

# Analytical Methods

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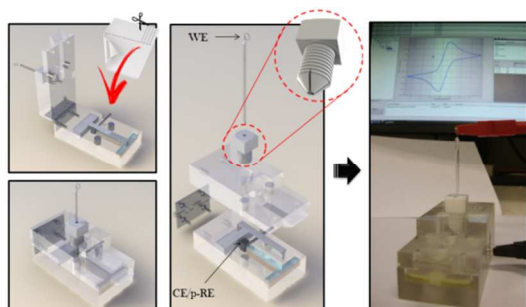
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## Technical Note



Graphical Abstract

Electrochemical device for using in point of care testing based on paper microfluidic platform.

## Technical Note

## Construction of a new versatile point-of-care testing device with electrochemical detection employing paper as microfluidic platform

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E. T. S. G. Silva,<sup>a</sup> M. Santhiago,<sup>a</sup> J. T. C. Barragan<sup>a</sup> and L. T. Kubota<sup>a</sup>

**A portable, low-cost, versatile and non-disposable electrochemical device has been proposed based on a simple design allowing easy change of the working electrode and piece of paper, for point-of-care testing.**

The use of paper as microfluidic platform for point-of-care testing (POCT) was firstly demonstrated by Whitesides and co-workers.<sup>1</sup> This new class of a POCT device, also known as microfluidic paper-based analytical device ( $\mu$ PAD), is becoming increasingly attractive in the last few years due to its portability, low-cost and easy to use system, that has low sample volume requirements and allows even unskilled people performing measurements. These characteristics are very important mainly in developing countries and remote regions.<sup>2</sup> Among the different detection systems usually coupled with paper microfluidic platforms, the electrochemical is one of the most promising due to its easy miniaturization, speed, simplicity, high sensitivity and quantitative response, making  $\mu$ PAD a powerful tool for the most different applications from environmental monitoring to healthcare diagnostics.<sup>3</sup>

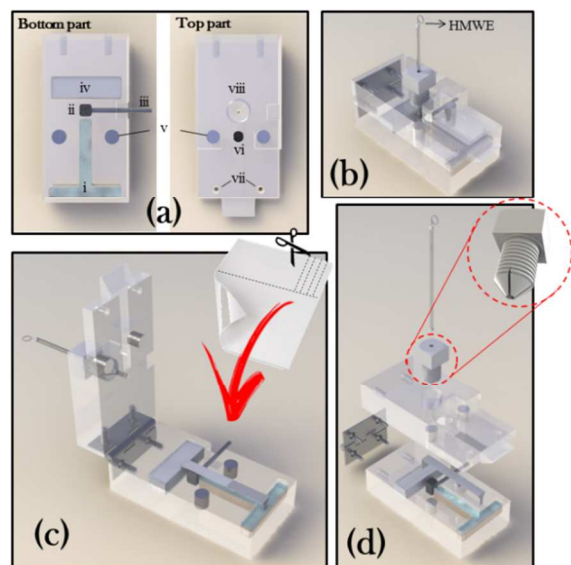
Generally these  $\mu$ PAD require some modifications to obtain the microfluidic channels and construction the electrochemical system. These modifications include the formation of hydrophobic walls (generally using wax or organic inks) and printing electrodes on the paper surface (generally metals and/or carbon). Nowadays, different techniques have been used to modify the paper and obtain a complete  $\mu$ PAD, as lithography,<sup>4a</sup> wax printing, screen printing,<sup>4b</sup> inkjet printing<sup>4c</sup> and others<sup>4def</sup>. However, considering the disposability of these devices, the discard of the paper with these modifications can increase de cost in large scale production and the problems with recycling to be environmentally friendly. In this context, some authors have been used the simple cut technique to obtain the well-defined hydrophilic channel with different sizes and designs,<sup>5</sup> others works published in the literature the authors have been used external electrodes to perform the electrochemical

measurements, avoiding to print metals and/or other materials as electrode increasing the cost of the device.<sup>6</sup> Herein, a novel POCT device that combines the simple cut technique using external simple home-made electrodes was developed. This device allows the use of paper without modifying, allowing its easy exchange, thus being a simple and versatile device.

