Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

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

Everson T. S. G. da Silva, Murilo Santhiago, Jose T. C. Barragan and Lauro T. Kubota*

Materials, equipment and chemicals.

All chemicals were of analytical grade. The Whatman chromatographic papers n1 and P81 were acquired from Merck, São Paulo, Brazil. Paracetamol was acquired from Synth, São Paulo, Brazil and acetic acid from TEDIA, Fairfield, USA. Sodium acetate, sodium phosphate, 4-Aminophenol, potassium ferricyanide and ferrocyanide were acquired from Sigma-Aldrich, São Paulo, Brazil. The potassium chloride was obtained from J. T. Baker, NJ, USA.

All the solutions were prepared by using purified water in a MilliQ Millipore system from Billerica, MA, USA. The pH values of the acetate and phosphate buffer solutions were determined with a Corning pH/Ion Analyser model 350 from Corning, NY, USA, and the concentration of both solutions were maintained at 0.1 mol L^{-1}, with pH 4.5 and 7.4, respectively.

For the experiments, the chromatographic paper was cut to obtain 5 mm width and 50 mm length, and 1 mL of eluent was added in a device cavity, as showed in Figure S1. Moreover, some chromatographic paper sheets with 1 mm width were used as absorbent pad.

Electrochemical experiments were performed with a potentiostat PGSTAT-30 Model from Autolab Eco Chemie (Utrecht, The Netherlands) connected to a PC (Software NOVA 1.9). For the electrochemical experiments, a two-electrode system was used with graphite/acrylic as a reference and counter-electrode, and platinum wire or graphite pencil as the working electrode.

Construction of the device.

The layout of device was firstly designed using SolidWorks^{TM} Premium 2010, and then was made, with acrylic material, by turner cutter in a Precision Mechanics Laboratory at State University of Campinas, São Paulo, Brazil (Figure S1).

