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Sensitive detection of endocrine disrupters using ionic liquid – Single walled carbon nanotubes modified screen-printed based biosensors

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ABSTRACT

Simple and low cost biosensor based on screen-printed electrode for sensitive detection of some alkylphenols was developed, by entrapment of HRP in a nanocomposite gel based on single-walled carbon nanotubes (SWCNTs) and 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]) ionic liquid. Raman and FTIR spectroscopy, CV and EIS studies demonstrate the interaction between SWCNTs and ionic liquid. The nanocomposite gel, SWCNT–[BMIM][PF_6] provides to the modified sensor a considerable enhanced electrocatalytic activity toward hydrogen peroxide reduction. The HRP based biosensor exhibits high sensitivity and good stability, allowing a detection of the alkylphenols at an applied potential of −0.2 V vs. Ag/AgCl, in linear range from 5.5 to 97.7 μM for 4-t-octylphenol and respectively, between 5.5 and 140 μM for 4-n-nonylphenol, with a response time of about 5 s. The detection limit was 1.1 μM for 4-t-octylphenol, and respectively 0.4 μM for 4-n-nonylphenol (S/N = 3).

1. Introduction

The endocrine disrupting chemicals (EDCs), a class of substances defined not by chemical nature but by biological effect, affecting the population health have attracted great interest [1,2]. They include pesticides, polycyclic aromatic hydrocarbons (PAHs), phthalate plasticizers, alkylphenols (APs), bisphenol A, polychlorinated biphenyls (PCBs), dioxins, surfactants, synthetic steroids, brominated flame retardants, parabens and are potentially present in food as phytoestrogens. From all these, nonylphenol has been designated as a member of the endocrine disrupters, more specifically, pseudoestrogens, which are suggested to be related to the decline of human and wildlife reproductive health. Octylphenol is very toxic to aquatic organisms, is not easily degraded in the environment and has the potential to cause significant endocrine disruption effects.

Some analytical techniques reported for EDCs detection are fluorescence immunoassays which are able to detect endocrine disrupting compounds in wastewater [3], liquid chromatography coupled with mass spectrometer (LC–MS) and ELISA technique which provide detection of the most important classes of EDCs in food and environment at levels of biological significance [4]. The levels of concentrations of EDCs in different sample are low (1–72,000 ng L^{-1}) and due to the complexity of the environmental matrices, pre-concentration of the samples is required before LC–MS analysis [5,6].

All the reported techniques are reaching the highest accuracy with low detection limits, but are expensive, time-consuming, and require the use of highly trained personnel. The development of biosensors as analytical devices for fast, low cost, reliable and sensitive determinations is a result of the current demand for field monitoring. These devices can be used both by regulatory authorities and by industry to provide enough information for routine testing and screening of samples [7].

Yin et al. [8] have developed an amperometric sensor based on CoTe quantum dots and poly (amidoamine) dendrimers (PAMAM) immobilized onto glassy carbon electrode using layer-by-layer assembly technique for determination of trace amounts of bisphenol A. Nonylphenol was determined by Evtugyn et al. [9], using an immunosensor obtained by immobilisation of specific antibodies together with horseradish peroxidase (HRP) on the surface of carbon screen-printed electrodes. The detection limit achieved was 10 μg L^{-1} for nonylphenol. A human recombinant estrogen receptor was used in the competitive assay mode by Usami and Ohno [10] for detection of 4-nonylphenol. Indirect ELISA and fluorescence polarization immunoassay protocols have been used for detection of 1 mg L^{-1} nonylphenol by Yakovleva et al. [11]. Detection of 10–100 μg L^{-1} 4-n-nonylphenol was achieved by competitive immunoassays in the format of microtitre

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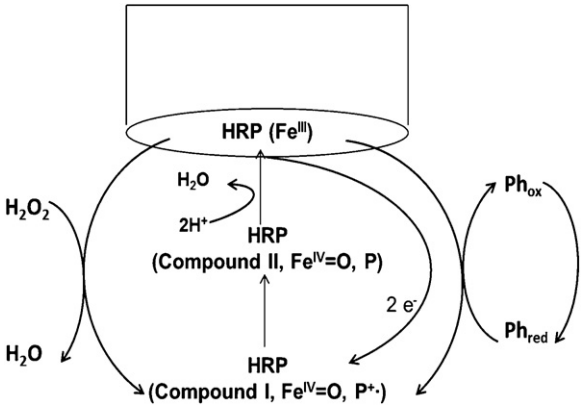

Fig. 1. Detection of phenolic compound using a HRP based biosensor.

plate ELISA and dipstick tests involving monoclonal antibodies [12].

Biosensors based on tyrosinase were also reported in literature for the detection of phenols [13,14]. The main drawbacks of these biosensors include the electrode fouling due to a radical polymerisation and the deactivation of enzyme by *o*-quinone generated in the enzymatic reaction. Other reported biosensors for the detection of phenolic compounds use laccase to catalyze the oxidation of analyte. Many of these are based on oxygen consumption that is not very convenient from analytical point of view. Systems using both laccase and tyrosinase have been reported as well [15].

