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Cysteine functionalized bio-nanomaterial for the affinity sensing of Pb(II) as an indicator of environmental damage

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ABSTRACT

This work aims at the development of an electrochemical affinity biosensor for Pb(II) quantification using a platform that combines glassy carbon electrodes (GCE) and an aqueous dispersion of single-walled carbon nanotubes (SWCNT) covalently modified with cysteine residues (Cys). The biosensing protocol includes the accumulation of Pb(II) at the electrode surface through the affinity interaction promoted by Cys residues at open circuit potential, followed by the reduction of the accumulated Pb(II) at -0.900 V and the transduction step performed by linear sweep-adsorptive stripping voltammetry (LSAdSV) in a 0.020 M acetate buffer solution pH 5.00. There is a linear relationship between Pb(II) oxidation peak current and Pb(II) concentration. The dynamic linear range extends from 5.0 to 125.0 $\mu g \cdot L^{-1}$, exhibiting a sensitivity of 0.061 $\mu A \mu g^{-1} L$ and a detection limit of 0.69 $\mu g \cdot L^{-1}$. In addition, the selectivity of the biosensor was evaluated in the presence of high concentrations of possible interferents such as Cu(II), Cd(II), Ni(II), Hg(II), Rh(II), Ru(II), Zn(II), Ir(IV), Co(II) and As (III) demonstrating a high discrimination of Pb(II) in complex samples. The sensor was challenged with tap and rain water samples enriched with Pb(II), demonstrating outstanding properties in terms of recovery percentages showing an excellent agreement with ICP- MS.

1. Introduction

The increasing industrialization and urbanization over the past few decades led to the pollution of water due to the discharge of several toxic agents into ecosystem. This issue has become a major global concern [1]. Effluents may contain different kinds of toxic wastes like heavy metals and synthetic chemicals such as pesticides, pharmaceuticals, among other harmful contaminants [2,3]. It is widely known that the main sources of heavy metals are derived from industrial and agricultural activities like the use of pesticides and fertilizers, mining processes, fossil fuel, metallurgical and plastics production [3,4]. Heavy metals are non-biodegradable and ubiquitously spread, in addition, they are leading the ranking of harmful environmental chemicals since human and cattle health could be seriously damaged by long-term exposure and accumulation due to the ingestion of contaminated food and drinking water [3,5–7].

Lead, chromium, cadmium, mercury, nickel, copper and zinc are present at high levels in wastewater, mainly in undeveloped countries [8,9]. Among them, lead is a general metabolic poison and enzyme inhibitor which can be accumulated in bones, liver, brain, kidney and muscles [6,7]. Consequently, long term incorporation of lead can cause serious disorders like anemia, kidney disease, severe damage to the nervous system and mental retardation, especially children who are more likely to absorb and retain lead [5,7,10,11]. According to World Health Organization (WHO), the provisional guideline value for lead in drinking water is 0.010 mg L^{-1} [12]. These issues emphasize the importance of developing reliable, highly sensitive, user-friendly and cost effective tools for detection of heavy metals in different samples.

Considering the fact that detection and removal of these toxic ions is essential for the human well-being, several analytical techniques have been developed in order to survey drinking water, wastewaters and other samples that could be potentially in contact with them [13–15]. The most commonly used analytical techniques for their quantification include gas chromatography/mass spectrometry (GC/MS), inductively coupled plasma-mass (ICP-MS) and atomic absorption spectroscopy (AAS), among others [16–19]. Though highly sensitive, these analytical techniques are time-consuming, expensive, require experienced operator and are not easy to be employed in the field due to their bulky

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size. Overcoming these limitations, chemical sensors have emerged as an alternative for a simple, rapid, cost-effective and portable tool to analyze environmental security threats [15,20]. Among them, (bio) sensors are self-contained integrated devices that are capable of providing analytical information by integrating a biorecognition element which is in direct spatial contact with a transduction element [21,22]. In particular, electrochemical biosensors are simple to operate, facile fabrication and device integrated. These features make them outstanding alternative tools for a wide range of analytical purposes [15,20–23].

Advancements in the field of nanotechnology, especially the development of nano(bio)materials, have opened a new era for Analytical Chemistry [15,24]. Nanomaterials exhibit unique characteristics which contribute to its extensive applications in electrochemistry [25,26]. Among them, multi-walled carbon nanotubes (MWCNTs) and single walled carbon nanotubes (SWCNTs) have interesting physical, chemical and electrical properties that make them excellent candidates for the development of electrochemical biosensors [27–29]. However, due to the strong π-π interactions between their aromatic rings, CNTs tend to aggregate in aqueous solutions, making difficult their dispersion and further use for the development of electrochemical (bio)sensors [30–32]. Therefore, covalent or non-covalent functionalization of CNTs is a key alternative to avoid this problem [33]. The covalent functionalization of CNTs is more robust and better controllable as compared to non-covalent based methods [34–39]. Covalent functionalization also offers the possibility of establish a selective interaction and the ability to pre-concentrate a given analyte before its quantification. Amino acids have been reported as possible biorecognition molecules for the selective determination of heavy metals due to the presence of sulfur, nitrogen and/or oxygen atoms that can be recognized via the cooperative metal–ligand interaction [40–43]. In this sense, the use of glassy carbon electrodes (GCE) modified with poly-histidine have demonstrated to be very useful for the highly selective quantification of Cu(II) [44]. Recently, we reported the covalent functionalization of SWCNT with Cys and the development of a sensitive and selective biosensor for Cd (II) quantification as a model analyte [45].