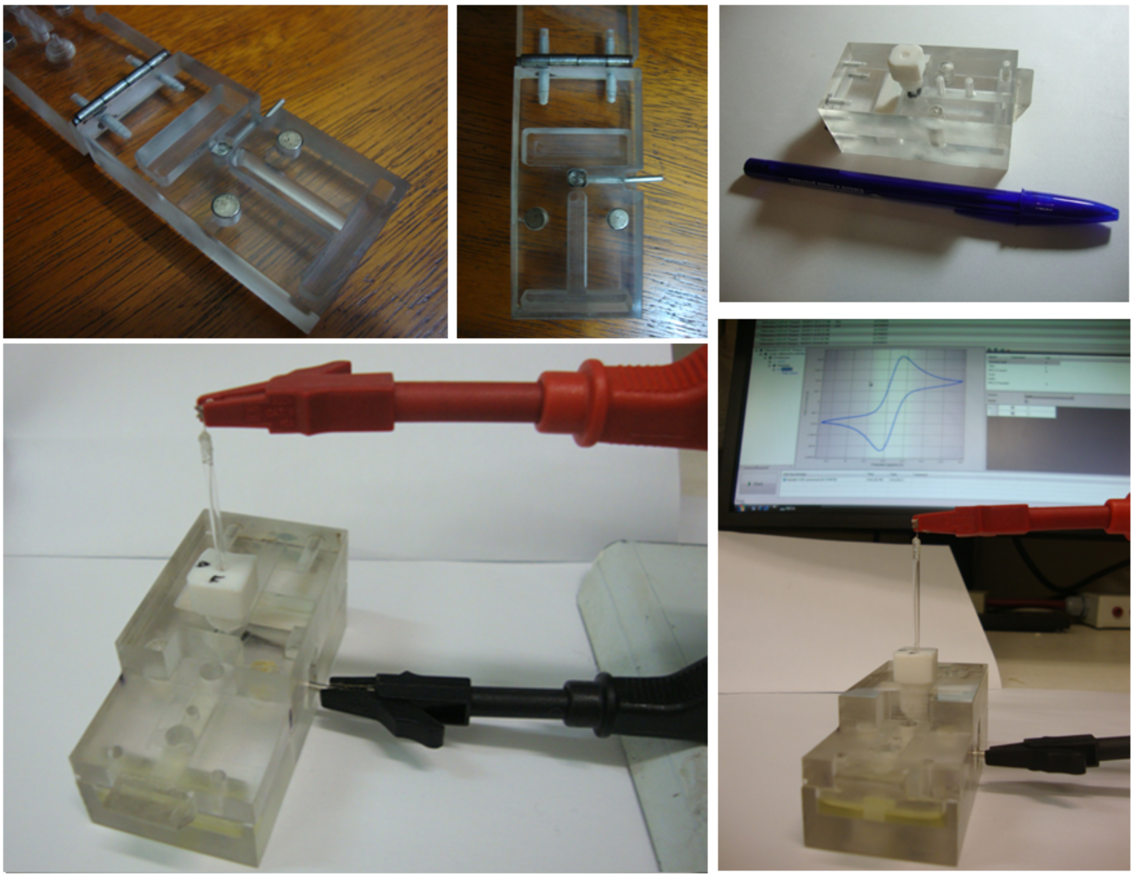

Figure S1. Photos of the device just after its construction and during measurement.

Construction of the electrodes

Working electrode (WE)

For the construction of the homemade working electrodes (WE), platinum wire (with diameter of 0.25 mm) by sigma Aldrich and commercially available type HB cylindrical graphite pencils (with diameter of 0.30 mm) were employed. The graphite pencils, initially 60 mm long, were cut in half and placed within a glass capillary tube (2 mm of external diameter). Then, a fast-drying epoxy resin (~2 min) was used to seal and attach the electrodes to the glass capillary. An alligator type connector was used to make electrical contact.

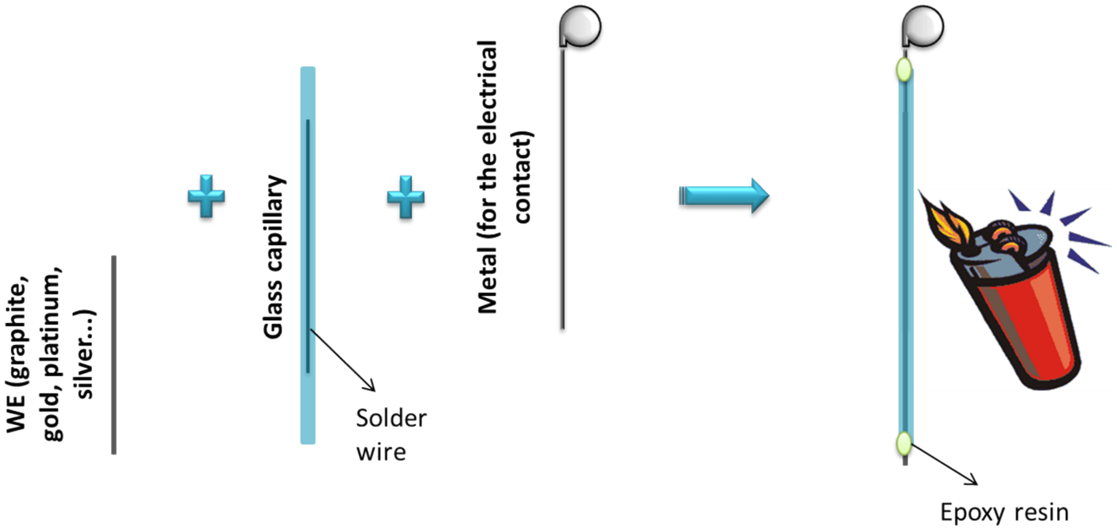

Figure S2. Scheme for the WE construction

Before the electrochemical experiments, the platinum and graphite pencil electrodes (geometric area of 0.05 and 0.07 mm^2, respectively) were carefully polished with 0.3 μm alumina slurry. Then, the surface of the electrodes was washed with water to remove weakly adsorbed alumina particles.

Counter/pseudo-Reference electrode (CE/pRE)

The CE/pRE was made from graphite/acrylic composite, with 60% of graphite weight in relation to acrylic. The solids were mixed and a small amount of acetyl acetate was added to promote the sintering of materials. Then, the composite was put in a cavity located in the bottom part of the device, with the aid of a syringe with the tip cut off (Figure S3), upon a metallic peace responsible for the electrical contact (see Figure S1), and left to dry at room temperature (12 hours). The resultant carbon-based electrode was polished before using and presented a low electrical resistance, about 14(± 3)Ω.

