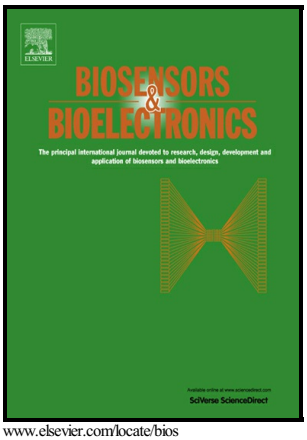

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Pin-based electrochemical glucose sensor with multiplexing possibilities

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Abstract

This work describes the use of mass-produced stainless-steel pins as low-cost electrodes to develop simple and portable amperometric glucose biosensors. A potentiostatic three-electrode configuration device is designed using two bare pins as reference and counter electrodes, and a carbon-ink coated pin as working electrode. Conventional transparency film without any pretreatment is used to punch the pins and contain the measurement solution. The interface to the potentiostat is very simple since it is based on a commercial female connection. This simple electrochemical system is applied to glucose determination using a bienzymatic sensor phase (glucose oxidase/horseradish peroxidase) with ferrocyanide as electron-transfer mediator, achieving a linear range from 0.05 to 1 mM. It shows analytical characteristics comparable to glucose sensors previously reported using conventional electrodes, and its application for real food samples provides good results. The easy modification of the position of the pins allows designing different configurations with possibility of performing different measurements simultaneously. This is demonstrated through a specific design that

includes four pin working-electrodes. Antibody labeled with alkaline phosphatase is immobilized on the pin-heads and after enzymatic conversion of 3-indoxylphosphate and silver nitrate, metallic silver is determined by anodic stripping voltammetry.

Keywords: pin-based electroanalysis, multiplexed detection, glucose sensor, enzymatic assay, glucose oxidase, alkaline phosphatase.

1. Introduction

Nowadays there is an increasing interest in new strategies for the rapid detection of analytes with clinical, food and environmental importance without the need of sophisticated instrumentation. Since Whitesides group proposed paper as material for fabricating low-cost microanalytical devices (Martinez et al., 2007, 2010) many microfluidic paper-based electroanalytical devices (EμPADs) have been developed to detect a wide variety of analytes (Dungchai et al., 2009; Liu et al., 2012; Nie et al., 2010; Wu et al., 2013). Electrochemical detection owns a particular interest for μPADs due to its low cost, portability, ability for miniaturization, low sample consumption and high accuracy at low analyte concentrations (Rungsawang et al., 2015). However, the fabrication and integration of electrodes in paper-based platforms in a rapid, efficient and cost-effective way is still a challenge. The most widespread methods are the deposition of carbon or metallic films, using either thick- or thin-film technologies. In most of the cases, stencils are required to deposit the conductive materials on the substrate (Cate et al., 2015; Tobjörk and Österbacka, 2011), and their fabrication implies an increase in cost and time. Graphite electrodes can be fabricated without

stencils using a pen-on-paper approach, by printing on the surface of hydrophobic paper following a template designed using a software (Glavan et al., 2014). However, as in the other cases, once the device is finished, the setting of the electrodes in the electrochemical cell cannot be altered.

Besides paper as substrate for low-cost electrode fabrication, commercial transparency film is also a practical option due to its chemical compatibility and disposability (Berg et al., 2015; Ruecha et al., 2015). Moreover, since paper is a porous media, the deposition of conductive materials can alter its porosity, wicking and interfacial energy.

In this context, the use of already mass-produced stainless-steel pins makes possible the development of low-cost electroanalytical devices with a versatile disposition of the electrodes. Pins provide a simple solution to the challenge of fabrication and integration of electrodes in miniaturized devices. Their use has only been reported previously in a work made in collaboration with Whitesides group (Glavan et al., 2016). We used them in systems fabricated on omniphobic R^F paper or thread to quantify lactate in human plasma. In this case, the enzymatic reaction occurs in solution and the product of the enzymatic reaction is deposited on the paper with the pins, or arrives through the thread at the pin surface. We also demonstrated the fabrication of thread-based arrays for performing multiple measurements, as well as the fabrication of a 96-well plate in paper to perform independent measurements in each well. Stainless-steel pins show several advantages as electrodes since they are: i) inexpensive, ii) available nearly worldwide, iii) disposable, iv) highly conductive, v) electrochemically stable in neutral or mildly acidic or basic aqueous solutions (Malik et al., 1992) and vi) easily modifiable with conductive ink by simple dip and dry, with proteins, in order to develop a biosensor, or with nanomaterials. Moreover, the shape of a pin and its different parts can be used for

different purposes; for example, the head can be used as electrode while the sharp tip allow to drill the substrate and to anchor the pins in a support making them easy to handle. Another advantage is that pins offer readily accessible connection points perpendicular to the plane where the solution is added (the shaft of the pins), and therefore, alligator clips, grabber clips or female standard connections are very appropriate. Besides this, pins can be easily combined with different materials such as paper, transparency or different polymers in order to incorporate electrochemical detection to analytical devices. In addition, pins allow the fabrication of devices with modifiable configurations that can be used for multiplexed analysis because of these readily accessible connection points to electrochemical readers.

