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Optical microfluidic system based on ionophore modified gold nanoparticles for the continuous monitoring of mercuric ion

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ABSTRACT

An optical microfluidic system based on the use of modified gold nanoparticles for monitoring Hg(II) is presented. The system is based on the specific recognition of the heavy metal by a new synthesized ionophore based on a modified thiourea, which is attached to the gold nanoparticles. This interaction generates a change on the gold Surface Plasmon Resonance (SPR) band. The sensitivity and selectivity of the procedure is firstly studied in batch. The obtained results demonstrate the mercury selective response over the different tested ions that can be found in environmental water samples. Due to the remarkable unusual rapid signal change observed during the interaction of the metal and the modified gold nanoparticles, the reaction can be easily performed in a microfluidic system. Results obtained by using the microfluidic system revealed improved analytical features compared to batch experiments such as a lower detection limit (11 ppb), higher sensitivity and faster analysis time, all this with an easy and automated procedure. Therefore, the approach has shown great potential for designing low cost instrumentation for automatic in-field discrete or continuous measurements of Hg(II).

1. Introduction

The growth of industrialization, urbanization and human population increases pollution problems. Industrial wastes, even in very small concentrations, are often extremely toxic. Among other heavy metals, mercury is one of the greatest concerns due to its bioaccumulation [1] and because it can be easily released in water, soil or air by some different industrial and anthropogenic sources [2,3], it produces toxic effects in living organisms [4]. Because of its importance for human health, there is a strong need to develop new methods for its determination and on-site monitoring.

Current analytical methods for Hg(II) detection are based on electrochemical methods [5], inductively coupled plasma mass spectrometry [6], cold vapor atomic absorbance [7] or fluorescence [8] spectrometry and high performance liquid chromatography [9]. All they are time consuming analytical approaches that frequently require qualified personnel and cannot perform in-field measurements. To overcome these limitations, other simpler methods based on the use of different (bio)sensors incorporating selective or specific recognition elements and optical detection have been proposed, which also show low detection limits [10,11]. Some of them are based on the use of DNAzymes, oligonucleotides or cells [12,13], which considerably increase the cost of the whole procedure, and usually cannot be reused. On the other hand, methods such as optodes, which are based on the use of fluoro/chromophores and iononophores, have also been widely used due to the high selectivity accomplished by the ionophores and their relative simple preparation [14,15]. However, the stability and life time of membranes is still a challenge, since dye leaching and/or photobleaching is a frequent problem.

The use of nanoparticles as signal transduction elements seems to offer great advantages. In concrete, metallic nanoparticles, such as gold colloidal, exhibit a strong SPR (Surface Plasmon Resonance) band of high molar absorption coefficient, which is highly sensitive to changes on the nanoparticles surface and the surrounding media [12]. Some analytical approaches based on the use of gold nanoparticles for heavy metals detection take advantage of the absorption wavelength shift, from red to blue, derived from the interaction of the capping ligand/modifier of the nanoparticles with the analyte, which produces the particle aggregation [16,17]. In this sense, there are several proposed optical sensors based on gold nanoparticles showing very low detection limits, which demonstrate the simplicity of the approach [18,19]. Despite this fact, the sensors have not been designed for the in situ monitoring.

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Miniaturization of analytical processes and microfluidics can provide several advantages over conventional methods. By reducing size of the developed analytical systems, it is possible to minimize the energy consumption, making feasible the development of low cost portable devices to perform in field measurements [20]. Moreover, microfluidics for continuous monitoring minimizes the amount of the required reagents and therefore the wastes generation, thus reaching a greener chemistry. Microfluidics offers also better control, in a very reproducible way, over certain mixture/reaction parameters than conventional methods, such as mass and heat transfer, thanks to the possibility of computer-controlled elements [21,22]. Furthermore, microfluidic platforms can integrate all the necessary steps of the analytical procedure, such as the sample collection, sample pretreatment, the required analytical separations, reactions and detection.

Very few papers have been presented until the date related to the monitoring of heavy metals in microfluidic systems based on nanomaterials, and they are based on the use of expensive reagents and costly and bulky instrumentation, as well as complicated pretreatment steps [23–25]. Herein, we purpose a simple, economic and miniaturized microfluidic system based on the use of modified gold nanoparticles for a rapid continuous monitoring of Hg(II).

Due to the optical properties of the thiourea-modified gold colloidal, it is possible to follow absorbance changes produced from the interaction of the ionophore with the heavy metal. On the other hand, analysis performed in the microfluidic system allows the easy and automatic manipulation of fluidics meanwhile changes on the absorption maximum of the nanoparticles is continuously monitored.

2. Materials and methods

2.1. Chemicals, materials and instruments

For the synthesis of gold nanoparticles, hydrogen tetrachloroaurate (III) hydrate was provided by Fluka (p.a. ACS, ≥49% Au); sodium borohydride by Panreac with 98% purity and tiopronin (N-(2-Mercaptopropionyl)glycine) (TP) was obtained from Sigma–Aldrich with 99% purity. All solutions were prepared in double distilled water. For the synthesis of the ionophore the following reagents were also purchased from Sigma–Aldrich: p-hydroxyacetanilide; potassium carbonate; 1,4-dibromobutane; carbon tetrachloride; thiourea; *n*-butanol; ethanol; NaOH; HCl; CH_2Cl_2; Na_2SO_4; sulfanilic acid; thiophosgene; THF and ether. Boron trifluoride dimethanol complex 50% was supplied from Acros.

