Microchemical Journal 121 (2015) 130–135

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Graphene and gold nanoparticles based reagentless biodevice for phenolic endocrine disruptors monitoring

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ARTICLE INFO

Article history:
Received 6 October 2014
Received in revised form 19 December 2014
Accepted 3 March 2015
Available online 10 March 2015

Keywords:
Reagentless biodevice
Tyrosinase
Reduced graphene oxide
Gold nanoparticles electrodeposition
Endocrine disruptors
Catalytic efficiency

ABSTRACT

The goal of this paper aimed the development of an inexpensive, reliable and easy-to-use biodevice for detection and monitoring of phenol and phenolic endocrine disruptors in water samples. X-Ray (XRD) diffraction technique was employed for physical characterization of the modified electrode surface, results revealing nanostructured layers assemblies of polycrystalline gold with (200) growth preferred orientation and gold crystallite size of 33.19 nm. Investigation of the modified surface charge transfer properties was performed using cyclic voltammetry technique highlighting a significant enhancement of the electron transfer rate. Dual signal amplification offered by synergistic effect of gold nanoparticle and reduced graphene oxide layers and tyrosinase led to competitive detection limits (7.2×10^{-8} mol $L^{-1}$for phenol and 4.8×10^{-7} mol L^{-1} for octylphenol) and sensitivities (416 nA/μmol for phenol and 155 nA/μmol for octylphenol). The obtained values of the K_m^{app} and I_{max}/K_m^{app} ratio confirmed a strongly dependence of the immobilized tyrosinase catalytic efficiency on the steric and electronic properties of the bulky side chain in the para position of the phenolic compound. The biodevice showed a percent recovery between 87 ± 8% and 94 ± 11% demonstrating a suitable degree of accuracy and confirming the application potential to the detection and monitoring of phenol and several endocrine disruptors in water samples.

1. Introduction

The core objective of the Water Framework Directive (WFD) [1] is to achieve good chemical and ecological status for all water bodies, both the surface water and the groundwater. Under this Directive, Member States of the European Union should prepare and implement program of measures and impose strict deadlines to improve the chemical and ecological status of all surface waters by 2015. A subsidiary objective of this Directive is to reduce pollution and to phase out discharges, emissions and losses of priority hazardous substances. First list of priority substances presented in 2001 in Annex X of the Directive was replaced in 2008 by Annex II which included environmental quality standards [2] thereby establishing maximum allowed concentration levels and maximum annual average concentration. Among these pollutants, alkylphenols and bisphenols known as endocrine disruptors are of great interest due to the persistence in water even after wastewater treatments and to the potential biological effects exerted at low concentrations [3,4]. Studies on the consequences of exposure of living organisms to different concentrations of phenolic endocrine disruptors are limited, identification of these compounds and monitoring their level of concentration representing the two major concerns of the World Health Organization and of the European Commission [5]. Phenol is known for its persistency in the environment and susceptibility for bio-concentration and bio-magnification [6]. Due to its toxicity, concentration levels higher than 50 ppb are harmful to aquatic species, whereas ingestion of 1 g phenol is fatal to humans [7,8]. Detection and quantification of phenol traces from industrial wastes (petrochemical, wood preservatives, textiles, plastics, dyes, paper, herbicides and pesticides) discharged into the ground waters or surface waters is of special importance for preserving quality of water intended for human consumption or for agriculture.

Liquid chromatography coupled with different detection systems (mass spectrometry, fluorescence, electrochemical) is the technique most widely used for separation and quantification of phenol and phenolic endocrine disruptors [9–13]. Gas chromatography coupled with flame-ionization or mass spectrometric detection has been successfully employed in quantification of volatile or nonvolatile derivatized phenolic compounds [14–16]. Although these techniques reach low limit of detection and high reproducibility, they involve large volumes of water samples and require preconcentration and extraction steps which increase the risk of sample loss. Therefore

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http://dx.doi.org/10.1016/j.microc.2015.03.002
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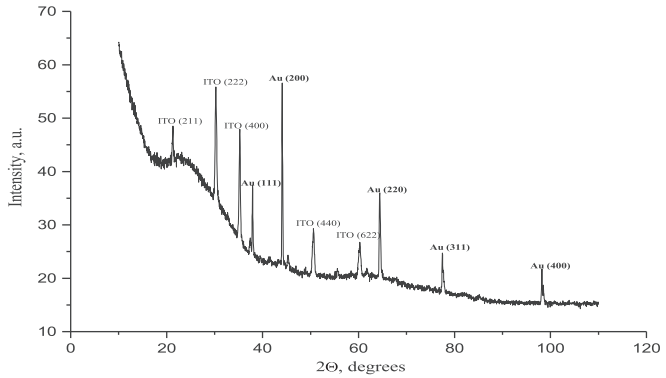

Fig. 1. XRD pattern of GNP layer assembly on ITO surface.

there is a great demand to develop inexpensive, reliable and easy-to-use devices able to detect different classes of environmental pollutants [17]. Enzymatic biosensors based on redox enzymes proved to be reliable analytical biodevices for phenolic endocrine disruptors due to lower costs, simpler operation, wide dynamic range and fast analysis times. The advantage of the redox enzymes used in electrochemical device development is the value of operational potential employed to monitor redox processes at the electrode surfaces which allow reaching detection free of interferences [18].

