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A biosensor based on gold nanoparticles, dihexadecylphosphate, and tyrosinase for the determination of catechol in natural water

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ABSTRACT

In this work, a biosensor using a glassy carbon electrode modified with gold nanoparticles (AuNPs) and tyrosinase (Tyr) within a dihexadecylphosphate film is proposed. Cystamine and glutaraldehyde crosslinking agents were used as a support for Tyr immobilization. The proposed biosensor was characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), and cyclic voltammetry in the presence of catechol. The determination of catechol was carried out by amperometry and presented a linear concentration range from 2.5×10^{-6} to 9.5×10^{-5} mol L^{-1} with a detection limit of 1.7×10^{-7} mol L^{-1}. The developed biosensor showed good repeatability and stability. Moreover, this novel amperometric method was successfully applied in the determination of catechol in natural water samples. The results were in agreement with a 95% confidence level for those obtained using the official spectrophotometric method.

1. Introduction

Metallic nanoparticles have been extensively used in recent years in several technologic devices [1–3]. In electroanalysis, nanoparticles have been applied for electrode modification, due to advantages, such as their electrocatalytic effect and improved active area and mass transport [2,4]. These advantages can provide a better analytical performance in comparison with bulk electrodes [5–8]. Furthermore, for the development of new biosensors, gold nanoparticles (AuNPs) show good mechanical resistance and electrical properties, and have large active surface areas for enzyme immobilization (which increases the number of attached biocomponents in the sensing surface), and good biocompatibility [5,9]. Thus, several studies have explored the use of AuNPs in combination with biomolecules for the development of new architectures for biosensors [10–12].

Some phenolic compounds are toxic pollutants that present a potential hazard for human health and aquatic life [13,14]. They often enter the aquatic environments *via* industrial residues from different kinds of production, such as plastics, dyes, drugs, resins, pesticides, and especially paper and cellulose [15,16]. The use of new biosensors for the detection of phenolic compounds has advantages, such as rapid response, shorter analysis times since there is no need sophisticated sample treatment, low cost, simplicity of preparation, and high sensitivity and selectivity. Different kinds of architecture for biosensors to detect phenolic compounds can be found in the literature, including dual-enzyme systems, immunosensors, nucleic acid biosensors, and enzyme-based biosensors (tyrosinase, laccase, and horseradish peroxidase) [17].

Tyrosinase (Tyr) is also known as polyphenol oxidase or catechol oxidase, and has two copper atoms in its active center [15]. This important enzyme catalyzes oxidation reactions, including the hydroxylation of monophenols to *o*-dihydroxy phenols, and subsequently, the oxidation-dihydroxy phenols to *o*-quinones in the presence of molecular oxygen [18].

A critical step in the development of biosensors is how to immobilize the enzyme efficiently without any modification to the active center or denaturation. In recent years, a wide range of surface-linking methods have been developed, including physical adsorption [19], occlusion via gel encapsulation [20,21], electrochemical entrapment of enzymes within a polymer or a composite

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matrix [22–24], covalent bonds [11,12,25], and covalent crosslinking [24,25].

Dihexadecylphosphate (DHP) is a surfactant that presents a negatively charged phosphatic polar head and two long hydrophobic tails, and does not form micelles [26]. This material can be dispersed in water by ultrasonic agitation, and its dispersion can form a very stable film on electrode surfaces, which is similar to stacks of biomembranes. The film formation probably occurs via hydrogen bonds, and has been used in the development of sensors [27–29] and biosensors [30–33].

In this paper, we report a new biosensor using Tyr enzyme immobilized covalently in a film containing AuNPs and DHP on a glassy carbon electrode (GCE) to determine catechol concentrations by amperometry in natural water samples.

2. Experimental

2.1. Chemicals and reagents

Hydrogen tetrachloroaurate (III), 25% (v/v) glutaraldehyde, cystamine, dihexadecylphosphate (DHP), sodium citrate, and tyrosinase 50 kU (from mushroom), 4-aminoantipyrine, and catechol (99%) were purchased from Sigma–Aldrich. All other chemicals were of analytical grade and were used as received. A 0.01 mol L^{-1} catechol stock solution (prepared daily) was made in a 0.1 mol L^{-1} phosphate buffer solution (pH 6.0), which was obtained using Na_2HPO_4 and NaH_2PO_4. All solutions were prepared using nanopure water (resistivity > 18.2 MΩ cm) from a Millipore Milli-Q system (Billerica, USA). The 0.1 mol L^{-1} phosphate buffer solution was employed as the supporting electrolyte in all the measurements with the biosensor.

2.2. Apparatus

The electrochemical measurements were recorded with a three-electrode system, including the biosensor Tyr-AuNPs-DHP/GCE as the working electrode, a platinum plate as the counter electrode, and Ag/AgCl (3.0 mol L^{-1} KCl) as the reference electrode at 25 °C, to which all potentials are hereinafter referred. Electrochemical measurements were carried out using an Autolab Ecochemie model PGSTAT12 (Utrecht, Netherlands) potentiostat/galvanostat controlled by GPES 4.9 software.

