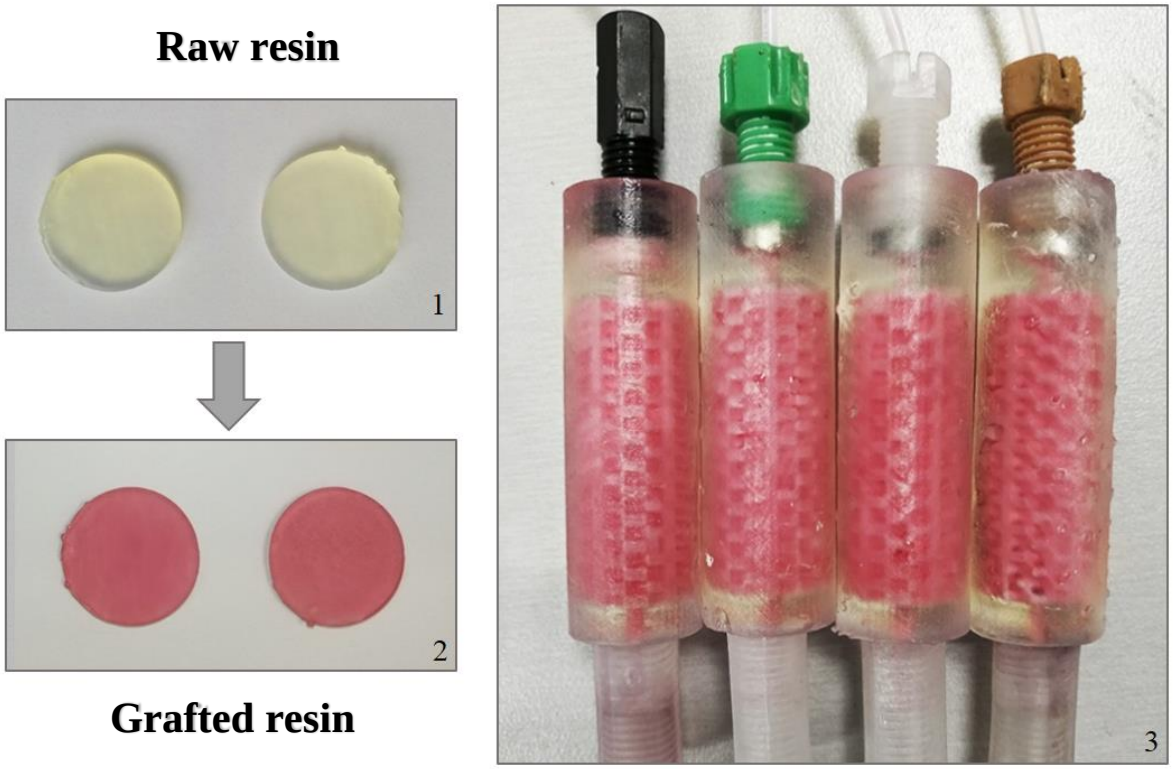

Figure S1: Pictures of 3D printed objects during grafting steps (1: raw resin disks; 2: grafted resin disks; 3: grafted cuboids columns).

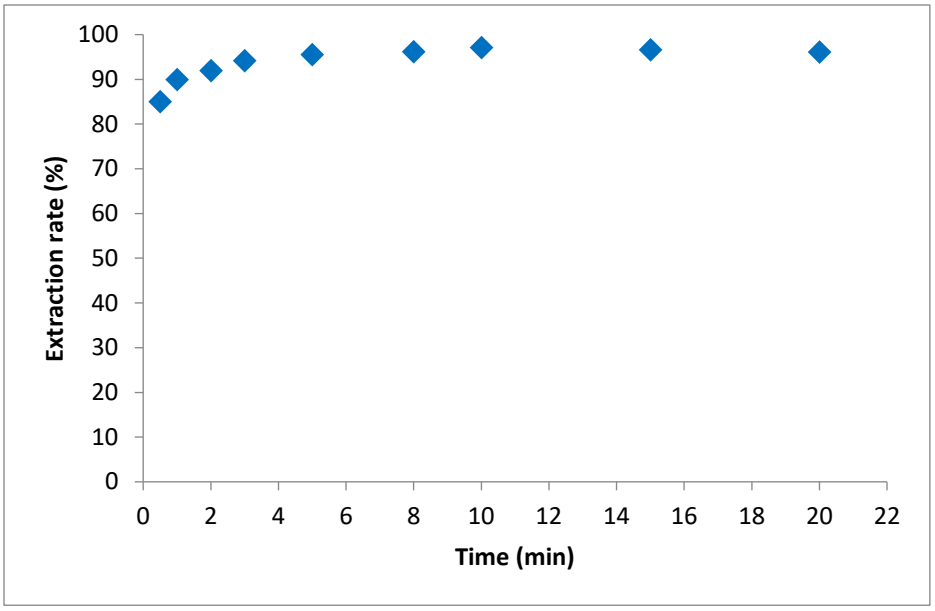

Figure S2: Extraction rate versus contact time at pH=2.

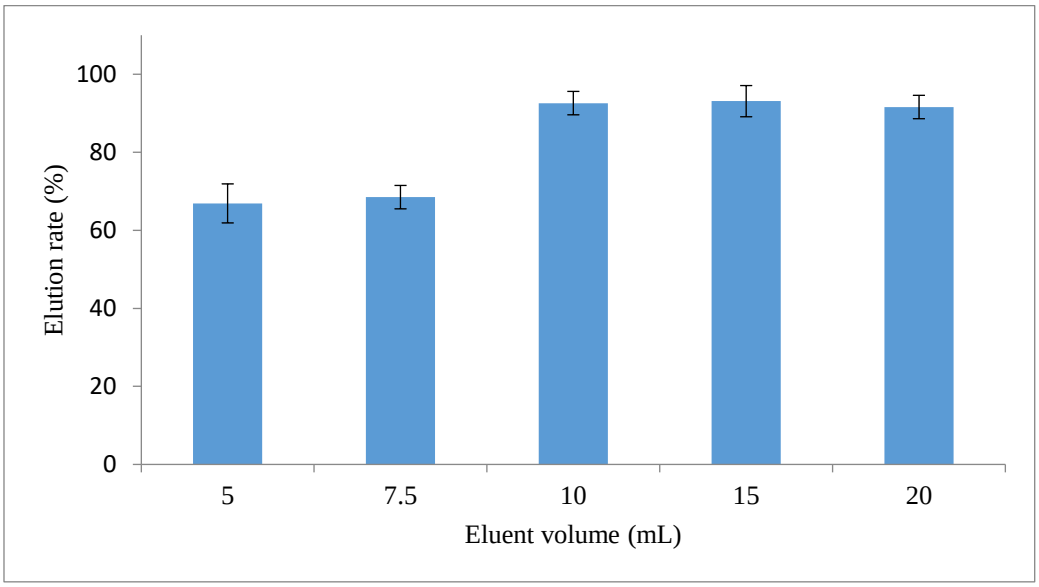

Figure S3: Elution rate by L-cysteine versus eluent volume (L-cysteine 0.5% m/v in 100 mM borate buffer pH=11).