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Electrocatalytic processes promoted by diamond nanoparticles in enzymatic biosensing devices

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ABSTRACT

We have developed a biosensing platform for lactate determination based on gold electrodes modified with diamond nanoparticles of 4 nm of nominal diameter, employing the enzyme lactate oxidase and (hydroxymethyl)ferrocene (HMF) as redox mediator in solution. This system displays a response towards lactate that is completely different to those typically observed for lactate biosensors based on other nanomaterials, such as graphene, carbon nanotubes, gold nanoparticles or even diamond nanoparticles of greater size. We have observed by cyclic voltammetry that, under certain experimental conditions, an irreversible wave ($E^0 = +0.15$ V) appears concomitantly with the typical Fe^{II}/Fe^{III} peaks ($E^0 = +0.30$ V) of HMF. In this case, the biosensor response to lactate shows simultaneous electrocatalytic peaks at +0.15 V and +0.30 V, indicating the concurrence of different feedback mechanisms. The achievement of a biosensor response to lactate at +0.15 V is very convenient in order to avoid potential interferences. The developed biosensor presents a linear concentration range from 0.02 mM to 1.2 mM, a sensitivity of 6.1 μA mM^{-1}, a detection limit of 5.3 μM and excellent stability. These analytical properties compare well with those obtained for other lactate-based biosensors that also include nanomaterials and employ HMF as redox mediator.

1. Introduction

Nowadays, the employment of different kinds of nanomaterials, such as metallic nanoparticles, carbon nanotubes or graphene, in the development of electrochemical biosensors is a field of great interest [1,2]. These nanomaterials present important characteristics that contribute to increase the performance of the biosensing devices. Among them, it can be highlighted a large specific surface area, a high electronic conductivity and an increase in the surface functionalization possibilities. From the point of view of electrochemical biosensing, these nanomaterials offer not only a large surface area for immobilizing a high amount of a wide variety of recognition biomolecules, but also promote the electron transfer between the electrode surface and the biomolecule [3].

Recently, a member of the carbon nanomaterials family, denoted as diamond nanoparticles (DNPs), has also become a promising alternative for biosensor development. Until now, although they present a wide range of interesting properties, DNPs have been less employed for this purpose than the rest of the carbon nanomaterials. Among the properties that make them suitable candidates for biosensing can be mentioned [4–7]: i) their production at large-scale by detonation methods with a narrow size distribution and a moderate cost, ii) the presence on their surface, as a consequence of the production and purification methods, of several oxygenated functional groups, which facilitate the immobilization of biomolecules (peptide nucleic acids, antibodies, enzymes) [8–11] and iii) their biocompatibility and noncytotoxic nature.

In contrast to these excellent properties, diamond material has an insulating character, with a band gap of 5.47 eV [12], which is, in principle, inadequate for electrochemical applications. However, several fundamental studies have shown that, despite the great bandgap value, electrodes modified with DNPs exhibit a high electrochemical response towards different charged redox probes in solution such as $Fe(CN_6)^{3-/4-}$, $Ru(NH_3)_6^{2+/3+}$, $Ru(CN)_6^{3-/4-}$, $IrCl_6^{2-/3-}$ [13–15], and they are also able to promote direct electron transfer between redox enzymes and the underlying electrode [16]. This unexpected behavior is due to the existence of discrete electronic states within the diamond band gap originated by the overlapping of molecular orbitals of the different functionalities with unsaturated bonding present on the diamond nanoparticles surface. Therefore, DNPs can be either oxidized or reduced depending on their potential relative to the underlying electrode or to a redox probe [13,14]. Recently, the electrochemical behavior of neutral redox probes in solution has also been studied

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employing DNPs modified electrodes, showing an oxidation/reduction current even higher than that obtained for charged species [17].

However, the employment of DNPs in biosensors development is still scarce. In this sense, in a previous work, we reported [18] the development of an electrochemical enzymatic biosensor nanostructured with diamond nanoparticles of 9 nm of nominal diameter (DNPs9), employing a neutral probe, (hydroxymethyl)ferrocene, as redox mediator in solution. The results obtained demonstrated the suitability of this nanomaterial to prepare high performance electrochemical biosensing platforms that allow lactate determination at +0.30 V. In the present work, we want to go one step further by developing a similar biosensing platform, but based on DNPs of smaller size (4 nm of nominal diameter), that allows diminishing the potential value for lactate determination. To undertake this study, we started delving into the effect of several factors, such as pH and scan rate on the HMF electrochemical behavior at 4 nm DNPs modified gold electrodes (DNPs4/Au). Once these parameters were optimized, lactate oxidase (LOx) was immobilized onto the DNPs4 modified electrode and the electrocatalytic response of the LOx/DNPs4/Au system towards lactate was studied and compared with those of the LOx/DNPs9/Au biosensor. Finally, a similar biosensor, but based on glucose oxidase, was fabricated in order to assess the broad applicability of the developed platform.