Mercuric ion stock solution is prepared with mercury (II) nitrate monohydrate 99% and sodium acetate and acetic acid. All purchased from Sigma–Aldrich.

The purification of the obtained colloidal was performed by centrifugal filtration in 4 mL centrifugal filter devices (CENTRIPLUS YM30, MICROCON, MWCO 30000) at 3000 rpm for 30 min. For UV–visible spectra, a Shimadzu UV-310PC UV–vis–NIR double-beam scanning spectrophotometer (Kyoto, Japan) was used between 800 and 200 nm. The shape and dimensions of the core of the particles were measured by a transmission electron microscope (TEM), JEOL 1400, and a high resolution electron microscope (HRTEM), JEOL 2011 (Tokyo, Japan). Samples were prepared by dipping a copper grid, coated with a thin carbon film, in the gold nanoparticles suspension at room temperature. A Malvern Instruments Ltd., Zetasizer Nano ZS (Worcestershire, UK) was used for Dynamic Light Scattering (DLS) and ζ potential measurements. For RMN measurements, a Bruker 250 MHz equipment (Karlsruhe, Germany) was used. Elemental analysis was performed by an Optima 4300DV, Perkin-Elmer Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Massachusetts, USA).

For the microfluidic platform construction a micromilling Computer Numerically Controlled (CNC) machine (Protomat C100/HF, LPKF Laser & Electronics, Garbsen, Germany) and a specially fabricated uniaxial hydraulic press (Talleres Francisco Camp S.A., Granollers, Spain) were used.

Different grades of COC Topas (Topas Advanced Polymers, Florence, KY, USA) were chosen as polymeric substrate: Topas 6013 layers (from 500 to 1000 μm) and Topas 8007 foils (25 μm).

2.2. Preparation of the selective recognition optical element

The first step was the synthesis of the gold colloidal suspension and, in order to make selective the response of the measurement system to Hg(II), a new ionophore based on a thiourea derivative was synthesized. This was later attached to the surface of the gold nanoparticles, the whole acting as the recognition and transducing element.

2.2.1. Synthesis and characterization of AuNPs@Tiopronin

The synthesis of gold nanoparticles (AuNPs) was performed in a ceramic microfluidic platform, thoroughly described elsewhere [26]. Tiopronin was chosen as the colloidal stabilizer to prevent aggregation and to make feasible the manipulation of AuNPs. Briefly, aqueous solutions of 1 mM $HAuCl_4$, 1.5 mM $NaBH_4$ and 1 mM tiopronin placed in Hamilton syringes (Hamilton series GASTIGHT 1000 TLL, Bonaduz, GR, Switzerland) were propelled by means of syringe pumps (540060 TSE systems, Bad Homburg Germany) to the ceramic microfluidic platform, which consisted of four reagent inlets and a three-dimensional serpentine micromixer. Once the chemical and hydrodynamic parameters of the reaction were optimized, the dispensing protocol consisted in the continuous pumping of the $NaBH_4$ solution at a 1 μl s^{-1} flow rate, where 0.7 μl of the $HAuCl_4$ solution was sequentially dispensed every 2 s at a 2.5 μl s^{-1} flow rate. These solutions were mixed in the serpentine and after that, the tiopronin solution was continuously pumped at 0.5 μl s^{-1}. The obtained particles were purified by centrifugation. The final solution was adjusted to pH 8.5, since it ensures the ionization of the tiopronin functional group, which confers an extra stabilization to the colloidal for the electrostatic repulsions generated. In this way, the colloidal can be stored for a long time. UV–vis spectroscopy characterization showed a stable SPR band centered at 518 nm. Transmission electron microscopy (TEM) and electron diffraction (SAED) pattern of the selected area revealed crystalline homogeneous particles of a mean core size of 3.4 nm. Meanwhile, dynamic light scattering (DLS) and ζ potential results gave particles of 10.1 nm hydrodynamic diameter and −36.1 mV. NPs characterization is shown in the Supporting Information (Fig. S1).

2.2.2. Synthesis and characterization of the ionophore

Since thiourea and its derivatives usually present chelating capability toward heavy metals, the goal was to synthesize a derivative, which could also be easily attached to the gold surface. For this purpose, different strategies can be selected, such as coupling by physical interactions (hydrophobic, electrostatic) or by using the specific recognition of biomolecules, one of them attached to the AuNPs (such as the avidin–biotin interaction). In this case, a simple covalent coupling has been preferred, since it confers higher stability to nanoparticles. Thus, a N-(4-sulfonatophenyl)-N′-[4-(4-mercaptobutoxy)-phenyl]thiourea was synthesized. Its structure has a short C_4 hydrocarbon chain with a -SH terminal group (a mercaptobutoxy group) to be linked to the gold surface and a sulfonate group to assure its solubility in water. A scheme from the synthetic route is depicted in Fig. 1 and consisted of five simple reactions.

