Published in final edited form as:

Anal Chem. 2013 May 21; 85(10): 5233–5239. doi:10.1021/ac400728y.

Construction and electrochemical characterization of microelectrodes for improved sensitivity in paper-based analytical devices

Murilo Santhiago^{†,‡}, John B. Wydallis[§], Lauro T. Kubota^{†,‡}, and Charles S. Henry^{§,*}

[†]Department of Analytical Chemistry, Institute of Chemistry – UNICAMP, P.O. Box 6154, 13084-971, Campinas, SP, Brazil

[‡]Instituto Nacional de Ciência e Tecnologia em Bioanalítica, Institute of Chemistry UNICAMP, P.O. Box 6154, 13084-971, Campinas, SP, Brazil

[§]Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523, United States

Abstract

This work presents a simple, low cost method for creating microelectrodes for electrochemical paper-based analytical devices (ePADs). The microelectrodes were constructed by backfilling small holes made in polyester sheets using a CO₂ laser etching system. To make electrical connections, the working electrodes were combined with silver screen-printed paper in a sandwich type two-electrode configuration. The devices were characterized using linear sweep voltammetry and the results are in good agreement with theoretical predictions for electrode size and shape. As a proof-of-concept, cysteine was measured using cobalt phthalocyanine as a redox mediator. The rate constant (k_{obs}) for the chemical reaction between cysteine and the redox mediator was obtained by chronoamperometry and found to be on the order of $10^5 \text{ s}^{-1} \text{ M}^{-1}$. Using a microelectrode array, it was possible to reach a limit of detection of $4.8 \text{ } \mu\text{M}$ for cysteine. The results show that carbon paste microelectrodes can be easily integrated with paper-based analytical devices.

Introduction

Microfluidic paper-based analytical devices (μ PADs) were introduced by Martinez et al. in 2007 as an alternative to first-generation microfluidic devices.¹ Paper is a useful substrate for developing simple, point-of-need assays due to low cost of materials, ease of device fabrication and the ability to modify paper with a variety of reagents.² The porous structure of the paper is also important because it creates flow *via* capillary forces without the addition of mechanical or electrical forces.^{1–3} μ PADs are different from traditional lateral flow assays in that they contain barriers to direct flow to multiple sites for chemical analysis. Device fabrication can be achieved using several methods, with photolithography and wax printing being most common.^{1,4–7} Detection is also key to the device functionality, and techniques ranging from monitoring color changes with the naked eye to the use of electrochemistry have been demonstrated. The most common form of detection with μ PADs is based on optical imaging for colorimetric reactions *via* cameras or scanners. In addition, other methods have been demonstrated,⁴ including chemiluminescence,⁸ Raman,⁹ colorimetry,¹⁰ mass spectrometry,¹¹ and electrochemistry.^{12,13}

*Corresponding author: chuck.henry@colostate.edu.

Electrochemical detection for paper-based microfluidic devices (ePADs) was demonstrated in 2009 by Dungchai et al.¹² To date, different strategies have been used to integrate electrodes with ePADs.^{12–15} First-generation electrodes were printed directly on the μ PAD using silk-screen technology and used carbon, silver, and gold as electrode materials.¹⁴ Dungchai et al. reported the construction of a three-electrode system by screen printing carbon and silver inks directly on paper.¹² Alternatively, pre-fabricated screen-printed carbon electrodes and strips of paper were used for the detection of glucose and Pb(II).¹³ Second-generation devices using electrodes fabricated on a separate substrate that is integrated with the paper in an attempt to further improve limits of detection and sensitivity have been demonstrated.¹⁵ To the best of our knowledge, however, there have been no attempts to integrate microelectrodes with ePADs. Here, a simple method for integrating microelectrodes with ePADs was developed that maintains the inherent low cost and ease of use associated with μ PADs.