In the present work, we develop the first amperometric glucose sensor using prefabricated stainless-steel pins as electrodes. The determination of glucose finds important applications in many different areas such as clinical diagnostics, biotechnology and food industry, all of them attracting extensive attention. Indeed, glucose biosensors account for approximately 85% of the world biosensor market, which has been estimated to be around $5 billion (Newman and Turner, 2005; Wang, 2008). In order to fabricate the pin-based device, transparency film was chosen as substrate for the fabrication of the electrochemical device because of its widely availability, lightness, disposability, flexibility and ease to drill it. Moreover, its hydrophobicity makes unnecessary to delimit the electrochemical cell. This work also describes the fabrication of a device with four pin working-electrodes in the same electrochemical cell. As proof-of-concept, different concentrations of an alkaline phosphatase labeled antibody were immobilized on each pin working-electrode. Thus, using 3-indoxyl phosphate and silver nitrate as enzymatic substrates, multidetection

possibilities were demonstrated by the oxidation of the silver enzymatically reduced on each working electrode. This is the first time pins are used as both, surface for immobilization of bioreagents and transducers in biosensors for enzymatic sensing.

2. Materials and methods

2.1 Chemicals, materials and apparatus

Glucose oxidase from Aspergillus niger (GOx), horseradish peroxidase, Type VI-A (HRP), antimouse IgG conjugated with alkaline phosphatase (antiIgG-AP), glucose assay kit (GAGO20), D-(-)-fructose, ascorbic acid, ferrocene carboxylic acid ($FcCO_2H$), potassium ferrocyanide ($K_4Fe(CN)_6$), silver nitrate ($AgNO_3$), magnesium nitrate ($Mg(NO_3)_2$) and tris(hydroxymethyl)aminomethane (Tris) were purchased from Sigma-Aldrich. D-(+)-glucose anhydrous was delivered by Merck and 3-indoxyl phosphate disodium salt (3-IP) by Biosynth. N,N-Dimethylformamide (DMF) and isopropyl alcohol were purchased from VWR International. Carbon ink (C10903P14) was provided by Gwent Electronic Materials Ltd and carboxylated multiwalled carbon nanotubes (MWCNTs) from Nanocyl. Ultrapure water obtained from a Millipore Direct-Q^{TM} 5 purification system was used throughout this work. All chemicals were of analytical reagent grade.

Stock solutions of $FeCO_2H$, glucose, ascorbic acid and fructose, as well as solutions of GOx/HRP/ferrocyanide were prepared daily in 0.1 M phosphate buffer of pH 7.0. The antiIgG-AP was diluted in 0.1 M Tris-HNO_3 pH 7.2 buffer. A solution containing 1.0 mM 3-IP and 0.4 mM $AgNO_3$ was prepared in 0.1 M Tris-HNO_3 pH 9.8 buffer containing 20 mM $Mg(NO_3)_2$ using opaque micro test tubes.

Pins (AIN265925) were supplied by Metalúrgica Folch. Transparency film for photocopier was purchased from Apli Paper S.A.U. The 3-pin Dupont female cable were supplied by Amazon.

A bench magnifier of 12 diopter and with a fluorescent lamp of 12 W purchased from RS Components Ltd. was used to take the photographs of the head of the pins.

Voltammetric and chronoamperometric measurements were performed using an ECO Chemie μAutolab type II potentiostat/galvanostat (Metrohm Autolab B.V.) interfaced to a Pentium 4 2.4 GHz computer system and controlled by the Autolab GPES software version 4.9. The voltammetric measurements performed with the multiplex device were recorded using a μStat 8000 potentiostat (DropSens) interfaced to a Pentium 4 2.4 GHz computer system controlled by DropView 8400 2.0 software.

2.2 Fabrication of pin-based devices

Stainless-steel pins with the following dimensions: 26 mm large, 0.59 mm shaft diameter and 1.5 mm head diameter were employed throughout the work. Transparency sheets were cut in rectangles (3 cm x 2 cm approximately) for the fabrication of the electrochemical devices.

For the pin-based device fabrication, the stainless-steel pins were cleaned by sonication in isopropyl alcohol for 20 min. Two of these pins were used without further treatment as reference (RE) and counter electrodes (CE). A stainless-steel pin coated with freshly prepared carbon ink was used as working electrode (WE). Two carbon inks were tested: one was prepared by dispersing carbon ink in DMF (50%, w/w), and the other was prepared adding to this, multiwall carbon nanotubes (in this case the ratio carbon :

MWCNTs : DMF was 49.8 : 0.2 : 50, w/w). Both inks were sonicated for 1 hour (37 kHz of frequency and 320 W of power) obtaining homogeneous inks. In order to coat the pins, their head was immersed in the corresponding ink, and then allowed to dry for 15 min in an oven at 70 ºC. This process was repeated 3 times. The effect of the drying time after the last immersion was evaluated.

