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# A novel hybrid platform for the preparation of disposable enzyme biosensors based on poly(3,4-ethylenedioxythiophene) electrodeposition in an ionic liquid medium onto gold nanoparticles-modified screen-printed electrodes

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ABSTRACT

A novel electrochemical platform for the preparation of disposable enzyme biosensors is reported in this work. This platform is constructed by electrodeposition of poly(3,4-ethylenedioxythiophene) (PEDOT) using the ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate ($BMIMPF_6$) as the electropolymerization solvent onto gold nanoparticles-modified screen-printed carbon electrodes (SPCE). The enzymes alcohol dehydrogenase (ADH) or Tyrosinase were entrapped onto the electrode surface during the electropolymerization step. The potentiostatic electropolymerization process of PEDOT on gold nanoparticles-modified SPCE was optimized and the resulting modified electrodes characterized voltammetrically and by electrochemical impedance spectroscopy (EIS). The NADH amperometric detection at PEDOT/nAu/SPCE was also optimized and compared with that produced at a PEDOT/SPCE. ADH/PEDOT/nAu/SPCEs were constructed. The measured current for ethanol was 30% larger than that obtained using ADH/PEDOT/SPCEs. At a detection potential of +300 mV, a calibration graph for ethanol with a linear range between 5 and 100 μM was obtained with a detection limit of 2 μM. The PEDOT/nAu/SPCEs electrodes were also tested for the preparation of Tyrosinase biosensors. Using a detection potential of −150 mV, a linear calibration graph for phenol was constructed over the 0.1–50 μM concentration range, with a limit of detection of 0.02 μM.



## 1. Introduction

Poly(3,4-ethylenedioxythiophene) (PEDOT) is a conducting polymer with a high electrochemical stability and low energy band-gap with a wide range of applications including the preparation of electrochemical sensors [1]. PEDOT has been usually synthesized in acetonitrile [2] or using aqueous surfactants – containing EDOT solutions [3]. Recently, ionic liquids (ILs) have been also employed as suitable media for EDOT polymerization [4]. The properties of the resulting polymers were characterized as a function of the IL used for this strategy [5]. ILs act simultaneously as the solvent and supporting electrolyte and their characteristics affect the rapidity of the electropolymerization process as well as the morphology and conductivity of the resulting polymer.

In spite of the PEDOT stability in both doped and un-doped states and the high electrical conductivity and low oxidation potential for the monomer, the use of PEDOT-coatings as enzyme immobilization matrices for the preparation of biosensors is scarce, on the contrary to that occurred with other conducting polymers such as polypyrrole. This is probably due to the fact that water is not adequate for EDOT polymerization because of the poor solubility of the monomer and the interaction of water molecules with the polymerization intermediate [6]. The use of ILs as the solvent for enzymes and the growth medium for electro-synthesis of PEDOT is an interesting approach to overcome these problems. Different ILs were used to prepare enzyme solutions [7]. In particular, alcohol dehydrogenase (ADH) and tyrosinase (Tyr) have proved to exhibit activity in such media [8]. Therefore, pure ILs can be used simultaneously as the media for electropolymerization and the enzyme entrapment onto the polymer network, thus improving the process for biosensor preparation.

Some recent enzyme electrochemical biosensors involving PEDOT-modified electrodes can be found in the literature. An alcohol oxidase biosensor prepared by electrodepositing the polymer at +800 mV onto a platinum electrode immersed into an EDOT solution in sodium dodecyl sulphate (SDS) was reported [9]. PEDOT synthesized in the presence of poly(styrene sulfonic acid) (PSS) was also used for the immobilization of enzymes such as acetylcholinesterase [10]. A glucose biosensor based on a glucose oxidase/PEDOT/Prussian blue bilayer and multi-walled carbon nanotubes has also been reported [11]. Furthermore, gold nanoparticles were electrochemically deposited onto a polymer film prepared from

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commercial PEDOT–PSS. The resulting modified electrode was used for the electrocatalytic oxidation of NADH and the fabrication of an ADH biosensor [12]. Moreover, Zanardi et al. [13] incorporated gold nanoparticles surrounded by anionic encapsulating agents to the electrode coating material. These PEDOT-modified glassy carbon electrodes were applied to the detection of ascorbic acid and dopamine.

In this work we have developed a novel configuration for the construction of enzyme electrochemical biosensors. Both dehydrogenase and oxidase type enzymes bioelectrodes were evaluated. As representative examples of these enzymes groups ADH or Tyr were entrapped during the electropolymerization of EDOT using 1-butyl-3-methylimidazolium hexafluorophosphate ($BMIMPF_6$) as the solvent, onto gold nanoparticles-modified screen-printed carbon electrodes. The electropolymerization process produces incorporation of the homogenously distributed enzyme molecules during its growth process [14,15]. The prepared modified electrodes resulted highly appropriate to obtain suitable electroanalytical responses for NADH and, therefore, for the preparation of dehydrogenase-based biosensors, as well as for phenol as an example applicable to oxidase bioelectrodes. The use of the ionic liquid as the working medium for both enzyme solubilization and polymer electro-synthesis allows taking advantage of its unique properties for biosensor design circumventing the problems found with traditional solvents. The analytical characteristics of the developed disposable biosensors were investigated in detail.

