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Graphitized carbon nanofiber–Pt nanoparticle hybrids as sensitive tool for preparation of screen printing biosensors. Detection of lactate in wines and ciders

Oscar A. Loaiza [a,*], Pedro J. Lamas-Ardisana [a], Larraitz Añorga [a], Elena Jubete [a], Virginia Ruiz [a], Maryam Borghei [b], Germán Cabañero [a], Hans J. Grande [a]

[a] Sensors Unit, Materials Division, IK4-CIDETEC, Parque Tecnológico de San Sebastián, 20009 Donostia-San Sebastián, Spain
[b] Department of Applied Physics, Aalto University School of Science, P.O. Box 15100, 00076 Aalto, Finland

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ABSTRACT

This work describes the fabrication of a new lactate biosensor. The strategy is based on the use of a novel hybrid nanomaterial for amperometric biosensors i.e. platinum nanoparticles (PtNps) supported on graphitized carbon nanofibers (PtNps/GCNF) prepared by chemical reduction of the Pt precursor at GCNF surfaces.
The biosensors were constructed by covalent immobilization of lactate oxidase (LOx) onto screen printed carbon electrodes (SPCEs) modified with PtNps (PtNps/GCNF–SPCEs) using polyethyleneimine (PEI) and glutaraldehyde (GA). Experimental variables concerning both the biosensor design and the detection process were investigated for an optimal analytical performance. Lactate biosensors show good reproducibility (RSD 4.9%, n = 10) and sensitivity (41,302 ± 546) μA/M cm^2, with a good limit of detection (6.9 μM). Covalent immobilization of the enzyme allows the reuse of the biosensor for several measurements, converting them in a cheap alternative to the solid electrodes. The long-term stability of the biosensors was also evaluated. 90% of the signal was kept after 3 months of storage at room temperature (RT), while 95% was retained after 18 months at −20 °C. These results demonstrate that the method provides sensitive electrochemical lactate biosensors where the stability of the enzymatic activity can be preserved for a long period of time in adequate storage conditions.

1. Introduction

Lactic acid or lactate detection has an important role in several fields. D- and L-lactic acid are found in many foods, beverages and fermented products such as yoghurt, butter, cheese wine and ciders [1–7]. An increase in L-lactate concentration in eggs is an indicator of spoilage by contamination or incubation. Contamination of fruit juices with lactic acid producing bacteria often remains unnoticed for a long time, allowing the bacteria to spread and infect huge volumes of juice [8]. On the other hand, alcoholic beverage production includes alcoholic fermentation conducted by yeast and a secondary fermentation performed by lactic acid bacteria, called malolactic fermentation. During this process, L-malic acid is transformed into L-lactic acid and CO_2. This transformation influences the quality and taste of the beverage. In addition to the deacidification, the malolactic fermentation is considered to contribute in the complexity of the flavor, the final taste and the microbiological stability of the beverage.

L-lactate concentration in blood is essential for the diagnosis of patient conditions in intensive care and during surgery [9–11]. An elevated lactate level in blood is a major indicator of ischemic conditions of the respective tissue. The ischemic situation can be caused by all types of shock, suffocation and respiratory insufficiency. Another reason for an altered lactate level is a disturbed lactate metabolism which may be caused by diabetes. In sport medicine, blood lactate levels during exercise are an indicator for training status and fitness [12–14]. During the demand of high intensity exercise, the cell utilizes a substantial amount of glucose and glycogen. The product of the anaerobic glucose breakdown is lactate. This increase of lactate coincides with an increase in blood and muscle acidosis. Therefore lactate is an excellent indirect marker of cellular fatigue. Lactate testing is considered to be the single most important determinant of success in endurance related activities.

The importance of lactate determination has generated much interest in developing lactate biosensors. Some techniques including automated lactate analyzers, HPLC or UV methods [10,12,15] have been developed for the detection method of lactate. Additionally, screen printed amperometric biosensors based on enzymes have been generally considered a simple, inexpensive and sensitive detection of lactate in aqueous solution [1,16–21]. Screen printed electrodes (SPEs) have overcome serious drawbacks of conventional disc electrodes such as the need of surface regeneration after each measurement [22,23], which prevents them from being used in routine analysis. In the last decade,

* Corresponding author. Tel.: +34 943309022; fax: +34 943309136.
E-mail address: oloaiza@cidetec.es (O.A. Loaiza).