Microelectrodes have many attractive properties for electroanalytical chemistry including: (i) the ohmic drop (IR) is minimized because of the small measured currents,^{16,17} (ii) a fast response time is achieved due to the low electric double layer capacitance¹⁸, (iii) an increase of the mass transfer rates to the electrode surface relative to macroelectrodes due to radial diffusion,^{16,17,19} and (iv) the ratio between faradaic and capacitive currents (I_f/I_c) is increased since microelectrodes have substantially smaller areas.¹⁷ In addition, electrochemical experiments can be conducted using two-electrode systems, because of reduced effects of uncompensated resistance.²⁰

Microelectrodes have been constructed from a variety of materials, including gold, platinum and carbon.²¹ Carbon is an attractive electrode material due to its broad potential window, low cost, and ease of chemical modification. These properties also make it an excellent choice for the fabrication of ePADs.^{12,13} Carbon paste microelectrodes are particularly attractive for low-cost applications because they can be readily modified by mixing catalytic agents into the paste before electrode fabrication.²² Additionally, carbon paste is nontoxic and can be easily manipulated, facilitating the construction of microelectrode arrays.²²

Here, a novel approach for constructing carbon paste microelectrodes for ePADs is presented where carbon paste is filled in small holes made in polyester (transparency film) sheets made using a laser cutting system. Polyester sheets were selected due to their low cost and broad availability. Once fabricated, electrical contact was made to contact pads printed on paper. The electrode performance was characterized with cyclic voltammetry using $\text{Fe}(\text{CN})_6^{4-}$ and the response compared to theory for elliptical electrodes. Next, single electrodes were compared to arrays of four working electrodes to show the ability to easily create small arrays. The electrode behavior gave good agreement between measured and predicted currents. To show application of the system, carbon paste was modified with the catalyst, cobalt phthalocyanine (CoPC), and used to measure cysteine. Catalytic constants were measured and found to be among the highest reported to date.

Experimental

Materials, equipment and chemicals

All chemicals were of analytical grade and used as received. Whatman #1 chromatography paper was acquired from Whatman (Buckinghamshire, UK). Potassium phosphate monobasic, acetic acid, sodium acetate, citric acid, sodium citrate, potassium chloride, sodium hydroxide and heavy mineral oil were acquired from Fischer Scientific, (NJ, USA). Potassium phosphate dibasic and phosphoric acid were acquired from EMD Chemicals Inc. (NJ, USA). Cysteine, potassium ferricyanide, cobalt phthalocyanine and graphite powder

(particle size <20 μm) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Potassium ferrocyanide was acquired from Mallinckrodt (St. Louis, MO, USA).

A XEROX Phaser 8860 printer was used to print the paper-based analytical devices following established protocols.¹ An Isotemp hot plate from Fischer Scientific set at 150°C was used to melt the wax on the paper. Silver paint from SPI Supplies (PA, USA) was used for the construction of the contact pads and reference/counter electrodes. Scotch-brand double-sided tape was used to mount the polyester sheet containing electrodes on the paper device. A Zygo ZeScope optical profilometer was used to measure the size and distance between the microelectrodes.

The unmodified carbon paste was prepared by hand mixing graphite powder and mineral oil (60:40, w:w) in a glass vial. For the modified carbon paste, varying amounts of cobalt phthalocyanine (0.5–10.0% w/w) were added to the graphite, while keeping the ratio of mineral oil constant. The modified and unmodified carbon pastes were kept in a closed glass vial and used as needed.

Solutions were prepared by using purified water (18.2 M Ω) from a MilliQ Millipore water purification system. Solution pH was determined with Denver Instrument UltraBASIC UB-5 pH Meter (Denver, CO, USA). Working standard solutions were prepared daily by appropriate dilution of the stock solutions with pH 4.0, 0.5 M acetate buffer. For pH studies in the range of 2.5–3.5, mixtures of citric acid and sodium citrate were used at a concentration of 0.5M.

Laser cutting process

Microelectrodes and screen-printing masks were fabricated using polyester transparency sheets (215 $\times$ 279 mm $\times$ 0.11mm thick) (Highland 901, 3M, Austin, TX, USA). The sheets were used as received and cut using a laser engraving system (Epilog, Golden CO, USA). The CO₂ laser system had a peak power of 30 W and was controlled by Epilog software after uploading drawing files.

