

Fabrication and modification of homemade paper-based electrode systems

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Abstract

This work reports the simple and inexpensive fabrication of homemade paper-based carbon-printed electrodes (HP C-PEs), aiming to produce an alternative way to generate electrochemical biosensors to all and promoting their wide use. This is especially important in times of pandemics, considering the excellent features of electrochemical biosensing, which may ensure portability, low-cost and quick responses.

HP C-PEs were fabricated using a standard cellulose filter paper that was first modified with wax, to make it hydrophobic. Then, the electrodes were manually printed on top of this cellulose/wax substrate. The electrodes were designed by having standard configurations for potentiometric and electrochemical readings, combining two or three electrodes. In general, both electrode systems showed excellent electrochemical and mechanical features, which were better in specific cases than commercial devices. The 3-electrode system displayed high current levels with low peak-to-peak potential separation, yielding highly stable signals after consecutive electrode bending that corresponded to high active areas. The possibility of modifying the devices with polymers produced *in-situ* was also explored and proven successful, providing also advantageous features when compared to other devices. The 2-electrode system was also proven highly stable and capable of subsequent use in potentiometric sensing development.

Overall, the fabrication process of the 2- and 3-electrode systems described herein may be employed in laboratories to produce successful electrochemical biosensors, with the final devices displaying excellent electrochemical and mechanical features. This procedure offers the advantages of being simple and inexpensive, when compared to other commercial devices, while using materials that are promptly available and that may undergo a worldwide use.

Keywords: Paper substrate; carbon-printed electrodes; 3-electrode system; 2-electrode system; electropolymerization.

1 Introduction

Easy-to-built electrochemical biosensors are gaining each time more importance in medical and clinical analysis, especially in times of pandemics where lack of availability of reagents and supplies such as commercial electrodes may be a significant problem. Because of its easiness of use, capability of miniaturization and low instrumentation cost, several commercial electrochemical biosensors are available for the detection of medically relevant molecules, such as glucose, cholesterol, creatinine, choline, lactate or ethanol, among many others. The use of printed electrodes for the fabrication of these biosensors, together with miniaturized and portable instrumentation, has also contributed to their widespread because of their reliability and low cost [1]. The printed electrodes can be drawn in different sizes and shapes, and can be made of different materials to be modified with a variety of biological recognition elements [2–4].

Photolithography is often used to fabricate the electrodes in many devices, but it is of limited use because of its high cost, long operational time and complex operations [5–7]. Other limitations of these techniques are that the electrodes fabricated can be easily damaged or even disabled when bent and/or folded, or photoresist residues can interfere in some electrochemical detections [8].

Low-cost, portable and robust electrodes that require small volumes of sample also responds to the needs of laboratories and point-of-care applications. Nowadays, electrochemical electrodes based on glassy carbon, gold or indium tin oxide on glass substrates, among others, are available. The classical configuration of conventional electrodes is the tubular configuration (as for instance in glass electrodes for pH monitoring), but the introduction of new materials favours other configurations such as the planar configuration, with many different sizes, even including the planar configuration, with all the necessary electrodes combined in a single support. Among these new materials, we can mention ceramics, glass or polyethylene terephthalate. More recently, paper-based supports have been used to prepared biosensors [4,9,10]. The use of paper in this context has gained increased attention, because it offers the advantages of including renewable, low cost and recyclable materials. Although this technology is currently promising, and it is becoming of regular use, its fabrication procedures have not been systematized in order to achieve maturation for commercialization [11]. The most common design to obtain planar electrodes using these new materials is screen-printing technology, where suitable masks are used for screen-printing the electrodes on a suitable support [12]. This may be achieved both in homemade or pilot scale approaches. Similarly to screen-printing, stencil-printing has also been used in electrode

fabrication [13]. Other used technologies to obtain planar electrodes are for instance inkjet printing [14], cutter printing [15], direct printing and pressure-assisted ball pen [16], with all of them needing the assistance of an electronic instrument to print.

Many applications also require a suitable electrode surface to include a bioreceptor layer. In this regard, polymeric materials over low cost electrodes are also gaining increased attention, with thin films achieving several technological applications such as coatings, adhesives, electronic devices or sensors, and detectors [17,18]. Great advantages are achieved when these films are made by electropolymerization on several types of surfaces. The film thickness can be subject to considerable control by varying the parameters of electropolymerization [19], and scaling-up is a possibility when driving into commercial devices. It is also important that scaling-up becomes feasible, in order to address basic commercial requirements and meet worldwide clinical needs.

