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Paper-based maskless enzymatic sensor for glucose determination combining ink and wire electrodes

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ABSTRACT

In this work we have developed an amperometric enzymatic biosensor in a paper-based platform with a mixed electrode configuration: carbon ink for the working electrode (WE) and metal wires (from a low-cost standard electronic connection) for reference (RE) and auxiliary electrodes (AE). A hydrophobic wax-defined paper area was impregnated with diluted carbon ink. Three gold-plated pins of the standard connection are employed, one for connecting the WE and the other two acting as RE and AE. The standard connection works as a clip in order to support the paper in between. As a proof-of-concept, glucose sensing was evaluated. The enzyme cocktail (glucose oxidase, horseradish peroxidase and potassium ferrocyanide as mediator of the electron transfer) was adsorbed on the surface. After drying, glucose solution was added to the paper, on the opposite side of the carbon ink. It wets RE and AE, and flows by capillarity through the paper contacting the carbon WE surface. The reduction current of ferricyanide, product of the enzymatic reaction, is measured chronoamperometrically and correlates to the concentration of glucose. Different parameters related to the bioassay were optimized, adhering the piece of paper onto a conventional screen-printed carbon electrode (SPCE). In this way, the RE and the AE of the commercial card were employed for optimizing the paper-WE. After evaluating the assay system in the hybrid paper-SPCE cell, the three-electrode system consisting of paper-WE, wire-RE and wire-AE, was employed for glucose determination, achieving a linear range between 0.3 and 15 mM with good analytical features and being able of quantifying glucose in real food samples.

1. Introduction

Analytical Chemistry follows, for almost two decades, some specific trends that are related to productivity. Miniaturization (size reduction), simplification (reduction of complexity), and automation (reduction of human activity) are well-known trends (Valcárcel et al., 1999) that are followed by the more recent reduction of costs. Developed countries ask for more analytical data that give information on diseases, food quality or environmental damage, which allows advancing in the general knowledge of society. This implies a decentralization of analysis, which can only be performed with low-cost simple analytical platforms. Then, Analytical Chemistry laboratories are suffering significant changes with the development of reliable and fast methodologies for *in-situ* analysis (also known as field analysis or point-of-care, point-of-use in a more general way). These, in turn, are very useful for resource-limited regions in developing countries, where power sources or highly qualified personnel (either for performing analysis or for repairing instrumentation) are scarce.

In practice, electrochemical detection can be connected to all these trends due to its simplicity and ease of miniaturization. In this context, from the early 1990s, screen-printing technology has been increasingly used for the mass production of low-cost small electrodes with good analytical features. In addition, they are very versatile owing to their facility of being modified in multiple ways depending on the application required. Specifically, those made of carbon (SPCEs) are the most widely used in the development of electrochemical sensors (Domínguez Renedo et al., 2007; Hayat and Marty, 2014). Correct choice of materials and automation of the production process have increased their precision and, nowadays, they are widely considered as analytical tools for development of new methodologies..

On the other hand, microfluidic paper-based analytical devices (μPADs) are one of the most studied fields of analysis, due to the necessity of fast, sensitive, affordable and, as we commented previously, simple and miniaturized methods for quantifying compounds of interest, either from the clinical sector, food industry or environmental monitoring (Lawrence et al., 2014). On this basis, paper is a very useful low-cost material for the development of point-of-care devices, which can produce results in a quick way by untrained staff

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(Jung et al., 2015).

From all the advantages of this material (Martinez et al., 2010), there are some that can be highlighted for electrobioanalytical applications, namely: i) storage and modification possibilities (either hydrophobic wax, conductive ink or biorreagents), ii) passive pumping of solutions by capillary forces (with low sample volumes) with the possibility of flowing to different layers of materials due to its porous nature, and iii) high interface electrode-solution in the case of using conductive inks. The most commonly used type of paper for the construction of these devices is chromatographic paper, which is made only of cellulose. It can be wax-printed to generate hydrophobic barriers (Carrilho et al., 2009; Martinez et al., 2010) in such a way that solutions flow only through the area designed for that purpose, with cellulose fibers behaving as channels (Yu et al., 2011). Alternatively, the working area can be delimited by drawing barriers with inexpensive hydrophobic markers (Nie et al., 2012).

Paper-based devices first used for electroanalysis were based on screen-printing technologies using carbon inks (Dungchai et al., 2009). Later on, although some metallic electrodes, wires or films (Berg et al., 2016; Scida et al., 2014) were employed, carbon is the material most commonly used (Cate et al., 2015), following different strategies: pencil drawing (Dossi et al., 2014; Li et al., 2016), pen-on-paper (Russo et al., 2011; Glavan et al., 2014) or screen/stencil-printing methodologies (Taleat et al., 2014). Most of the technologies based on printing need a stencil or a screen for patterning the whole electrochemical cell in order to separate the three electrodes required, namely working (WE), reference (RE) and auxiliary (AE). If a mask is not used, electrodes can connect in between producing short circuits. Drawing with pencils or pens, directly or following the pattern previously designed with a software allows avoiding the use of masks. However, the geometry has to be carefully optimized to obtain adequate analytical features. On the other hand, if only the WE is printed on the paper meanwhile RE and AE are located in a different place, there is no need to use stencils nor a computer design. But where in a paper platform? We have designed here a device where a volume of carbon ink is deposited on a working area delimited by hydrophobic wax. This is a very simple and precise procedure for constructing the working electrode. Reference and auxiliary are then placed externally in a very simple way using racks of gold-plated pins that form low-cost standard electronic connections. Thus, the standard connection acts as a multifunctional component that is used as both, electrode (RE and AE), as well as an interface between the three electrodes and the potentiostat. This simplifies the procedure since screen/stencil printing is not needed and the connections are commercial, can be used without modifications and allow using a commercial connector for SPCEs. Hence, the use of alligator clips or similar is not needed and changing the biosensors for different measurements is as easy as in the case of a SPCE. The clip holder can be reused with different paper platforms, although the low cost and precision makes possible to dispose it.

