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Self-assembled films containing crude extract of avocado as a source of tyrosinase for monophenol detection

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ABSTRACT

This paper reports on the use of the crude extract of avocado (CEA) fruit (*Persea americana*) as a source of tyrosinase enzyme. CEA was immobilized via layer by layer (LbL) technique onto indium tin oxide (ITO) substrates and applied in the detection of monophenol using a potentiometric biosensor. Poly(propylene imine) dendrimer of generation 3 (PPI-G3) was used as a counter ion in the layer by layer process due to its highly porous structure and functional groups suitable for enzyme linkage. After the immobilization of the crude CEA as multilayered films, standard samples of monophenol were detected in the 0.25–4.00 mM linear range with approximately 28 mV mM^{-1} of sensitivity. This sensitivity is 14 times higher than the values found in the literature for a similar system. The results show that it is possible to obtain efficient and low-cost biosensors for monophenol detection using potentiometric transducers and alternative sources of enzymes without purification.

1. Introduction

Phenols and some of their derivatives, such as chlorophenol and related aromatic compounds are present in industrial residues from the production of plastics, dyes, drugs, resins, pesticides, detergents, disinfectants and especially paper and cellulose [1]. Classically, the analyses of phenolic species have been carried out by spectrophotometric and chromatographic methods [2,3]. However, these techniques do not allow continuous monitoring and analyses in a short period of time. In this context, analytical devices, i.e., biosensors are promising for this purpose.

Various types of biosensors have been used for the detection of phenolic compounds, including optical [4], amperometric [5] and potentiometric [6] transducers. In most biosensors, the tyrosinase enzyme (also known as polyphenol oxidase) is used as a biological recognition element because it catalyzes several types of phenolic compounds [7].

Particularly, electrochemical transducers based on electric field-effect, such as ion-sensitive field-effect transistors (ISFETs) [8] are interesting for the construction of biosensors because their fabrication on a large scale is favored by microelectronic processes. For example, ISFET-based biosensors that use tyrosinase immobilized on silicon nitride (Si_3N_4) have been used for the determination of phenols in water [9]. On the other hand, the measurement system of ISFET sensors may be perturbed since the FET part is not isolated from the chemical environment. In addition, the immobilization of enzymes on a very small platform may be an obstacle. As an alternative, a separated extended gate field-effect transistor (SEGFET) [10–12] or a sensitive layer connected to the input pin of a high-impedance buffer like an instrumentation amplifier (IA) can be utilized as a readout circuit [13]. Besides the reuse of the FET or IA part in new measures, the flexibility of the extended sensitive layer facilitates the process of enzyme immobilization [14,15].

The use of the alternating physical adsorption of oppositely charged materials or layer by layer technique (LbL) enables the construction of several thin films with a rigorous control of the film architecture at molecular level [16]. Different materials, including biomolecules can be easily immobilized on solid substrates by the LbL technique for the use in biosensors [17]. Therefore, the LbL technique combined with separative platforms enables the production of sensitive membranes to be used as field-effect based biosensors. In other words, enzymes can be combined with suitable materials and immobilized via physical adsorption without compromising their catalytic site and keeping high activity [14].

Vegetal tissue or crude extracts have been alternatively utilized as sources of tyrosinase in order to reduce costs. In these cases, fruits, such as avocado (*Persea americana*) and jack (*Artocarpus integrifolia*) are used as a source of tyrosinase without purification [18,19]. Thereby, biosensors for the determination of phenolic compounds can be constructed in a simple and low-cost way by combining the LbL technique, field-effect biosensors and crude extract of fruits.

This paper reports on the construction of a biosensor for the monophenol determination based on the immobilized crude extract of avocado (CEA) as a source of tyrosinase on indium tin oxide films (ITO) in conjunction with poly(propylene imine) dendrimers (PPI) as

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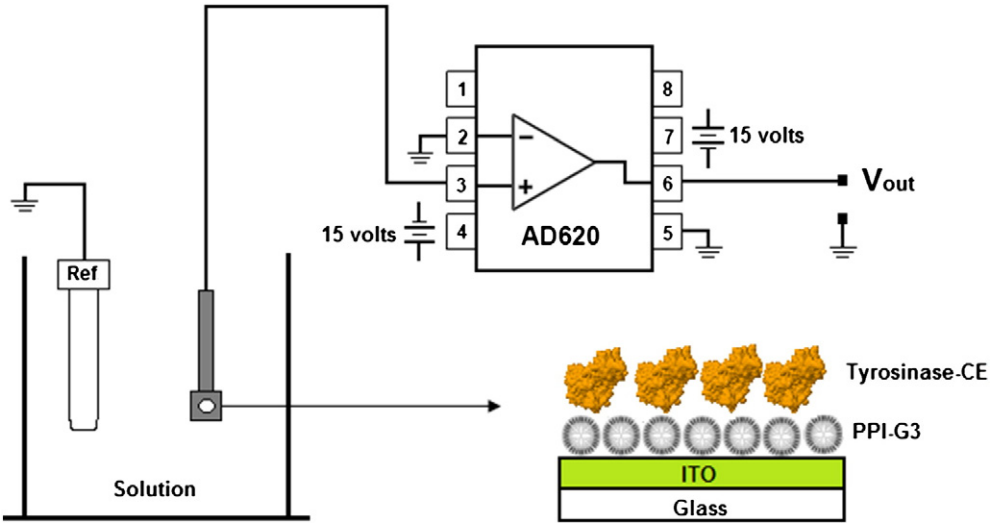

