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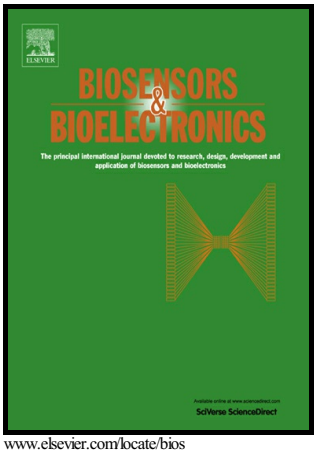

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Paper-based maskless enzymatic sensor for glucose determination combining ink and wire electrodes

*O. Amor-Gutiérrez, E. Costa Rama, A. Costa-García, M. T. Fernández-Abedul**

Departamento de Química Física y Analítica, Facultad de Química, Universidad de Oviedo, 33006 Oviedo, Spain

**Tel.: +34 985 10 29 68. e-mail: mtfernandeza@uniovi.es*

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, AE and flows by capillarity through the paper and contacts 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 capable of quantifying glucose in real food samples.

Keywords: Paper-based electroanalysis, paper-based biosensor, bioelectroanalytical platforms, enzymatic analysis, glucose, low-cost analysis.

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 more recent reduction of costs. Developed countries ask for more and continuous information about analytical parameters 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 solved with low-cost simple analytical platforms. Then, Analytical Chemistry labs suffer 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 can be considered nowadays as analytical tools for reference in the 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 (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 type of paper most used 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 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 (or 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, 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 the different measurements is as easy as in the case of a SPCE. It can be a reusable clip holder for the paper platform, although the low cost and precision makes possible to dispose it.

One of the main fields of electrochemical devices is devoted to biosensing. It also allows real-time transduction in many cases, and the limits of detection that provides are commonly low enough for the detection of a large number of analytes. Combined with microfluidics, it generates powerful analytical tools (Bunyakul and Baeumner, 2015). As a

proof-of-concept, in this work we developed an enzymatic biosensor for glucose. Although it is 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, and 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, screen-print 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) in all the three electrodes (Rungsawang et al., 2016; LOD = 0.86 mM), ii) in 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 the 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 electron transfer due to the reduction of ferricyanide, that takes place on the electrode surface (Murphy, 2006) and is proportional to glucose concentration. Initially, 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 generate 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. Both, 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^{o}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 x 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 from the SPCE. To prepare the working electrode, it was necessary to wax-print a pattern and then let it diffuse one minute at 100^{o}C on a heating plate. After that, 2 μL of carbon ink dilution (23 % of carbon ink in DMF) was deposited on the electrode and left to dry 12 hours. This procedure is detailed in Figure 1a. Then, the area with the carbon ink is adhered onto the SPCE, which integrates a silver pseudo-reference electrode and a carbon auxiliary electrode. The carbon ink of the paper contacts the carbon of the SPCE and the solution is applied on the opposite side.

With the recognition phase optimized, all the following studies were done using a paper-based working electrode and gold-plated connector headers as reference and auxiliary electrodes and also as connection to the potentiostat. The procedure for printing the

pattern, diffusing the wax and depositing the carbon ink was the same as before, but, instead of pasting the paper-based electrode onto the SPCE, it was connected with the gold-plated connector headers as seen in Figure 1b. Since the solution is deposited on the opposite side of the ink, the connection of the working electrode does not touch the other two pins, employed as reference and auxiliary electrodes. The paper layer is inserted in between three gold-plated pins in such a way that the one in the middle serves as a connection for the WE and is placed below the paper and the other two at the upper side, avoiding short circuits in between. Those at the ends are bent one to the other in order to both be inside the working hydrophilic area (Figure S1 in Supplementary information). They are also separated from the plane (those at the ends in a different sense to that at the middle) to form a clip that can support the paper. Solution is added on the side of the RE and the AE. The paper-based WE has a diameter of 4 mm, which accounts for an area of 12.6 mm^2. There is no need to use conductive adhesives, cables, clips or similar elements for connection because the rack separates the pins into two parts, the gold-plated one is used as stated before, and the other tin-based is inserted in the commercial connector.

