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Bismuth nanoparticles for phenolic compounds biosensing application

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ABSTRACT

The rapid determination of trace phenolic compounds is of great importance for evaluating the total toxicity of contaminated water samples. Nowadays, electrochemical tyrosinase (Tyr) based biosensors constitute a promising technology for the *in situ* monitoring of phenolic compounds because of their advantages such as high selectivity, low production cost, promising response speed, potential for miniaturization, simple instrumentation and easy automatization. A mediator-free amperometric biosensor for phenolic compounds detection based on the combination of bismuth nanoparticles (BiNPs) and Tyr for phenol detections will be hereby reported. This is achieved through the integration of BiNPs/Tyr onto the working electrode of a screen printed electrode (SPE) by using glutaraldehyde as a cross-linking agent. BiNPs/Tyr biosensor is evaluated by amperometric measurements at −200 mV DC and a linear range of up to 71 μM and 100 μM and a correlation coefficient of 0.995 and 0.996 for phenol and catechol, respectively. The very low DC working potential ensures the avoidance of interferences making this biosensor an advantageous device for real sample applications. In addition, the response mechanism including the effect of BiNPs based on electrochemical studies and optical characterizations will be also discussed. The obtained results may open the way to many other BiNPs applications in the biosensing field.

1. Introduction

The determination of trace phenol compounds released into the ground and surface water is of special importance. Phenolic compounds are widely used in petrochemical products and in wood preservatives, textiles, plastics, dyes, paper, herbicides and pesticides (Shan et al., 2009; Lu et al., 2010). Most of them can be highly toxic and many health problems are related to them, as they could be absorbed through oral, dermal, or respiratory tracts (Shan et al., 2009; Apetrei et al., 2011). The development of methods to identify and quantify phenolic compounds in various samples to evaluate their total toxicity in environmental and human health problems is of great relevance. Therefore, numerous analytical methodologies for detecting phenolic compounds, such as, ultraviolet spectrophotometric analyses, gas chromatography, liquid chromatography, or capillary electrophoresis have been developed. These methods offer good limits of detection (LODs) and wide working concentration ranges. However, they need the use of sophisticated and relatively costly apparatus and they generally require complicated pretreatment procedures. Recently, large efforts have been focused on the development of simple and effective analytical methods for the determination of phenolic compounds (Tsai and Chiu, 2007; Lu et al., 2010).

Nowadays, electrochemical enzyme-based biosensors constitute promising technology for the *in situ* monitoring of phenolic compounds because of the advantages that they present, such as high selectivity, low production cost, promising response speed, potential for miniaturization, simple instrumentation and easy automation (Apetrei et al., 2011; Lu et al., 2010). Among the developed electrochemical methods, amperometric biosensors based on tyrosinase (Tyr) and polyphenol oxidase (PPO) have proved to be sensitive and convenient for the determination of phenolic compounds (Song et al., 2011; Tsai and Chiu, 2007; Shan et al., 2009). In particular, tyrosinase (Try) enzyme catalyzes the hydroxylation of monophenols to o-diphenols (monophenolase activity) by oxygen, and also catalyzes the oxidation of o-diphenols to o-quinones (catecholase activity). O-quinones can be later electrochemically reduced to catechol without any mediator on the electrode surface. The detection of phenol derivates thus relies on monitoring the catechol product (Tsai and Chiu, 2007; Apetrei et al., 2011; Cosnier and Popescu, 1996). The effective immobilization of tyrosinase on the electrode surface is considered a key step in the development of tyrosinase biosensors for phenolic compounds determination (Lu et al., 2010). More recently, various supporting materials have been successfully used to immobilize tyrosinase (Tyr) on the electrode. In addition, there are several related works reporting the development of

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biosensors where tyrosinase is immobilized onto the screen printed electrode (SPE) (Song et al.,2011) that can be implemented in portable systems as an alternative detection method for the direct on-site analysis.

Nanomaterials have also been used to fabricate tyrosinase-based biosensor, especially nanoparticles, such as ZnO nanoparticles (Li et al., 2006), calcium carbonate nanoparticles (Shan et al., 2007), gold nanoparticles (Song et al., 2011; Pingarrón et al., 2008), or hydroxyapatite nanoparticles (Lu et al., 2010). On the other hand bismuth (Merkoçi et al., 2010), and bismuth oxide (Shan et al., 2009) films have recently been reported for the detection of phenolic compounds by using amperometric biosensors. These materials applied to electroanalysis have opened a new range of possibilities for the construction of phenolic electrochemical biosensors based on these nanomaterials.

In addition to its regular size bismuth nanoparticules (BiNP) shows other advantages such as a larger specific surface area and biocompatibility. These properties provide an excellent platform for enzyme and protein immobilizations.

In the current study, we propose the development of an amperometric BiNP/Tyr-based biosensor. This is achieved through BiNP/Tyr integration onto the working electrode of a screen printed electrode (SPE) by using glutaraldehyde as a cross-linking agent. The resulting BiNP/Tyr-based biosensor exhibited high sensitive response toward phenol and catechol detection with very low detection limits (26 nM for catechol and 62 nM for phenol) and showing a linear response up to 100 μM and 71 μM for catechol and phenol, respectively.

