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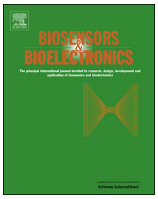

A very low potential electrochemical detection of L-cysteine based on a glassy carbon electrode modified with multi-walled carbon nanotubes/gold nanorods

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ABSTRACT

A nanohybrid platform built with multi-walled carbon nanotubes and gold nanorods, prepared via a cationic surfactant-containing seed-mediated sequential growth process, in aqueous solution, on a glassy carbon substrate has been successfully developed to be used in the electrocatalytic oxidation of L-cysteine (Cys). The nanohybrid was characterized by transmission electron microscopy, Raman spectroscopy and electrochemical measurements. Cyclic voltammetry results had shown that the modified electrode allows the oxidation of Cys at a very low anodic potential (0.00 V vs. Ag/AgCl). The kinetic constant k_{cat} for the catalytic oxidation of Cys was evaluated by chronoamperometry and provided a value of 5.6×10^4 L mol^{-1} s^{-1}. The sensor presents a linear response range from 5.0 up to 200.0 μmol L^{-1}, detection limit of 8.25 nmol L^{-1} and a sensitivity of 120 nA L μmol^{-1}.

1. Introduction

The design of multifunctional nanostructures has attracted the imagination and efforts of many researchers in the last few years. The combination of two (or more) types of materials, such as carbon nanotubes (CNTs) and gold nanoparticles might add new functionalities or extend the practical applications of these nano-objects. CNTs are the ideal building blocks for molecular nanoelectronics and are very robust materials, already incorporated into light reinforced composites. On the other hand, noble metal nanostructures, such as gold or palladium nanoparticles (AuNPs and PdNPs), are of general interest mainly due to their remarkable high catalytic activity and their special electronic and optical properties. The technological perspectives of the hybrid structures formed by these materials in electrochemical sensing have attracted a widespread interest in the scientific community. Several groups have successfully synthesized hybrids of AuNPs on the surface of CNTs. They have mostly used covalent linkage through bifunctional molecules (Sainsbury et al., 2005; Raghuveer et al., 2006; Hu et al., 2005), while others have prepared hybrids taking advantage of electrostatic interaction between a polyelectrolyte layer absorbed on the nanotubes wall and charged gold nanoparticles (Kim and Sigmund, 2004). These new Au-CNT structures could be employed in optoelectronic devices as well as in biomedicine, allowing the possibility to carry and selectively deliver, into the body, different therapeutic drugs (Ou and Huang, 2006; Tello et al., 2008; Li et al., 2006), or by using them in combination with electromagnetic irradiation to provoke localized heating which could eventually destroy surrounding cancer cells (Khaled et al., 2005).

Cysteine (Cys) is an important aminoacid present in natural proteins and plays key roles in biological systems. It is known to be an active site in the catalytic function of certain enzymes called cysteine proteases and in many other peptides and proteins (Ardakani et al., 2007; Lai et al., 2010), being critical for their proper metabolism. It acts as cancer indicator, radioprotective agent, antitoxin, antioxidant and free radical scavenger. It is also considered as a model for thiol derivatives (Fei et al., 2005). Cys deficiency is associated with a number of syndromes, such as

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slowed growth in children, hair depigmentation, edema, liver damage, muscle and fat loss, skin lesions, and weakness (Shahrokhian, 2001; Wang et al., 2005). Sensitive detection of Cys is thus of interest for physiological and clinical diagnoses (Lawrence et al., 2001; Chen et al., 2010).

Numerous research efforts have been performed for determination of Cys (Heli et al., 2009; Herzog and Arrigan, 2007; Molnar-Perl, 2003; Chen et al., 2010). The focus on Cys reflects the central role this species plays in the chemistry of biological thiols. Typically, the intracellular Cys concentrations are on the order of 30–200 μmol L^{-1} (Chung et al., 1990). The low absolute concentrations of Cys require the use of analytical methods with high inherent sensitivity.

Electrochemical techniques have the inherent advantages of high sensitivity, relatively low cost, simple instrumentation and selectivity by proper choice of the electrochemical potential. However, the electrochemical detection of Cys has some disadvantages. The electrooxidation of Cys on the conventional solid electrodes (Pt, Au, graphite, and carbon) is usually followed by sluggish electron transfer kinetics. Additionally, it needs a large overpotential for the electrooxidation process at a desirable rate to attain reasonably good sensitivity (Lock and Davis, 2002). Moreover, the necessity of high overpotential significantly reduces the detection selectivity. On the other hand, electrooxidation of Cys on these electrodes at highly positive potentials causes surface oxide formation as well as fouling effects. In order to circumvent these inherent difficulties, the surface of bare electrodes is being modified with suitable electrocatalysts to improve interfacial electron transfer (Silva et al., 2012a).

In this contribution, the results of the preparation and electrochemical characterization of hybrid nanostructures composed by gold nanorods and multi-walled carbon nanotubes by using a simple non-covalent synthetic route are presented as well as its use for the quantitative analysis of Cys alone and in the presence of other biologically relevant antioxidants, like other thiols, NADH, uric and ascorbic acids. Development of nanotechnology has ground-breaking applications in (electro)catalysis and chemical analysis (Wang et al., 2013).