2.4. Electrode biomodification

When using the paper-based electrodes combined with the SPCE, 10 µL of a mixture of enzymes (GOx and HRP) and ferrocyanide were deposited on the paper-based working electrode (on the opposite side of the carbon ink). This mixture, prepared in 0.1 M Tris-HNO_3 buffer pH 7.0, was left to dry for 1 hour and then pasted onto the SPCE. One SPCE can be used for approximately 10 measurements, while the paper-based working electrode is disposable and changed for each measurement.

In the case of the paper-based electrodes combined with gold-plated connector headers, the volume of mixture of enzymes and ferrocyanide was 5 µL, prepared in the same way as before. Each paper is used only once, but the connector header can be used many times without affecting the signal.

2.5. Analytical signal recording

To obtain analytical signals an aliquot of 40 µL, in the case of the paper-based electrode combined with the SPCE and of 10 µL for the paper-based electrode with connector headers was deposited on the sensor, covering all the three electrodes. The chronoamperograms were recorded applying a potential of -0.1 V during 100 s. The intensity at this time is due to the reduction of the ferricyanide previously generated on the

surface of the electrode and is related to the concentration of glucose in the sample. This chain of enzymatic reactions is represented in Figure S2 (in Supplementary information).

3. Results and Discussion

3.1. Optimization of the concentration of enzymes

First of all, using the paper-based working electrode pasted onto the SPCE, several biosensors were prepared using different concentrations of enzymes for a 2.5 mM glucose solution. To that end, different mixtures of GOx and HRP (0.8 and 1.6 U/μL for GOx and 2.5 and 5 U/μL for HRP), and a fixed concentration of ferrocyanide (0.1 M) were tested. A chronoamperogram was recorded for 100 s at -0.1 V *vs.* silver pseudo-reference electrode. A cyclic voltammogram was done previously in a ferrocyanide solution to fix this potential, as seen in Figure S3 of Supplementary information. The results of this optimisation are reported in Figure 2.

As it can be seen, there was a slight increase of the signal of the background with HRP concentration as happens also with glucose signals. Therefore, the lower concentration, 2.5 U/μL of HRP was chosen for the remainder of the work. Since the precision is better for 1.6 U/μL of GOx, this concentration of enzyme was employed in the enzyme mixture.

The SPCEs used here serve as an excellent connection to the potentiostat, as well as to provide the RE and the AE. Between measurements only the paper-based WE is changed, and one SPCE can be used for at least 10 measurements (washing it properly before adhering a new WE). Nevertheless, to facilitate and simplify the working methodology, it is preferred to use a self-contained platform which uses paper-based WE and gold-plated connector headers.

3.2. Integrated bioelectroanalytical platform: analytical characteristics

Once optimized the concentration of the enzymes of the sensing phase, it was studied the response of the paper-based platform combined with gold-plated connector headers as described in Section 2.3 and Figure 1b. The solution is added on the opposite side of the carbon ink and capillarity forces the solution to go through the porous paper and bring it to the interface electrode-solution. Reference and auxiliary pins are also wetted by the electrolyte, but without any contact with the working electrode. This easy-to-use platform, where the paper is inserted in between three pins (one at the bottom for WE connection and two at the top for acting as electrodes) can be used for different paper cards (all the measurements done in this article were done with the same connector headers). Once

the paper working electrode is used, it is disposed and a new one is placed in between the gold-plated pins.

The use of this simple paper-based device allows decreasing the sample volume considerably using at the same time a low-cost connection. Cutting a piece of paper is not needed in this case because the paper is directly inserted in between the pins. Biomodification is performed depositing the mixture of enzymes (GOx and HRP) and ferrocyanide on the paper-based working electrode (on the opposite side of the carbon ink). Different electronic properties of the electrode surface (with and without sensing phase) were observed by electrochemical impedance spenctroscopy, as shown in Figure S4 of the Supplementary information. With this platform, which works independently of the SPCE, the signal was recorded for increasing concentrations of glucose. Chronoamperograms corresponding to additions of 10 µL of different glucose concentrations were recorded. As can be seen in Figure 3, this method to construct the glucose biosensor allows quantifying it in a range of concentrations comprised between 0.3 and 15 mM, and the limit of detection, calculated as three times the standard deviation of the intercept divided by the slope, is 0.12 mM. The correlation coefficient is 0.996 (n = 9).

