NEW ELECTROCHEMICAL CELL IN MICROFLUIDICS ALLOWS NAKED-EYE DETECTION WITH RENEWABLE ELECTRODES AND SIMULTANEOUS MULTIPLE ANALYSES FROM A SINGLE WORKING ELECTRODE

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Abstract: *This work describes a microfluidic platform with electrochemical detection that transforms the signals of current in a visible readout, representing promising alternative to the deployment of rapid testing and in-situ analytical technologies. The amount of species reduced at the working electrode was indirectly estimated by detecting the extent of oxidation of the Ag-coated auxiliary electrodes with naked-eye. We herein outline an inedited electrolytic cell in microfluidics that requires a simple, low-cost, solventless, bondless, and cleanroom-free prototyping and assures the reuse of both the microdevice and electrodes. It was possible to perform a concept proof for the proposed electrochemical cell for both single and simultaneous analysis besides the possibility of attain different sensitivities by simply modifying the flow rate and to make simultaneous multiple analyses from a single working electrode.*

Key-Words*: electrochemical; microfluidic; naked-eye; renewable; simultaneous*

Introduction: Point-of-use assays have currently played a crucial role in modern analytical chemistry by considering the challenges of continuous control in many industrial processes. The relevance of this quality monitoring is associated with the increase in population awareness, incidence of lifestyle-related diseases, demand for processed products, and requirements from regulatory agencies for more precise chemical tests.[1] The Point-of-use methods involve low-cost, simple, and fast analyses by the bypass the need of sophisticated instruments or skilled operators as well these approaches are portable presenting great potential for in-situ tests, including also clinical diagnoses at patient's home and assays at remote regions and developing countries.[2] Oh and Chow[3] reported other promising naked-eye technique, which is based on faradaic electrochemical reactions in microfluidics. Succinctly, the concentration of analyte reduced at the working electrode (WE) under faradaic reaction condition is indirectly obtained from the monitoring of the length of silver (Ag) auxiliary electrodes (AE) that is oxidized. When the AE operates as anodic pole, the Ag layer into contact with the solutions in channel starts to dissolve. The contrast between non-oxidized and oxidize Ag allows the naked-eye detection of the length of oxidized AE that represents the analytical response of technique. Positively, this method presents low consumption of chemicals and high analytical frequency, simplicity, precision, robustness, sensitivity, miniaturization compatibility, and selectivity. Hence, the ability to convert the electrochemical sensing phenomenon in visible readout ensures the creation of high-performance point-of-use methods, combining great analytical performance with low-cost and simple tests. Otherwise, such electrochemical technique shows limitations that undermine the construction of rapid testing analyses. The device was disposable once it irreversibly attachés the AEs that undergo oxidation during the measurements. This is the only aspect that represents a disadvantage once the device fabrication was laborious, time-consuming, and high-cost, requiring conventional steps of photolithography and bonding as well as successive processes of film deposition and lift-off. Such downsides are relevant because it is imperative the naked-eye detection-based platforms. Considering the AE dissolution in faradaic electrolytic cells, can be a promising sensing event concept toward the construction of point-of-use test, we herein address a naked-eye faradaic electrochemical platform based on low-cost and reusable devices and electrodes. Reusable microchips composed of a single piece of polydimethylsiloxane (PDMS) had different channels to put external electrodes and to pump the samples. Its fabrication relied on simple, low-cost, solventless, and bondless sequential steps of PDMS polymerization and scaffold removal (PSR)[4].

Experimental: PDMS was prepared mixing monomers with curing agent at 10:1 w/w ratio. Then, the bubbles were removed at vacuum for 30 min. The resist (AZ® 4620) added on the three stainless steel microwires was coated at 2.000 rpm for 30 s and dried at room temperature for 30 min. The PDMS, in turn, was cured at 60 ºC for 1 h. After withdrawing the scaffold, the microchip was hard baked at 120 °C for 20 min. The gap between the three sample channels was 8.3 mm, whereas this distance between the electrodes was 1.3 mm. The chip showed the dimension 34.0 mm x 56.0 mm x 10.0 mm. The stainless steel and silica scaffolds presented diameters of approximately 700.0 and 670.0 µm, respectively. In accordance with the stereoscopy and microscopy images shown in Fig. 1(e, f), the channels had well-defined edges and circular cross section. The three sample channels had global diameter of 717.3 ± 3.7 µm (n = 15; all the confidence intervals were calculated for $\alpha = 0.05$). This result indicates the coating of the positive resist on the stainless steel microwires (scaffold) was uniform. Stainless steel microwires (720 µm in diameter and 10.0 cm in length) coated with thin films were used as electrodes. These films were deposited by electron beam vapor deposition. The electrical short circuit between the Ag-coated AEs was achieved using a crocodile-clip connector. The reuse of the AEs after their oxidation at each assay was assured by renewing the surface of such electrodes. This renewal relied on manually pulling the microwires in the presence of sample into the channel.

