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# Diamond nanoparticles based biosensors for efficient glucose and lactate determination

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ABSTRACT

In this work, we report the modification of a gold electrode with undoped diamond nanoparticles (DNPs) and its applicability to the fabrication of electrochemical biosensing platforms. DNPs were immobilized onto a gold electrode by direct adsorption and the electrochemical behavior of the resulting DNPs/Au platform was studied. Four well-defined peaks were observed corresponding to the DNPs oxidation/reduction at the underlying gold electrode, which demonstrate that, although undoped DNPs have an insulating character, they show electrochemical activity as a consequence of the presence of different functionalities with unsaturated bonding on their surface. In order to develop a DNPs-based biosensing platform, we have selected glucose oxidase (GOx), as a model enzyme. We have performed an exhaustive study of the different steps involved in the biosensing platform preparation (DNPs/Au and GOx/DNPs/Au systems) by atomic force microscopy (AFM), field emission scanning electron microscopy (FE-SEM) and cyclic voltammetry (CV). The glucose biosensor shows a good electrocatalytic response in the presence of (hydroxymethyl)ferrocene as redox mediator. Once the suitability of the prototype system to determine glucose was verified, in a second step, we prepared a similar biosensor, but employing the enzyme lactate oxidase (LOx/DNPs/Au). As far as we know, this is the first electrochemical biosensor for lactate determination that includes DNPs as nanomaterial. A linear concentration range from 0.05 mM to 0.7 mM, a sensitivity of 4.0 μA $mM^{-1}$ and a detection limit of 15 μM were obtained.



## 1. Introduction

In recent years, a wide range of carbon nanomaterials has been explored as potential based platforms for developing biosensing systems. Among them, carbon nanotubes and graphene are the most employed due to their unique mechanical, electrical, thermal and optical properties (Merkoçi et al., 2005; Pumera, 2009; Yang et al., 2010; Kochmann et al., 2012). Recently, a new member of the carbon nanomaterials family, denoted as diamond nanoparticles (DNPs), has gained attention since it presents some additional advantages compared to other carbon nanomaterials such as an excellent biocompatibility, a noncytotoxic nature, a narrow size distribution and a moderate price since it can be produced at large-scale by detonation methods. Furthermore, as a consequence of the purification procedures employed after fabrication, DNPs possess several oxygenated functional groups on its surface, including hydroxyl and carboxyl groups, which facilitate the immobilization of biomolecules. Due to this interesting surface chemistry, it has been possible to conjugate DNPs with peptide nucleic acids (Gaillard et al., 2014), anticancer-drugs (Li et al., 2010), antibodies (Zhang et al., 2014) and proteins such as glucose oxidase (Zhao et al., 2006), hemoglobin (Zhu et al., 2007), alcohol dehydrogenase (Nicolau et al., 2012), cytochrome c (Chang and Lora Huang, 2004), lysozyme (Chang et al., 2007), and bovine seroalbumine (Wang et al., 2011). The possibility of immobilizing a wide variety of biomolecules in conjunction with the excellent properties mentioned above, makes DNPs good candidates for several biomedical applications such as protein separation, drug delivery and biosensing (Man and Ho, 2012; Puzyr et al., 2007). Particularly, in the field of biosensors, DNPs have been incorporated in analytical platforms for glucose (Zhao et al., 2006),

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alcohol (Nicolau et al., 2012) or nucleic acids (Gaillard et al., 2014) determination. Furthermore, it has been proven that DNPs are able to promote direct electron transfer between redox enzymes and the underlying electrode (Zhu et al., 2007).

Concerning the employment of DNPs for electrochemical biosensors, it should be highlighted that, in contrast with the advantages mentioned above, undoped diamond has an insulating character, with a band gap of 5.47 eV (Clark et al., 1964). An approach commonly used to overcome this problem consists in the employment of diamond doped with different elements such as boron, as a way to increase its conductivity (Cunci and Cabrera, 2011). However, several studies have shown that, despite the great bandgap value, undoped diamond can also be considered as a potential material for electrode fabrication. In this sense, several strategies to incorporate DNPs into electrodes can be found in the literature. In one of the first reports concerning the employment of DNPs as electrode (Novoselova et al., 2004), they were sintered at high pressure and temperature to form pellets. In this case, the resistance of the powder was so high that the electrochemical response of a redox probe in solution was superimposed on a linearly sloping background, similar to that obtained for a resistor. However, some later reports show that undoped DNPs immobilized onto an electrode surface enhance the electrochemical response of different redox probes in solution, such as $Fe(CN)_6^{3-/4-}$, $Ru(CN)_6^{3-/4-}$, $Ir(Cl)_6^{2-/3-}$, $Ru(NH_3)_6^{3+}$ and (hydroxymethyl)ferrocene (Holt et al., 2009; Holt, 2010; Scholz et al., 2011; Varley et al., 2014). According to these authors, the enhancement of the oxidation/reduction current is a consequence of i) electron transfer between diamond nanoparticles and the redox probe in solution, evidencing the existence of DNPs surface redox states at given pH-dependent potentials and ii) electron transfer mediated by the redox probe adsorbed onto the diamond nanoparticles surface. The surface redox states are related to the presence of a considerable number of functional groups at the DNPs surface, as a consequence of the synthesis procedure and the subsequent purification methods. Therefore, although bulk diamond is known to be a good insulator, its properties cannot be generalized at the nanoscale since new phenomena may appear. In this case, it can be assumed that although DNPs are non-conducting in a conventional manner, they do display redox activity.