One of the main fields of electrochemical devices is devoted to biosensing. Biosensors allow real-time transduction in many cases, and the limits of detection that provide are commonly low enough for a large number of analytes. Combined with microfluidics, they generate powerful analytical tools (Bunyakul and Baeumner, 2015). As a proof-of-concept, in this work we have developed an enzymatic biosensor for glucose. Apart from being a good model for evaluating the performance of this type of catalytic sensors, glucose is a very important biomolecule, present in many reactions of living organisms. An abnormal glucose concentration in blood and tissues can produce a disease that is suffered by hundreds of millions of people all over the world (Heller and Feldman, 2008): *diabetes mellitus*. Methodologies for its determination are being developed continuously not only for clinical but also for food samples. Following the trend of the development of paper-based devices, recently, different paper-based electrochemical sensors for glucose determination have been reported: most of them combine paper with SPEs or, alternatively, are based on screen-printing the electrodes on paper. Paper has been combined with SPEs for preconcentrating the analyte, avoiding matrix effects or immobilizing reagents (Kong et al., 2014; Noiphung et al., 2013; Sekar et al., 2014), but its use as electrode has not been reported. When electrodes have been screen-printed on the paper, carbon ink was employed: i) for all the three electrodes (Rungsawang et al., 2016; LOD=0.86 mM), ii) for the working and counter electrodes, using a Ag/AgCl as pseudoreference electrode (Nie et al., 2010; LOD=0.22 mM) or iii) modified with nanomaterials (zinc oxide nanowires) (Li et al., 2016; LOD=59.5 μM).

According to all these ideas, in this work we develop a paper-based glucose electrochemical biosensor including external RE and AE. The recognition phase of the biosensor is composed by the bienzymatic system (glucose oxidase / horseradish peroxidase) which uses potassium ferrocyanide as mediator for the electron transfer, previously studied in our research group (Biscay et al., 2011). Glucose can be electrochemically detected through the reduction of ferricyanide, electron transfer that takes place on the electrode surface (Murphy, 2006) and is proportional to glucose concentration. Initially, the reference and auxiliary electrodes of a SPCE card were employed; the paper working electrode was then adhered reversibly over the circle of the screen-printed working electrode. Once the enzymatic phase was optimized, a low-cost gold-plated connector header was used: this provides the RE and the AE and, at the same time, allows for a connection between the device and the potentiostat. This is the first time, to the best of our knowledge, that a paper-platform modified with carbon ink is inserted in a standard connector like this, without needing any additional step to constitute the electrochemical cell. Although it has been evaluated for glucose biosensing, its simplicity, size, disposability and low cost makes it very promising for all type of applications, especially decentralized analysis.

2. Materials and methods

2.1. Chemicals

Glucose oxidase (GOx), horseradish peroxidase (HRP), potassium ferrocyanide, Trizma® base, *D*-(-)-fructose, ascorbic acid and the Glucose Assay Kit were purchased from Sigma-Aldrich. *D*-(+)-glucose anhydrous was delivered by Merck and nitric acid 65% and dimethylformamide by Normapur. The carbon sensor ink used was from Gwent Group. All chemicals were of analytical reagent grade, and the water used was obtained from a Millipore Milli-Q purification system (Millipore Direct-Q™ 5). Stock solutions were prepared daily in 0.1 M Tris-HNO_3 buffer, pH 7.0. This solution, 0.1 M Tris-HNO_3 buffer, pH 7.0, and solutions of GOx and HRP enzymes in Tris-HNO_3 buffer were prepared weekly and stored at 4 °C.

2.2. Apparatus and Measurements

Chronoamperometric measurements were performed using a μAutolab type II potentiostat controlled by the Autolab GPES software. All measurements were carried out at room temperature. Screen-printed carbon electrodes (SPCEs, ref. DRP-110) and an edge connector (ref. DRP-DSC) were purchased from DropSens S.L. Whatman™ paper (dimensions of 100×300 mm) and a wax printer XEROX ColorQube 8570 were used for the fabrication of paper-based electrodes. Gold-plated connector headers were purchased from Digikey. Electrochemical Impedance Spectroscopy measurements were performed with an Autolab PGSTAT12 potentiostat/galvanostat controlled by FRA software.

2.3. Electrochemical cells

First, optimization studies were done by combining the paper-based working electrode with the reference and auxiliary electrodes of the