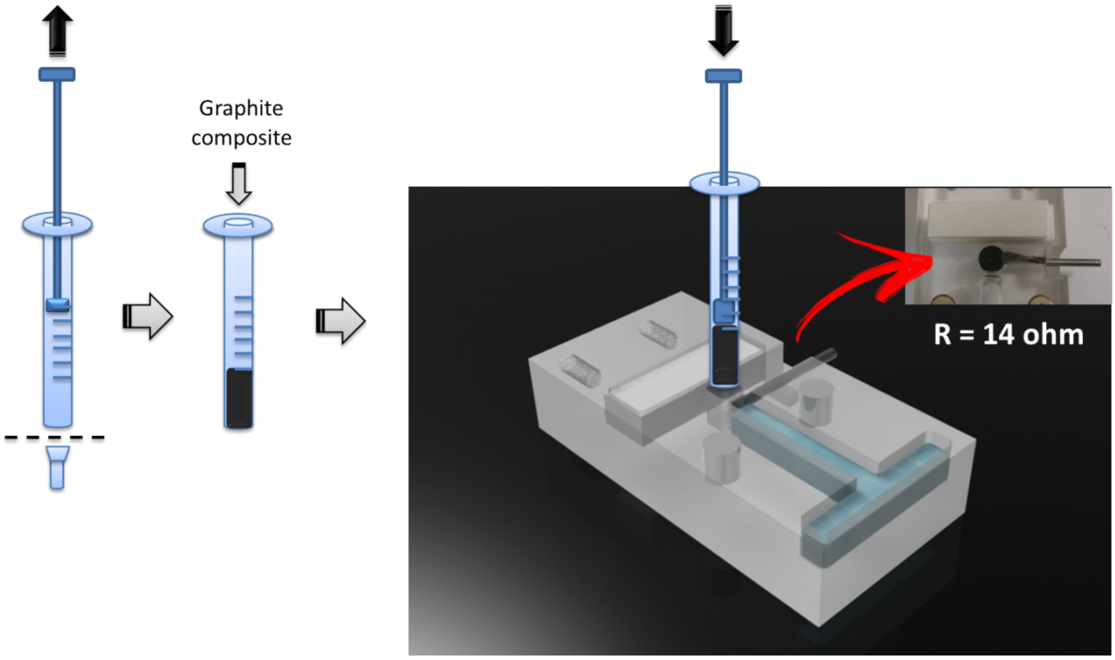

Figure S3. Scheme for the CE/pRE construction

Electrochemical characterization of the device

Before the application of device for the detection of paracetamol (PA) and 4-aminophenol (4-AP), it was evaluated the functioning of the homemade electrodes on the device. For this experiment, cyclic voltammetry (CV) was carried out in a background solution of 5.0 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ in 0.1 mol L^{-1} KCl, without paper, as showed in Figure S4*a*. As can be observed in Figure S4*a inset*, the WE was placed near the CE/pRE without touching the surface. The same experiment was carried out with a conventional electrochemical system (three electrodes) for comparison. For the conventional electrochemical system, the same WE was used (platinum wire into glass capillary), however the reference electrode used was Ag/AgCl and auxiliary was a platinum wire. The obtained results are presented in Figure S4*b*.

