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Paper-based enzymatic electrode with enhanced potentiometric response for monitoring glucose in biological fluids

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ABSTRACT

A novel paper-based potentiometric sensor with an enhanced response for the detection of glucose in biological fluids is presented. The electrode consists on platinum sputtered on a filter paper and a Nafion membrane to immobilize the enzyme glucose oxidase. The response obtained is proportional to the logarithm of the concentration of glucose, with a sensitivity of -119 ± 8 mV·decade^{-1}, a linear range that spans from 10^{-4}M to $10^{-2.5}$ M and a limit of detection of $10^{-4.5}$ M of glucose. It is shown that Nafion increases the sensitivity of the technique while minimizing interferences. Validation with human serum samples shows an excellent agreement when compared to standard methods. This approach can become an interesting alternative for the development of simple and affordable devices for point of care and home-based diagnostics.

1. Introduction

Enzymatic biosensors show attractive analytical features, such as high specificity, good reproducibility, stability, low limits of detection in complex matrices (such as biological fluids), a broad range of applications and a simple detection scheme (Anzai et al., 1998; Evtugyn et al., 1998). For this reason, they are extensively used in clinical, environmental, forensic and food analysis, etc. (Karube and Nomura, 2000; Khan et al., 2008; Wang, 2006; Wang et al., 2009; Wilson and Hu, 2000). Oxidase-type enzymes are widely used since the generation of hydrogen peroxide as a byproduct (Ansari and Husain, 2012; Wilson and Hu, 2000) can be detected using different approaches (Anh et al., 2003; Xiao et al., 1999; You et al., 2011).

Electrochemical detection –in particular amperometric techniques- is widely used because of the outstanding performance and a simple, compact setup (Li et al., 2015; Wang, 2008). Interferences from matrix components are a major challenge that has been solved by the use of permselective membranes. Nafion, for example, is a polyelectrolyte with negatively charged sulfonate groups employed to overcome the interferences of negatively charged species. The success of amperometry is reflected on the commercial implementation of home glucometers (Invernale et al., 2013). However, emerging social needs –such as the development of wearable (Bandodkar et al., 2016) and low-cost sensors (Maxwell et al., 2013)- are creating a growing demand for alternative approaches combining good analytical performance, robustness, simplicity and low costs. For this reason, potentiometric techniques are attracting a renewed interest, since they display an unrivalled simplicity (Ismail and Adeloju, 2014; Psychoyios et al., 2013; Yang et al., 2014), robustness and low-cost.

Enzyme-based biosensors based on the potentiometric detection of the reaction byproducts were first proposed several decades ago (Pasto et al., 1969). Later, a glucose sensor (Caras and Janata, 1985; van der Schoot and Bergveld, 1988) and a coated-wire sensor to detect urea and penicillin (Anzai and Osa, 1986) were proposed, but never widely adopted. More recently, the use of enzymes and potentiometric detection was explored by Willander *et al.* for the determination of glucose and cholesterol (Israr et al., 2010; Usman Ali et al., 2010), and by Adeloju *et al.* for glucose and phosphate (Adeloju and Moline, 2001; Ayenimo and Adeloju, 2014; Lawal and Adeloju, 2013). Despite of their advantages (Asif et al., 2010; Shukla et al., 2012), most of them have not been validated with real samples.

Finding alternative approaches for the determination of glucose in blood is still a very relevant topic, considering the growing number of people affected by diabetes, particularly in poor regions of the planet (Shaw et al., 2010). Therefore, in line with the cost-reduction approaches using screen-printed techniques (Renedo et al., 2007), nanomaterials and flexible electronics (Guinovart et al., 2013; Novell et al., 2012, 2014), developing a paper-based potentiometric biosensor for glucose may bring significant benefits.

Low-cost paper-based platforms to make affordable analytical tools have been proposed many years ago (Mabey et al., 2004), especially in combination with new materials (Kim et al., 2014; Liana et al., 2012). Although most of these devices are colorimetric assays (Curto et al., 2013; Yetisen et al., 2013), paper-based enzymatic electrodes (Nie

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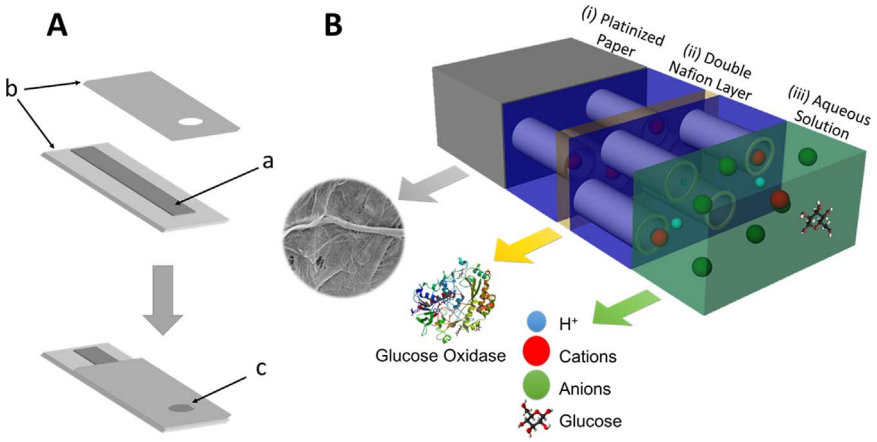