The morphology and the size of AuNPs were characterized by scanning electron microscopy (SEM) using a Zeiss DSM 940A and transmission electron microscopy (TEM) using a FEG Zeiss Supra 35-VP equipment with electron beam energy of 20 keV. Histograms were constructed using the public-domain ImageJ image-processing software.

For comparison, the catechol determination was also performed spectrophotometrically [34] using a Femto spectrophotometer (model 435, Brazil) with a quartz cuvette (optical path length of 10 mm) set at 500 nm.

2.3. Synthesis of AuNPs

The AuNPs colloidal solution was prepared by citrate reduction of $HAuCl_4$ in aqueous solution by the Turkevich method [35]. Initially, we added 4.0 mL of 0.05 mol L^{-1} $HAuCl_4$ in 200 mL of water at 90 °C under stirring. Then, 2.0 mL of 0.3 mol L^{-1} sodium citrate solution was added, and the mixture was stirred for 4 min at 90 °C until the appearance of a red color. Finally, the reaction was rapidly cooled to room temperature in an ice bath.

2.4. Gold nanoparticles dispersion

AuNPs dispersion was prepared by dispersing 1.0 mg of DHP in 1.0 mL of AuNPs colloidal solution. The dispersion was subjected to ultrasonication for 30 min. In addition, for comparison, we constructed one film containing 1.0 mg of DHP in 1.0 mL of nanopure water in the same way.

2.5. Preparation of the Tyr-AuNPs-DHP/GCE biosensor

The GCE (diameter 5 mm) was polished sequentially with metallographic abrasive paper (No. 6) and slurries of 0.3 μm alumina microparticles to a mirror finish. After being rinsed with nanopure water, the electrode was sonicated in isopropyl alcohol for 3 min and then with nanopure water for a further 3 min, and then dried at room temperature. Afterwards, 20 μL of AuNPs-DHP dispersion was casted onto the GCE, and the solvent was allowed to evaporate at room temperature for 2 h. The electrode was named AuNPs-DHP/GCE. After that, the AuNPs-DHP/GCE was placed in the electrochemical cell containing 0.1 mol L^{-1} KCl, and 20 cycles in the potential range from −0.3 to 0.7 V at a scan rate of 100 mV s^{-1} were applied using cyclic voltammetry (CV). The electrode was carefully washed with nanopure water. Then, the AuNPs-DHP/GCE was modified with cystamine (CYS) and glutaraldehyde (GA) to link the Tyr covalently. Next, 20 μL of 10 mmol L^{-1} CYS solution was casted onto the electrode surface, and the solvent was evaporated at room temperature for 1 h, and was then rinsed with 0.1 mol L^{-1} phosphate buffer solution (pH 6.0). Subsequently, 20 μL of 2.5% (v/v) GA solution was cast onto the surface of the AuNPs-DHP/GCE, and the solvent was evaporated at room temperature for 1 h. The electrode was washed with 0.1 mol L^{-1} phosphate buffer solution (pH 6.0). Finally, 20 μL of a solution containing 2000 units of Tyr in phosphate buffer solution (0.1 mol L^{-1}, pH 6.0) was cast onto the surface of the AuNPs-DHP/GCE, and the solvent was evaporated at room temperature for 2 h. Thereafter, the electrode was thoroughly washed with phosphate buffer solution. The schematic process of the fabrication of the biosensor is presented in Fig. 1. When it was not in use, the Tyr-AuNPs-DHP/GCE was stored in phosphate buffer solution (0.1 mol L^{-1}, pH 6.0) in a refrigerator at 4 °C.

2.6. Samples and reference method

The samples from natural water (A–C) were collected from the lake at the Federal University of São Carlos, São Carlos city, Brazil. The collection points were recorded using a GPS (A = 21°59′08.06″s 47°52′55.13″W, B = 21°59′10.96″s 47°52′52.16″W, and C = 21°59′11.54″s 47°52′42.63″W). Each sample was prepared using an aliquot of 50 mL of the sample, which was transferred to different calibration flasks. Then, aliquots of catechol standard solution was carefully added to the samples and they were stirred in order to homogenize the solutions. The 4-aminoantipyrine spectrophotometric method [34] was performed to compare the results with those obtained by the Tyr-AuNPs-DHP/GCE method employing amperometry.

3. Results and discussion

3.1. Characterization of AuNPs-DHP/GCE

Firstly, we characterized the stability of AuNPs-DHP. The addition of DHP to the AuNPs solution led to the production of a stable solution (data not shown), and this solution became an adherent film when it was casted onto the GCE. We employed SEM to characterize the AuNPs-DHP/GCE and GCE. Fig. 2 shows the GCE (a) and AuNPs-DHP/GCE (b) images, in which it can be observed that the