The design of the one-WE pin-device is shown in Figure 1A. The transparency was drilled with the pins; a pin header without the pins (Fig. S1, in Supplementary information) was used as alignment tool for placing the pins at the same distance (1.5 mm approx.) in between. The interface between the pins and the potentiostat was a 3-pin Dupont female cable. This allowed the easy, quick and reproducible connection of the pins with the potentiostat for signal recording.

The small size of the pins and the modifiable configuration allow designing multiplex devices. Using the Dupont female cable as interface between pins and potentiostat, a multiplex pin-based device was constructed. This (Fig. 1B) consisted of four pins acting as working electrodes sharing reference and counter electrodes (bare pins). Two standard pin headers (without including the corresponding pins) were stuck in order to use them as alignment tool for the insertion of the pins. Similarly, two 3-pin Dupont female cables were stuck to obtain six connection points for the six pins (4 WEs, 1 RE and 1 CE).

2.3 Cyclic voltammograms (CVs) using pin-based electrodes

Using one-WE pin-devices, cyclic voltammograms (i-E curves) in solutions of ferrocene monocarboxylic acid or potassium ferrocyanide were recorded by dropping a 70-µL aliquot covering all the three pins. The potential was scanned between 0 and 0.5

V for ferrocene solutions and between -0.2 and 0.7 V for ferrocyanide solutions, at 50 $mV \cdot s^{-1}$ in both cases.

When a four-WE pin-device was used, voltammograms in solutions of ferrocene monocarboxylic acid were performed dropping a 90-µL aliquot of the measuring solution on the transparency film covering the six pins. The potential was scanned between 0.1 and 0.6 V at 50 $mV \cdot s^{-1}$.

For both devices, different pins serving as working, counter and reference electrodes were used for each measurement.

2.4 Procedure for fabrication of and measurement with the enzymatic glucose sensor

The preparation of the glucose sensor phase is based on a previous work reported by our group (Biscay et al., 2011). The first step of the procedure for fabricating the single-use glucose sensor was the deposition of 3 µL of a mixture of GOx (3 U/µL), HRP (5 U/µL) and potassium ferrocyanide (20 mM) onto the head of the pins coated with carbon ink (working electrodes). Then, after drying at room temperature (approximately 30-40 min), sensors were ready to use (Fig. S2 shows a pin unmodified, one modified with carbon ink and one modified with carbon ink and with the mixture of GOx/HRP/ferrocyanide).

To record the analytical signal, a 70-µL aliquot of the glucose solution was deposited on the transparency film covering all the three electrodes. Glucose determination was carried out applying a potential of -0.2 V and the chronoamperogram (i-t curve) was recorded for 50 s. A different pin-based sensor was used for each measurement.

2.5 Procedure for the assay performed in a multiplexed device

Antibody conjugated with the enzyme alkaline phosphatase (IgG-AP), usually employed in the last step of many immunoassays, was immobilized by physical adsorption depositing 3 μL of an IgG-AP solution in 0.1 M Tris-HNO_3 pH 7.2 buffer onto the head of each pin working-electrode, and then leaving until dryness (30-40 min approx.). The enzymatic reaction was performed covering the 6 pins with a 90-μL drop of a 1.0 mM 3-IP / 0.4 mM silver nitrate solution and leaving to react for 20 min. Then, metallic silver generated enzymatically was anodically stripped, recording the linear voltammogram between 0.05 and 0.50 V at 50 $mV \cdot s^{-1}$.

2.6 Real sample analysis

The pin-based sensor designed was tested for use in real food samples: honey and orange juice. In both cases the only sample treatment needed was dilution in phosphate buffer in order to obtain adequate glucose concentration, comprised in between the limits of the linear calibration range. The accuracy of the results given by the enzymatic pin-based sensor was evaluated by analyzing the samples using a commercial glucose enzymatic kit with spectrophotometric detection.

3. Results and discussion

Pins are very promising tools for electroanalysis. One forward step is the demonstration of their use as substrate for bioimmobilization and transducers for sensor fabrication. The characteristics and advantages of pins pave the way to their use for the development not only of enzymatic assays but also of immune, DNA or other affinity-based assays.

In this work, the arrangement employed is the basic three-electrode potentiostatic configuration with three stainless-steel pins, one of them coated with carbon ink and acting as working electrode. The pins were drilled on a transparency sheet employing the head of the pin as electrode surface.