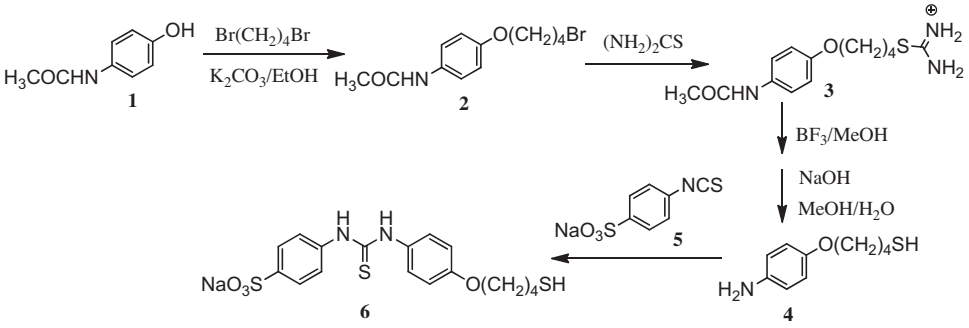

Fig. 1. Scheme from the synthetic route of the ionophore N-(4-sulfonatophenyl)-N'-[4-(4-mercaptobutoxy)-phenyl]thiourea sodium salt (**6**).

^{1}H RMN and mass spectroscopy data are given next (also shown in Supporting Information, Fig. S2). The total yield of the reaction was 9.3%.

Synthesis of 4-(4-bromobutoxy)-acetanilide (2) [27]: A mixture of p-hydroxyacetanilide (**1**) (7.57 g, 50 mmol), potassium carbonate (6.90 g, 50 mmol) and 1,4-dibromobutane (16.2 g, 75 mmol) in ethanol (250 mL) was heated at reflux. When the reaction was completed (about 8 h) the mixture was poured into crushed ice and crystallized from carbon tetrachloride. Yield 6.72 g, 47%. m.p. 100–102 °C (lit.[24] m.p. 101–102 °C).

^{1}H NMR (DMSO-d6): 1.74–1.88 (m, 2H), 1.89–2.03 (m, 2H), 2.00 (s, 3H), 3.59 (t, *J* = 7 Hz, 2H), 3.94 (t, *J* = 7 Hz, 2H), 6.85(d, *J* = 9 Hz, 2H), 7.46(d, *J* = 9 Hz, 2H), 9.76 (s, 1H).

*Synthesis of 4-(4-acetamidophenoxy)-butylisothiuronium bromide (**3**)*: A mixture of (**2**) (5.72 g, 50 mmol) and thiourea (1.52 g, 50 mmol) was heated at reflux for 5 h in *n*-butanol (50 mL). After cooling, the precipitate was filtered out and washed with cold ethanol and ether. After drying on air, it yielded 6.00 g, 83%. ^{1}H NMR (DMSO-d6): 1.77 (br.s, 4H), 2.00 (s, 3H), 3.22 (t, *J* = 6 Hz, 2H), 3.94 (t, *J* = 6 Hz, 2H), 6.85 (d, *J* = 10 Hz, 2H), 7.48 (d, *J* = 10 Hz, 2H), 8.97 (br.s, 2H), 9.10 (br.s, 2H), 9.84 (s, 1H).

*Synthesis of 4-(4-aminophenoxy)-butanethiol (**4**)*: 5.43 g (15 mmol) of (**3**) and 10.0 mL (95 mmol) of boron trifluoride dimethanol complex 50% in 60 mL of methanol were heated at refluxed for 10 h (NMR of the probe showed absence of NH-protons). The mixture was transferred to a screw-capped Shott 100 mL bottle, and argon was bubbled while a solution of 5.0 g (125 mmol) NaOH in 20 mL of water was added on cooling in ice water. The bottle was closed and heated in a boiling water bath for 4 h. Then the solution was neutralized with HCl up to pH 5 (under argon), the solvents were evaporated in vacuo and the residue was taken up with CH_2Cl_2. The extract was dried with Na_2SO_4. The solvent evaporated in vacuo and the residue was stirred with hot hexane, filtered and after evaporation of hexane in vacuo, it yielded 1.27 g (43%) of yellowish oil. ^{1}H NMR ($CDCl_3$): 1.39 (*t*, J = 7 Hz, 1H), 1.70–2.00 (m, 4H), 2.60 (q, *J* = 7 Hz, 2H), 3.91(t, *J* = 6 Hz, 2H), 6,40 (d, *J* = 9 Hz, 2H), 6.75 (d, *J* = 9 Hz, 2H).

*Synthesis of 4-isothiacyanatobenzoic acid sodium salt (**5**) (Modified procedure from* [28]*)*: Sulfanilic acid (1.73 g, 10 mmol) was dissolved in 15 mL of water, containing 2.46 g (30 mmol) of sodium acetate. Thiophosgene (1.15 g, 10 mmol) in 1.5 mL of THF was added. After stirring at room temperature for 2 h the precipitate was filtered, washed with cold ethyl acetate and dried in vacuo. Yield 1.47 g (62%). ^{1}H NMR (D_2O): 7.34 (d, *J* = 8 Hz, 2H), 7.72 (d, *J* = 8 Hz, 2H).