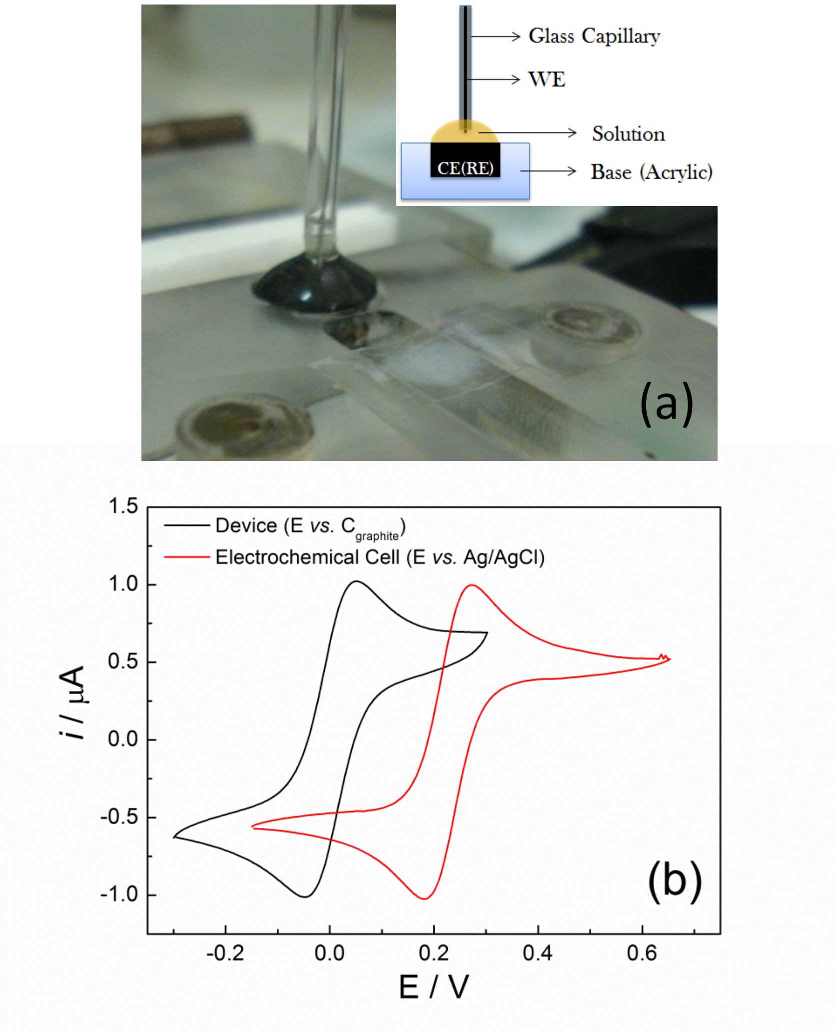

Figure S4. (a) Illustrative photo of the experimental setup and (b) the CV obtained with the device proposed and with a conventional 3 electrodes cell in a background solution of 5.0 mmol L^{-1} [Fe(CN)$_6$]$^{3-/4-}$ in KCl 0.1 mol L^{-1}.

As can be observed in Figure S4*b* the cyclic voltammogram obtained from the proposed device showed a good response with no differences for the anodic and cathodic peak currents and separation of peaks when compared with the conventional three electrodes electrochemical system (ΔE = 85 mV). However, a cathodic shift was observed due to the distinction of the reference electrodes used in each case. This result demonstrates a great performance of the homemade electrodes on the device as compared with conventional electrochemical system.

Detection of 4-aminophenol (4-AP) and paracetamol (PA)

Using graphite pencil as WE

The graphite pencil working electrode was used, as mentioned previously, to demonstrate the versatility of the device proposed and to decrease its cost, as well as take advantages of the good properties of the carbon-based material, as great conductivity, low cost and high potential window. The 4-aminophenol (4-AP) was used here as example to verify the performances of the electrode and the device. First of all, cyclic voltammetry was obtained in static conditions of flow and without paper, same as the Figure S4 experiments, with 10 mmol L^{-1} of 4-AP in a buffer solution. In this case, it was used acetate buffer to maintain the pH approximately at 4.5, in order to difficult the oxidation of this analyte, that is very fast in neutral or basic medium, just by the presence of light and/or oxygen (pK_a = 5.3). The obtained result is presented in Figure S5, where is possible to observe an anodic peak in approximately 0.2V (*vs.* $C_{graphite}$) referent to oxidation of 4-AP (red line). The same experiment was carried out with only acetate buffer and, as expected, no anodic peak was observed in the interval of potential evaluated (0.00 V to 0.45 V *vs.* $C_{graphite}$).

