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An amperometric biosensor based on multiwalled carbon nanotube-poly(pyrrole)-horseradish peroxidase nanobiocomposite film for determination of phenol derivatives

Seyda Korkut, Bulent Keskinler, Elif Erhan*

Department of Environmental Engineering, Gebze Institute of Technology, 41400 Cayirova, Kocaeli, Turkey

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ABSTRACT

An amperometric biosensor based on horseradish peroxidase (HRP) and carbon nanotube (CNT)/polypyrrole (PPy) nanobiocomposite film on a gold surface has been developed. The HRP was incorporated into the CNT/PPy nanocomposite matrix in one-step electropolymerization process without the aid of cross-linking agent. Amperometric response was measured as a function of concentration of phenol derivatives, at a fixed bias voltage of −50 mV. Optimization of the experimental parameters was performed with regard to pH and concentration of hydrogen peroxide. The linear range, sensitivity and detection limit of the biosensor were investigated for eighteen phenol derivatives. The sensitivity in the linear range increased in this order: 4-methoxyphenol > 2-aminophenol > guaiacol = *m*-cresol > 2-chlorophenol = 4-chlorophenol = hydroquinone = pyrocatechol > 2,6-dimethoxyphenol > 3-chlorophenol > *p*-cresol > *p*-benzoquinone = 4-acetamidophenol > catechol > phenol = pyrogallol = 2,4-dimethylphenol. CNTs was shown to enhance the electron transfer as a mediator and capable to carry higher bioactivity owing to its intensified surface area. The biosensor exhibited low detection limits with a short response time (2 s) for the tested phenolics compared to the reported working electrodes. It retained 70% of its initial activity after using for 700 measurements in 1 month.

1. Introduction

The determination of phenol and its derivative compounds is of the environmental importance, since these species are toxic and evolved in many industrial processes. They are present in many wastewater streams of the oil, paint, polymer and pharmaceutical industries [1]. Electrochemical methods have been widely used for measuring these compounds due to their advantages such as good selectivity in the presence of phenol oxidases, relatively low cost of realization and storage and the potential for miniaturization and automation [2–4]. Regarding the amperometric enzyme biosensors, tyrosinase has been the most currently used enzyme for the detection of phenolic compounds [5–9]. However, these tyrosinase biosensors are restricted to the monitoring of phenolic compounds having at least one *ortho*-position free [10]. On the other hand, laccase biosensors give response to phenolic compounds with free *para*- and *meta*-position with a complicated catalytic cycle [11]. HRP having less selectivity to phenolics is capable of giving response to a large number of phenol derivatives [12], and shows a high stability and efficiency for different biosensor designs [3,13].

The selectivity and sensitivity of the modified electrodes depends on the stability of the phenoxy radicals produced in the enzyme reaction, electrode material, immobilization method, and the magnitude of the applied potential [11]. In addition to this, the performance of biosensor is mainly affected by the electrocatalytic activity of modified electrode material and composites. CNTs have emerged as a new class nanomaterials that are receiving considerable interest owing to their ability to promote electron transfer reactions with enzymes [14,15]. The high conductivity of this carbon material leading to level of $10^2\ \Omega^{-1}\,cm^{-1}$ improves electrochemical signal transduction, while its nano-architecture imposes the electron contact between the redox centres, deeply inlaided in enzyme structure, and the smooth surface of the electrode [16]. Sotiropoulou et al. reported that CNTs have a metallic character in the range of potentials between −1.5 and +1.5 V, since there are no apparent oxidation or reduction peaks. Based on this, CNTs can donate and accept electrons in a wide range of potentials, and could therefore be used as mediators in biosensor systems [17]. A key barrier for developing CNT-based biosensors is the insolubility of CNTs in most solvents [18]. Functionalization of CNTs has been achieved by an oxidation process, which involves extensive

* Corresponding author. Tel.: +90 262 6053217; fax: +90 262 6053205.
E-mail address: e.erhan@gyte.edu.tr (E. Erhan).

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ultrasonic treatment in a mixture of concentrated hydrogen peroxide and sulfuric acid [19]. The ends and sidewalls of the treated CNTs are mainly decorated with carboxyl groups. CNTs functionalized in this manner obtain good solubility in water and retain their pristine electronic and mechanical properties [20]. Recently, there has been growing interest in using CNT-based electrode configurations with tyrosinase and laccase polyphenol oxidases for detection of phenolics [21,22]. However, the use of HRP, alternative polyphenol oxidase, in modified CNT electrodes has been less reported for the measurement of phenol derivatives.

The aim of this study is to develop a CNT/PPy/HRP nanobiocomposite film for the bioanalytical applications. The working electrode was constructed in one-step by the electropolymerization process of multiwalled CNT, pyrrole and HRP. The parameters such as operating potential, pH level and concentration of hydrogen peroxide were investigated and were tested using a large set of eighteen phenol derivatives.