For microelectrode fabrication, 25 to 100 μm circles were drawn using CorelDRAW X5. The circles were cut using the laser engraving system. The devices were denoted according to the intended size of the circles: ePAD1 (25 μm), ePAD2 (50 μm), ePAD3 (75 μm) and ePAD4 (100 μm). Finally electrode sizes were measured with an optical profilometer and found to be larger but dependent on the input size. The same procedure was adopted for the construction of the electrode arrays with the center-to-center distance varied from 300 to 1000 μm .

We recently described the use of transparency sheets as masks for screen printing carbon electrodes.¹⁵ Here, the laser engraving system was used to cut the transparency and prepare two masks, one for the construction of the contact pad for the working electrode and a second one for the fabrication of the reference/counter electrode (RE/CE). The size and shape of the masks are shown in the Supporting Information (Figure 1S(a)).

Fabrication of the ePAD

Whatman #1 chromatographic paper is the most common porous substrate used for construction of μPADs and was selected for fabrication of the ePADs reported here. Wax patterns were created to constrain liquid to the region directly around the electrodes. In the absence of the wax, solution free flowed away from the electrodes. Black patterns were drawn to create hydrophobic zones on the paper (Fig. S1(b)). The sheets of paper were cut into letter size (216 $\times$ 297 mm) and the black patterns were printed using a solid ink printer (XEROX Phaser 8860). The printed paper was placed on a hot plate at 150 °C for 2 min to

melt the wax. The paper was allowed to cool to room temperature before screen-printing electrodes.

Contact pads for the working and counter electrode were constructed using transparency masks and silver paint. The masks were positioned on the paper and the silver paint was spread over the surface. The mask was carefully removed and the device containing the conductive tracks (Fig S1(c)) baked at 60°C for 15 minutes. After curing the silver paint, the devices were allowed to cool to room temperature. A 4 mm biopsy punch was used to make a hole in the counter electrode (Fig. S1(d)) to allow solution contact between the working and counter electrode. Double-sided tape was used to attach the device parts. The attachment procedure is shown in the supplementary section, Fig. S2.

Electrochemical detection

Electrochemical experiments were performed with a model 660B potentiostat (CH Instruments, Austin, TX). For the electrochemical experiments, a two-electrode system was used with silver paint as the counter electrode and the carbon paste microelectrode(s) as the working electrode (WE). Alligator-type connectors were connected to contact pads on the edge of the device as shown in Figures 1 and S2(c). For characterizing the ePAD, 30 μ L of solution was used to wet the active area of the electrodes. Chronoamperometric detection of cysteine was performed by applying 0.6 V vs. the silver counter electrode for 20 s. Detection limits were calculated as the current giving rise to signal three times the standard deviation of the background (n=10).

Results and discussion

Electrochemical characterization of the ePAD with microelectrodes

As can be seen in Figure 2(a), the electrodes designed to have an elliptical shape after laser cutting despite being designed to be circular. Furthermore, the electrode size was increased relative to the intended size, and a raised ridge formed around the hole. The observed electrode shape and size relative to the intended shape and size may arise from melting effects due to the contact of the laser with the polyester sheet. The raised ridge observed around the carbon paste is the result of polyester being redeposited during the cutting process.

Microelectrodes were initially characterized with linear scan voltammetry using $\text{Fe}(\text{CN})_6^{4-}$ as the redox probe. Figure 2(b) shows a well-defined voltammogram with a typical sigmoidal shape for a microelectrode. The diffusion current of elliptical shape microelectrodes was described by Bruckenstein and Janiszewska,²³ where the limiting current for the oxidation of electroactive species A can be defined as:

$$I_{\text{ell,lim}} = 2\pi n F D_A C_A^b a/K \left(\left[1 - b^2 a^{-2} \right]^{1/2} \right), \quad a \geq b \quad (1)$$

where a and b are the major and minor semi-axes, respectively, of the ellipse, n is the number of transferred electrons, F is the Faraday constant, D_A is the diffusion coefficient of the species A (cm^2/s), C_A is the concentration of the electroactive species A (mol/cm^3) and K is the complete elliptical integral of the first kind. By solving K , the elliptical limiting current can be obtained and compared with experimental results for the microelectrodes. For more information see Supporting Information S3.