In order to evaluate the reproducibility of this biosensor, three different calibration graphs were made in three different days and with different solutions of Tris-HNO_3 buffer, GOx, HRP, ferrocyanide and glucose. Thus, as Table 1 illustrates, this glucose biosensor shows a very good reproducibility with a relative standard deviation (RSD) for the slopes of 4.4 %.

The precision obtained when different papers are inserted in the same connector header or in different ones was studied for a concentration of glucose of 2.5 mM. Therefore, different paper-based working electrodes were evaluated with i) the same connector and, hence, the same RE and AE and ii) different RE and AE. As seen in Figure S5 in Supplementary information, the RSD (%) values for both studies were below 5 %, demonstrating that the biosensor developed is very precise, even when working electrodes are hand-made.

The analytical performance and some other characteristics of the biosensor developed here were also compared with other glucose biosensors found in the bibliography. As seen in Table S1 (Supplementary information), it can be noted that the linear range for the biosensor developed here is wider than others, which were fabricated with different methods. Related to sensitivity, it is in the same order of magnitude than that obtained

with the rest of biosensors. However, the use of nanomaterials (here not employed and possible option) increases clearly the sensitivity. Although lower limits of detection have been achieved with previous biosensors, this presents a LOD low enough for the samples that were analyzed (cola beverages and orange juice). In addition, glucose concentration in blood samples is comprised between 80 and 120 mg/dL (4.4 – 6.6 mM) for healthy people and between 150 and 300 mg/dL (8.3 – 16.6 mM) for diabetes patients (Wang et al., 2008), concentrations that are in the range of calibration of this biosensor. On the other hand, the volume of the sample employed (10μL) is among the lowest values, as well as the size.

3.2.1. Specificity of the biosensor

The effect of different interferences that can affect the signal of the glucose biosensor here constructed was studied. For instance, the most important sugars that are present in many real samples are glucose (G) and fructose (F). Another compound that can interfere, especially present in juices, is ascorbic acid (AA). In this manner, individual solutions of 2.5 mM of glucose, fructose and ascorbic acid, a mixture of glucose and fructose (both in concentration 2.5 mM) and another of glucose and ascorbic acid, in the same proportion as in samples such as orange juices (Barberis et al., 2015) were prepared. Ascorbic acid is approximately in a concentration fifty times lower than glucose, so a mixture of 2.5 mM glucose and 0.05 mM ascorbic acid was tested. Chronoamperograms were recorded in the paper-based biosensor that uses the gold-plated connector headers. Results are shown in Figure 4.

As can be seen, both fructose and ascorbic acid in their own did not produce any interference, since they give a signal very similar to the one obtained when measuring 0.1 M Tris-HNO_3 buffer, pH 7.0 (background). In the case of the mixtures, the presence of fructose and ascorbic acid in different concentrations hardly affect the glucose signal. Therefore, enzymes specifity can be demonstrated with this study of interferences.

3.2.2. Stability of the biosensor

Several glucose biosensors were prepared and kept in a refrigerator at 4 ºC and protected from light until use. Measurements were recorded in 0.1 M Tris-HNO_3 buffer and 2.5 mM glucose solutions just after fabrication and after 24 h, 48 h and a week. Results are shown in Figure S6 in Supplementary information. As it can be seen, the glucose biosensor here developed is stable for at least 1 week.

3.3. Real sample measurements

Glucose was determined in real food samples like commercial orange juice and cola beverages using the paper-based glucose biosensor. The only sample pretreatment in the case of the cola beverage was degasification with the help of a stirrer. Next, both samples were diluted in 0.1 M Tris-HNO_3 buffer solution, pH 7.0 until its concentration entered into the linear range of the biosensor (in this case, a dilution of 1:20 in both cases). When this is done, a chronoamperogram was recorded in the same way as was for the rest of the measurements. The results obtained were compared to those acquired following a spectrophotometric assay commercially available (Table 2). A Student *t*-student test was performed to compare the values obtained with the paper-based glucose biosensor and the spectrophotometric assay. It was concluded, with a 0.05 significance level, that there were no systematic errors in the methodology.