Synthesis of N-(4-sulfonatophenyl)-N′-[4-(4-mercaptobutoxy)-phenyl]thiourea sodium salt (**6**): 4-Isothiacyanatobenzoic acid sodium salt (**5**) (173 mg, 1 mmol) was dissolved in 2 mL of dry dimethylacetamide under stirring at room temperature. 4-(4-Aminophenoxy)-butanethiol (**4**) (197 mg, 1 mmol) was added, and stirring was continued for 24 h. The resulting solution was poured into 30 mL of dry ether. The precipitate was washed for several times with ether and dried in vacuo. Yield 386 mg, 89%. m.p. > 300 °C ^{1}H NMR (DMSO-d6): 1.60–1.90 (m, 5H), 2.54 (q overlapped with DMSO, 2H), 3.90–4.05 (m, 2H), 6.89 (d, *J* = 9 Hz, 2H), 7.31 (d, *J* = 9 Hz, 2H), 7.42 (d, *J* = 9 Hz, 2H), 7.54 (d, *J* = 9 Hz, 2H), 9.71 (s, 1H), 9.77 (s, 1H). NMR H1 (D_2O): 1.64 (br.s, 2H), 1.75 (br.s, 2H), 2.47 (br.s, 2H), 3.98 (br.s, 2H), 6.92 (br.s, 2H), 7.13 (br.s, 2H), 7.35 (br.s, 2H), 7.69 (br.s, 2H).

ES-MS(-): 411.0347 (calc 411.0507).

2.2.3. Synthesis and characterization of AuNPs@Ionophore

The final step was the ligand exchange on the AuNPs surface, which was accomplished by just mixing a solution of purified AuNPs@TP with an aqueous solution of the ionophore, taking into account that an excess of ionophore (1:10 of AuNPs:Ionophore) is necessary in order to obtain the desired exchange. The complete reaction took place in 12 h. Then, the solution was filtered repeated times in order to eliminate the excess of ionophore and the tiopronin present in the solution. Since the ionophore absorbs at 260 nm, the purification process could be followed by UV–vis spectroscopy of the filtered solution. The spectra of the modified gold colloidal show the characteristic SPR band at around 525 nm and a new band at 260 nm appears demonstrating the surface modification (Fig. 2). The obtained ionophore-activated AuNPs (AuNPs@Ionophore) were characterized by DLS and ζ potential at slightly basic pH values (7.5) to ensure its stability for a long time, which revealed stable (−31.4 mV) and larger particles (17.8 nm) (Supporting Information, Fig. S3). Results concerning TEM analysis revealed a mean size of 3.7 nm AuNPs@Ionophore (Fig. 2b). Inductively coupled plasma optical emission spectrometry (ICP-OES) results gave the calculated ratio of S:Au before and after the ligand exchange, which were 0.1 and 0.3 respectively (Supporting Information, Fig. S3). This also ensured that the ligand exchange was done.

2.3. Microfluidic system and set-up

2.3.1. Design and construction of the microfluidic platform

The microfluidic platform was fabricated with Cyclic Olefin Copolymer (COC), an amorphous polymer, which permits the construction and integration of bi and three-dimensional structures using a multilayer approach [29]. Polymers offer some advantages over other materials [30] as simple, cheap and rapid prototyping and the possibility of developing disposable devices due to their low cost. Furthermore, they are compatible with further integration of other components by means of UV curing adhesives. In concrete, COC, as a thermoplastic polymer, is in addition found at different polymerization grades, which entails that it is possible to use the ones with lower glass transition temperatures (Tg) as sealing layers. Thus, a simple lamination process permits obtaining specific 3D structures by a simple multilayer approach.

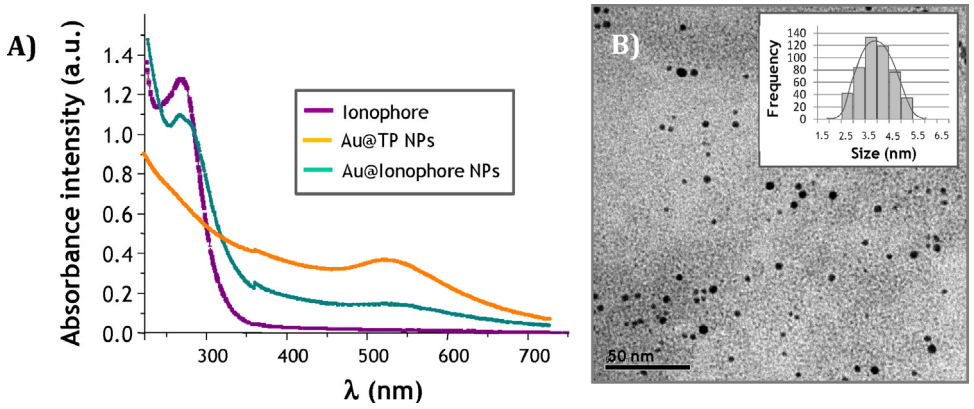

Fig. 2. (A) UV–vis spectra of the ionophore, the gold nanoparticles stabilized with tiopronin (Au@TP NPs) and with the ionophore (Au@Ionophore NPs) in solution (not at the same concentration). (B) TEM image from 3.7 nm Au@Ionophore NPs.

The constructed microfluidic platform consisted of three layers of the polymeric substrate. Once the design was created by means of Computer-Aided Design (CAD) software, it was transferred to a micromilling CNC machine in order to mechanize the polymeric substrate. Different grades of COC Topas were used. Topas 6013 layers (from 500 to 1000 μm) were used to mechanize the microfluidic structures, while Topas 8007 foils (25 μm) were used as adhesive layers. Once the pattern was mechanized by means of different End Mill and Spiral Drill Tools, the different layers were aligned, using fiducial holes, and laminated through a thermo compression process to completely seal the microfluidic platform. This lamination consisted in the following temperature profile at 4–6 bar pressure: a first drop temperature ramp from room temperature to 108 °C, a 10–15 min of temperature stabilization and a temperature decrease until room conditions.