Fig. 1. Schematic diagram of the biosensor.

ligands. Commercial ITO films exhibit Nernstian sensitivity to pH variations [13] and dendrimers are porous macromolecules permeable to H^+ diffusion [11,14] and ideal for biosensors. The activity of the CEA was assessed by measuring the o-quinone formation in reaction with catechol. The CEA immobilization was monitored via UV–vis absorption spectroscopy after each PPI/CEA bilayer had been formed. The PPI/CEA biosensor presented good analytical sensitivity to monophenol in the 0.25 to 4.00 mM linear range.

2. Experimental

2.1. Chemicals

Avocado fruit was utilized as a source of tyrosinase. The methodology of extraction is the same used by da Cruz Vieira and Fatibello-Filho [20]. The formation of o-quinone due to the reaction with catechol was used to determine the activity of as-obtained CEA. A generation 3 of poly(propylene imine) dendrimer (PPI-G3) was synthesized by divergent route from ethylenediamine (EDA) core as described in details elsewhere [11]. ITO-covered substrates (160 nm) were purchased from Delta Technologies and cleaned by immersion in a mixture of HNO_3:HCl:H_2O (1:3:20) for 10 min followed by washing in Milli-Q water. Monophenol was purchased from Sigma-Aldrich. All other reagents were of analytical grade and were used as received.

2.2. PPI/CEA multilayer assembly

The PPI/CEA films were deposited onto a hydrophilic quartz substrate for adsorption studies and onto ITO-covered substrates for biosensor construction. The substrates were immersed in a polycationic PPI solution ((1.0 ± 0.1) mg mL^{-1}) for 5 min and in an anionic tyrosinase solution (CEA) for 10 min. After each immersion, the pre-coated substrate was washed with a phosphate buffer solution (pH 7.0) for few seconds and gently dried by nitrogen flow. The desirable number of PPI/CEA bilayers was achieved upon the repetition of the methodology previously described. The growth of PPI/CEA films was monitored by UV–vis absorption spectroscopy (Hitachi U-2001 spectrophotometer).

2.3. Biosensor configuration

The phenolic biosensor was based on the concept of Vanderspiegel et al. [12] improved by Chi et al. [10]. A sensitive membrane formed by ITO-PPI/CEA was connected to a commercial high-input impedance circuit. An AD620 instrumentation amplifier was utilized as a unit gain buffer. A silver/silver chloride (Ag/AgCl) reference electrode was used to maintain a constant voltage. The PPI/CEA membrane was immersed in a phosphate buffer solution ((10 ±1) mM, pH 6.0) and solutions containing monophenol were added in the measuring cell. The output voltage of the operational amplifier was recorded along the time using a digital multimeter. Fig. 1 illustrates the schematic diagram of the proposed biosensor.

3. Results and discussion

3.1. Characterization of the CEA and PPI/CEA multilayer assembly

Fig. 2 shows the UV–vis absorption spectrum of a catechol solution ((100 ± 10) mM) after exposure to the as-obtained crude extract of avocado. A wide band at around 405 nm, typical of o-quinone structures and formed due to the catalytic oxidation of catechol by tyrosinase enzyme can be observed. This result indicates that the extract obtained from the avocado fruit contains tyrosinase in the active form [20].

Fig. 3 shows the UV–vis absorption spectrum of a diluted solution of CEA (black line) and the UV–vis absorption spectrum of a PPI/CEA in the LbL film (10 bilayers, gray line). As expected for enzymes, a band was observed at around 280 nm due to the presence of aromatic amino acids, such as tryptophan, tyrosine and phenylalanine. When in

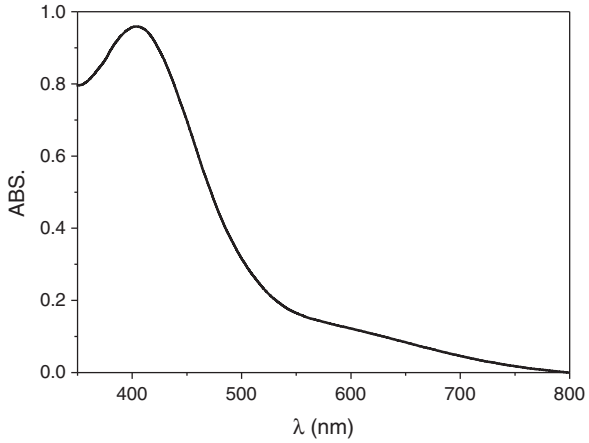

Fig. 2. UV–vis absorption spectrum of a catechol solution (1 mL) after exposure to the crude extract of avocado (0.2 mL).