2. Materials and methods

2.1. Apparatus and reagents

Electrochemical measurements were performed with a model CH-Instrument potentiostat 660 A electrochemical workstation from CH Instruments Inc., Austin, TX. Impedance analysis of the prepared SPE modified electrodes was performed by using an Autolab302 potentiostat/galvanostat/frequency-response analyzer PGST30, controlled by GPES/FRA Version 4.9. Magnetic stirrer was used to provide the convective transport during the amperometric measurements. Scanning electron microscope (SEM) analysis were performed by using a EVO (Carl Zeiss NTS GmbH. Germany). Transmission electron microscope (TEM) images were taken with a JEM-2011 (Jeol, Ltd., Japan). X-ray powder diffraction patterns were collected on a Siemens D-5000 diffactometer (German) by using Cu Kα radiation. Patterns were recorded over the 2θ range 20–65° at 40 kV and 40 mA. Z-Potential measurements were made with a Malvern ZetaSizer Nano ZS instrument operating at a light source wavelength of 532 nm and a fixed scattering angle of 173° for detection.

Glutaraldehyde (at 25%), tyrosinase from mushroom ($\geq$ 1000 unit/mg), phenol, potassium dihydrogen phosphate, potasium monohydrogen phosphate dehydrate and potassium chloride were purchased from Sigma Aldrich. For the synthesis of bismuth nanoparticles, bismuth (III) nitrate pentahydrate (reagent grade, 98%) from Aldrich was used. Potassium hydroxide, ACS-ISO-For analysis and isopropanol (synthesis grade) were obtained by Carlo Ebra reagents. Poly (vinyl pirrolidone) (PVP, MW$\sim$30.000) was acquired from Aldrich. J. T. Baker Chemicals B. V. supplies ethylene glycol. All solutions were prepared with ultra-pure water from a Millipore-MilliQ system.

2.2. Synthesis of bismuth nanoparticles

One-step bismuth (III) nitrate reduction method has been used in order to obtain bismuth nanoparticles (Wang and Kim, 2008). In this synthesis, 0.1 g of $Bi(NO_3)_3$, 0.19 g KOH and 0.05 g of Poly(vinyl pirrolidone) (PVP, MW $\approx$ 30.000) were dissolved in 25 mL of ethylene glycol (EG). The mixture was heated up to 185 °C and kept under nitrogen atmosphere and constant stirring during 2 h. The obtained nanoparticles were cleaned with isopropanol and milli-Q water by centrifugation. The obtained nanoparticles were dried by a nitrogen airflow. These nanoparticles can also be partially dispersed in water and ethanol.

2.3. Preparation of Screen Printed Electrode (SPE) and modification with BiNP and Tyr

Screen printing electrodes fabrication is based on the sequential deposition of a graphite ink, Ag/AgCl ink and insulating ink on a polyester substrate. After the deposition of each layer, a drying process is followed by keeping the polyester substrate at 120 °C for 45 min (graphite) and 30 min (Ag/AgCl and insulating). A 5 μL BiNP at 1 mg/ml solution drop was deposited onto the working SPE electrode surface and allowed to dry at room temperature for 20 min. 1 mg of tyrosinase (Tyr) enzyme was dissolved in 50 μL of 0.1 M phosphate buffer at pH 6.5. A 7 μL drop of Tyr solution was deposited onto the working SPE/BiNP electrode surface and allowed to dry at room temperature for 3 h. Finally, 5 μL drop of glutaraldehyde (Glu) solution at 1% was casted onto the SPE/BiNP/Tyr electrode surface and let to dry at 40 °C for 30 min. The prepared SPE/BiNP/Tyr/Glu sensor was kept at 4 °C.

2.4. O-quinone synthesis

As to determine phenol detection mechanism o-quinone was synthesized following the method described by Laird et al. (Aebisher et al., 2007). A solution composed of catechol (0.1 g), acetone (10mL) and silver oxide (0.4 g) was stirred during 10 min. The obtained solution was immediately filtered and dried. To purify this product it was dissolved in water and extracted again by using chloroform. After the extraction, the product is dried under vacuum.

2.5. Electrochemical experiments

All electrochemical experiments were carried out at room temperature. In order to study the characteristics of the BiNPs/Tyr modified electrodes, electrochemical impedance spectroscopy (EIS) studies were performed in 1 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) mixture with KCl 0.1 M as redox probe. The AC frequency range studied was from 0.1 Hz to 100 kHz, with logarithmic scale of 10 points per decade and an applied potential of 50 mV of AC Potential. Nyquist (imaginary impedance versus real impedance) diagrams were also recorded. CV measurements were carried out at the potential range of -0.8 to 0.8 V vs. Ag/AgCl with an scan rate of 50 mv/s. All electrochemical experiments were carried out in 0.1 M phosphate buffer (PB) at pH 6.5 with 0.1 M KCl.

3. Results and discussion

3.1. Morphological studies

A homogeneous distribution of the BiNP on the electrodes surface can be observed by Scanning Electron Microscopy (SEM) image (see Fig. 1A). Morphological and structural studies of bismuth nanoparticles were performed by Transmission Electron Microscopy (TEM). Highly regular and uniform average diameter of around 244 nm (see Fig. 1B) can be measured.

Z-potential (surface charge) measurements are a commonly used tool to determine the stability of a colloidal suspension of