The constructed microfluidic device consists of two initial inlets for reagents, a bidimensional micromixer of 400 μm width (total micromixer volume of 95 μL), a 2 cm × 1 mm × 1 mm (20 μL) optical detection chamber and a collection channel to the outlet. The total volume of the microfluidic platform is of 130 μL. Optical fibers are directly connected to the optical chamber by means of their insertion and gluing into two mechanized guides (1 cm × 1 mm × 1 mm) at both sides. A picture of the microfluidic device is shown in Fig. 3.

2.3.2. *Microsystem set-up and optical system*

The whole set-up was also composed of a flow injection analysis (FIA) system, which includes a peristaltic pump (Gilson Minipuls® 3, Middleton, USA) and an injection valve (Hamilton MVP, Bonaduz, GR, Switzerland). PTFE tubes (i.d. 0.9 mm) were used to connect the microsystem to chemical solutions.

On the other hand, a low Numerical Aperture Polymer Optical Fiber (NA POF) (Toray PMU-CD 1002–22-E, Tokyo, Japan) is used for light injection and a high NA POF (Mitsubishi ESKA Premier GH4001, Tempe, USA) for light collection from the optical window (Fig. 3). These fibers were directly glued (using a UV-Curing Norland Optical Adhesive: NOA61, Cranbury, NJ, USA) to the microsystem.

The optical detection system consists of the following components: a 525 nm Light Emitting Diode (LED) (Roithner Lasertechnik B5B-433-B525, Vienna, Austria), which is mounted in a Printed Circuit Board (PCB) and matches the absorption maximum of the AuNPs SPR band; a photomultiplier (H6780-03, Hamamatsu, Japan) and a Data Acquisition Card (National Instruments NI USB-6211, Austin, USA), which is connected to a PC that controls the system and modulates the LED signal. Details from the optical system are described elsewhere [31].

3. Results and discussion

In order to demonstrate the general features of the proposed microsystem, in terms of enhanced sensitivity, adequate selectivity toward Hg(II), easy handling of low volumes of reagents, adequate analysis time, automation and portability for in situ monitoring, and for comparison purposes, modified AuNPs were initially characterized with a conventional spectrophotometer in a batch mode. Sensitivity and selectivity were determined by mixing different aliquots of the colloidal solution AuNPs@Ionophore with increasing concentrations of mercuric ion, which were prepared in a 0.01 M acetic acid/sodium acetate buffer adjusted to pH 5.5. The modification of the absorption signal of the resulting SPR band was followed.

With the aim of achieving the maximum sensitivity and detection limit, different concentrations of the colloidal, obtained by dilution of the purified AuNPs@Ionophore, were tested. Solutions of about 1.5×10^{-3}% $HAuCl_4$ (4.7×10^{-8} M), 2.3×10^{-3}% (7.5×10^{-8} M) and 3.0×10^{-3}% (9.6×10^{-8} M), (calculated by a calibration curve using a commercial gold colloid of a 5 nm mean size (Aldrich), with an extinction coefficient of 2.3×10^{6} M^{-1} cm^{-1}) were prepared. The intermediate concentration gave the best results in terms of sensitivity and was chosen as the optimized to perform the following studies. Response to mercury (II) at low concentration range (0, 20, 40, 60, 80, and 100 ppb) and at high concentration range (0, 20, 40, 60, 80, and 100 ppm) was tested by performing three different calibration experiments with each concentration range, demonstrating the interaction of the metal with the ionophore. As it is already reported, this change is probably due to a change in the refractive index on the surface of the AuNPs generated from the interaction metal-ionophore [32,33]. Linear least squares fitting of the calibration curves gave a mean sensitivity (as the mean of the three slopes) of $4.03 \times 10^{-4} \pm 0.06 \times 10^{-4}$ a.u. ppm^{-1} Hg^{2+} and $6.3 \times 10^{-5} \pm 0.6 \times 10^{-5}$ a.u. ppb^{-1} Hg^{2+} for high and low concentration ranges respectively (Fig. 4) [34]. A detection limit, calculated as three times the standard deviation of the blank signal (AuNPs@Ionophore), of 67 ± 3 ppb can be achieved in batch conditions.

The interaction time of the metal-ionophore to achieve a stationary signal appears to be of few seconds. This signal could not be reversed despite the addition of chelating compounds, such as ethylenediaminetetraacetic acid (EDTA) or thiourea into the reaction cuvette.

Selectivity of the method was also determined by comparison of the response of the AuNPs@Ionophore to mercury with the one obtained with different potential interfering ions such as Ni^{2+}, Pb^{2+}, Cd^{2+}, Fe^{2+}, Mg^{2+}, K^{+}, Ba^{2+}, Cu^{2+} and Al^{3+} all at 100 ppm