As conventional electrodes need suitable cleaning approaches prior to any electrochemical reading, the use of such miniaturized and compact solutions also demand suitable cleaning strategies. These strategies are of major importance when the bioreceptor layer stands in the electrode, because the devices should be cleaned until reproducible measures are achieved, but especially in cases of electropolymerization. These cases demand clean surfaces with high and reproducible conductivity features. As far as we know, and although it has been mentioned in some papers [20], proper cleaning and conditioning of homemade paper-based electrode systems using electropolymerization for creating the bioreceptor layer has not been clearly studied.

Thus, this paper presents the details of fabrication/characterization of paper-based carbon-printed electrodes (HP C-PEs) on wax-modified standard filter paper, already with successful application, as indicated in a previous work [21]. This study includes a proper strategy for suitable cleaning, and study the electropolymerization of the working electrode and the pseudo-reference characteristics of the reference electrode. Two different device configurations are presented: two electrodes (to be used for instance in potentiometric measurements) or three electrodes (to be used for instance in voltammetric experiments). The electrochemical characterization of these electrodes shows that they have very good electrochemical properties obtaining a low peak separation and high current peak difference, similar to commercial electrodes. Low peak separation and high current peak difference are key parameters for evaluating the quality of electrochemical electrodes [22]. These homemade electrodes are also robust and flexible, since they can also be bent without losing their performance characteristics.

2 Material and methods

2.1 Reagents and chemicals

All chemicals were of analytical grade and water was de-ionized or ultrapure Milli-Q laboratory grade. Potassium hexacyanoferrate III, potassium hexacyanoferrate II trihydrate, and sodium acetate anhydrous were obtained from Riedel-deHäen; 2-(*N*-morpholino)ethanesulphonic acid monohydrate 98% (MES) from Alfa Aesar; phenol, 3- amino phenol(AP), 3- aminophenyl boronic acid (ABA), bovine serum albumin (BSA), paraffin wax were from Sigma Aldrich, and potassium chloride from Merck. Carbon and Ag/AgCl ink were from Creative Materials, USA. Standard filter paper (cellulose, size 20 cm × 20 cm) was from Fanoia, Barcelona, Spain.

2.2 Solutions

The electrodes were electrochemically cleaned and stabilized with KCl solution (0.1M KCl in Milli-Q), and MES buffer (1.0×10^{-2} mol/L, pH 5.0, KCl 0.1M), respectively. Standard solutions of phenol (3.55×10^{-3} mol/L), AP (1.0×10^{-2} mol/L) and ABA (1.0×10^{-2} mol/L) were prepared in sodium acetate buffer (1.0×10^{-2} mol/L, pH 5.0) for the electropolymerization. Electrochemical assays were performed with 5.0×10^{-3} mol/L $[\text{Fe}(\text{CN})_6]^{3-}$ and 5.0×10^{-3} mol/L $[\text{Fe}(\text{CN})_6]^{4-}$, prepared in MES buffer. The pseudo-reference characteristics of the reference electrode and the stability of the working electrode were studied in HEPES buffer (1.0×10^{-4} mol/L, pH 7.2).

2.3 Instrumentation

Plastic tape (0.3 mm thick) coated with acrylic adhesive on one side (Arcare 8565) was obtained from Adhesives Research Inc., (Limerick, Ireland). A plastic mask was fabricated using a laser marker from Fenix Flyer (Synrad, USA). The rolling ball used was from Schmidt Technology, type EF117. The electrochemical measurements were made with a potentiostat/galvanostat from Metrohm Autolab and a PGSTAT302N (Utrecht, The Netherlands), equipped with a FRA module and controlled by Nova software. The homemade paper-based carbon-printed electrodes (HP C-PEs) were connected to a portable switch box, from DropSens (DRP-DSC), interfaced with the potentiostat/galvanostat. The commercial single-walled carbon nanotubes screen-printed electrodes (SWCNTs-SPEs) of which the working electrode diameter is 4 mm (DRP-110SWCNT) were obtained from DropSens (Oviedo, Spain). Electromotive force (EMF) was measured with a

high input impedance (1015 W) EMF16 multichannel data acquisition device (Lawson Laboratories, Inc., Malvern) at room temperature. The standard reference electrode used was a Ag/AgCl 3 M KCl double-junction electrode (type 6.0726.100, Metrohm AG) containing a 1 M LiAcO salt bridge.