In addition, a sugar-free cola beverage was analyzed as a negative control, being the positive one the cola beverage with sugar. As can be seen in the results shown in Figure S7 of the Supplementary Information, the value for the current in the sugar-free beverage is very similar to that of the background (0.1 M Tris-HNO_3 pH 7.0).

3.4. Analysis time and cost

These features are very relevant nowadays because they are related to productivity and allow the disposal of devices and the performance of decentralized analysis. In Table S2 (Supplementary information) a cost analysis for a glucose biosensor made with paper-based working electrodes and gold-plated connector headers is shown. Excluding labor and capital expenses, the cost of fabricating the biosensor described in this work is less than $ 0.52 (taking into account the prices for research quantities of materials and reagents). Considering, for instance, a 5-point calibration graph, with 5 replicates, the number of biosensors required is 25, and therefore it costs $ 13. As can be seen, the enzymes are the most expensive part of this biosensor (the header costs $ 0.3 per device but can be reused many times).

Referring to the analysis time, in Table S3 (Supplementary information), detailed information about the time taken for the different steps required for the determination of glucose with the combination of paper-based working electrodes and gold-plated connector headers is included. The time needed for sample detection is less than 38 minutes, but this time can be reduced further taking into account that biosensors can be mass-produced, thereby saving time considerably and also costs. The time for each chronoamperogram could also be reduced from 100 s to e.g. 50 s.

4. Conclusions

Glucose biosensors account for a high percentage of the whole biosensor market, being among the analytes for which more analytical methodologies are still being developed. Here, its determination is employed as a proof-of-concept of a new, simple and low-cost electroanalytical biodevice. The novelty of this biosensor lies in the transducer based on paper-based working electrodes and low-cost commercial connections, and uses a minimum sample volume and as a result a minimum generation of waste. This interface provides reference and auxiliary electrodes and, at the same time, allows the connection of the three electrodes to the potentiostat through a commercial connector for SPCE. Considering the enzymes are immobilized by adsorption, the fabrication of this glucose biosensor is inexpensive and quick, and the measurement step is very simple and easy. Moreover, the biosensor achieved shows a wide concentration range and very good reproducibility when doing different calibration graphs in several days. Glucose can be determined in real samples with a good accuracy and pretreatment of the sample is not required. To note, the good results achieved for this glucose sensor allow to think of the development of other (bio)sensors based on the transducer here developed.

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Supplementary material

Supplementary data associated with this article can be found in the online version.

References

Barberis, A., Spissu, Y., Fadda, A., Azara, E., Bazzu, G., Marceddu, S., Angioni, A., Sanna, D., Schirra, M., Serra, P.A., 2015. Simultaneous amperometric detection of ascorbic acid and antioxidant capacity in orange, blueberry and kiwi juice, by a telemetric system coupled with a fullerene- or nanotubes-modified ascorbate subtractive biosensor. Biosens. Bioelectron. 67, 214–223.

Berg, K.E., Adkins, J.A., Boyle, S.E., Henry, C.S., 2016. Manganese detection using stencil-printed carbon ink electrodes on transparency film. Electroanalysis 28, 679–684.

Biscay, J., Rama, E.C., García, M.B.G., Carrazón, J.M.P., García, A.C., 2011. Enzymatic sensor using mediator-screen-printed carbon electrodes. Electroanalysis 23, 209–214.

Bunyakul, N., Baeumner, A.J., 2015. Combining electrochemical sensors with miniaturized sample preparation for rapid detection in clinical samples. Sensors 15, 547–564.

Carrilho, E., Martinez, A.W., Whitesides, G.M., 2009. Understanding wax printing: a simple micropatterning process for paper-based microfluidics. Anal. Chem. 81, 7091–7095.

Cate, D.M., Adkins, J.A., Mettakoonpitak, J., Henry, C.S., 2015. Recent developments in paper-based microfluidic devices. Anal. Chem. 87, 19–41.

Domínguez Renedo, O., Alonso-Lomillo, M.A., Arcos Martínez, M.J., 2007. Recent developments in the field of screen-printed electrodes and their related applications. Talanta 73, 202–219.

Dossi, N., Toniolo, R., Impellizzieri, F., Bontempelli, G., 2014. Doped pencil leads for drawing modified electrodes on paper-based electrochemical devices. J. Electroanal. Chem. 722–723, 90–94.