2. Experimental

2.1. Reagents

Horseradish peroxidase (E.C.1.11.1.7) with an activity of 10,000 U/vial (according to pyrogallol method performed by the supplier), aqueous solution of hydrogen peroxide (30%), lithium chloride, di-potassium hydrogen phosphate, citric acid, tri-sodium citrate, acetic acid (96%), sodium acetate tri-hydrate and potassium di-hydrogen phosphate were purchased from Merck. Phenol, *ρ*-benzoquinone, hydroquinone, 2,6-dimethoxyphenol, 2-chlorophenol, 3-chlorophenol, 4-chlorophenol, 2-aminophenol, 4-methoxyphenol, pyrocatechol, guaiacol, *m*-cresol, *o*-cresol, *p*-cresol, catechol, 4-acetamidophenol, pyrogallol, 2,4-dimethylphenol, pyrrole (99%), CHES buffer and sodium dodecyl sulfate (SDS) were obtained from Sigma. The phenol reagents were used as purchased without any further pre-treatment. Stock solutions of various phenols were daily prepared in 0.1 M phosphate buffer solution (pH 7.0). Multiwalled CNTs were obtained from Nanocs, Inc., NY, USA.

2.2. Apparatus

Electrochemical experiments were performed by using a CHI Model 800B electrochemical analyzer. A gold working electrode (2 mm diameter), a Platinum wire counter electrode, an Ag/AgCl (3 M NaCl) reference electrode, and a conventional three-electrode electrochemical cell were obtained from CH Instruments.

2.3. Preparation of CNT/PPy/HRP nanobiocomposite film coated gold electrode

Gold electrode was polished with slurries of fine alumina powders (0.3 and 0.05 mm) on a polishing microcloth pad. The electrode was then rinsed with distilled water. The facile routine for preparation of water-soluble CNTs was a modification of the acid oxidative method developed by Smalley's group [23]. Firstly, 14 mg of multiwalled CNTs were added into 5 mL of a 9:1 concentrated H_2SO_4/H_2O_2 (30%) aqueous solution and the mixture was stirred for 30 min for CNTs oxidation. After the reaction, 15 mL of the 9:1 concentrated H_2SO_4/H_2O_2 (30%) aqueous solution was added into the mixture. The mixture was placed in an ultrasonic bath (Elma 460-H) and sonicated for 5 min. Resulting CNTs dispersion was diluted using 1 L of distilled water, then was filtered through a 0.45 μM cellulose membrane. After, the filtrate was washed with 10 mM NaOH solution and distilled water till the pH level reaching to 7, the filtrate was separated from the membrane and dispersed in distilled water (0.03 mg/L). The resulting CNTs solution was sonicated for 2 min to obtain a homogeneous CNTs solution [19].

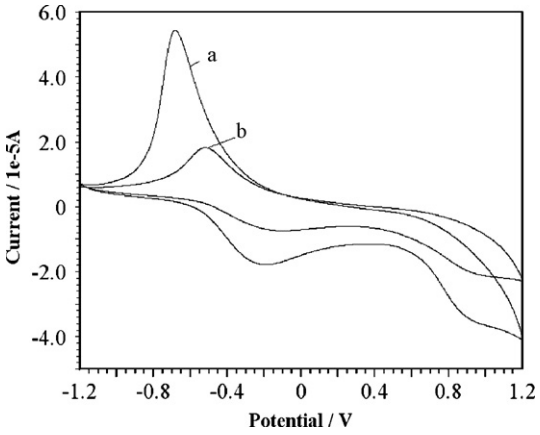

Fig. 1. Multiscan cyclic voltametric curves for CNT/PPy/HRP nanobiocomposite film (a), CNT/PPy film (b) in 0.1 M phosphate buffer (pH 7.0). Potential was scanned between −1.2 and +1.2 V at the scan rate of 100 mV s^{-1}.

CNT/PPy/HRP nanobiocomposite film was coated onto the surface of the gold working electrode by electrochemical polymerization in a three-electrode cell. The polymerization medium contained 5 mL of oxidized CNTs solution, 5 mL of 50 mM pH 6.5 citrate buffer including 0.01 M pyrrole, 0.6 mg/mL SDS and 0.3 mg/mL. HRP used in this study is a water-soluble enzyme. SDS is one of the best supporting electrolyte for electropolymerization of pyrrole in aqueous medium [24]. Since the anion of the SDS represents the dopant ions that stabilize the cationic sites in the polypyrrole, it might have a certain effect on the amount of the immobilized enzymes as well as its activity. Cyclic voltammogram of the nanobiocomposite film was scanned between 0 and 1.2 V for 4 min.

2.4. Electrochemical measurements

Electrochemical batch measurements were carried out in a 0.1 M phosphate buffer solution (pH 7.0) in the presence of 0.7 mg/mL lithium chloride with an applied working potential of −50 mV and a continuous stirring at 600 rpm in three-electrode cell mentioned in Section 2.2. Various phenol derivatives were tested to produce *i*–*t* curves of chronoamperometric measurements.

3. Results and discussion

3.1. Characterization of the CNT/PPy/HRP nanobiocomposite film

Fig. 1 shows the CV of the CNT/PPy film and CNT/PPy/HRP nanobiocomposite film in 0.1 M phosphate buffer (pH 7.0) at the scan rate of 100 mV s^{-1}. The peak current increased by the introduction of HRP into the film, indicating the synergy effect between HRP and CNT similar to the previous study [25]. In addition to this, the electrocatalytic sites placed in the active centre of HRP would join into the CNT/PPy nanobiocomposite film. There was virtually no change in the shape or peak potentials of the CV suggests that there was no hindrance for electron transfer process between electrode and HRP. The well-defined peaks indicate that films are highly homogeneous.

3.2. Effect of applied potential and pH on biosensor response

The response of the peroxidase biosensors to phenolic compounds is based on the so-called double displacement or