To compare experimental results to theoretical predictions, elliptical disks of different sizes were constructed. The limiting currents were compared with theoretical values for the same size elliptical electrodes (Table 1). We used an optical profilometer to measure the semi-

axes of the working area of the microelectrodes. As can be seen in the Table 1, the relative error for the correlation between theoretical and experimental values is lower than 5.0% for the microelectrodes that were drawn in the range of 50 – 100 μm . The higher relative error observed electrodes drawn with a 25 μm diameter was caused by the inability of the laser cutting system to consistently cut smaller openings. Thus, the smallest elliptical microelectrodes that can be fabricated with good repeatability are the electrodes that have a drawn diameter of 50 μm and a final size of $\sim 190 \times 240 \mu\text{m}$.

Cyclic Voltammetry

The electrocatalytic activity of the ePAD with microelectrodes toward cysteine electrooxidation was investigated by cyclic voltammetry. The process associated with the electrochemical oxidation of cysteine (Cys) is illustrated in Figure 3a by cyclic voltammograms of 500 μM Cys in 0.5 M acetate buffer, pH 4.0. As can be seen in Figure 3a, the unmodified carbon paste microelectrode gives a small response for Cys electrooxidation. On the other hand, a well-defined oxidation peak and significant peak current increase are observed when CoPC is added as redox mediator. The modified electrode shows a shoulder at 0.4 V and a peak at 0.6 V *vs.* the Ag counter electrode for Cys electrooxidation. Additional experiments used the peak current at 0.6 V. The phenomenon giving rise to the shoulder at 0.4 V is currently unknown and out of the scope of this investigation.

Cysteine is a zwitterionic compound and present in three different forms depending on solution pH,²⁴ thus the effect of pH on peak current was measured. Figure 3b shows that higher currents are observed at pH 4.0 – 4.5. Above pH 4.5 the peak current drops dramatically and the peak potential shifts to more positive values (Supporting information S4). One of the reasons for the lower responses observed above pH 4.5 can be due to the deactivation of the redox mediator as the solution pH increases,²⁵ disfavoring the reaction between cysteine and redox active sites.

The amount of CoPC catalyst added was optimized next. It has been reported that the amount of CoPC adsorbed onto glassy carbon electrodes influences the current and peak splitting of the anodic oxidation peak for 2-mercaptoethanesulfonic acid.²⁶ In order to check both parameters, cyclic voltammograms as a function of CoPC percentage were obtained (Figure 3c). A linear correlation between the %CoPC and the peak current from 0.5 % to 8.0 % was found. For higher percentages, the peak current remains constant due to a saturation of available redox active sites. We found that even at lower CoPC concentrations the cyclic voltammograms maintained the same general shape shown in Figure 3a (Figure S5).

Finally, the influence of the scan rate was evaluated. Figure 3d shows a linear dependence of the anodic peak current for the process at 0.6 V *vs.* square root of the scan rate, indicating that the electrochemical process is controlled by diffusion.¹⁹ The electrochemical oxidation process at 0.6 V shifts toward more positive potentials as the scan rate is increased indicating a kinetic limitation between cysteine and cobalt phthalocyanine. Also, a plot of the sweep rate normalized current ($I_p/\nu^{1/2}$) *versus* scan rate shows a typical shape for electrochemical-chemical catalytic (EC_{cat}) process¹⁹ (Supporting Information S6). A deeper examination of the electrocatalytic process between cysteine and modified ePAD was done using chronoamperometry.

Chronoamperometry

Single step chronoamperometry was employed to determine the catalytic constant between the redox active sites of the redox mediator and cysteine. Figure 4a shows the chronoamperometric current as a function of the applied potential. The current maximum

was found $E_{\text{appl.}} = 0.6 \text{ V vs. Ag}$. This applied potential was used to obtain the chronoamperometric curves for different cysteine concentrations, and current increased as a function of cysteine concentration as expected (Figure 4b). The reaction of cysteine and the redox mediator is as follows:

The equations above described are consistent with the type of mechanism (EC_{cat}) obtained by cyclic voltammetry. The overall reaction can be written as:

The kinetics of the above reaction can be obtained by measuring the rate constant (k_{obs}), and it is expected that higher constants will give lower detection limits. The rate of the chemical reaction between cysteine and the redox active sites confined on the electrode surface can be evaluated by chronoamperometry:²⁷

$$\frac{I_{\text{cat}}}{I_L} = \gamma^{1/2} \pi^{1/2} = \pi^{1/2} (k_{\text{obs}} C t)^{1/2} \quad (5)$$

where I_{cat} is the catalytic current of the modified microelectrode in the presence of cysteine, I_L is the background current and $\gamma = k_{\text{obs}} C t$ (C is the bulk concentration of cysteine and t is the elapsed time). In order to calculate the rate constant, plots of I_{cat}/I_L versus $t^{1/2}$ were initially obtained (Figure 4c). The slopes of I_{cat}/I_L versus $t^{1/2}$ were plotted as a function of cysteine concentration (Figure 4d) and a value of $3.92 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ obtained for the heterogeneous rate constant. The high catalytic constant for this ePAD is most likely the result of a combination of the fast reaction between cysteine and cobalt phthalocyanine with the reduced electrode size.

The chronoamperometric response-time experiments described in Figure 4(b) can be used to construct the analytical curve. The linear response range was from $50 - 600 \mu\text{M}$ (Figure S7), which can be expressed by the following equation: $\Delta I(\text{nA}) = 0.38(\pm 0.34) + 0.061(\pm 0.001) [\text{Cys}] (\mu\text{M})$ with a correlation coefficient of 0.998 ($n = 3$).

Array of microelectrodes

To further improve detection limits, electrode arrays were constructed using simple methods and materials. Microelectrode arrays have been reported for many applications.^{28–32} In general, the construction of traditional microelectrode arrays involves many steps and complicated fabrication tools making them diametrically opposed to the overall goals of most μPADs . Microelectrode arrays constructed using our methods should provide even lower detection limits. In fabricating electrode arrays, diffusion layer overlap is a significant concern and results in linear diffusion dominating mass transport.³³ Arrays of four microelectrodes with varying spacing were fabricated to determine diffusional overlap. Figure 5a shows limiting current for the oxidation of Fe(CN)_6^{4-} as a function of the distance between the microelectrodes in the array. For a distance of $300 \mu\text{m}$, a peak shape voltammogram is obtained, indicative of linear diffusion (Figure S8) and as a consequence, the response of the ePAD is lower when compared with the other distances. When the

distance between electrodes is increased, the limiting current increases significantly (Figure 5a). In addition, the increase in limiting current from a single electrode to the four electrode array is easily seen for the oxidation of $\text{Fe}(\text{CN})_6^{4-}$ using one and four microelectrodes (distance = 500 μm) (Figure 5b). As can be observed in Figure 5b, the voltammograms have the same sigmoidal shape but with a significant current increase for the array of microelectrodes. The theoretical values for the electrochemical response of the array were calculated using Equation 1 and are in good agreement with the experimental results.

In order to show the current increase in the presence of the analyte, chronoamperograms were obtained after successive additions of cysteine and the current was plotted as a function of Cys concentration (Figure 5c). The results from the microelectrode array and a comparison with a single electrode of the same size are given in Table 2. A significant improvement in the analytical figures of merit was obtained for the array relative to the single electrode.

Conclusions

We reported here for the first time the construction and electrochemical characterization of carbon paste microelectrodes and microelectrode arrays for paper-based analytical devices. We demonstrated that polyester transparency sheets provide a low cost alternative substrate for microelectrode fabrication and can be easily combined with paper-based devices in a sandwich-type configuration. The electrochemical response of the ePAD is similar when compared with well-established microelectrode systems and can be predicted by theoretical predictions. CoPC modified microelectrodes showed an excellent response toward cysteine electrooxidation with one of the highest heterogeneous rate constants reported so far. Modified carbon paste microelectrodes are a promising tool for ePADs since they are easy to make, use, modify, and can improve the limit of detection of ePADs. We also have demonstrated that microelectrode arrays can be used in order to obtain lower limits of detection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank financial support from Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Instituto Nacional de Ciência e Tecnologia de Bioanálítica (INCTBio). MS is indebted to FAPESP for the fellowship. This work was also supported by the National Institute of Environmental Health Safety of the National Institutes of Health (R21ES19264). We also would like to thank Colorado State University for all the support in the development of this work.