Dungchai, W., Chailapakul, O., Henry, C.S., 2009. Electrochemical detection for paper-based microfluidics. Anal. Chem. 81, 5821–5826.

Glavan, A.C., Christodouleas, D.C., Mosadegh, B., Yu, H.D., Smith, B.S., Lessing, J., Fernández-Abedul, M.T., Whitesides, G.M., 2014. Folding analytical devices for electrochemical ELISA in hydrophobic RH paper. Anal. Chem. 86, 11999–12007.

Hayat, A., Marty, J.L., 2014. Disposable screen printed electrochemical sensors: tools for environmental monitoring. Sensors 14, 10432–10453.

Heller, A., Feldman, B., 2008. Electrochemical glucose sensors and their applications in diabetes management. Chem. Rev. 108, 2482–2505.

Jung, W., Han, J., Choi, J.-W., Ahn, C.H., 2015. Point-of-care testing (POCT) diagnostic

systems using microfluidic lab-on-a-chip technologies. Microelectron. Eng. 132, 46–57.

Kong, F.Y., Gu, S.X., Li, W.W., Chen, T.T., Xu, Q., Wang, W., 2014. A paper disk equipped with graphene/polyaniline/Au nanoparticles/glucose oxidase biocomposite modified screen-printed electrode: Toward whole blood glucose determination. Biosens. Bioelectron. 56, 77–82. doi:10.1016/j.bios.2013.12.067

Lawrence, C.S.K., Tan, S.N., Zerda Floresca, C., 2014. A “green” cellulose paper based glucose amperometric biosensor. Sensors Actuators B Chem. 193, 536–541.

Li, W., Qian, D., Li, Y., Bao, N., Gu, H., Yu, C., 2016. Fully-drawn pencil-on-paper sensors for electroanalysis of dopamine. J. Electroanal. Chem. 769, 72–79.

Martinez, A.W., Phillips, S.T., Whitesides, G.M., Carrilho, E., 2010. Diagnostics for the developing world: Microfluidic paper-based analytical devices. Anal. Chem. 82, 3–10.

Murphy, L., 2006. Biosensors and bioelectrochemistry. Curr. Opin. Chem. Biol. 10, 177–184.

Nie, J., Zhang, Y., Lin, L., Zhou, C., Li, S., Zhang, L., Li, J., 2012. Low-cost fabrication of paper-based microfluidic devices by one-step plotting. Anal. Chem. 84, 6331–6335.

Nie, Z.H., Nijhuis, C.A., Gong, J.L., Chen, X., Kumachev, A., Martinez, A.W., Narovlyansky, M., Whitesides, G.M., 2010. Electrochemical sensing in paper-based microfluidic devices. Lab Chip 10, 477–483. doi:10.1039/B917150A

Noiphung, J., Songjaroen, T., Dungchai, W., Henry, C.S., Chailapakul, O., Laiwattanapaisal, W., 2013. Electrochemical detection of glucose from whole blood using paper-based microfluidic devices. Anal. Chim. Acta 788, 39–45. doi:10.1016/j.aca.2013.06.021

Rungsawang, T., Punrat, E., Adkins, J., Henry, C., Chailapakul, O., 2016. Development of electrochemical paper-based glucose sensor using cellulose-4-aminophenylboronic acid-modified screen-printed carbon electrode. Electroanalysis 28, 462–468. doi:10.1002/elan.201500406

Russo, A., Ahn, B.Y., Adams, J.J., Duoss, E.B., Bernhard, J.T., Lewis, J.A., 2011. Pen-on-paper flexible electronics. Adv. Mater. 23, 3426–3430.

Scida, K., Cunningham, J.C., Renault, C., Richards, I., Crooks, R.M., 2014. Simple,

sensitive, and quantitative electrochemical detection method for paper analytical devices. Anal. Chem. 86, 6501–6507.

Sekar, N.C., Shaegh, S.A.M., Ng, S.H., Ge, L., Tan, S.N., 2014. A paper-based amperometric glucose biosensor developed with Prussian Blue-modified screen-printed electrodes. Sensors Actuators, B Chem. 204, 414–420.