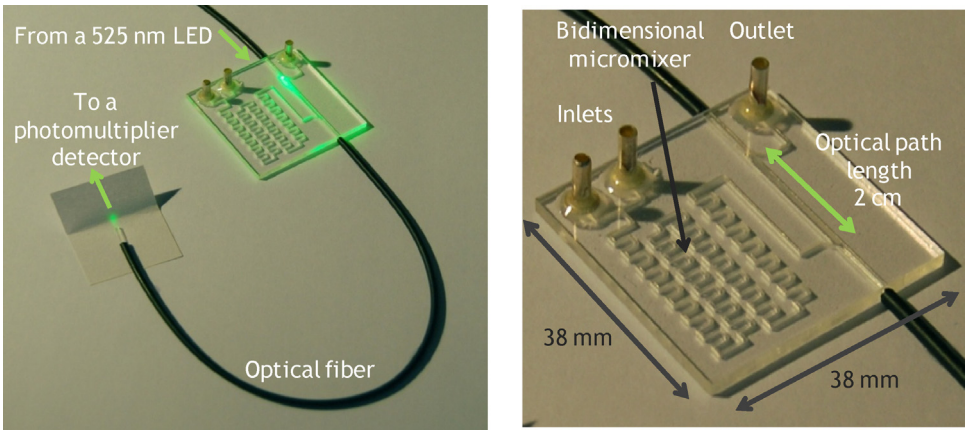

Fig. 3. Pictures from the constructed microfluidic platform for the continuous monitoring of Hg(II).

concentration. As Fig. 5A shows, only mercuric ion gave a significant change in the absorption signal of the SPR band. Furthermore, a simplification of the Fixed Interference Method (FIM) was performed since it reproduced the final situation of the analytical measurement more closely. The signal of a solution containing 1 ppm of Hg^{2+} was compared with the signal of the same solution containing a higher concentration of an interfering ion as a background. 10 ppm Ni^{2+} and Cu^{2+} were selected, while 1 ppm of Pb^{2+} and Cd^{2+} were used, since a lower concentration of these ions is expected to be found in water samples. Again, the presence of the tested metal ions does not result in a significant increase in the absorption signal (Fig. 5B).

3.1. Microfluidic system for the monitoring of Hg(II)

One of the aims of the work was to develop an optical microfluidic system, which exploited and improved the physicochemical properties of the AuNPs@Ionophore as a selective recognition optical element and provided a simple and portable method for the in situ monitoring of Hg(II). The use of a microfluidic system to perform an analytical measurement can avoid some of the problems related with the lack of reproducibility of the analytical methods when using nanomaterials, while allows controlling and managing small volumes of fluids. As it is well known, some quality parameters as sensitivity and response time of AuNPs are highly dependent on the size and concentration of the colloidal. This has been proved to be overcome by the use of microreactors [26]. As these quality parameters are also highly affected by kinetics, microfluidics is an excellent tool for the optimization of mass-transfer rates and other mixture parameters, which also affect sensitivity and improve the analysis time. In this sense, the hydrodynamic parameters of the system must be first optimized.

A continuous flow injection analysis (FIA) system was employed as the analytical method for the microfluidic platform operation. A certain plug of sample was injected by means of an external injection valve into a continuous flowing carrier solution of 0.01 M acetic/acetate buffer at pH = 5.5, as a conditioning solution, and this flow was mixed inside the microfluidic platform with a continuously flowing solution of AuNPs@Ionophore before reaching the detection area. A T-shape confluence point is preferred to assure an efficient mixture of both solutions, which are also mixed throughout a bidimensional meander micromixer before reaching the detection chamber, where the absorbance signal of the SPR band maximum is continuously recorded. The blank signal is taken when

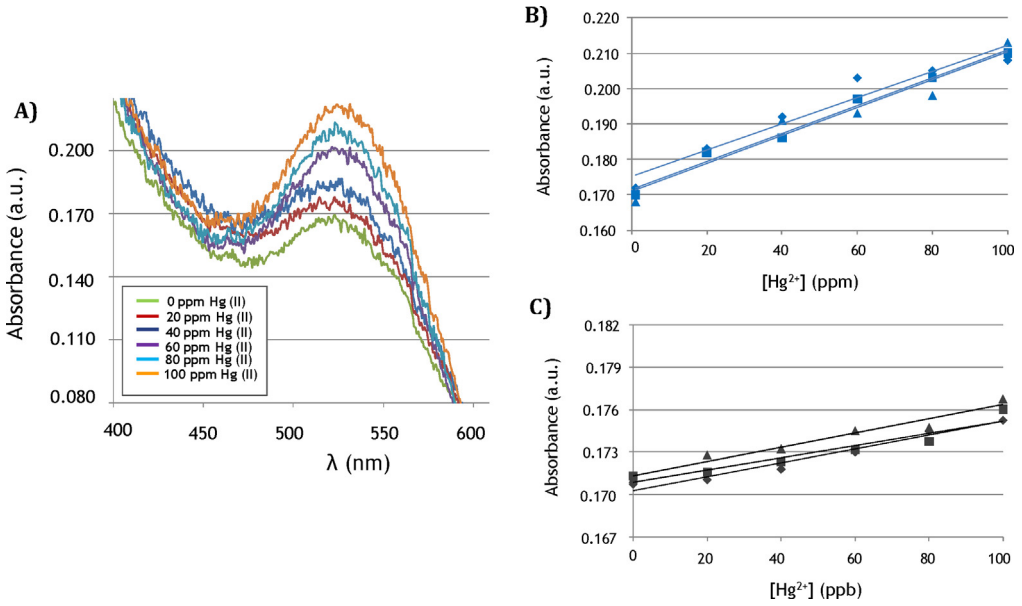

Fig. 4. Response from AuNPs@Ionophore to Hg(II) (A) Example of the absorbance spectra. (B) Response at high concentration range (0, 20, 40, 60, 80, and 100 ppm). (C) Response at low concentration range (0, 20, 40, 60, 80, and 100 ppb). Plotted calibration curves from B and C graphics correspond to three different calibration experiments.