3.1 Evaluation of the coating of the pins with carbon ink

In order to obtain an adequate surface area of the working electrodes, stainless-steel pins were coated with carbon ink. The coating is performed by immersion of pins in the carbon ink. The number of these immersions has not been optimized here since in our previous work about electroanalytical application of pins, it has been demonstrated that three immersions offer a fully covering by the ink without producing a possible detachment due to a too high amount of ink layers.

In this work, modification with carbon nanotubes was considered since they have demonstrated to improve the analytical signal (Fanjul-Bolado et al., 2007b; Fernández-Abedul and Costa-García, 2008) and have been employed in the first electroanalytical pin-application (Glavan et al., 2016). Apart from the composition of the ink, the time of drying is also important. Both were evaluated using a redox probe with well-characterized electrochemical behavior: ferrocene monocarboxylic acid ($FcCO_2H$). Pins were coated with carbon ink and with MWCNTs modified carbon ink. For each one, different times of drying were tested: 15 min, 12, 24 and 48 h. Fig. 2A and 2B show the corresponding cyclic voltammograms recorded in a 0.5 mM $FcCO_2H$ solution in phosphate buffer pH 7.0. Using both inks, the capacitive current decreased considerably when the time of drying increased from 15 min to 12 h. It could be due to changes in the thickness an uniformity of the ink layers on the pin-head. No further improvement is

produced as seen in cyclic voltammograms recorded for higher drying time. Therefore, 12 hours was chosen as drying time for the pin coating. When carbon ink and carbon ink modified with MWCNTs were compared (for a 12-hours drying time), the improvement using MWCNTs was very slight. Capacitive current was very similar and the faradaic current changed from 3.7 to 4.1 μA (Fig. 2C). Therefore, for the sake of economy and simplicity, the use of MWCNTs in the ink was discarded. Using carbon ink and a drying time of 12 hours, precision between 5 independent pin devices (with different WE, RE and CE) was studied. The redox probe presented in all these devices a well-defined almost reversible process, with potentials of 269 ± 6 mV (RSD 2.3%) for the anodic and 200 ± 10 mV (RSD 4.9%) for the cathodic process. The difference between potentials is ≈ 69 mV indicating a fast electron transfer. The mean value of anodic and cathodic peak currents was 3.5 ± 0.1 μA (RSD 3.6%) and -3.8 ± 0.1 μA (RSD 3.9%) respectively (CVs shown in Figure 3D). Therefore, the precision and low cost of the pins allow considering them as disposable elements.

3.2 Glucose sensor

The sensor phase of the pin-based glucose sensors consisted of enzymes GOx and HRP, and ferrocyanide as mediator. The system ferro/ferricyanide shows a redox process according to the following reaction:

$$[\mathrm{Fe(CN)_6}]^{4-} \rightleftharpoons [\mathrm{Fe(CN)_6}]^{3-} + 1\,\mathrm{e}^{-}$$

We chose chronoamperometry as detection technique since it is very adequate for portable equipments and allows the determination of glucose concentration by measuring the concentration of the ferricyanide enzymatically generated (Fig. S3 shows

a scheme of the reactions involved). For each mole of glucose, two moles of ferrocyanide are oxidized to ferricyanide (Ruzgas et al., 1996; Wang, 2008). Applying an appropriate potential, ferricyanide is reduced, and the current measured (at a fixed time) is proportional to its concentration and therefore to this of glucose.

Then, first of all, a cyclic voltammogram was recorded in a 1.0 mM ferrocyanide solution in 0.1 M phosphate buffer of pH 7.0 (Fig. S4) with the aim of determining anodic and cathodic peak potentials (vs. a stainless-steel quasi-reference electrode) and of setting the most adequate potential for recording the chronoamperograms. A -0.2 V potential was chosen in order to assure the electrochemical reduction of ferricyanide.

Chronoamperograms were recorded for evaluating the response of the bienzymatic sensor in presence of different concentrations of glucose. Fig. 3B shows the response of the sensor for concentrations of glucose comprised between 0.05 and 5 mM. When the potential is applied, the capacitive current decreases faster than the faradaic current, and therefore measuring the current at an appropriate time warranties an adequate faradaic/capacitive current ratio. The analytical signal in this case was the current measured at a time of 50 s. A linear relationship between current and glucose concentration was obtained in the range of 0.05 - 1 mM, with a R^2 of 0.9998, according to the equation $|i|$ (μA) = 1.44 $C_{glucose}$ (mM) + 0.14 (Fig. 3C). The limit of detection (LOD) and the limit of quantification (LOQ) were calculated as $3s_b/m$ and $10s_b/m$ respectively, where m is the slope of the calibration plot, and s_b is the standard deviation of the intercept. LOD and LOQ values thus obtained were 0.03 and 0.10 mM respectively. It is interesting to remark that this simple electrochemical biosensor design using pins as electrodes shows an LOD and linear dynamic range similar, or in some cases better, than those obtained with other glucose sensors previously developed, even

those employing carbon nanomaterials or nanoparticles on glassy carbon electrodes (Su et al., 2014; Ye et al., 2015): 0.01 - 0.3 mM and 0.05 - 1.2 mM respectively), using a combination of a paper disk with commercial screen-printed electrodes (Chandra Sekar et al., 2014; Kong et al., 2014): LOD of 0.01 and 0.1 mM respectively), or employing screen-printed electrodes based on paper (Rungsawang et al., 2015): LOD of 0.86 mM) (Table S1).