A schematic representation of the proposed device is shown in Scheme 1. As can be observed the structure of the device was based on two acrylic pieces (7.0 cm x 3.6 cm x 1.5 cm), bottom and top part, which were used as support for the home-made electrodes, the eluent reservoir, the absorbent pad and the paper microfluidic platform (details and photos in ESI†). Acrylic was chosen due to its transparency, weight and low reactivity, allowing visualising the experiment inside the closed device, facilitating its use in POCT, without causing chemical interference in the analysis. As can be observed in the Scheme 1a, the eluent reservoir, with seating for 1.2 mL, is located on the base of the device (Scheme 1a<sub>i</sub>). It was designed in inverted "T" form, with the aim to allow the eluent replacement or change keeping its atmosphere inside the device, avoiding errors related to eluent evaporation and/or environment change (temperature), which is common in paper chromatography. A two-electrodes system was used to simplify the device, where the counter/pseudo-Reference home-made electrode (CE/pRE) was prepared from a graphite/acrylic composite containing 60% of graphite in weight, and placed inside the cavity also located on the bottom of the device (Scheme 1a<sub>ii</sub>). In the bottom of this cavity, a piece of metal makes the electrical contact between the carbon electrode and the connector outside of the device (Scheme 1a<sub>iii</sub>). The absorbent pad cavity (Scheme 1a<sub>iv</sub>) was designed to place 12 sheets of absorbent paper (CFSP001700, Millipore) to be sufficient to run more than two hours of measurement without needing to change, rendering the use for a long period of time at the same experiment.

To connect both bottom and top parts, a hinge was used allowing opening and closing the device with no longitudinal and transversal

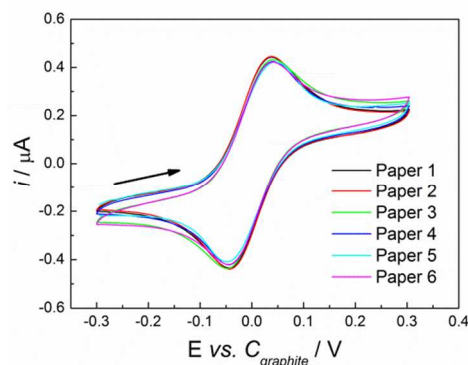
changes on the position with the aim to always keep the device closed with the same force during the measurement. Two neodymium magnetics with 6.0 mm of diameter and 6.0 mm of height were used (Scheme 1*a*<sub>v</sub>), minimizing the errors and simplifying the use of the device.



**Scheme 1.** Schematic representation of the (a) bottom and top parts, where: *i*) is the eluente reservoir, *ii*) is the CE/pRE, *iii*) is a metallic piece responsible for the electrical contact of the CE/pRE outside the device, *iv*) is a place for the sorbent pad, *v*) is the neodymium magnetic used to close the device, *vi*) is the entrance of the analyte, *vii*) is the holes used to replace the eluent, *viii*) is the entrance for the threaded Teflon piece and WE. (b) View of the device closed, with the home-made WE on top; (c) paper placement process; (d) exploded view (detail: Teflon threaded piece).