References

1. Martinez AW, Phillips ST, Butte MJ, Whitesides GM. *Angew Chem Int Ed.* 2007; 46:1318–1320.
2. Pelton R. *Trends Anal Chem.* 2009; 28:925–941.
3. Martinez AW, Phillips ST, Carrilho E, Thomas SW III, Sindi H, Whitesides GM. *Anal Chem.* 2008; 80:3699–3707. [PubMed: 18407617]
4. Carrilho E, Martinez AW, Whitesides GM. *Anal Chem.* 2009; 81:7091–7095. [PubMed: 20337388]
5. Dungchai W, Chailapakul O, Henry CS. *Analyst.* 2011; 136:77–82. [PubMed: 20871884]
6. Abe K, Suzuki K, Citterio D. *Anal Chem.* 2008; 80:6928–6934. [PubMed: 18698798]
7. Songjaroena T, Dungchai W, Chailapakul O, Laiwattanapaisa W. *Talanta.* 2011; 85:2587–2593. [PubMed: 21962687]

8. Yu J, Wang S, Ge S. *Biosens Bioelectron.* 2011; 26:3284–3289. [PubMed: 21257303]
9. Yu WW, White IM. *Anal Chem.* 2010; 82:9626–9630. [PubMed: 21058689]
10. Dungchai W, Chailapakul O, Henry CS. *Anal Chim Acta.* 2010; 674:227–233. [PubMed: 20678634]
11. Liu J, Wang H, Manicke NE, Lin J, Cooks RG, Ouyang Z. *Anal Chem.* 2010; 82:2463–2471. [PubMed: 20158226]
12. Dungchai W, Chailapakul O, Henry CS. *Anal Chem.* 2009; 81:5821–5826. [PubMed: 19485415]
13. Nie Z, Nijhuis CA, Gong J, Chen X, Kumachev A, Martinez AW, Narovlyansky M, Whitesides GM. *Lab Chip.* 2010; 10:477–483. [PubMed: 20126688]
14. Shiroma LY, Santhiago M, Gobbi AL, Kubota LT. *Anal Chim Acta.* 2012; 725:44–50. [PubMed: 22502610]
15. Santhiago M, Kubota LT. *Sens Act B.* 2013; 177:224–230.
16. Štulík K, Amatore C, Holub K, Marek V, Kutner W. *Pure Appl Chem.* 2000; 8:1483–1492.
17. Wang, J. *Analytical Electrochemistry.* 3. Wiley; New Jersey: 2006.
18. Schulte A, Schuhmann W. *Angew Chem Int Ed.* 2007; 46:8760–8777.
19. Bard, AJ.; Falkner, L. *Electrochemical methods – Fundamentals and Applications.* 2. Wiley; New York: 2001.
20. Turcu F, Schulte A, Schuhmann W. *Anal Bioanal Chem.* 2004; 380:746–741.
21. Huang X, O'Mahony AM, Compton RG. *Small.* 2009; 5:776–788. [PubMed: 19340821]
22. Satpati AK, Bard AJ. *Anal Chem.* 2012; 84:9498–9504. [PubMed: 23030705]
23. Bruckenstein S, Janiszewska J. *J Electroanal Chem.* 2002; 538:3–12.
24. Lehninger, AL.; Nelson, DL.; Cox, MM. *Principles of Biochemistry.* 2. Worth Publishers; New York: 2003.
25. Schutten JH, Beelen TPM. *J Mol Catalysis.* 1981; 10:85–97.
26. Gulppi M, Griveau S, Bedioui S, Zagal JH. *Electrochim Acta.* 2001; 46:3397–3404.
27. Galus, Z. *Fundamentals of Electrochemical Analysis.* Ellis Horwood Press; New York: 1976.
28. Huang X, Aldous L, O'Mahony AM, del Campo FJ, Compton RG. *Anal Chem.* 2010; 82:5238–5245. [PubMed: 20469834]
29. Lee C, Tan Y, Bond AM. *Anal Chem.* 2008; 80:3873–3881. [PubMed: 18412371]
30. Lin Y, Trouillon R, Svensson MI, Keighron JD, Cans A, Ewing AG. *Anal Chem.* 2012; 84:2949–2954. [PubMed: 22339586]
31. Lin Z, Takahashi Y, Kitagawa Y, Umemura T, Shiku H, Matsue T. *Anal Chem.* 2008; 80:6830–6833. [PubMed: 18665613]
32. Kalantari R, Cantor R, Chen H, Yu G, Janata J, Josowicz M. *Anal Chem.* 2010; 82:9028–9033.
33. Davies TJ, Compton RG. *J Electroanal Chem.* 2005; 585:63–82.