In order to evaluate the reproducibility, seven sensors were prepared in different days to carry out seven different measurements in a 0.5 mM glucose solution. The reproducibility estimated in terms of the RSD was 7.8% (the mean value of the current was 0.83 ± 0.06 μA). Precision compares to other electrochemical devices based on paper or transparency (Dungchai et al., 2009; Rungsawang et al., 2015) (Table S1).

Furthermore, the sensor shows Michaelis-Menten kinetic behaviour. The apparent Michaelis-Menten constant (K_M) was calculated using the Lineweaver-Burk linearization obtaining a value of 2.2 ± 0.6 mM (equation: $1/I = 0.590 \cdot 1/C_{glucose} + 0.007$; $R^2 = 0.9990$). This K_M value is comparable, or in some case lower, than those calculated with other enzymatic glucose sensors based on screen-printed (Biscay et al., 2011): 1.7 mM) or glassy carbon electrodes (Ye et al., 2015; Zou et al., 2008) 2.39 and 14.4 mM respectively) (Table S1). Since a low K_M value indicates strong affinity between the enzyme and its substrate, the value obtained with our sensor demonstrates adequate immobilization of enzyme in the active form and high bioaffinity to glucose.

The selectivity of this sensor in presence of potential interferences presented in food samples, such as fructose and ascorbic acid, was evaluated (Table 1). As can be seen, the sensor did not respond to the presence of fructose, even at the same concentration

than glucose (signal change is <10%). In the case of ascorbic acid, when ascorbic acid is present at a very high concentration, it is an interference. But, in food samples, such as juices, the concentration of ascorbic acid is present at much lower concentration (in order of mg/mL) than glucose (in order of g/mL) (Barberis et al., 2015; Pisoschi et al., 2011). Thus, we tested the potential interference of ascorbic acid when it is present in a 5-time lower concentration than glucose. As it is showed in Table 1, from this ratio of concentrations of glucose/ascorbic acid, the ascorbic acid is not an interference for this sensor (signal change is <10%).

This sensor was applied to glucose determination in two real samples with different matrix and appearance: honey and orange juice. The only pretreatment needed was an adequate dilution in phosphate buffer pH 7.0 in order to obtain a signal in between the linear range. Samples were also analyzed using an enzymatic kit assay with spectrophotometric detection to compare the results, which are summarized in Table 2. Comparing the mean values obtained by both methodologies through the Student's t-test, we can conclude there are no significant differences between the values labeled at a 0.05 significance level, thus demonstrating the good precision and accuracy of the sensor developed. The total cost of this sensor is less than 0.7 $ (Table S2), being enzymes the higher contribution (HRP is the most expensive component of the sensor).

Although the preparation of the sensor is easy and requires short time (30-40 min), it is worth to note that in our previous work we developed a glucose sensor based on screen-printed electrodes with the same sensing phase and its stability was at least three months. So, the pin-based glucose sensor could show similar stability.

3.3 Multiplex pin-based device

It is still a challenge to be able to determine several analytes with the same device (multianalyte determination), or alternatively, perform simultaneous measurements. With this aim, using pins as electrodes, we fabricated a multiplex device consisting of four pins acting as working electrodes shown in Fig. 1B. In order to evaluate its performance the redox probe $FcCO_2H$ was used. Since the counter electrode should present an area similar to that of the working electrode (commonly is designed to be three times the WE) and now we have four working electrodes, the increase of its area was evaluated. CVs were recorded in 0.5 and 1.0 mM $FcCO_2H$ solutions on two different types of multiplex devices: in one, the counter electrode was a common stainless-steel pin and in the second one, an extra stainless-steel piece was placed surrounding the head of the counter-electrode pin resulting in an area ca. four times bigger. The peak currents were similar in both devices and no other differences were found, indicating there was no limitation of the current due to a small area of the counter electrode. Therefore, it was decided to use only the stainless-steel pin as counter in the multiplex device due to the easier handling. Fig. 4A shows the four CVs obtained with this device. It can be observed that the peak currents and potentials are very similar for the four working electrodes, with a RSD value of 2.5% and 1.7% for anodic and cathodic peak currents respectively. They are very low, even considering that pin coating and device fabrication are hand-made.