Fig. 1. Illustration of the paper-based biosensor. (A) Fabrication of the electrode, using a strip of platinized paper (a), sandwiched between two plastic masks (b), with a window of electroactive surface(c). (B) Scheme of the enzymatic membrane: (i) Pt-Paper Substrate; (ii) enzyme (GOx) sandwiched between two layers of Nafion, one at Pt-interface and the other at the (iii) solution interface.

et al., 2010) for amperometric detection have been recently proposed. In this work, a novel, simple, robust and sensitive enzymatic paper-based biosensor for the potentiometric determination of glucose is presented. The method is based on the detection of the H_2O_2 generated as a result of the enzymatic oxidation of glucose. A platinum-sputtered paper sensor is used as a redox-sensitive substrate and membrane of Nafion is employed to eliminate interferences and increase the sensitivity of the technique. The results show that this device can accurately predict levels of glucose in body fluids such as serum. Some limitations and potential future applications of these novel sensors in real life scenarios are discussed.

2. Experimental

2.1. Materials and methods

Whatman® Grade 5 qualitative filter paper, Nafion® 117 solution (ca. 5% in a mixture of lower aliphatic alcohols and water), glucose oxidase (GOx) from *Aspergillus niger* type X-S, lyophilize powder (100,000–250.000 units/g) D-glucose, hydrogen peroxide (30% wt in water), sodium urate, sodium ascorbate and D-Fructose were purchased from Sigma-Aldrich. In all cases, the Nafion solution was used as received. All reagents used were analytical grade and were purchased from Sigma-Aldrich. Phosphate buffer saline (PBS) was prepared at 0.1 M and used in all the experiments. All solutions were prepared using 18.2 MΩ cm^{-1} double deionized water (Milli-Q water systems, Merck Millipore).

Platinum sputtering was performed using a radiofrequency sputtering process (ATC Orion 8-HV, AJA International) operated at 3 mTorr, for 65 s at 200 W. Filter paper strips were placed inside the sputtering chamber to generate the electrodes. An adhesive plastic mask (0.3 mm thick) coated with an acrylic adhesive on one side (Arcare 8565, Adhesives Research Inc., Limerick, Ireland) was used to expose a given area of the coated paper and isolate the rest of the conductive surface.

Details of the characterization analysis can be found in the Supplementary material.

2.2. Electrochemical measurements

Potentiometric measurements were performed using a standard two-electrode (i.e., working and reference) cell configuration, using the paper sensor as a working electrode and commercial reference electrode, in a 4 mL cell in 0.1 M PBS (pH 7.4) at 25 °C. A double junction Ag/AgCl/3 M KCl reference electrode (type 6.0726.100, Metrohm AG) containing a 1 M LiAcO bridge was used in all the experiments. Electromotive force (EMF) was measured using a high input impedance (10^{15} Ω) EMF16 multichannel data acquisition device (Lawson Laboratories, Inc. Malvern).

2.3. Fabrication of glucose biosensor

Electrodes were made by sputtering Pt on one side of a filter paper, which was then cut into rectangular pieces (20 mm× 5 mm) sandwiched between two rectangular plastic masks. The top mask (15 mm×10 mm) had a 3 mm diameter circular window and the bottom mask was slightly larger (20 mm×10 mm), as shown in Fig. 1A. The exposed top of the conductive paper was connected with the reading instrument, and the circular window that will be used as the electrochemically active surface.

These bare Pt electrodes are then functionalized with the biosensing membrane, which is made using Nafion as polymeric coating and glucose oxidase enzyme as the biological receptor. First, the window of each electrode was rinsed with double-distilled water and air-dried. Thereafter, a first layer of Nafion was made by drop casting 4 µL of the Nafion solution air-dried for 60 min at room temperature. Thereafter, 20 µL of a solution containing 20 mg/mL^{-1} of glucose oxidase (GOx) in distilled water was drop cast on top of the Nafion membrane and the system was left drying overnight at 4 °C. Finally, 2.5 µL of the same solution of Nafion was applied in order to make a second layer that entraps the enzymatic layer and let dry overnight at 4 °C (See Fig. 1B) This electrode was kept at 4 °C when not in use.

2.4. Analysis of real samples

Serum samples of patients were obtained at a local hospital (Hospital de Sant Pau i Santa Tecla). Values from serum samples were provided by the hospital using hexokinase/glucose-6-phosphate dehydrogenase colorimetric test as a standard method for further validation of the paper-based potentiometric system.

3. Results and discussion

3.1. Characterization of the platinized paper-based electrodes

Environmental scanning electron microscopy (ESEM) images of the electrodes shows the cross-linked cellulose fibers completely covered by a thin layer of sputtered platinum (Fig. S1A). While it is difficult to assess the thickness of the metal layer on paper, on a flat surface the thickness of the platinum layer is of approximately 100 nm. When a drop of water is added on top of the Pt layer, no percolation through the metallic layer (i.e. no wetting of the paper) is observed. Additionally, this thin layer of Pt shows an electrical resistance of a few ohms,