Figure 1.
Picture of the paper-based analytical device with electrochemical detection.

Figure 2.

(a) Optical profilometry image of the working microelectrode filled with carbon paste and
(b) linear scan voltammogram at 10 mV s^{-1} using ePAD2 containing 8% of CoPC.

Figure 3.

(a) Cyclic voltammograms obtained using the modified (CPE+CoPC) and unmodified (CPE) ePAD2 in the presence and absence of the analyte. Background electrolyte: 0.5 M Ac. buffer pH 4.0. (b) Graph of anodic peak current (I_{pa}) versus solution pH for the modified ePAD2 (8% of CoPC). (c) Plot of I_{pa} versus percentage of CoPC. The cyclic voltammograms for the graphs (a) – (c) were obtained using ePAD2 at 5 mV s^{-1} and $500 \mu\text{M}$ of cysteine. (d) Graph of I_{pa} versus $v^{1/2}$ for ePAD2 (8% of CoPC) and $500 \mu\text{M}$ of cysteine in 0.5 M Ac. buffer pH 4.0.

Figure 4.

(a) Effect of the applied potential on the response of the device in the presence of 500 μM of cysteine. (b) Chronoamperograms obtained at 0.6 V after successive additions of cysteine. (c) Graph of I_{cat}/I_L versus $t^{1/2}$. (d) Plot of the slopes versus square root of cysteine concentration. All the plots were obtained using ePAD2 containing 8% of CoPC in 0.5 M Ac. buffer pH 4.0.

Figure 5.

(a) Plot of the electrodes response *versus* centre-to-centre distance between the microelectrodes of the array. (b) Linear scan voltammograms for the comparison between the array of microelectrodes and ePAD2. The response of plots (a) and (b) were evaluated by measuring peak or limiting current of the linear scan voltammograms in the presense of 5 mM $Fe(CN)_6^{4-}$ in 0.5 M KCl solution at 10 mV s^{-1} . (c) Analytical curve of the array of microelectrodes obtained in 0.5 M Ac. buffer pH 4.0 applying 0.6 V on the WE. Figures (a) – (c) were obtained using carbon paste modified with 8% of CoPC

Table 1

Theoretical and experimental values for the characterization of the microelectrodes.

Drawn ¹ d/ μ m	Observed ² d/ μ m	I _{ell,lim} /nA (theoretical)	I _{ell,lim} /nA (experimental)	Relative error/%
25	a = 189.7 $\pm$ 24.6 b = 136.7 $\pm$ 13.1	177.9 $\pm$ 17.7	145.4 $\pm$ 25.2	18.2
50	a = 245.7 $\pm$ 1.1 b = 189.3 $\pm$ 4.2	238.7 $\pm$ 2.6	228.7 $\pm$ 7.3	4.2
75	a = 246.7 $\pm$ 19.7 b = 208.0 $\pm$ 7.0	253.1 $\pm$ 8.4	264.1 $\pm$ 1.5	4.3
100	a = 280.0 $\pm$ 22.6 b = 225.3 $\pm$ 2.1	279.2 $\pm$ 8.1	276.1 $\pm$ 4.9	1.1

¹Circular shape.²Elliptical shape (a and b are the major and minor semiaxes of the ellipse). n = 3 for experimental and theoretical experiments.

Table 2

Analytical characterization of the devices (1 and 4 microelectrodes).

Number of microelectrodes	Sensitivity/nA μM^{-1}	Linear range/ μM	R^2	L.D./ μM
1	0.061	50 – 600	0.998	52.0
4 (array)	0.21	10 – 500	0.998	4.8