Once the precision of the measurements performed in different WEs has been evaluated, the possibility of using this device for multianalyte immunoassays has been tested by immobilizing an immunoreagent commonly employed in the last step of many immunoprocedures. As a proof-of-concept the following experiment was carried out: 3

μL of antiIgG-AP solutions of different concentrations (1:2500, 1:5000, 1:10000 and 1:50000 dilutions) in 0.1 M Tris-HNO_3 pH 7.2 buffer were deposited onto the head of four pins coated with carbon ink, leaving there until dryness. Thus, different amounts of AP were adsorbed in each pin. In order to measure the amount of AP, the enzymatic reaction was performed by covering the six pins with a 90-μL drop of the 1 mM 3-IP / 0.4 mM silver nitrate solution, and after 20 min, the analytical signal is recorded (i.e. the peak current obtained recording the anodic stripping linear sweep voltammogram from 0.05 to 0.5 V at a scan rate of 50 $mV \cdot s^{-1}$). Since silver is reduced and deposited on the electrode, no cross talk between electrodes is produced. The enzymatic silver deposition catalyzed by alkaline phosphatase has been already reported (Fanjul-Bolado et al., 2007a) and this procedure was used in several electrochemical (multiplexed) immunosensors (Escamilla-Gómez et al., 2009; Neves et al., 2013; Rama et al., 2014). Fig. 4B shows the linear voltammograms obtained for each working electrode. As it can be seen, peak current increases with the amount of AP adsorbed. This opens the possibility for using this device in multianalyte determination by immobilizing different recognition elements on each working-electrode pin. Moreover, Dupont female cables for more (and less) pins are commercially available, and then their use as well as utilizing transparency as substrate provides an enormous versatility.

4. Conclusions

Mass-produced stainless-steel pins provides the basis for fabricating simple, portable, low-cost and versatile bioelectroanalytical sensors. These devices are based on materials readily available and avoid the use of stencils for electrode fabrication. The use of Dupont female cables allows a quick and reproducible interface between the pins and the potentiostat. The system here developed can be used to fabricate a glucose sensor

with satisfactory results when applied to real food sample analysis. The combination of pins and transparency allows a versatile modification of the electrodes position in the electrochemical cell and the possibility of fabricating multiplex electrochemical devices. We demonstrated the fabrication of a device with four working electrodes in the same cell that could be used for multianalyte determination.

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Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org.

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Caption of Figures

Fig. 1. Photographs of electrochemical cells fabricated using transparency film, stainless-steel pins as reference and counter electrodes (RE and CE) and one (A) or four (B) stainless-steel pins coated with carbon ink as working electrodes (WE). (C) Pin header used as alignment tool and 3-pin Dupont female cable used as interface between the pins and the potentiostat.

Fig. 2. Comparison of CVs recorded in 0.5 mM $FcCO_2H$ solution in phosphate buffer pH 7.0 at a scan rate of 50 $mV \cdot s^{-1}$: (A, B) using as working electrode a pin coated with (A) carbon ink and (B) carbon ink modified with MWCNTs, and dried for different times: 15 min, 12, 24 and 48 hours; (C) in electrochemical cells using as working electrode a pin coated with carbon ink and a pin coated with carbon ink modified with MWCNTs, both dried for 12 hours; (D) using 5 different devices using as working electrode a pin coated with carbon ink and dried for 12 hours.

Fig. 3. (A) Chronoamperograms recorded at -0.2 V vs. quasi-reference pin electrode for different glucose concentrations using the proposed glucose sensor. (B) Calibration curve and (inset) linear dynamic range obtained by applying -0.2 V (vs. stainless-steel quasi-reference electrode) for 50 s. Error bars correspond to the standard deviation of 5 measurements.

Fig.4. (A) Cyclic voltammograms recorded in 1 mM $FcCO_2H$ solution in phosphate buffer pH 7 at a scan rate of 50 $mV \cdot s^{-1}$ in an electrochemical cell consisting of four pins coated with carbon ink acting as working electrodes. (B) Linear sweep voltammograms from 0.05 V to 0.5 V at a scan rate of 50 $mV \cdot s^{-1}$ for the anodic stripping of metallic silver enzymatically deposited on the electrode surface by adsorption of different concentrations of antiIgG-AP (1:2500 (WE1), 1:5000 (WE2), 1:10000 (WE3) and 1:50000 (WE4)).