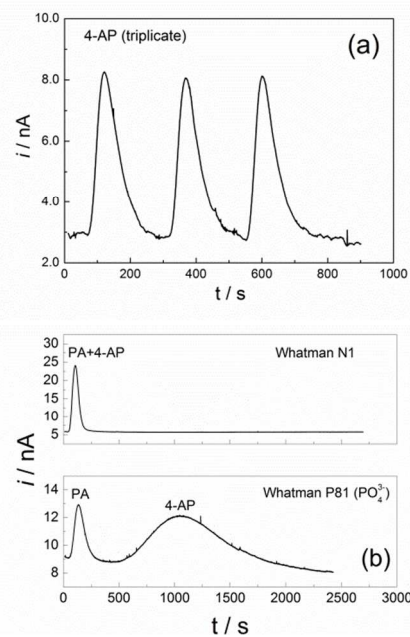
In the top part, also showed in Scheme 1*a*, it is possible to observe three small openings: one with 5.0 mm of diameter located in the middle at 1.0 cm from the electrodes place (Scheme 1*a*<sub>vi</sub>), where the sample is injected; and other two with 3.0 mm of diameter located in opposite to the eluent reservoir at the bottom part (Scheme 1*a*<sub>vii</sub>), where the eluent is added and replaced; in addition, these two openings can probably be used to add gas (nitrogen or argon, for example) and perform experiments in absence of oxygen; however this kind of testing was not performed in this work. A threaded hole with 9.0 mm of diameter, and having a funnelling with a 2.0 mm hole at the end, was also made in the area of the top part immediately opposite to the CE/pRE (Scheme 1*a*<sub>viii</sub>), with the aim to place the HMWE and make a simple two electrodes system with a “sandwich” contact, with the microfluidic paper between the electrodes. To keep the HMWE motionless during the measurement and avoid errors caused by different positioning of the electrode, a special Teflon piece was built (detailed in Figure 1*d* and ESI†), which after threading, blocks the movement of the electrode in the desired position and maintain gently contact with the paper surface (red circle in the Scheme 1*b*). To confirm the motionless of the HMWE and the success of this procedure, a cyclic voltammetry (CV) in a background solution of 5.0 mmol L<sup>-1</sup> [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup> in 0.1 mol L<sup>-1</sup> KCl was carried out with a consecutive change of paper strip (Whatman #1, 5.0 x 50.0 mm), opening and closing the device in each change, and using a graphite pencil as example for the HMWE (details for the construction of the cheap graphite pencil and a platinum HMWE are presented in ESI†). The result obtained is presented in Figure 1, where the cyclic voltammograms showed very good repeatability even after six changes of paper, with coefficient of variation related to anodic peak current in approximately 2.5%. This result shows the importance of the special Teflon piece and

suggests that the novel device proposed is robust and stable, and there is a great perspective that this device can be used by non-professionals without affecting the final results.



**Figure 1.** CV curves obtained in 5.0 mmol L<sup>-1</sup> [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup> + 0.1 mol L<sup>-1</sup> KCl ( $v = 50$  mV s<sup>-1</sup>) with six replacement of paper microfluidic platform.

In order to evaluate the performance of the device, it was carried out the electrochemical detection of 4-aminophenol (4-AP) and paracetamol (PA) by chronoamperometry (CA) using graphite pencil as working electrode. This material was chosen due to its great properties as high conductivity, low cost and large electrochemical window. For the amperometric measurement, a solution of 0.1 mol L<sup>-1</sup> acetate buffer (pH 4.5) was used as eluent and a constant lateral flow, with  $0.155 (\pm 0.003)$  mm/s, was observed by capillarity. The applied potential for this detection was 0.30 V (vs C<sub>graphite</sub>) and the results are shown in Figure 2*a*.



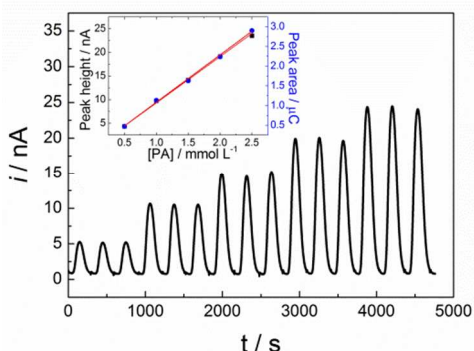
**Figure 2.** CA curves obtained in acetate buffer (pH 4.5) as eluent for the detection of 0.5 mmol L<sup>-1</sup> 4-AP (a) and (b) for the separation and detection of 4-AP and PA, using two different chromatographic papers. Applied potential was 0.65 V vs C<sub>graphite</sub>.

As can be observed in Figure 2*a*, three peaks were obtained from the consecutive additions of 0.5 mmol L<sup>-1</sup> 4-AP (2.0 μL). The peak currents obtained showed a great repeatability regarding the analyte injection, with coefficient of variation of 1.2% and 1.5% considering the peak height and the peak area, respectively. In addition, a very



low baseline (approximately 3.0 nA) and low intensity of noise ( $\pm 0.1$  nA) were observed in this experiment.