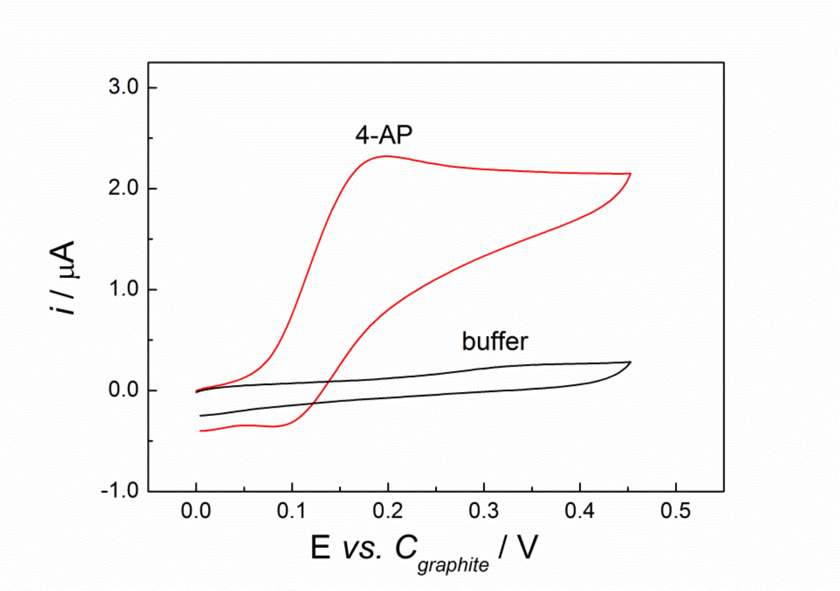

Figure S5. CV curves of a 10 mmol L^{-1} 4-AP in 0.1 mol L^{-1} acetate buffer (red line) and the blank (black line) with scan rate of 50 mV s^{-1}.

Regarding the experiment related to the separation and detection of 4-AP and PA using different kind of paper, it was prepared a solution with 5 mmol L^{-1} of PA and 4-AP in acetate buffer, pH 4.5, and a cyclic voltammetry was carried out with this solution to observe the oxidative potential for both analytes. The result is presented in Figure S6 and was also carried out without paper, similarly to the previous experiment presented in this work. Two anodic peaks in the voltammogram with the analytes solution (red line) are observed in the same potential of the previous results, 0.20V and 0.55V (*vs.* $C_{graphite}$) to 4-AP and PA, respectively, showing the reliability of this device. Thus, the potential 0.65V (*vs.* $C_{graphite}$) could be used to detect both analytes simultaneously by chronoamperometry.

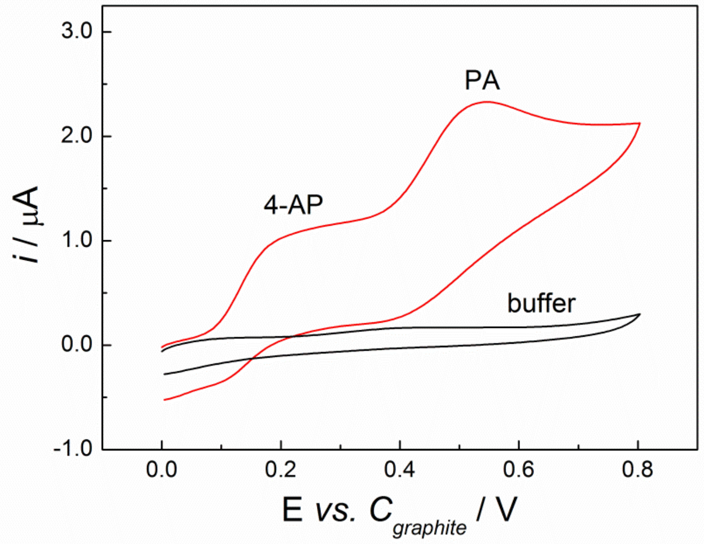

Figure S6. CV curves of a 5 mmol L^{-1} 4-AP and PA in 0.1 mol L^{-1} acetate buffer (red line) and the blank (black line) with scan rate of 50 mV s^{-1}.

Using Pt wire as WE

Before the chronoamperometric experiments for PA detection, cyclic voltammograms were obtained in static conditions of flow and without paper, same as before. Figure S7*a* shows the cyclic voltammograms obtained for the 10 mmol L^{-1} PA solution and the blank, phosphate buffer at pH 7.4. As can be observed, the voltammogram obtained for phosphate buffer, without the analyte (black line), showed no peak in the interval of potential investigated (from 0.0 V to 0.9 V, *vs.* carbon graphite). However, in the presence of 10 mmol L^{-1} *PA* (red line) an anodic peak can be observed with maximum in 0.56 V (*vs*. carbon graphite) referent to oxidation of PA. Considering that experiment, the potential chosen for chronoamperometric measurements was 0.65 V, in order to ensure the oxidation of PA.