This work proposes SWCNT-Cys dispersion as a suitable platform with unique properties for Pb(II) selective and sensitive quantification based on the high affinity of Cys residues towards Pb(II) [40]. SWCNT-Cys dispersion and the obtained biosensor (SWCNT-Cys/GCE) were critically studied. The optimal dispersion conditions as well as the correct transducer functionalization were investigated by electrochemical impedance spectroscopy zeta potential and linear adsorptive sweep voltammetry (LAdSV) measures. Experimental parameters, such as the deposition time and reduction time, were optimized by LAdSV. Finally, the electrochemical sensor was applied to the quantification of Pb(II) in real samples demonstrating outstanding capabilities in comparison with reference methods such as ICP-MS.

2. Experimental

2.1. Reagents

SWCNTs (AP-SWNT grade) were purchased from Carbon Solutions Inc., Riverside, California. The supporting electrolyte for the electrochemical assays was 0.020 molL^{-1} acetate buffer solution pH 5.00 (ABS), prepared by mixing stock standard solution of acetic acid and sodium acetate obtained from J.T. Baker. $Hg_2(NO_3)_2H_2O$ and As_2O_3 was obtained from Biopack; $Pb(CH_3COO)_2Pb(OH)_2$ and $(CdSO_4)_3.8H_2O$ were obtained from Anedra. Sodium dodecylbenzenesulfonate (SDBS), anhydrous *N,N′*-dimethylformamide(DMF), o-(benzotriazol-1-yl)-N,N,N´,N´-tetramethyluroniumhexafluorophosphate (HBTU), N,N-diisopropylethylamine (EDIPA), *S*-triphenylmethylcysteine (S-Trt-Cys), trifluoroacetic acid (TFA), $NiCl_2.6H_2O$, $Zn(NO_3).6H_2O$ were purchased from Sigma-Aldrich. Atomic absorption standard solutions of copper, rhodium, ruthenium, iridium were provided by Merck, while $CoSO_4.7H_2O$ was purchased from Dalton. Ultrapure water (p = 18.2MΩ cm) from a Millipore-MilliQ system was used for preparing all the solutions.

2.2. Apparatus

Electrochemical experiments were performed with an Autolab PGSTAT 128 N potentiostat (EcoChemie). Glassy carbon electrodes (GCE, CH Instruments, 3 mm diameter) were used as working electrodes. A platinum wire and Ag/AgCl, 3 M NaCl (BAS, Model RE-5B) served as counter and reference electrodes, respectively. All potentials are referred to the later. The electrodes were inserted into the conventional electrochemical cell through holes in its Teflon cover. A magnetic stirrer set at 800 rpm provided the convective transport during the pre-concentration step (previous to stripping experiments).

Electrochemical Impedance Spectroscopy (EIS) measurements were performed with an Autolab PGSTAT 128 N potentiostat (EcoChemie). The redox probe was 0.020 mol L^{-1} hydrogen peroxide prepared in a 0.050 mol L^{-1} phosphate buffer solution (PBS) pH 7.40 and the working potential was −0.100 V.

The zeta potential (Z) was determined by electrophoretic light scattering (ELS) measurements, using a Delsa Nano C instrument (Beckman Coulter).

Infrared spectroscopy (FTIR) measurements were performed with a Bruker Vertex 70 spectrometer. The samples were prepared with spectroscopic-grade KBr.

2.3. Cysteine-covalent functionalization of SWCNT (SWCNT-Cys) and preparation of SWCNT-Cys dispersion

The obtention of SWCNT covalently modified with Cys was published elsewhere [45]. Briefly, SWCNTs were purified in-house by air oxidation (350 °C, 2 h) followed by reflux in HCl (3 M, 2 h). Later, SWCNT were oxidized in a H_2SO_4/HNO_3 mixture (50:50 ratio, during 3 h), then they were dispersed in SDBS by ultracavitation and purged in an Ar atmosphere. The carboxylic activation was performed using HBTU/EDIPA for later interaction with L-S-Trityl-Cys dissolved in DMF. The deprotection of the thiol group was accomplished by using TFA and a silane derivative acting as scavenger. The final product was filtered, rinsed and dried under vacuum. Modified SWCNT were characterized by FTIR, TGA, XPS and Raman [45].

SWCNT-Cys dispersion was obtained by mixing 0.50 mg of covalently modified SWCNT-Cys with 1.0 mL of ultrapure water followed by 5 min of ultracavitation. Similar procedure was followed in order to prepare SWCNT and oxidized-SWCNT (SWCNT-Ox) dispersions for control experiments.

2.4. Preparation of SWCNT-Cys-modified GCE (SWCNT-Cys/GCE)

Prior to modification, GCEs were polished with alumina slurries of 1.0, 0.30 and 0.05 qm for 2 min each and then sonicated in ultrapure water for 10 s. The pretreated electrodes were dried with a stream of nitrogen. After that, GCEs were modified with SWCNT-Cys dispersion in the following way: an aliquot of 20 μL was dropped on top of each polished GCE and then the solvent was allowed to evaporate at room temperature.

2.5. Procedure

The capture of Pb(II) at SWCNT-Cys/GCE through complex formation was carried out at open circuit potential (OCP) for 7.0 min under stirring conditions. After the accumulation step, the electrode was thoroughly rinsed with 0.020 mol L^{-1} acetate buffer solution (ABS) pH 5.00 for 10 s and then transferred to a cell containing fresh 0.020 mol L^{-1} ABS pH 5.00. At this stage, the potential of the electrode was kept at −0.900 V for 3.0 min without stirring in order to reduce the