Taleat, Z., Khoshroo, A., Mazloum-Ardakani, M., 2014. Screen-printed electrodes for biosensing: A review (2008-2013). Microchim. Acta 181, 865–891.

Valcárcel, M., Cárdenas, S., Gallego, M., 1999. Sample screening systems in analytical chemistry. TrAC - Trends Anal. Chem. 18, 685–694.

Yu, J., Ge, L., Huang, J., Wang, S., Ge, S., 2011. Microfluidic paper-based chemiluminiscence biosensor for simultaneous determination of glucose and uric acid. Lab Chip 11, 1286–1291.

Caption of Figures

Figure 1. Scheme of the preparation of electrochemical cells: **a)** based on SPCEs and **b)** using gold-plated connector headers.

Figure 2. Current intensities obtained for 0.1 M Tris-HNO_3 buffer pH 7.0 (background) and 2.5 mM glucose solutions with the concentrations of enzymes indicated. Three measurements were made for each concentration.

Figure 3. Chronoamperograms recorded for different glucose concentrations (up to down: 0, 0.3, 0.5, 1, 3, 5, 7.5, 10 and 15 mM) using the paper-based WE combined with the gold-plated connector headers. Inset: Calibration plot of the paper-based glucose sensor using the gold-plated connector headers. Detection potential = - 0.1 V *vs.* pseudoreference, applied for 100s. Data are given as average ± SD (n = 5).

Figure 4. Study of the possible interference caused by fructose and ascorbic acid. Data are given as average ± SD (n = 5).

Tables

Table 1. Study of reproducibility: characteristics of the three calibration graphs carried out with the glucose biosensor.

Calibration plot	Slope (μA/mM)	R^2	Linear range (mM)
Day 1	- 1.13	0.996	0.3 – 15
Day 2	- 1.19	0.993	0.3 – 15
Day 3	- 1.26	0.996	0.3 – 15
Average	- 1.19		
Standard Deviation	± 0.06		
% RSD	5.4 %		

Table 2. Measurement of glucose in orange juice with the biosensor here developed and comparison with a commercial kit for glucose. Data are given as average ± SD (n = 5 for the paper-based glucose biosensor and n = 3 for the spectrophotometric assay)

Sample	Paper-based biosensor	Spectrophotometric assay
Orange Juice	2.73 ± 0.2 g/100 mL	2.72 ± 0.04 g/100 mL
Cola beverage	3.01 ± 0.3 g/100 mL	3.12 ± 0.03 g/100 mL

Highlights

- Paper-working electrode with gold-plated auxiliary and reference electrodes for glucose biosensing
- Paper microfluidic electroanalytical device with combined metallic and carbon-ink electrodes
- Simple carbon ink-based paper platform with low-cost pin electrodes for decentralised bioanalysis

Figure(s)