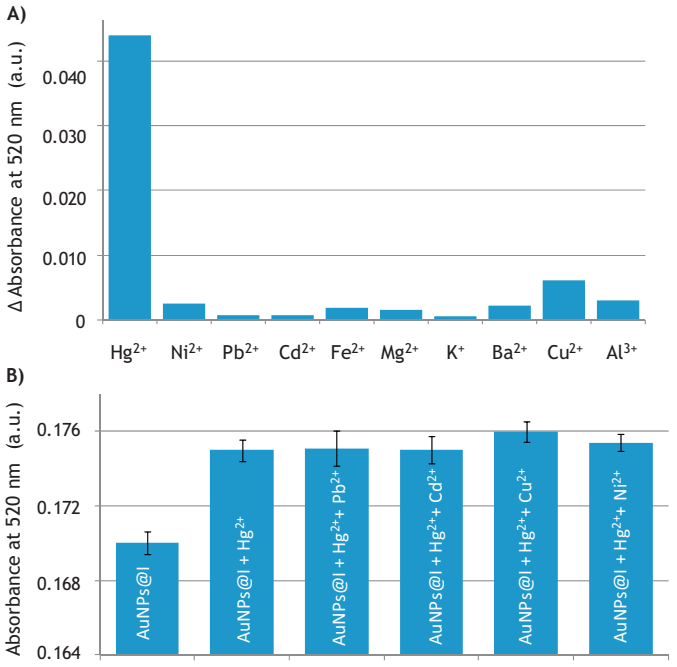

Fig. 5. Absorbance signals at 525 nm from the selectivity tests performed with the AuNPs@Ionophore. (A) Absorbance signal variation at 100 ppm concentration level of all the metals. (B) Signal (each one per triplicate) from blank and 1 ppm Hg^{2+} solutions containing 1 ppm Pb^{2+}, 10 ppm Ni^{2+}, 10 ppm Cu^{2+}, 1 ppm Cd^{2+}.

AuNPs@Ionophore, mixed with the conditioner solution, reach the detection chamber. Therefore, a transitory signal is obtained due to the sample crossing, which corresponds to the absorption signal increase of the SPR band due to the metal-AuNPs@Ionophore interaction. With the purpose of optimizing the injection volume and flow rate, an intermediate concentration of mercuric ion (100 ppb) was selected to ensure a significant response of the system. 100, 300 and 500 μL of sample volumes were injected into the pumped carrier at 0.8 mL min^{-1}. The flow rate of AuNPs@Ionophore was also 0.8 mL min^{-1}. For the optimization of flow rate, 0.8, 1.6 and 3.2 mL min^{-1} flows, measured at the outlet, were tested. As it can be seen in Fig. 6A and B, a 300 μL injection volume and a total flow rate of 1.6 mL min^{-1} were the optimum values as a compromise between sampling rate and analytical sensitivity.

Sensitivity and detection limit were evaluated from calibration curves at low mercuric ion concentrations. Thus, solutions of 20, 40, 60, 80 and 100 ppb of Hg^{2+} were injected into the microsystem at the optimized hydrodynamic conditions. Fig. 7A shows an example of the obtained recorded results. As it can be observed, peaks showed a very high signal to noise ratio and are totally recovered, which demonstrated the complete renewal of the volumes on the detection chamber. Fig. 7B shows the comparison of three calibration experiments performed in bath with three other ones performed with the microsystem. The sensitivity (mean slope of the three calibration curves) was of $2.07 \times 10^{-4} \pm 0.06 \times 10^{-4}$ a.u. ppb^{-1} Hg^{2+} and the detection limit, calculated as three times the standard deviation of the blank signal, was of 11.0 ± 0.6 ppb.

As it can be observed, a clear sensitivity improvement in terms of slope and detection limit is achieved when using the proposed microfluidic system. The later should be further improved to reach the regulations from the Environmental Protection Agency (EPA) (2 ppb) [35]. In this sense, the microfluidic platform allows increasing the optical path length and thus, sensitivity can be enhanced. Other systems already described in the literature based on the use of modified gold nanoparticles show better sensitivity in terms of limit of detection but on the other hand, they require costly bioreagents [36–38] or a large reaction time [39–42], or are not fully described for comparison purposes. Selectivity of the present approach is comparable or even better to other methods [17,40]. On the other hand, a working range of 11–100 ppb was achieved,