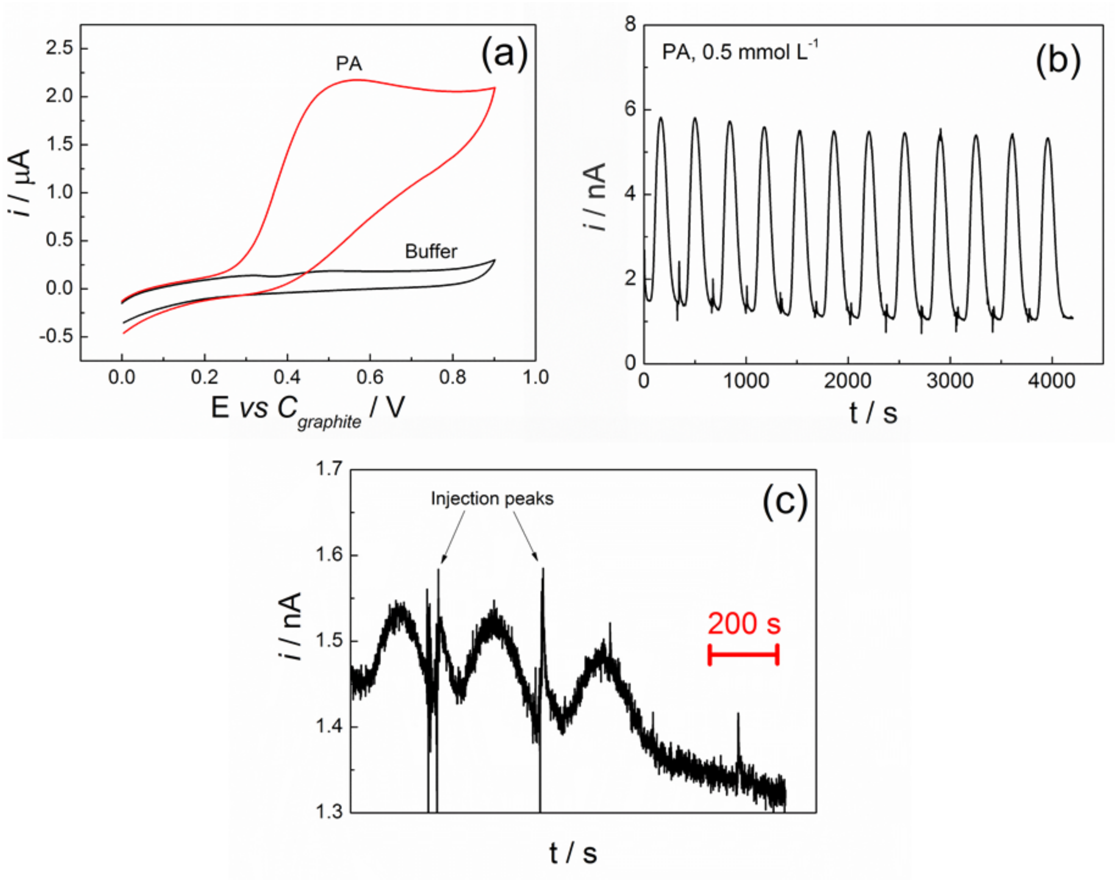

Figure S7. (a) CV curves of a 10 mmol L^{-1} PA in 0.1 mol L^{-1} phosphate buffer (red line) and the blank (black line) with scan rate of 50 mV s^{-1}; (b) chronoamperogram obtained after consecutive additions of 0.5 mmol L^{-1} PA under 0.65 V (versus $C_{graphite}$) of applied potential and (c) the LOD obtained for PA detection (10 µmol L^{-1}) at the same conditions considering the s/n ratio approximately 3.