Results and discussion: Initially, p-BQ standards ranging from 0.5 to 9.5 mmol L^{-1} were ascertained at a flow rate of 75.0 µL min^{-1}. The i_{lim} values were recorded after a tome of 180 s. Analyses in triplicate were realized with the same electrodes by the renewing the AE surfaces. The limiting currents linearly varied with the concentration of p-BQ (R^2 larger than 0.99). The confidence intervals changed of 0.2 to 0.8 µA only, showing the electrode renewal was reproducible. In addition, the analytical sensitivity (S) and the limit of detection (LOD) were of 1.6 µA $mmol^{-1}$ and 114.7 µmol L^{-1}, respectively. Such data show our naked-eye electrochemical detection can be successfully applied in semi-quantitative tests. Herein, the ranges of concentration os species reduced at WE (under faradaic reaction conditions) are defined from the values of n_{ox}. As an advantage, the use of the AE electrodissolution as sensing event provides a straightforward way to improve the detectability and sensitivity levels, which are based on increase of the sample flow rates. In this case, the matter transport is enhanced by convection, thus increasing the oxidation rate at AEs. Such results were obtained at 500 µL min^{-1}. The dynamic range was shifted to lowest contents of p-BQ, 0,5 to 2.5 mmol L^{-1}. S and LOD were improved to 4.1 µA $mmol^{-1}$ L and 44.8 µmol L^{-1}, respectively, R^2 was greater than 0.99. The confidence intervals in analytical curve ranged from 0.1 to 0.4 µA only. Hence, we can state the electrode fabrication was reproducible. The n_{ox} once again linearly changed with I_{lim} (data now shown) and then with the content of p-BQ (R^2 larger than 0.99). In this case as shown in Fig. 2(h), S was four times greater than the value at 75.0 µL min^{-1}. Chronoamperometry assays to standards of p-BQ in different concentrations were performed using the chip with three sample channels. Analytical curves recorded at 500 µL min^{-1} showed the inter-channel precision was poor. In fact, the data obtained in the three channels exhibited different sensitivity and detectability levels. All of the confidence intervals were lower than 0.2 µA, confirming once again the good precision of the process of electrode renewal. In the three cases, the Ag oxidation extents linearly changed with the amounts of p-BQ reduced at WEApplying the Randles-Sevcik equation, the electroactive areas of Wes in C_1 (1.5) and C_2 (1.6) were greater than the value attained in C_3 (1.2 10^{-6} cm^2). Finally, we ascertained an unprecedented ability provided by our naked-eye microfluidic electrolytic cell, namely, the possibility to make simultaneous multiple quantitative analyses from a single WE. Media containing 0.5, 6.5 and 4.5 mmol L^{-1} p-BQ were pumped at 500 µL min^{-1} into C_1, C_2 and C_3, respectively. Once the charge on WE is punctually balanced by the AEs, it is expected the Ag electrolysis extents on three channels be independent of each other. The values of n_{ox} were in agreement with the previous signals that were obtained in individual measurements to such contents at 500.0 µL min^{-1}, more specifically, 0.5 (C_1), 6.5 (C_2), and 4.5 mmol L^{-1} p-BQ (C_3) resulted in n_{ox} values equal to 1, 5 and 2, respectively. The i_{lim} acquired from such simultaneous multiple assays was approximately the sum of the individual currents recorded at each channel.

Conclusion: Our naked-eye electrolytic cell ensures the construction of cost-effective and simple measurements, addressing key challenges in the field of point-of-use methods. Its signal readout is effectively accessible for non-skilled users. In application steps, the comprehension of the quantitative data would be simply based on a table correlating the analyte concentration ranges with the number of oxidized Ag-coated microwires.

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