2. Material and methods

2.1. Chemicals and solutions

All the chemicals were of analytical grade and used as received, with no further purification. Multi-walled carbon nanotubes (MWCNT > 98% carbon basis, O.D. × L 6–13 nm × 2.5–20 μm, lote MKBF1048V), chloroauric acid ($HAuCl_4 \cdot 3H_2O$, 99.9%, L-cysteine (Cys)), D,L-homocysteine, L-glutathione, L-ascorbic acid and uric acid were acquired from Sigma-Aldrich (St. Louis, USA). Sodium borohydride ($NaBH_4$, > 98%, Acros Organics), cetyltrimethylammonium bromide (CTAB, > 99%, Acros Organics), silver nitrate (> 99%, Reagent) and ethanol (96%, Dinamica) were also used, as received.

2.2. Gold nanorods (AuNR) synthesis

AuNR were prepared via a surfactant-containing seed-mediated sequential growth process, in aqueous solution; seed nanoparticles with average size between 3.5 and 4.0 nm are prepared from Au (III) source ions from the metal precursor $HAuCl_4 \cdot 3H_2O$, in the presence of a strong reducing agent, such as $NaBH_4$. Briefly, 2.5 mL of CTAB 0.20 mol L^{-1} were poured into 5 mL of an aqueous solution of 0.5 mmol L^{-1} $HAuCl_4 \cdot 3H_2O$, which produced a dark yellow color. After that, 0.6 mL of ice-cold $NaBH_4$ 0.01 mol L^{-1} solution, freshly prepared, was added, and the resulting solution was subsequently stirred for two minutes. In the presence of CTAB, Au (III) is reduced to Au (0), producing a brownish color, indicative of seed nanoparticles formation. The solution was cooled to room temperature and used immediately for all experiments. It is stable for three days. The growth solution was prepared with a slight modification from previously reported procedure (Murphy et al., 2005; Da Silva et al., 2013).

2.3. Construction of the sensors

After cleaning the electrode, a GCE (3 mm in diameter), a suspension was prepared by sonication, mixing 1.0 mg of MWCNT in 1 mL of DMF. Then 10 μL of this suspension were deposited directly on the GCE surface and dried at 80 °C to form a MWCNT layer on the GCE surface (MWCNT/GCE). After that, 12.5 μL of the AuNR solution were directly added on the MWCNT/GCE, and also dried at 80 °C. The volume of the AuNR solution was chosen based on the highest current and lowest potential for the oxidation of 50 μmol L^{-1} of cystein, in phosphate buffer solution pH 7.0 and scan rate of 5 mV s^{-1} (see Supplementary material, S1, Figs. S1-1 and S1-2).

2.4. TEM, UV–vis/near-IR, Raman and electrochemical characterization

Transmission Electron Microscopy—TEM measurements on a Microscope FEI 200 kV, model Tecnai 20, issue LAB6 or W, EDAX module, module Xplore 3D tomography, support of heating control, point resolution of 0.2 nm and line resolution of 0.1 nm, with magnification of 1 million times. The samples were prepared with a drop of the gold nanorods solution on a copper grid (200 mesh), coated with CNT film at least 24 h before analysis, kept in a dissector, to be dried.

The analyses of UV–vis/near-IR measurements were performed on a Varian Model Cary 50 Scan UV–vis/near-IR spectrophotometer (see Supplementary material, S2, Fig. S2-1). The Raman spectra were obtained in a Renishaw in Via Raman Microscope with laser of 785 nm and using a glassy carbon plate as support.

The electrochemical measurements were performed in an Echo Chemie Autolab PGSTAT-30 potentiostat (Utrecht, The Netherlands). All the measurements were run using an electrochemical cell with three electrodes, with an Ag/AgCl, Cl^- 0.1 mol L^{-1} electrode as reference, a Pt wire as auxiliary and unmodified or modified glassy carbon, in the latter case, with MWCNT/GCE and AuNR/MWCNT/GCE as working electrodes. The actual surface area (0.091 cm^2) of the AuNR/MWCNT/GCE electrode was estimated as reported before (Silva et al., 2012b).

2.5. Real sample analysis

The standard addition technique was used for the determination of Cys in human serum samples according to described procedures (Wu et al., 2012; Kannan and John, 2011). Briefly, the blood samples were obtained from healthy volunteers from the Nutrition Faculty, UFAL. After collected, the samples were centrifuged for 5 min at 5000 rpm and 10 °C, then, the serum was separated. A sample volume of 50 μL of the serum sample was diluted to 5 ml with 0.1 mol L^{-1} PBS (pH 7.0), to perform the analysis.

3. Results and discussion

3.1. Sensor characterization

The sensor was prepared by a simple deposition process (Yang et al., 2010). Prepared gold nanorods have a bilayer of CTAB, which provides the surface of nanorods with a positive effective surface charge, preventing, thus, the aggregation of nanorods in water via