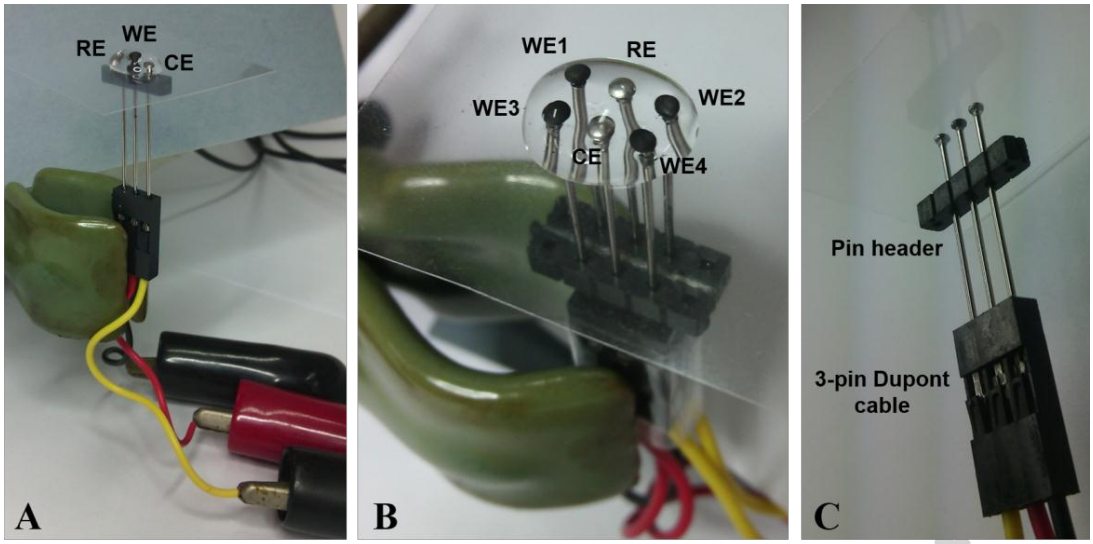
Tables

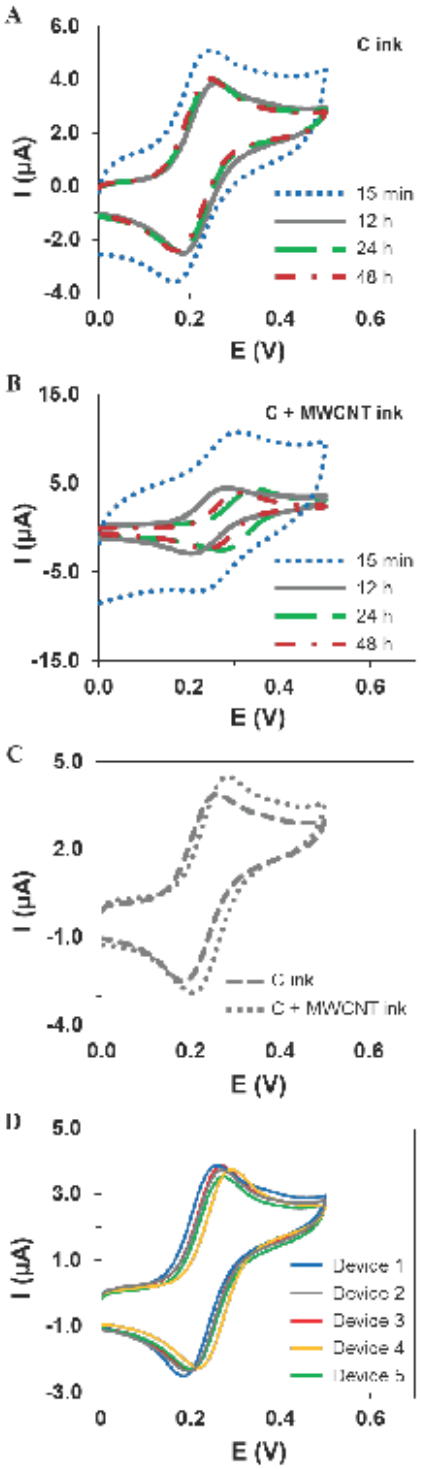
Table 1. Analytical signal recorded for different solutions: PBS solution pH 7.0 (background), 0.5 mM glucose, 0.5 mM fructose, 0.5 mM ascorbic acid, mixture of glucose/fructose 0.5 mM / 0.5 mM and mixture of glucose/ascorbic acid 0.5 mM / 0.1 mM. Data are given as average ±SD (n = 3).