In this article, we address some issues concerning the employment of diamond nanoparticles as a promising nanomaterial for electrochemical biosensing applications. As a model system, we develop a DNPs-based biosensing platform employing glucose oxidase. This first prototype was employed to perform an exhaustive study of the system, helping us to extend further the acquired knowledge to the development of lactate oxidase biosensors. In a first step, we have immobilized DNPs on a gold electrode and we have characterized the resulting surface by atomic force microscopy (AFM), field emission scanning electron microscopy (FE-SEM) and cyclic voltammetry (CV). These studies demonstrate the suitability of a direct adsorption strategy to immobilize DNPs onto metallic surfaces. Afterwards, glucose oxidase (GOx) was directly adsorbed onto the DNPs modified gold electrode. The morphology of GOx/DNPs/Au surfaces was studied by AFM and FE-SEM, the enzyme layer thickness was determined by force spectroscopy measurements and the electrochemical response towards a redox probe in solution was obtained. Finally, the response of the biosensor towards glucose was studied. Once the suitability of the prototype system to determine glucose was verified, in a second step, we prepared a new biosensor based on the same procedure but employing the enzyme lactate oxidase. To our knowledge, this is the first electrochemical biosensor for lactate determination that includes DNPs as nanomaterial.

## 2. Experimental section

### 2.1. Materials

Diamond nanoparticles (DNPs) are obtained from SkySpring Nanomaterials, Inc. (Product 0512HZ) (Houston, TX). 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 U/mg solid and 15,200 U/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 250 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 acetate buffer solution (pH=4.5), 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%), acetaminophen, uric acid, ascorbic acid and (hydroxymethyl)ferrocene (HMF) were obtained from Aldrich Chemical Co. (Milwaukee, WI). Sodium phosphate and sodium acetate (Merck) were 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

The Atomic Force Microscopy (AFM) data were obtained with a Nanoscope IIIa equipment (Veeco). For those studies performed in air, silicon tips, with a nominal spring constant in the 1–5 N/m range and a nominal tip radius of 8 nm (Bruker) were employed. For those measurements performed in buffer conditions oxide sharpened silicon nitride tips, with a spring constant of 0.24 N/m and nominal tip radius of 10 nm, were used. All images have been taken in the intermittent contact mode.

We have also performed force spectroscopy measurements by recording the approach force curves where the cantilever deflection is registered as a function of the vertical *z*-displacement. Under this configuration, the tip is at a fixed point over the surface and approaches it vertically. Typically, when the tip is far from the surface there is no any deflection (zero force) until the tip contacts the surface. At this point, if the surface is more rigid than the cantilever, such as nanodiamond, there is a new region in the force curve characterized by a linear dependence, with slope one, of the deflection with the *z*-displacement. This behavior is due to the fact that the tip does not indent the rigid surface and, therefore, the whole *z*-displacement is transferred as deflection to the tip. In contrast, when a soft material, such as an enzyme, is sampled the tip does indent it to some extent. This fact is reflected in the force curve by the appearance of curved regions (with smaller slopes) once the tip contacts the surface. In addition, the force curve is shifted with respect to that obtained on the rigid surface in such way that for a given deflection (i.e. force) this horizontal shift corresponds to the indentation. Eventually, if the soft layer is thin enough the underlying hard substrate is reached and the linear regime with slope one is attained. In this case, the horizontal gap measured for the same force between both linear regions is an estimation of the thickness of the soft layer deposit on top of the hard surface.

The field emission scanning electron microscope (FE-SEM) was an ISI (DS-130C). Measurements were carried out under high vacuum conditions.

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