HRP based biosensors represent an alternative to the above systems. HRP is a very stable and active enzyme as it was previously demonstrated after immobilization on the surface of SWCNT-Prussian blue modified screen-printed electrode [16]. The general principle for detection of phenolic compounds using biosensors based on horseradish peroxidase is presented in Fig. 1. HRP catalyzes the oxidation of electron donor substrates (e.g. phenols, amines, aminophenols, etc.) with hydrogen peroxide in three steps, as following [17,18].

$$HRP(Fe^{3+}) + H_2O_2 \rightarrow CompoundI + H_2O \quad (1)$$

$$CompoundI(Fe^{IV}{-}O, P^{+\bullet}) + S + H^+ \rightarrow CompoundII(Fe^{IV}{-}O) + S^\bullet \quad (2)$$

$$CompoundII(Fe^{IV}{=}O) + S + H^+ \rightarrow HRP(Fe^{3+}) + S^\bullet + H_2O \quad (3)$$

The H_2O_2 is reduced in a first step and an oxidized enzyme intermediate, called Compound I, is formed (reaction (1)). Compound I consisting in an oxyferryl iron (Fe^{IV}=O) and a radical, a porphyrin π–cation radical, is reduced in two steps, when a donor substrate (S) is oxidized to a radical product ($S^\bullet$) (reactions (2) and (3)). The formed radicals ($S^\bullet$) are reduced by the electrode, at an applied potential vs. Ag/AgCl, the current reduction being proportional with the substrate concentration (reaction (4)) [18].

$$S^\bullet + e^- + H^+ \rightarrow S \quad (4)$$

In the last years, a series of biosensors based on carbon nanotubes modified electrodes or different composites based on ionic liquids has been reported for H_2O_2 detection, but none was used further for alkylphenols detection. The high stability, high electrical conductivity and very low vapour pressure, make ionic liquids versatile and promising for electrochemistry of enzymes and a suitable media for supporting biocatalytic processes [19,20].

Chen et al. [21] have used Nafion as binder to form Nafion-ionic liquids composite film, helping [BMIM][PF_6] to adhere on the glassy carbon electrode, and using further this composite film as immobilisation matrix to entrap HRP. Fan et al. [22] developed an electrochemical biosensor for direct electrochemistry of HRP, using a glassy carbon electrode modified with Nafion, agarose hydrogel and [BMIM][PF_6] ionic liquid composite as sensing platform. This biosensor detected the H_2O_2 with a detection limit of 0.12 μM. A third generation biosensor for hydrogen peroxide was constructed by Xu et al. [23] through crosslinking horseradish peroxidase (HRP) onto a glassy carbon electrode modified with multiwall carbon nanotubes (MWNTs). Glutaraldehyde and bovine serum albumin were used to cross-link HRP and the MWNTs, and the detection limit achieved with this biosensor was 0.4 μM H_2O_2.

Others have used nonenzymatic systems for electrocatalytic reduction of hydrogen peroxide (H_2O_2) [24,25]. Li et al. [25] electropolymerized Prussian blue (PB) on a carbon ionic liquid electrode (CILE), while Wang et al. [24] electro-polymerized aniline and single-walled carbon nanotubes on a platinum electrode in a room temperature ionic-liquid.

Our work has been focused on development of an amperometric biosensor based on screen-printed electrodes modified with a nanocomposite film, consisting in SWCT-[BMIM][PF_6] and HRP, for sensitive detection of 4 n-nonylphenol and 4-t-octylphenol. This biosensor combines the advantages of both ionic liquids and CNTs.

The modified screen-printed electrodes were characterized by cyclic voltammetry (CV), amperometry and electrochemical impedance spectroscopy (EIS). The nanocomposite film facilitates the electrochemistry of HRP and the reduction of hydrogen peroxide (H_2O_2), allowing further the sensitive detection of endocrine disrupters, 4-n-nonylphenol and respectively, 4-t-octylphenol.

2. Experimental

2.1. Chemicals and reagents

Single-walled carbon nanotube (0.5–100 μm length, 1.1 nm diam.), N,N-dimethylformamide (DMF) (99%), horseradish peroxidase 25 kU (E.C.1.11.1.7, RZN3), 1-butyl-3-methylimidazolium hexafluorophosphate, potassium hexacyanoferrate (III), potassium hexacyanoferrate (II), tetramethoxysilane (TMOS), polyethyleneglycol (PEG600), 4-t-octylphenol (97%) were supplied from Sigma–Aldrich. The Nafion (perfluorinated ion-exchange resin, 5% solution in a mixture of lower aliphatic alcohols and water), hydrogen peroxide solution (30%), acetonitrile (>99.9%, GC) and methyltrimethoxysilane (MTMOS) were provided by Fluka, while the phenol (97%), HCl 37% and 4-n-nonylphenol (99.9%) were provided by Riedel-de Haën. All other chemicals were of analytical grade.

All aqueous solutions were prepared with ultrapure water, obtained with a Millipore system (18.2 MΩ cm). A 0.05 M phosphate buffer solution (PBS, pH 7.4) containing 0.1 M KCl was used as supporting electrolyte. The pH 7.4 of buffer solution has been optimized for HRP in our previous work [16]. A fresh stock solution of 20 mM H_2O_2 was prepared daily. Standard solutions of 5 mM alkylphenols were prepared in acetonitrile.

2.2. Apparatus and measurements

Electrochemical measurements were carried out using a BAS 100B Electrochemical Workstation (BAS, West Lafayette, USA) in a conventional three-electrode electrochemical cell of 5 mL. All the experiments were carried out at room temperature, using a system of screen-printed electrodes (SPE) Dropsens – DRP110. The working (4 mm diameter) and the counter electrodes are made of carbon and the reference electrode is made of Ag. Cyclic voltammetry experiments have been performed in the potential range of −0.6 to 1 V, with a scan rate of 0.1 V s^{-1}. Amperometric measurements were carried out under constant stirring (500 rpm). All potentials are referred to Ag pseudo-reference electrode. Electrochemical impedance spectroscopy (EIS) measurements were performed with