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Disposable amperometric biosensor based on lactate oxidase immobilised on platinum nanoparticle-decorated carbon nanofiber and poly(diallyldimethylammonium chloride) films

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ABSTRACT

A novel biosensor for lactate has been developed, using screen-printed carbon electrodes (SPCE) and lactate oxidase (LOx). The active surface of the electrodes was modified using a dispersion of platinum nanoparticle decorated carbon nanofibers (PtNp-CNF) in poly(diallyldimethylammonium) chloride (PDDA) solution. In this way, sensitive, disposable, low cost and reliable hydrogen peroxide sensors were obtained. The immobilisation of LOx on top of these PtNp-CNF-PDDA/SPCEs resulted in amperometric biosensors with high operational stability. The sensitivity of the optimised lactate biosensor was 36.8 (mA/M cm^2) with a linear range of 25–1500 μM. The limit of detection was 11 μM ($S/N=3$). Reproducibility, selectivity and storage stability were also evaluated. Additionally, the stability of the biosensor was also predicted by a model based on thermal degradation. Finally, lactate in sweat and blood samples was determined in a sport test using LOx/PtNp-CNF-PDDA/SPCEs and commercial biosensors respectively. Based on these data, the validity of the sweat lactate for the determination of the lactate threshold is discussed.

1. Introduction

Lactate is the key metabolite of the anaerobic glycolytic pathway. Lactate concentration in blood is an excellent indirect marker of anaerobic glucose breakdown, and so, cellular fatigue. Development of reliable methods of lactate determination is of great importance in clinical analysis because the concentration of lactate in blood is a fundamental parameter for the prevention and diagnosis of a number of pathological disorders such as hypoxia (Artiss et al., 2000; Frost and Meyerhoff, 2006), some acute heart diseases and drug toxicity (Pfeiffer et al., 1997; Przybyt et al., 2010). Lactate monitoring is also important in sport medicine because it is used to evaluate the maximum performance of an athlete in intensive and endurance-based activities. In this case, the physical condition is directly related to the lactate threshold i.e. the maximum steady state effort that can be maintained without increase in blood lactate (Faude et al., 2009).

Different techniques have been used for the determination of blood lactate such as HPLC (Simonides et al., 1988), enzymatic colorimetric assays (Saunders et al., 2005) or portable analysers (Buckley et al., 2003). Latest ones, based on finger-stick blood sampling and disposable sensor strips, could be considered the most adequate to obtain real temporal lactate profiles during physical training (Andrzejewski et al., 2012; Faridnia et al., 1993; Guilbault et al., 1995). However an inherent drawback of this methodology is the intrusiveness and inconvenience in the exercise routines. Hence researchers have indeed made efforts in order to develop non-invasive methods and new designs have been proposed such as lactate biosensors in a contact lens (Thomas et al., 2012) or in tattoos (Jia et al., 2013). The latest is connected with the concentration of lactate in sweat which has been proposed to evaluate the exercise intensity and the fitness level. Perspiration may thus be conveniently utilised for the analysis of physical performance in individuals without the need for an invasive blood sampling approach (Faridnia et al., 1993; Guilbault et al., 1995). Nevertheless, there is controversy over the validity of this parameter in sport training methods (Derbyshire et al., 2012). On the other hand, sweat lactate can also serve as a sensitive marker of tissue viability and may provide warning for pressure

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ischaemia, reflecting the insufficient oxidative metabolism and a compromise of tissue viability (Polliack et al., 1997).

Several amperometric lactate biosensors based on screen-printing have been previously reported (Albareda-Sirvent and Hart, 2002; Collier et al., 1996; Perez and Fabregas, 2012; Perez et al., 2012; Piano et al., 2010; Rawson et al., 2009; Shimomura et al., 2012). This technology has been successfully employed for the fabrication of electrodes in the last decade, allowing mass production of disposable low-cost sensors (Avramescu et al., 2002; Fanjul-Bolado et al., 2008; Tudorache and Bala, 2007). Lactate dehydrogenases (Piano et al., 2010) or lactate oxidases (LOx) (Albareda-Sirvent and Hart, 2002; Collier et al., 1996; Perez and Fabregas, 2012; Perez et al., 2012; Rawson et al., 2009; Shimomura et al., 2012) are immobilised on these electrode surfaces in order to obtain biosensors. LOx, the enzyme most widely used for lactate biosensors, catalyses the conversion of lactate to pyruvate and H_2O_2, which is subsequently detected by amperometry. This detection is limited in analytical applications by slow electrode kinetics and high overpotentials which may cause large interferences from other electroactive species in real samples. Therefore the current research on H_2O_2 detection is mainly focused on electrode modifications in order to overcome the limitations above mentioned (Chen et al., 2012). In this regard, nanomaterials have attracted tremendous interest in biosensor research. Some of them have shown great advantages over conventional materials for H_2O_2 detection such as ZnO nanorod arrays (Wang et al., 2011), MnO_2-modified vertically aligned multiwalled carbon nanotubes (Xu et al., 2010a), nitrogen-doped carbon nanotubes (Xu et al., 2010b) or platinum nanoparticles (PtNp) (Chikae et al., 2006). Carbon nanomaterials, especially carbon nanotubes and carbon nanofibers (CNFs), have also attracted considerable attention in H_2O_2 detection (Fanjul-Bolado et al., 2007; Wang et al., 2003). Carbon nanotubes are more used than CNFs, however the utility of the CNFs in biosensors has been recently reviewed (Huang et al., 2010). On the other hand, a new trend in biosensor design is the combination of two or more nanomaterials i.e. hybrid nanomaterials (Xiao and Li, 2008). In this sense, carbon nanotubes decorated with PtNp have been recently exploited (Huang et al., 2008; Male et al., 2007). CNFs decorated with PtNp (PtNp-CNF) are more extensively studied in fuel-cell research (Andersen et al., 2013; Wang et al., 2012). Nevertheless, these nanohybrid materials have been recently used for H_2O_2 detection and their applications in biosensor have been proposed (Liu et al., 2011).