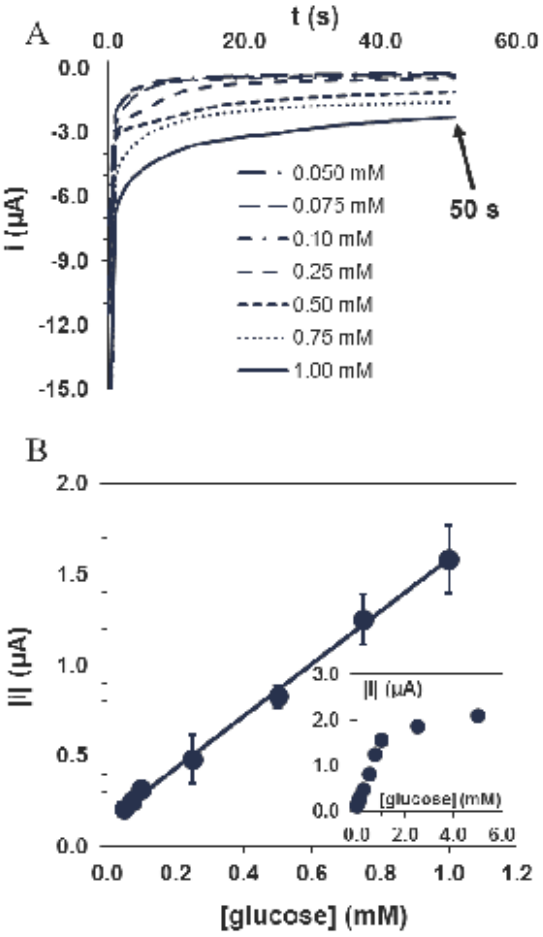
	Background	Glucose	Fructose	Ascorbic acid	Glucose/ Fructose	Glucose/Ascorbic acid
I (nA)	165 ± 6	830 ± 62	150 ± 13	500 ± 37	880 ± 57	890 ± 41

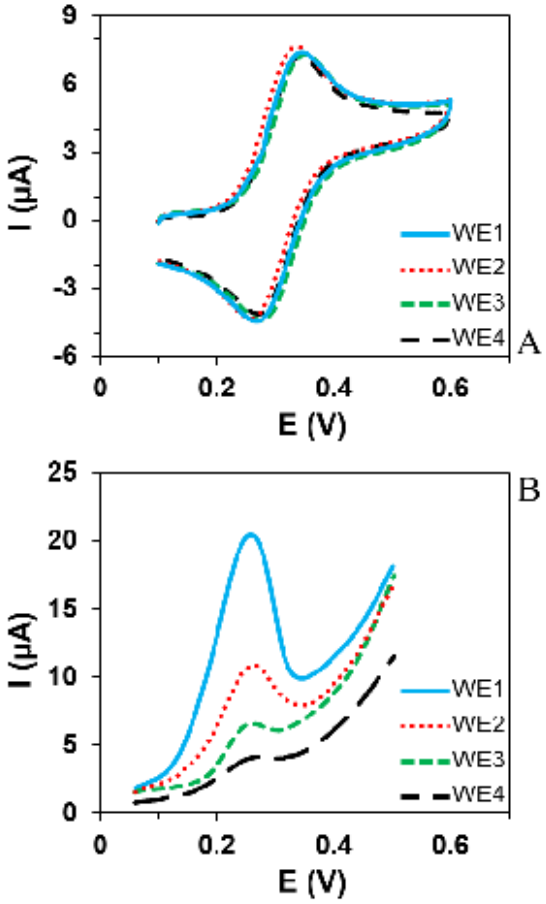
Table 2. Determination of glucose in real samples with the proposed sensor and with the enzymatic kit with spectrophotometric detection. Data are given as average ±SD (n = 5 for the sensor and n = 3 for the enzymatic kit).

Real sample	Glucose sensor	Enzymatic kit
Honey (g/100g)	35 ± 2	36.7 ± 0.3
Orange juice (g/100 mL)	3.7 ± 0.2	3.47 ± 0.04




A
C ink
15 min
12 h
24 h
48 h
I (μA)
E (V)
B
C + MWCNT ink
15 min
12 h
24 h
48 h
I (μA)
E (V)
C
C ink
C + MWCNT ink
I (μA)
E (V)
D
Device 1
Device 2
Device 3
Device 4
Device 5
I (μA)
E (V)


A
t (s)
0.0
20.0
40.0
60.0
0.0
-3.0
-6.0
-9.0
-12.0
-15.0
I (μA)
0.050 mM
0.075 mM
0.10 mM
0.25 mM
0.50 mM
0.75 mM
1.00 mM
50 s
B
2.0
1.5
1.0
0.5
0.0
|I| (μA)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
[glucose] (mM)
3.0
2.0
1.0
0.0
|I| (μA)
[glucose] (mM)
0.0
2.0
4.0
6.0


9
6
3
0
-3
-6
I (μA)
0
0.2
0.4
0.6
E (V)
WE1
WE2
WE3
WE4
A
25
20
15
10
5
0
I (μA)
0
0.2
0.4
0.6
E (V)
WE1
WE2
WE3
WE4
B

Highlights

- Stainless-steel pins are employed in electrochemical biosensing platforms
- A pin-based glucose amperometric enzymatic sensor is developed
- A multiplexed amperometric system is designed for pin-based enzymatic assays
- Low-cost electrochemical biosensor based on mass-produced common pins is evaluated
- Carbon-coated stainless-steel pins are employed as working electrodes in biosensing devices