Figure 1 MTFA et al

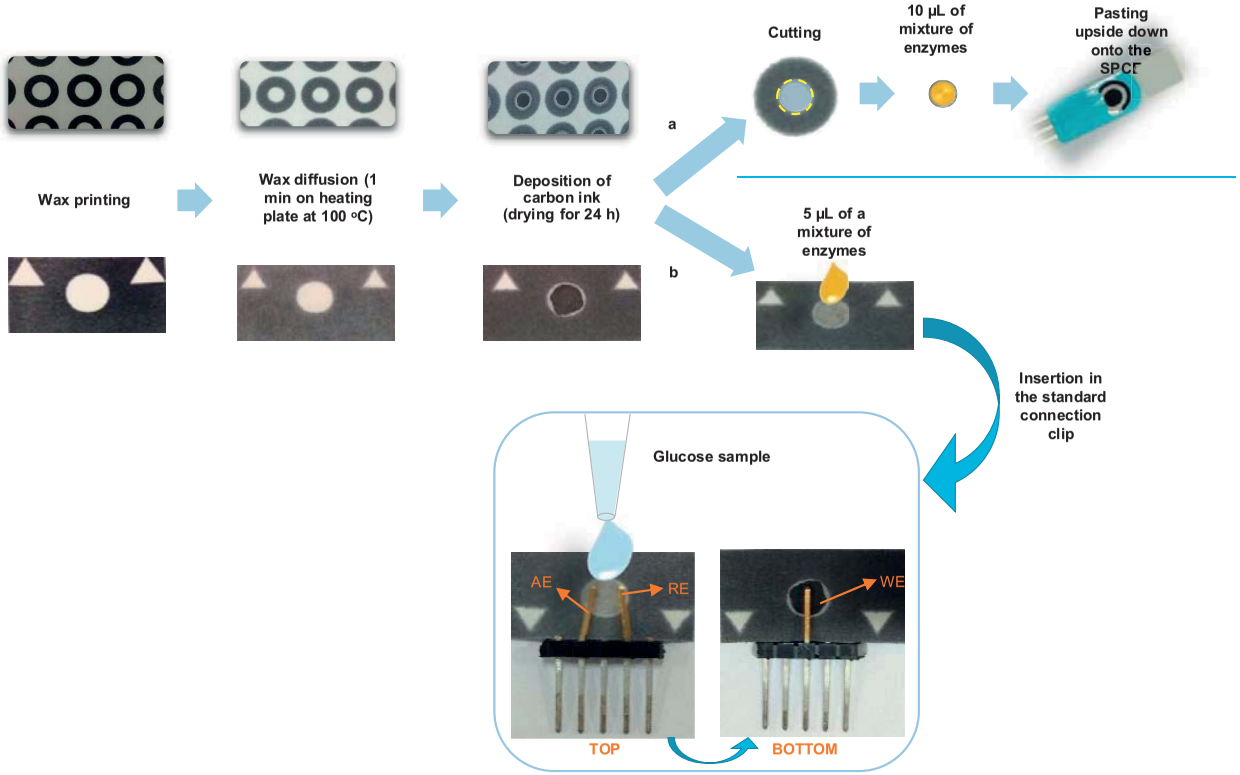

Figure 2. MTFA et al

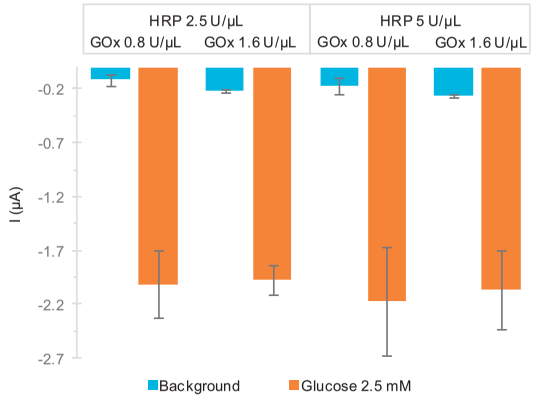

Figure 3 MTFA et al

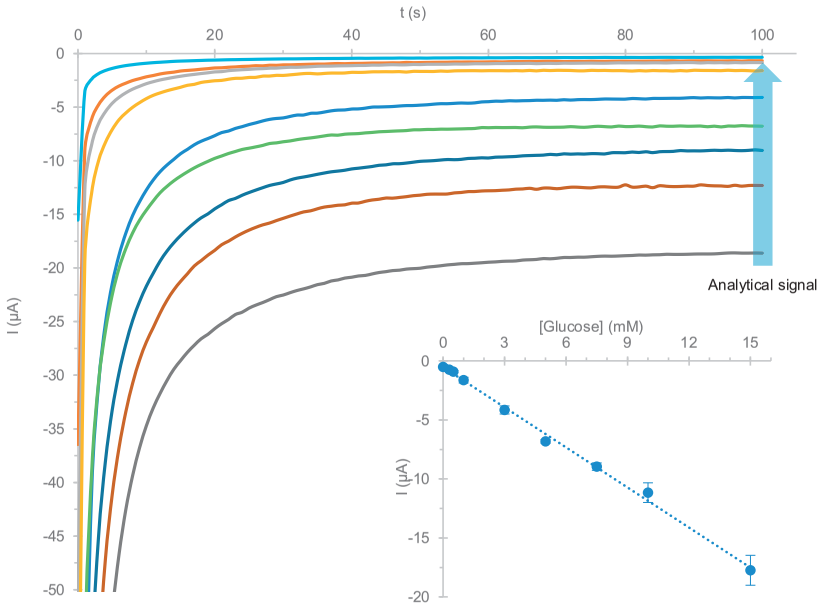

Figure 4 MTFA et al