## 2. Experimental

### 2.1. Reagents and solutions

3,4-Ethylenedioxythiophene (EDOT) (Aldrich) was used. For the polymer synthesis, a 0.03 M EDOT solution in $BMIMPF_6$ (IoLiTec, 99%) was prepared. Colloidal gold (Sigma, 20 nm $\phi$) was also used. β-Nicotinamide adenine dinucleotide (reduced form, NADH), β-nicotinamide adenine dinucleotide ($NAD^+$) and alcohol dehydrogenase (ADH) from bakeŕs yeast (451 U/mg), were all from Sigma–Aldrich. Tyrosinase (Tyr) from mushroom (Fluka, 2034 U/mg), ethanol (Scharlau) and phenol (Prolabo) were also used. All the other reagents and solvents were of analytical grade. Phosphate buffer solutions (PBS) were prepared from $Na_2HPO_4$ and $NaH_2PO_4$ (Scharlau).

### 2.2. Apparatus and electrodes

Voltammograms were registered with a BAS (West Lafayette, IN, USA) 100B potentiostat provided with a BAS C2 EF-1080 cell stand. Amperometric measurements were carried out using an Epsilon Multichannel detector from BAS. Electrochemical impedance measurements were performed using a μ-Autolab type III with FRA2 software (Ecochemie). Screen-printed carbon electrodes (SPCE) Dropsens DRP-154 (4 mm in diameter) were used as working electrodes. These electrodes are provided with an Ag/AgCl pseudo-reference electrode and a platinum counter electrode. Screen printed gold electrodes (SPAuE) Dropsens DRP-250AT (4 mm in diameter) provided with a Ag pseudo-reference electrode and a platinum counter electrode, were also used. BAS VC-2 10-mL electrochemical cells were also used. All electrochemical experiments were made at room temperature.

### 2.3. Preparation of PEDOT–gold nanoparticles–modified SPCEs

The SPCEs were firstly modified with gold nanoparticles. The appropriate volume of the colloidal gold suspension was dropped on the SPCE surface and the modified electrode was let to dry under darkness at room temperature. Finally, the electrode was rinsed by mechanically stirring at 250 rpm for 30 s in distilled water. Polymer growth was performed by depositing 40 μL of a 0.03 M EDOT solution in pure $BMIMPF_6$ onto the active surface of the SPCE or the gold nanoparticles-modified SPCE. Then, a potential value of +800 mV vs. Ag/AgCl was applied for 5 min.

### 2.4. Biosensors preparation

The SPCE were modified by depositing 15 μL of the colloidal gold suspension and the same drying and rinsing procedure mentioned above was followed. Then, 40 μL of a $BMIMPF_6$ solution containing 0.03 M EDOT and 2.25 U/μL ADH or 2.5 U/μL Tyr were deposited onto the nAu/SPCE surface. A potential value of +600 mV for the preparation of ADH biosensors or +200 mV in the case of Tyr biosensors were applied for 5 min. Thereafter, the prepared biosensors were rinsed as described in Section 2.3.

### 2.5. Determination of ethanol in beer samples

Approximately 20 mL of beer were degasified by ultrasonic stirring during 20 min. Then, 2 mL were diluted up to 50 mL with 0.1 M phosphate buffer solution of pH 7.4. An aliquot of 20 μL (commercial free-alcohol beer) or 40 μL (certified beer) of the diluted sample was added to 10 mL of the same buffer solution. The determination of ethanol was carried out by amperometry in stirred solutions at +300 mV and by applying the standard additions method, involving the addition of ethanol aliquots from a stock $1.5 \times 10^{-2}$ M solution.

## 3. Results and discussion

### 3.1. Electrodeposition of PEDOT in $BMIMPF_6$ on gold nanoparticle-modified SPCEs

The ionic liquid $BMIMPF_6$ was selected as an appropriate medium for PEDOT electro-synthesis based on literature data [16]. Moreover, it was also reported that ADH [17] and Tyr [18] exhibited a good enzymatic activity and stability in this ionic liquid. As described in Section 2.3, PEDOT was synthesized under potentiostatic conditions from EDOT solutions in $BMIMPF_6$. The polymer electro-synthesis process was optimized using NADH as the target compound evaluating its electrochemical response at the modified electrode. Therefore, once the PEDOT–gold nanoparticles–modified SPCE was prepared, the effect of the different variables on the electrochemical response of NADH at the modified electrode was evaluated. Regarding the electropolymerization potential, a bell-shaped current response, measured by amperometry at a constant potential of +300 mV was obtained using 5 min as the electropolymerization time, with the highest signal at +800 mV (not shown). This latter potential value was selected for further work. Concerning the electropolymerization time, a sharp increase in the NADH oxidation current measured by amperometry at +800 mV was observed up to 2 min while it remained practically constant between 2 and 15 min (results not shown). We selected 5 min as the electrodeposition time because, as it will be commented below, this was the time selected to carry out the enzymes entrapment during electrodeposition.

### 3.2. Characterization of the modified electrodes

Firstly, cyclic voltammograms of SPCEs modified with different loadings of the colloidal gold suspension were recorded in sulfuric acid medium. Conversely to that occurred at a PEDOT/SPCE (without gold nanoparticles) the electrodes modified with gold