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Magnetic silica/titania xerogel applied as electrochemical biosensor for catechol and catecholamines

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ABSTRACT

A magnetic silica/titania material with planned textural properties was obtained by sol-gel method through hydrolysis and condensation of tetraethyl orthosilicate and titanium isopropoxide in the presence of magnetite particles. The resultant material (SiTiMPs) was used as support for direct immobilization of tyrosinase (SiTiMPs-Ty) avoiding additional modification steps. This was possible due to the chemical reactivity of titania due to its surface acidity and also to the textural properties of the material like pore size near 20 nm and specific surface area of 158 $m^2 g^{-1}$. These characteristics combined to the presence of magnetite lead to possibility to prepare carbon paste electrodes with SiTiMPs-Ty material with high electroactive area when tested in the ferricyanide/ferrocyanide redox system. This electrode was applied as efficient electrochemical biosensor for catechol and catecholamines, such as dopamine and epinephrine. It can be observed great sensitivities for all analytes tested in wide linear range. The calculated detection limits were 0.23 μmol L^{-1}, 0.72 μmol L^{-1} and 2.94 μmol L^{-1} for catechol, dopamine and epinephrine, respectively. This electrode presented excellent response for simultaneous analyses of catechol and epinephrine, with high sensitivity and low detection limits. The present sensor showed good reproducibility for catechol detection with a relative standard deviation of 6.9% (n = 5).

1. Introduction

Catechol is an organic aromatic compound widely used as antiseptic, antifungal and antioxidant. It is found in natural products and can be easily adsorbed from the gastrointestinal tract being considered harmful to human health [1]. Catecholamines, such as dopamine and epinephrine, are bioamines with catechol skeleton, though acting as neurotransmitters in human body. Catechol and catecholamines detection and quantification are high value subjects of research and several techniques have been used, such as solid phase extraction [1], High-performance liquid chromatography [2], fluorescence [3], electrophoresis [4] and electrochemical sensors [5]. Electrochemical devices are a highlighted alternative due to its easiness, low cost, considerable sensitivity and selectivity for detection of these compounds. The redox processes of these species are based on the oxidation of two adjacent OH groups to the phenyl ring [6]. In this context, it is desirable to study the development of electrochemical sensors that are able to discriminate these very similar structures.

Electrochemical devices containing tyrosinase are enzymatic systems known by catalyzing oxidation of phenolic compounds, and as typical of biosensors, has prominent selectivity [7,8]. The tyrosinase immobilization and stability are considered critical steps to build these devices, therefore, different supports have been developed as for example polymeric membranes [9], polypyrole films [10] and graphene nanocomposites [11]. Carbon paste electrodes (CPE) are widely known in sensor and biosensor fields mainly due to their facile and simple manufacture, and they can be modified with different electroactive species for sensitive and simultaneous determinations [12,13].

The satisfactory response of enzymes in electrochemical biosensors is dependent on the immobilization approach, and the usual methods involve several activation steps that could affect the reproducibility of the system and also increases manufacturing

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time and costs [14]. Direct immobilization of tyrosinase on matrices could avoid these features and to perform it the matrices must present adequate surface chemical reactivity to interact with enzyme groups that are not part of the catalytic site, maintaining the activity of the enzyme. However, the activity of some enzymes decreases significantly even after direct immobilization, indicating that more efforts are required to explore direct immobilization techniques [15]. Due to the large size of the enzyme macromolecules, materials with adequate surface area and pore size are necessary to allow enzyme immobilization maintaining the availability of active sites and also the diffusion of the analyte [16–18].

Mixed silica/titania oxides can be an excellent choice to prepare electrochemical sensors, due to its chemical reactivity afforded by the surface acidity of titania moiety. These acid sites can interact with basic nitrogen atom in NH_2 or carboxylic groups of biomolecules or enzymes enabling the direct immobilization onto titania surface [19]. Besides, the physical properties combining mechanical strength and thermal stability are adequate to prepare the electrodes. Silica/titania material has been applied in other scientific areas as solar devices [20,21] or photocatalysts [22–24], however the total potential of this material for development of electrochemical sensors has been few explored [25,26]. As far as we have observed only one report is found dealing about the direct immobilization of laccase enzyme in titania matrix [27] and there is no previous report about direct immobilization of tyrosinase in these supports.

The sol-gel method is an excellent strategy to achieve homogeneous and polycondensed compounds with large specific surface area and planned porosity, making silica/titania a promising porous mixed oxide to host biomacromolecules as tyrosinase enzyme.

