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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 $\mu l\, s^{-1}$ flow rate, where 0.7 μl of the $HAuCl_4$ solution was sequentially dispensed every 2 s at a 2.5 $\mu l\, s^{-1}$ flow rate. These solutions were mixed in the serpentine and after that, the tiopronin solution was continuously pumped at 0.5 $\mu l\, s^{-1}$. 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.