The detection of PA by chronoamperometry was carried out with the new device, in paper platform and in continuous lateral flow. For that, the working electrode was placed and locked in contact with paper, and then the eluent was added for the wicking experiments. The applied potential was maintained constant at 0.65 V (*vs.* carbon graphite) and successive additions of *PA* solution were added at 10 mm of distance from the detection zone on the paper channel (see Scheme 1*a*). The obtained results are presented in Figure S7*b* where can be observed 12 consecutive peaks relating to successive additions of 2.0 µL of 0.5 mmol L^{-1} PA. Through this measurement it was possible to observe the great repeatability of analyte addition, with a coefficient of variation approximately 1.2% (peak height) and 1.8% (peak area). This result suggests that the device is stable even after one hour of experiment, without the necessity to exchange the absorbent pad, and with a constant flow rate of 0.155 (± 0.003) mm/s. As both the parameters (peak height and peak area) are reliable, it was chosen the one with smaller coefficient of variation as the main parameter for the construction of the analytical curve presented in Figure 3.

Figure S7*c* shows the chronoamperometric response for the injection of 10 μmol L^{-1} PA under constant lateral flow. As can be observed, the signal obtained in triplicate for PA is approximately 3 times higher than the noise, in other words, the LOD considering the s/n = 3 is 10 μmol L^{-1} for this system. The LOD obtained for PA using the new device proposed is more than 2 times better than the obtained previously using paper-based device and amperometric detection. This better LOD is probably related to the use of electrodes with reduced area that can be easily coupled with this device, highlighting its advantages.

Layout of the device and the piece that kept the working electrode motionless

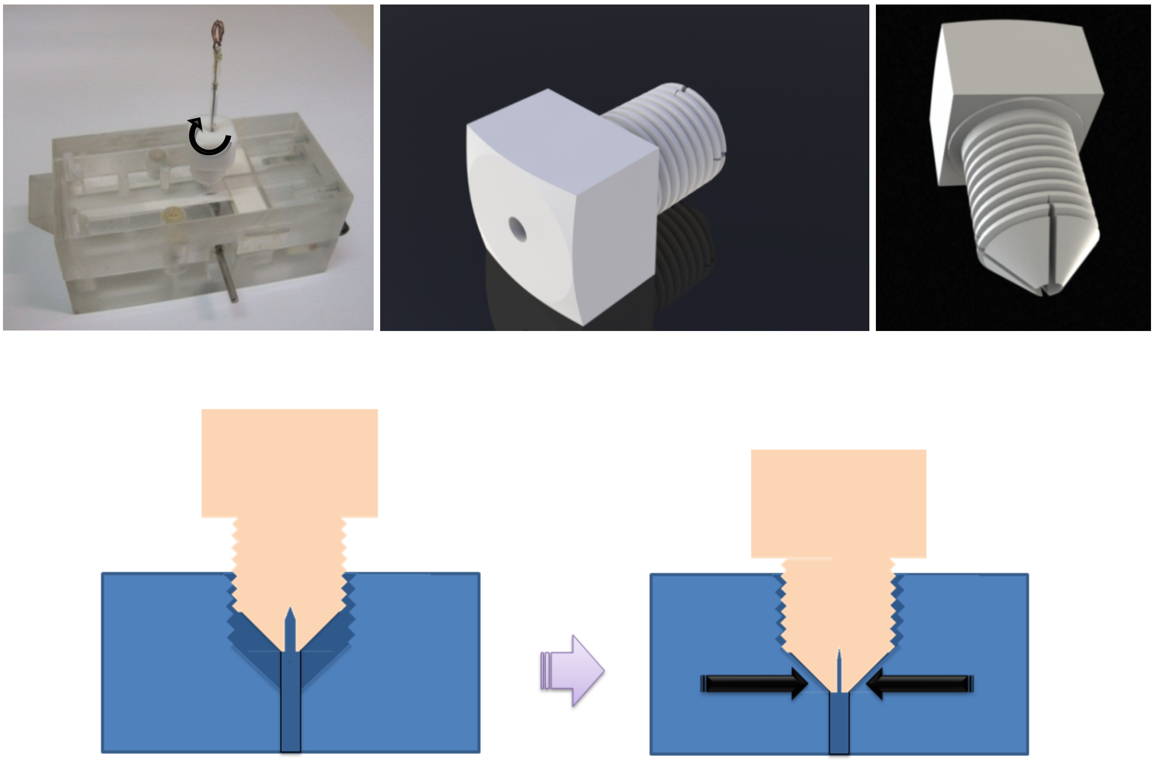

Figure S8. Photos and schematic representation of the Teflon threaded peace operation**.**