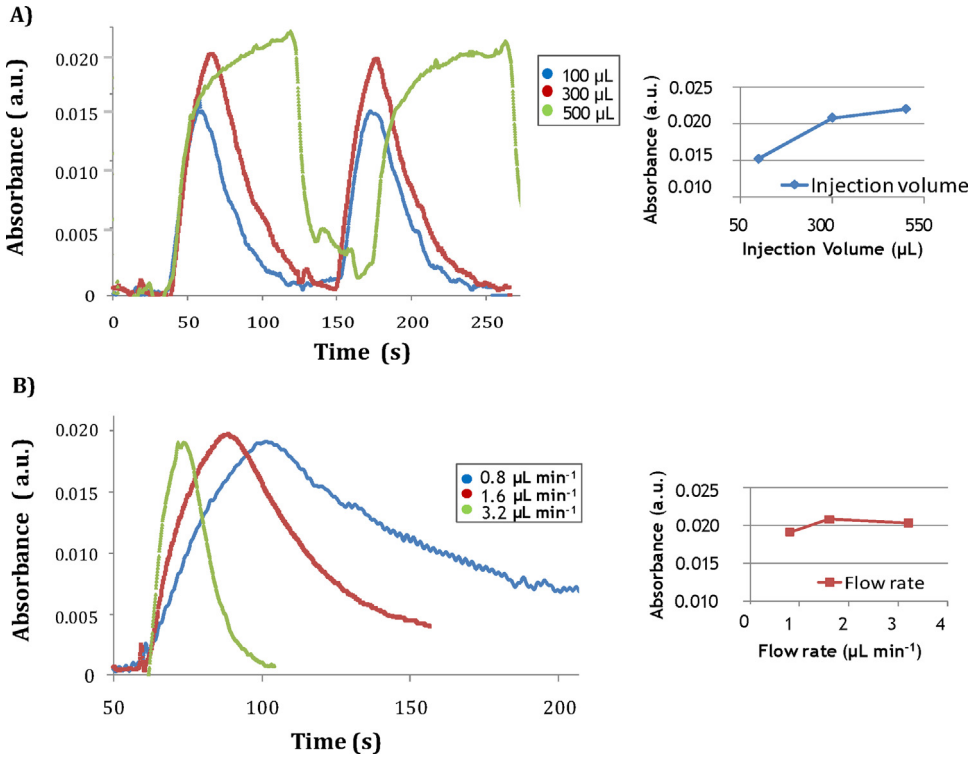

Fig. 6. Hydrodynamic parameters optimization of the microfluidic system for 100 ppb mercuric ion. (A) Injection volume optimization at a total flow rate (measured at the outlet) of 1.6 mL min^{-1}. (B) Flow rate optimization for 300 μL sample injection volume.

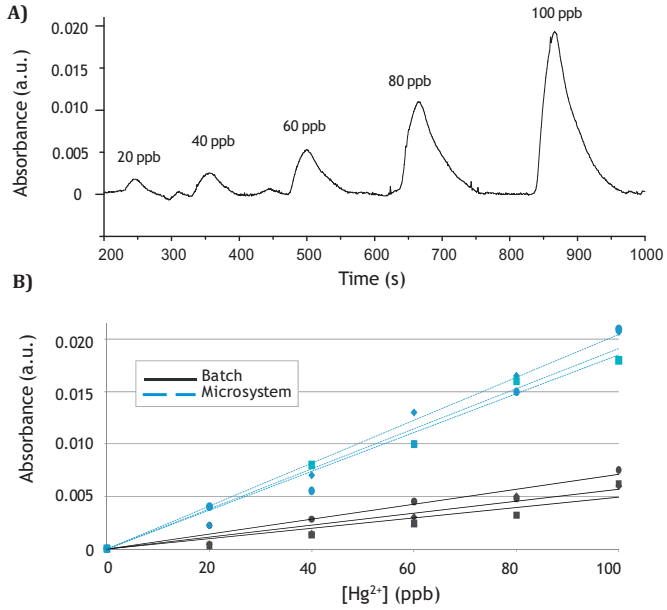

Fig. 7. (A) Example of the obtained signal from a mercury (II) calibration. (B) Comparison of the calibration curves obtained with the batch procedure and using the microfluidic system at the low concentration range.

which is shorter than other reported values [36,38,40,43] but may be enlarged because no signal saturation is observed till the highest concentration tested. Furthermore, the experiments performed with the proposed modified AuNPs in a batch mode analysis showed that it would be possible to detect mercury also in the range of ppm. Therefore, the calibration procedure of the microsystem should be optimized depending on the level of mercury to be determined. The remarkable advantage of the microsystem approach is the possibility of performing in situ monitoring. Actually, a sample throughput of 18 samples h^{-1} has been calculated, which can also be increased by reducing the stabilization time to the baseline signal between standards/sample injections.

4. Conclusions

A simple optical microfluidic system for continuously monitoring Hg(II) is presented. The detection method is based on the selective recognition of mercury by a thiourea derivative, which was specifically designed and synthesized for this purpose and which was attached onto gold nanoparticles surface. The synthetic process of the ionophore is simple and based on low cost reagents, and AuNPs are proposed as optical transducer due to their excellent properties; their SPR band is highly sensitive to changes derived from the metal–ionophore interaction. Moreover, they do not present photostability problems as commonly used organic dyes. This represents a long life-term reagent, which can be stored once synthesized. Results on the characterization of the optical recognition element revealed a high sensitivity and selectivity to mercury over Ni^{2+}, Pb^{2+}, Cd^{2+}, Fe^{2+}, Mg^{2+}, Ba^{2+}, Cu^{2+} and Al^{3+}, which demonstrate the suitability of the recognition element to the proposed Hg(II) detection. In order to exploit and improve the properties of the AuNPs@Ionophore and to develop a simple and portable method for the in situ monitoring of Hg(II), a polymeric microfluidic system was designed, constructed and tested. After the optimization of the hydrodynamic parameters, it was possible to improve sensitivity and detection limit, while taking advantage from microfluidics. Miniaturization enables the possibility of performing in-field measurements at lower cost and at lower reagents consumption. Thus, the proposal emerges as an appropriate approach for in situ unattended Hg(II) monitoring with a high sample throughput. It is of easy operation and the analytical process is automated.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2013.12.076.

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