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there have been a great number of reports on electrochemical lactate biosensors based on screen printed technologies [24–26].

Lactate biosensors can be based on two enzymes, either lactate oxidase (LOx) [1,16–18,20,21] or lactate dehydrogenase (LDH) [3,19,27]. However, the latter requires the presence of cofactor NAD^+ which complicates analysis and increases the cost. Moreover, a supplementary enzyme, glutamate pyruvate transaminase, is necessary for an efficient reaction [28], thus increasing the analysis price. LOx, the most widely used enzyme, catalyzes the conversion of lactate to pyruvate and H_2O_2, which is subsequently detected by amperometry. However, these detections are hindered in analytical applications by slow electrode kinetics and high overpotentials which may result in large interferences from other electroactive species in real samples. The current research on H_2O_2 detection is mainly focused on electrode modifications in order to improve those overpotentials and electron transfer kinetics [29–31].

It is well established that electro-catalytic properties of platinum nanoparticles (PtNps) are better than those of bulk platinum, due to their large specific surface area, which results in several electroanalytical advantages [32]. This characteristic is strongly dependent of the size, shape and distribution of PtNps on the electrode [33,34]. In addition to their higher mass specific activity, PtNps deposited onto SPE electrodes offer several advantages compared to bulk Pt electrodes such as mass production and low cost (disposability).

Carbon nanostructures, particularly carbon nanotubes (CNTs) and carbon nanofibers (CNFs) have become very popular electrode materials for electrochemical sensing due to their unique properties such as high conductivity, very large surface area, easy functionalization, biocompatibility, fouling resistance and high electrocatalytic activity towards many important electron transfer reactions. In fact, decoration of these carbon nanostructures with metal nanoparticles has attracted huge interest in biosensor research. The capability to promote electron transfer reactions at low overpotentials has motivated extensive coupling of these hybrid nanomaterials [35] with enzymes in biosensors [31,36–39]. In particular, Pt is widely employed in electrochemical biosensors due to its excellent electrocatalytic activity towards H_2O_2 oxidation/reduction which enables the amperometric detection of H_2O_2 at low overpotentials. The use of CNFs in biosensors is less common than CNTs although the usefulness of this family of nanomaterials has been recently reviewed [40]. CNFs decorated with PtNp (PtNp–CNF) have been used for biosensing applications [41] although these nanohybrids have been more extensively studied in fuel-cell research due to the high electrochemical stability of the CNFs [42,43].

In this paper a stable and sensitive lactate biosensor, based on the use of PtNps/GCNF–SPCE, is reported. The biosensor was constructed by covalent immobilization of LOx onto the PtNps/GCNF–SPCEs using polyethyleneimine (PEI) and glutaraldehyde (GA). The results concerning the structural and electrochemical characterization of the PtNps/GCNF are presented and discussed. In addition, the effect of each component in the biosensor design is studied. Furthermore, the analytical performance of the developed lactate biosensor is determined in terms of sensitivity, reproducibility and stability. Finally, the behavior of the sensor for lactate determination in Basque ciders and certified wines was evaluated. Data were compared with results of a reference method, obtaining an excellent agreement.

2. Experimental

2.1. Reagents and solutions

Ethylene glycol, ethanol absolute, hydrogen peroxide 30% (w/v), ortho-phosphoric acid 85% (w/w) and glycerol were purchased from Scharlab (Sentmenat, Spain). Glacial acetic acid was purchased from Panreac. Sodium L-lactate, poly(diallyldimethyl ammonium chloride) solution (PDDA) 20% (w/w) in water (Mw 100,000–200,000), polyethyleneimine branched (PEI) (Mw ~25,000), glutaraldehyde (GA) 50% (w/w) in water, chloroplatinic acid hydrate ≥99.9% (w/w), sulphuric acid, succinic acid, D-(−)-fructose, (+)-catechin, caffeic acid, L-(−)-malic acid, D-sorbitol, nafion® perfluorinated resin solution 20% (w/w) in lower aliphatic alcohols and chitosan (Mw 110,000–150,000) were purchased from Sigma-Aldrich (Madrid, Spain) and used as received.