2. Experimental section

2.1. Materials

Diamond nanoparticles (DNPs4 and DNPs9) are obtained from SkySpring Nanomaterials (Products 0510HZ and 0512HZ, respectively), Inc. (Houston, TX). According to data provided by the manufacturer, DNPs contain —OH, —CN, —COOH, C—O—C and C═O as functionality groups and their nominal diameters are 3–4 nm and 4–15 nm, respectively. DNPs suspensions (1 mg mL^{-1}) were prepared in water. Lactate oxidase (LOx, EC 232-841-6 from *Pediococcus* species) and glucose oxidase (GOx, EC 1.1.3.4 from *Aspergillus niger*) lyophilized powder containing 41 units/mg solid and 15,200 units/g solid, respectively were obtained from the Sigma Chemical Co. (St. Louis, MO). Stock solutions were prepared dissolving 1.3 mg of the LOx lyophilized powder in 500 μL of 0.1 M phosphate buffer solution (pH = 7.0) and 7.5 mg of the GOx lyophilized powder in 250 μL of 0.1 M phosphate buffer solution (pH = 7.0). The enzyme solutions were aliquoted (10 μL) and stored at −30 °C. Under these conditions, the enzymatic activity remains stable for several weeks. L-(+)-Lactic acid lithium salt 97%, D-(+)-glucose (99.5%) and (hydroxymethyl)ferrocene (HMF) were obtained from Aldrich Chemical Co. (Milwaukee, WI). An enzymatic assay kit for lactate determination (K-LATE 07/14) was purchased from Megazyme (Ireland). Sodium phosphate (Merck) was employed for the preparation of buffer solutions. Other chemicals used in this work were reagent grade quality and used as received without additional purification steps. Water was purified with a Millipore Milli-Q-System. All solutions were prepared just prior to use.

2.2. Experimental techniques

Electrochemical measurements were carried out with an Ecochemie Autolab PGSTAT12 system (Utrecht, The Netherlands) employing a three-compartment cell with a working gold electrode and a platinum wire as counter electrode. All potentials were reported with respect to a Ag/AgCl reference electrode. All solutions were deaerated with nitrogen gas before use, keeping the gas flow over the solutions during experiments.

FT-IR measurements were performed employing a Bruker IFS66v spectrometer. All the spectra were obtained from 7000 to 550 cm^{-1} using a 4 cm^{-1} resolution. 250 scans were recorded for each spectrum and background was subtracted in all the cases. FT-IR measurements were carried out from KBr pellets.

Atomic Force Microscopy (AFM) data were obtained with a Nanoscope IIIa equipment (Veeco) under ambient conditions by using silicon tips, with a nominal spring constant in the 1–5 N/m range and a nominal tip radius of 8 nm (Bruker). Images have been taken in the intermittent contact mode.

2.3. Procedures

2.3.1. Preparation of the electrochemical biosensing platforms

Prior to each experiment, gold electrodes were polished with 1 μm diamond paste (Buehler) and rinsed with water. Then, they were conditioned to obtain a proper baseline and stable response by holding the potential at +2.0 V (vs Ag/AgCl) for 5 s in 0.1 M H_2SO_4 and then at −0.35 V (vs Ag/AgCl) for 10 s, followed by potential cycling from −0.20 to +1.5 V (vs Ag/AgCl) at 5 V/s for 2 min. Subsequently, the cyclic voltammogram characteristic of a clean polycrystalline gold electrode was recorded, from −0.2 to +1.5 V (vs Ag/AgCl), at 100 mV s^{-1}. The electrode was subsequently rinsed with water, air-dried and modified with 5 μL of the DNPs suspension (system denoted as DNPs/Au). Finally, 5 μL of the GOx or LOx stock solution was placed onto the DNPs/Au electrode surface (GOx/DNPs/Au or LOx/DNPs/Au). For control experiments, LOx/Au and GOx/Au platforms were also developed by placing 5 μL of the enzyme stock solution onto a bare Au electrode surface.

2.3.2. Samples for AFM measurements

Samples for determining the average diameter of diamond nanoparticles were prepared in a particular way due to the great tendency of DNPs to agglomerate [19]. It is necessary to deposit single nanoparticles on a flat substrate, such as silicon (surface roughness: 0.2 nm), in order to obtain their height distribution unambiguously. Silicon substrates were located at the bottom of a vase containing the suspension of DNPs in water and sonicated during one hour. In this way, the aggregates, thanks to the shaking process, were able to disaggregate to some extent [20], leading to deposits on the substrate with both aggregates and isolated DNPs. Afterwards, the DNPs modified surface was dried under nitrogen and measured by AFM in the intermittent contact mode. Note that to avoid tip convolution effects, the size distribution was obtained by measuring the height of the isolated DNPs.

2.3.3. Determination of L-lactic acid in real samples

The concentration of L-lactic acid in a white wine (purchased in a local market) was determined by the standard addition method. For this, 2.0 mL of wine sample, after dilution 1:100 in distilled water, were mixed with increasing volumes (0, 50, 100, 150, 200 and 250 μL) of a 0.010 M lactic acid standard solution and diluted to 10.0 mL in a volumetric flask with 0.1 M phosphate buffer (pH = 7.0) containing HMF (final concentration of HMF 1.0 mM). The L-lactic acid concentration obtained employing the developed biosensor was compared to those obtained by a commercial enzymatic assay kit according to the manufacturer's instructions. This enzymatic assay is based on L-lactate dehydrogenase and D-glutamate-pyruvate transaminase. The NADH formed was measured by the increase in absorbance at 340 nm and correlated with the L-lactic acid concentration.

3. Results and discussion

Diamond nanoparticles are available from different commercial sources. Depending on the specific synthesis conditions and purification procedures employed in their fabrication, the resulting nanomaterials can present different sizes and physicochemical properties and, therefore, differences in their surface chemistry [14], which clearly affect their subsequent behavior. In particular, previous studies have shown that DNPs diameter has a great influence on their reactivity [17,21]. Thereby, the percentage of the number of atoms on the DNPs surface, the number of functionality groups and the sp^2/sp^3 carbon ratio are