In this work, a novel biosensor for lactate was developed, using screen-printed carbon electrodes (SPCE) and LOx. PtNp-CNFs were obtained by the polyol method and dispersed in poly(diallyldimethylammonium) chloride (PDDA) solution. The modification of SPCEs was optimised for H_2O_2 detection. Then, the LOx was immobilized on top of these PtNp-CNF-PDDA/SPCEs. The resulting biosensor was characterised and sensitivity, linear range, limit of detection, reproducibility, selectivity and storage stability were determined. Finally, the biosensor was applied to the quantification of lactate in real sweat samples.

2. Experimental

2.1. Reagents and solutions

Ethylene glycol, absolute ethanol, hydrogen peroxide 30% (w/v) and ortho-phosphoric acid 85% (w/w) were purchased from Scharlab (Sentmenat, Spain). Sodium L-lactate, poly(diallyldimethyl ammonium chloride) (PDDA) solution 20% (w/v) in water (Mw 100,000–200,000), chloroplatinic acid hexahydrate and the other chemicals 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) from *Pediococcus sp* was purchased from Sorachim S.A (Lausanne, Switzerland). LOx stock solution 1 U/μl was prepared in 0.1 M phosphate buffer pH 7.0 and aliquots of 20 μl were frozen until used. Other LOx solutions were prepared by dilution of thawed aliquots with 0.1 M phosphate buffer pH 7.0. The lactate stock solutions were prepared in 0.1 M phosphate buffer and 0.05 M NaCl pH 7.0 (PBS). H_2O_2 solutions were freshly prepared in PBS before each study, to avoid changes in the peroxide concentration by decomposition. All solutions were prepared with ultra pure water.

2.2. Apparatus and material

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. Screen printed carbon electrodes (SPCEs) and edge-connector were produced at IK4-CIDETEC facilities. The configuration of the SPCEs was three-electrode printed on a plastic substrate. The electrodes were Ag/AgCl (pseudoreference electrode) and carbon (working and counter electrodes). An insulating layer served to delimit the working area (∅=4.4 mm) and electric contacts. All potentials reported here refer to this Ag/AgCl pseudoreference electrode. Scanning electron microscopy with energy dispersive spectroscopy module (SEM) JEOL JSM-5500LV (Tokyo, Japan), transmission electron microscopy (TEM) Tecnai 12 Bio Twin (Oregon, USA) and thermogravimetric balance model Q500-TGA TA Instruments (Delaware, USA) were used for the PtNp-CNF characterisation. Thieme 110E screen printing machine from Thieme GmbH & Co (Teningen, Germany), Lactate Scout analyser and Lactate Scout test strip from SensLab GmbH (Leipzig, Germany), Ultrapure Water Synthesis A10 from Millipore (Darmstadt, Germany), oven UNE 200 from Memmert (Winsconsin, USA), pHmeter GLP 2 from Crison (Barcelona, Spain), microfiltration vacuum system from Scharlab (Sentmenat, Spain), polytetrafluoroethylene membrane discs with mean pore size 0.22 μm from Millipore (Massachusetts, USA), microwave LG 700W-19L Touch Control from LG (Madrid, Spain) and ultrasonic bath Ultrasons HD-5L from J.P. Selecta (Barcelona, Spain) were also employed.

2.3. Deposition of PtNp on CNF

CNFs are highly graphitised nanofibers with diameters and lengths around 150 nm and 10 μm respectively. An oxidative treatment was carried out to improve the anchoring of the PtNp onto the CNFs. This procedure consisted of refluxing the CNFs in acidic media (1 M HNO_3, 0.5 M H_2SO_4, 120 °C, 6 h). Then, the CNFs were filtered on polytetrafluoroethylene membrane discs and washed with water. PtNp were deposited on CNFs by the microwave-assisted polyol method i.e. reduction of the metal precursor, H_2PtCl_6, by ethylene glycol in basic media (Chen et al., 2004). Finally, the PtNp-CNFs were filtered and washed again. The characterisation of PtNp-CNFs was carried out by SEM, TEM and TGA.

2.4. Preparation of the PtNp-CNFs-PDDA dispersions

PtNp-CNFs were weighed in a glass vial and suspended in 0.5% PDDA (w/v) solution for a final concentration of 0.5 mg/ml. The suspension was immersed in the ultrasound bath until a stable and homogeneous dispersion was achieved (around 1 h). Other PtNp-CNF-PDDA dispersions were prepared in the same way.