Carbon nanofibers were kindly supplied by Showa Denko (Tokyo, Japan). L-lactate oxidase (LOx) 91.2 units/mg from *Pediococcus* sp. lyophilized powder was purchased from Sorachim S.A. (Lausanne, Switzerland). 20 μl LOx stock solutions (nominally 1 U/μl) were prepared in 0.1 M phosphate buffer pH = 7.0 solution (0.1 M PB, pH = 7.0) and kept frozen until used. Diluted solutions of the enzyme were prepared in the same buffer solution.

Unless stated otherwise, all solutions were prepared with ultrapure water of Synthesis A10 from Millipore (18.2 Ω·cm) (Billerica, MA, USA). Wine samples with certified content of lactate were supplied from the Centre Oenologique de Bourgogne. A L-lactic acid spectrophotometric-enzymatic kit (Megazyme®) for the determination of lactate in cider was also used.

2.2. Apparatus and electrodes

All electrochemical measurements were carried out with an Eco Chemie Autolab PGSTAT 128N potentiostat–galvanostat (KM Utrecht, The Netherlands), using the software package NOVA 1.9. SPCEs were printed on a plastic substrate using a Thieme 110E screen printing machine, following a three electrode configuration. The SPCEs are composed of a carbon counter and disc working electrode (Ø = 4.4 mm) and a silver/silver chloride (Ag/AgCl) pseudo-reference electrode. The potential values were referred to the screen printed Ag/AgCl pseudo-reference electrode unless otherwise stated. Sensitivity of the calibration plots was related to the geometrical area of the working electrodes i.e. 15.2 mm^2.

Field Emission Scanning Electron Microscopy (FESEM) images were acquired with a JEOL JSM-5500LV. The diameters of the PtNps in the SEM images were determined with Digital Micrograph (TM) 3.7.0 (Gatan Inc.). Transmission Electron Microscopy (TEM) images were acquired from a Tecnai 12 Bio Twin with a 120 kV LaB6 gun (Oregon, USA). Thermogravimetric balance model Q500-TGA TA Instruments (Delaware, USA), a UNE 200 oven from Memmert (Wisconsin, USA), a pH meter GLP 2 from Crison (Barcelona, Spain), a microfiltration vacuum system from Scharlab (Sentmenat, Spain), polytetrafluoroethylene (PTFE) membrane discs with mean pore size of 0.22 mm from Millipore (Massachusetts, USA), a microwave model LG 700 W–19 L Touch Control from LG (Madrid, Spain) and an ultrasonic bath model Ultrasons HD-5L from J.P. Selecta (Barcelona, Spain) were also employed.

2.3. Procedures

2.3.1. Preparation of PtNps/GCNF

Hybrid nanomaterial was prepared according to a previously reported procedure [30]. Briefly, highly graphitized nanofibers with a diameter of ~150 nm and 13 m^2/g surface area were oxidized by refluxing them in a 1:1 mixture of HNO_3 2 M and H_2SO_4 1 M at 120 °C for 6 h. The oxidized GCNF was filtered through a PTFE membrane disc and washed with water. PtNps were then deposited on GCNFs by the microwave-assisted heating polyol reduction of the metal precursor. Briefly, appropriate amount of H_2PtCl_6, NaOH, ethylene glycol and GCNFs was stirred for 20 min and sonicated for 20 min. After heating in microwave for 1 min, PtNp/GCNF hybrids were cooled down, filtered, washed and dried.

2.3.2. Characterization of PtNps/GCNF and PtNps/GCNF–SPCEs

The morphological characterization of PtNps/GCNFs was carried out by SEM, TEM and TGA. PtNp size distribution on GCNFs was examined by TEM. The samples were prepared by ultrasonic dispersion of PtNps/