The goal of this paper is to develop a reagentless amperometric device based on tyrosinase and reduced graphene oxide-gold nanoparticles functionalized indium tin oxide (ITO) electrode for detection and monitoring of phenol and phenolic endocrine disruptors in water samples. In spite of low capacitive currents over a wide range of potentials, the electrochemical activity of ITO is low compared with most of other types of electrodes [19]. This drawback amplifies when electrodes are covered with biosensing layers. Consequently numerous studies have been conducted toward modulation of charge transport properties using nanodimensional materials e.g. nanoparticles, nanotubes, nanofibres, nanorods and graphenes [20–23].

Since their report in 2007 by Geim and Novoselom [24,25] graphenes have attracted increased attention owing to their physical, optical and chemical properties such as their interesting molecular structure, high accessible surface area and high electrical conductivity and capacity for immobilizing enzymes leading to surfaces with enhanced electron properties.

Gold nanoparticles endowed with good biological compatibility and catalytic activity play a significant role in electrochemical biodevices improving the communication between the enzyme and the electrode material while preserving protein conformational specificity [26]. However it is worth noting that size, shape and crystallographic orientation of gold nanoparticles deposits are factors that drastically influence electronic and catalytic behavior of modified surfaces [27,28]. The reported methods for ITO electrodes functionalization with gold nanoparticles (GNP) are generally based on the electrochemical deposition at negative potentials using gold salts or chloroauric acid, the procedure leading to nanostructures of different shapes and dimensions.

In our study gold nanoparticles (GNP) assembly on ITO electrode surface was performed at positive potentials (0.9 V vs. Ag/AgCl) through in situ generation of H^+ species according to the method recently developed in our laboratory.

2. Materials and methods

2.1. Chemicals

Indium tin oxide (ITO), tyrosinase (Tyr) (EC 1.14.18.1) from mushroom (1881 U/mg), potassium ferricianyde ($K_3[Fe(CN)_6]$), phenol, octylphenol, 4-tert-octylphenol, gold (III) chloride trihydrate solution ($HAuCl_4$), sulfuric acid, potassium chloride (KCl) were purchased from Sigma-Aldrich. Graphite flakes (SP1) were obtained from Bay Carbon, Inc., USA. Glutaraldehyde was purchased from Merck. 4-Aminoantipyrine (4-AAP) was purchased from Fluka. Stock solutions of phenolic compounds were freshly prepared by solubilizing the appropriate amount in either buffer solution or in acetonitrile, depending on the phenolic compounds solubility. Diluted solutions were prepared in McIlvaine buffer (pH = 7.00). All other chemicals were of analytical grade.

2.2. Water samples preparation

Surface water samples from different monitoring sites along Danube River and Murighiol lake were collected in amber glass bottles of 1 L capacity according to procedures described in ISO 5667-14 standard [29]. Before collecting the samples, the bottles were properly washed and several times rinsed with the water that had to be sampled. Suspended particle matter was removed by filtration using Chromafil PTFE syringe filters pore size 0.20 μm. Analysis was performed within 24 h after sampling.

2.3. Equipment

Electrochemical measurements were performed on an Autolab System PGSTAT 302 N (Metrohm-Autolab) controlled by GPES (version 4.9, Eco Chemie B.V.) or NOVA (version 1.8, Eco Chemie B.V.) software. Experiments were carried out at room temperature using a three electrode cell operating with an Ag/AgCl (3 mol L^{-1} KCl) as reference electrode, a platinum wire as counter electrode and unmodified ITO, GNP-ITO, RGO-GNP-ITO, Tyr-RGO-GNP-ITO as working electrodes. Cyclic voltammetry (CV) was employed to assess the electrochemical behavior of the modified electrodes. Performance characteristics of the bioanalytical device were evaluated using chronoamperometry technique. Assembly of GNP on ITO electrode surface was performed using chronocoulometry technique. Spectrometric measurements were performed using an UV–VIS spectrophotometer Thermo Evolution 260 Bio (Thermo Fischer Scientific).

XRD measurements were performed using D8 Discover diffractometer (Bruker) configured in vertical theta/2theta Bragg-Bretano geometry, equipped with a Cu Kα source (λ = 1,5406 Å), a rotating stage, and a high speed 1D Lynxeye detector. To estimate the crystallite size and discard eventual cross-contaminations during the depositions, X-ray diffraction (XRD) measurements of the GNP layer assembly were performed in Panalytical X'Pert PRO with Cu Ka line radiation (l51.540598 Å) using the Bragg-Brentano configuration with a 2θ range of 10°–100° and a step size of 0.04°.

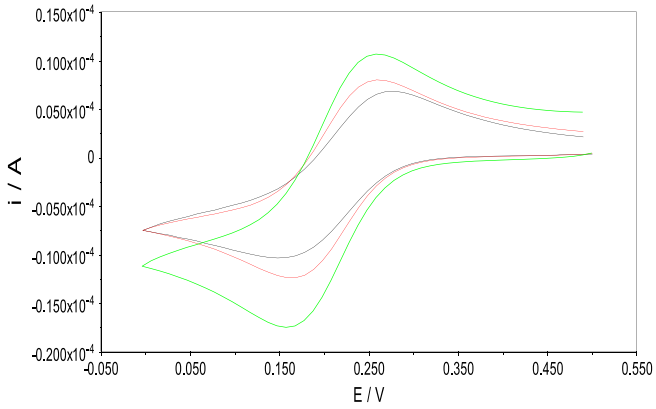

Fig. 2. Cyclic voltammograms for 5 mmol L^{-1} $K_3[Fe(CN)_6]$ in 0.1 M KCl at ITO bare electrode (black curve), GNP-ITO electrode (red curve), RGO-GNP-ITO electrode (green curve).