To add magnetic properties to silica/titania materials could improve their performance as electrochemical sensors [28]. Recently magnetic silica/titania materials were prepared mainly as core shell systems to be applied as photocatalysts or adsorbents [29–32]. When applied in the preparation of sensors, magnetic materials can provide signal amplification, improving the electroactive area although the mechanism is not well known [33]. Iron-oxide particles also provide a favorable microenvironment for electrochemical devices where enzymes may exchange electrons directly with the transducer, improving the sensitivity and the selectivity of electrochemical biosensors. Besides, magnetite particles provide biocompatibility and benefit the development of electrochemical biosensors, which is ideal to be applied in different analytical bio recognition [34–36]. Sol-gel technique is an excellent alternative to obtain a good dispersion of magnetite particles in inorganic matrices and also to help in their stabilization. As far as we know, there are no biosensors based on magnetic silica/titania materials obtained by the sol-gel method.

In this work, it was synthesized a magnetic silica/titania by sol-gel method with planned surface area and controlled porosity where tyrosinase was directly immobilized. This new material was employed in the preparation of CPE, characterized by cyclic voltammetry and applied as biosensor for catechol and catecholamine using differential pulse voltammetry.

2. Materials and methods

2.1. Materials and reagents

Iron chloride hexahydrate (Vetec, 97%), ethylene glycol (Merck, 99,5%), sodium acetate trihydrate (Nuclear, 99%), ammonium hydroxide (Merck, 25%) tetraethyl orthosilicate (TEOS) (Aldrich, 98%), titanium (IV) isopropoxide (TIPOT) (Aldrich, 97%), ethanol (Merck, 99.9%), hydrofluoric acid (HF) (Merck, 40%), hydrochloric acid (HCl) (Merck, 37%), tyrosinase (Ty) (Sigma, E.C. 1.14.18.1 with activity of 1000 U/mg), pyrocatechol (Sigma Aldrich, 99%), (−)-epinephrine (Sigma, >99%), dopamine (Sigma, 98%), potassium chloride (Sigma Aldrich, 99%), all analytical grade, were used without previous purification. Phosphate buffer solution (PBS) (0.1 mol L^{-1}, pH 7.0) was prepared from NaH_2PO_4 (FMaia, 98%) and Na_2HPO_4 (FMaia, 99%). All the solutions were prepared in distilled water.

2.2. Synthesis of magnetic particles (MPs)

Synthesis of magnetite particles was performed through solvothermal method by dissolving 2.16 g of iron chloride in 100 mL of ethyleneglycol and then adding 10.88 g of sodium acetate trihydrate. The mixture was stirred for 1 h, transferred to a Teflon lined stainless steel autoclave and heated up to 180 °C for 10 h. After, the black precipitate was separated using a magnet, washed thoroughly with ethanol and vacuum-dried for 2 h.

Silica shell was obtained using an adapted Stöber method [37]. Magnetite particles (300 mg) were dispersed in a mixture of ethanol (90 mL), distilled water (22.5 mL) and ammonium hydroxide (2.7 mL) in ultrasonic bath for 1 h. Then, 0.670 mL of TEOS was added and the system remained in ultrasonic bath for two additional hours. At this point, magnetite particles covered with silica (MPs) were separated with a magnet, washed in sequence with ethanol, water and ethanol, then vacuum-dried for 2 h.

2.3. Synthesis of magnetic silica/titania xerogel (SiTiMPs)

Magnetic silica/titania was synthesized by sol-gel method using TEOS and TIPOT as precursors with a TEOS/TIPOT mass ratio of 2.3. For this purpose, TEOS (1.56 mL) were hydrolyzed in ethanol (2.25 mL) in presence of water (0.23 mL) and hydrochloric acid (0.15 mL). The solution was kept under magnetic stirring at room temperature for 1 h, and then TIPOT (0.66 mL) was added. When the mixture became completely homogeneous, it was added to 1.5 mL of an ethanolic suspension of MPs (0.8 g). In the last step, to perform the gelation, a mixture of 0.9 mL of HF and 0.4 mL of HCl was used as catalyst. After seven days, the obtained monolith was powdered, washed with distilled water and ethanol, and vacuum-dried for 2 h, at room temperature.

2.4. Tyrosinase enzyme immobilization

Tyrosinase enzyme was immobilized in SiTiMPs material using a PBS Ty solution containing 0.25 mg mL^{-1} of protein. For this, 0.5 g of material was immersed in 10 mL of Ty solution, at room temperature, under shaking by 12 h. The resultant material was washed with PBS, vacuum-dried for 4 h at room temperature and it was called SiTiMPs-Ty. Fig. 1 shows an illustrating scheme of the SiTiMPs synthesis steps and the immobilization of tyrosinase enzyme in this material.

2.5. Materials characterization

X-ray diffraction measurements were performed using a Siemens diffractometer model D500 with CuKα as X-ray source (λ = 0.154056 nm) at a generator voltage of 40 kV and a generator current of 17.5 mA.

Scanning Electron Microscopy images were obtained using a scanning electron microscope model Zeiss EVO MA10, equipped with a backscattered electron detector. The materials were dispersed on a double side conducting tape on an aluminum support and coated with a thin film of gold using a BaltedSCD 050 Sputter Coater apparatus. The images were obtained at an accelerating voltage of 10 kV.

The magnetic properties were investigated by using an