Recently in our group, it was demonstrated the separation, detection and quantification of PA and 4-AP using a paper-based device constructed with an ion exchanger paper (Whatman P81).<sup>7</sup> This chromatographic paper has phosphate groups ( $\text{PO}_4^{3-}$ ) on the surface, responsible to interact and retard cationic species present in the mobile phase. Since at pH 4.5, 4-AP is cationic and PA is still neutral, both analytes can be separated by using this kind of paper. To highlight the simplicity and versatility of the proposed device, the same experiment was carried out by chronoamperometry (applying 0.65 V vs  $\text{C}_{\text{graphite}}$ ) using the Whatman #1 and P81 papers as microfluidic platforms. The results obtained for these experiments are presented in Figure 2b, where it is possible to observe that only using the cationic exchanger paper (Whatman P81) the species could be separated and detected, as expected. The result obtained is similar to the previous one reported by our group,<sup>7</sup> however both curves were obtained using the same device and changing only the type of paper, with no need to construct other device for that. This easy and simple change of the microfluidic platform using the proposed device can render it a powerful tool for several applications in the field of POCT, including the use of many types of paper with different porosity, wettability, properties for separation of analytes, and more. Besides the easy exchange of the microfluidic platform, the device was constructed to facilitate the exchange of WE. To show this possibility a platinum wire, inside a glass capillary, was used as HMWE to detect PA by chronoamperometry (details in ESI†). An analytical curve can be plotted using this system and the results are presented in Figure 3.



**Figure 3.** CA curve obtained after successive injections of standard solutions containing PA (from 0.5 to 2.5  $\text{mmol L}^{-1}$ ) in a constant flow of 0.1  $\text{mol L}^{-1}$  phosphate buffer (pH 7.4) at an applied potential of 0.65 V vs  $\text{C}_{\text{graphite}}$ . Inset: analytical curve.

Figure 3 shows the chronoamperogram obtained with successive additions of PA with five different concentrations in triplicate. Figure 3 inset present the analytical curve obtained from this measurement, where in the “Y” axis were plotted both peak height and peak area; which showed a great linearity considering the range (0.5 to 2.5  $\text{mmol L}^{-1}$ ), with the linear regression equation expressed as  $\text{Area}/\mu\text{C} = -0.12 (\pm 0.03) + 1.20 (\pm 0.03) [\text{PA}]/\text{mmol L}^{-1}$ , and correlation coefficient  $r = 0.999$ . The LOD calculated considering the signal/noise ratio ( $s/n = 3$ ) was 10  $\mu\text{mol L}^{-1}$ , which is more than two times better than the obtained previously using paper-based device and amperometric detection.<sup>7</sup> This low LOD obtained is probably related to the use of a WE with reduced area, which increase the  $s/n$  ratio and improve the detection.

## Conclusions

As a conclusion of this work, we have demonstrated the construction of a novel and versatile analytical device with electrochemical

detection that allows the use of different papers as microfluidic platform without needing to change the detection system. The use of two different WE for two analytes were also demonstrated to highlight the versatility of the proposed novel device. The main advantages of this approach include: (i) possibility to use different WE and paper as microfluidic platform at the same device. These aspects increase the possibilities for its use in environmental monitoring and healthcare diagnostics; (ii) a closed structure with a coupled eluent reservoir that maintain the system saturated with the eluent vapour inside the device avoiding errors related to environmental changes and external contamination for in field analysis; (iii) good reproducibility and (iv) the microfluidic channel delimited only cutting paper. Regarding the working electrodes used in this work, the pencil graphite can be more interesting for this kind of device due to its low cost and good electrical properties. However, the possibility for the use of WEs constructed with other materials, as gold for example, and with smaller sizes, can be very attractive for sensing applications. Finally, we believe that this novel approach can open the possibilities for the construction of new versatile POC devices that are robust and may work in different areas of the analytical field (environmental and healthcare diagnostics).

## Notes and references

Department of Analytical Chemistry, Institute of Chemistry - UNICAMP, P.O. Box 6154, 13084-974, Campinas-SP, Brazil.

Email: (L. T. Kubota) [kubota@iqm.unicamp.br](mailto:kubota@iqm.unicamp.br); Tel: +55 19 3521-3127

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