Raman measurements were performed in a Thermo Scientific DXR Raman spectrometer with confocal microscopy (Waltham, USA), with a 10 mW 532 nm excitation laser. Fourier transform infrared spectroscopy (FTIR) measurements were performed using a Thermo Scientific Smart iTR Nicolet iS10, coupled to a SAGA smart accessory, also from Thermo Scientific (Waltham, USA). Environmental scanning electron microscope (ESEM) images were obtained using a Quanta 600 (FEI Company) and atomic force microscope (AFM) images were obtained by a Keysight 500 (Keysight, USA).

2.4 Plastic mask fabrication

The plastic mask was designed and drawn using CleWin 4.0.1 Layout Editor (Phoneix Software, Netherlands). 30% laser power was applied to the plastic tape to make the plastic mask, which was used to draw the electrodes over the wax-modified hydrophobic paper.

2.5 Design and fabrication process of HP C-PEs

The design of the HP C-PEs (three-electrode system) is presented in Figure 1A. The diameter of the working electrode (WE) was ≈ 3.50 mm and the spacing between electrodes was ≈ 1 mm. The whole electrode system was 2.5 cm long and 0.75 cm wide.

The fabrication process of the HP C-PEs consists of the following steps. 1. Modify the filter paper (Figure 1B) with wax to make it hydrophobic and avoid electrical short-circuit between the electrodes by moisture [23]. To do this, ground wax was scattered on the filter paper ($0.037\text{g}/\text{cm}^2$) and another piece of filter paper was placed below the previous one, while the wax was being melted. The whole system was put in the oven at 95°C for about 3h, and then cooled down to room temperature (Figure 1C). 2. Placing the plastic mask above the hydrophobic paper (Figure 1D) and drawing the electrode system using a standard 2B graphite pencil. The sketch can be seen in Figure 1E. 3. Manually print the electrodes on the pencil sketch using conductive carbon ink soaked onto the tip of a non-use rolling ball pen and keep in the oven at 36°C overnight (Figure 1F). 4. Print Ag/AgCl ink on the reference electrode (using a rolling ball pen as in step 3) and dry in the oven

at 36°C for 30 minutes (Figure 1G). 5. Finally, the device was partly covered using plastic tape (Figure 1H) to make a specific working area that was isolated from the connection part. The connection of the electrodes with the portable switch box is shown in Figures 1I and J.

It is worth mentioning that curing the carbon ink would be faster at higher temperatures, but the best condition corresponds also to a compromise between the melting point of the wax (that is already on the substrate) and the physical properties of the cellulose paper. If the temperature goes too high, the ink undergoes the formation of a mixed layer of wax/carbon and therefore the conductivity properties of the original carbon ink are worsened. In addition, this may also change the hydrophobic properties of the substrate as the wax layer is also affected. As a compromise, we found that using a lower temperature (~36 °C) with an overnight curing time would be more appropriate.

A laser was used in the fabrication of the plastic mask, but then a single mask was used to make more than 100 electrodes and the mask was still in perfect condition. A virtually unlimited use of a mask significantly decreases the costs associated with the mask fabrication. Furthermore, the mask can also be made manually without the use of a laser using for instance a simple cutter, which decreases even more the associated costs.

2.6 Electrosynthesis of polymers on HP C- PEs

Prior to electropolymerization, the HP C-PEs were cleaned using cyclic voltammetry (CV) (-2.0 to +2.0 V, 40 cycles, scan rate 100 mV/s, using KCl 0.1M in Milli-Q water) and stabilized electrochemically with MES buffer (conditioning) by cyclic voltammetry (potential from -0.2 and 0.8 V, 10 cycles, scan-rate of 50 mV/s). Then 75 μ L of phenol solution (3.55×10^{-3} mol/L prepared in acetate buffer 1.0×10^{-2} mol/L, pH 5.0) was deposited onto the HP C-PE to cover the three-electrode for around 10s. Polymerization was then performed by CV between -0.2 and 1.2 V at a scan rate of 50 mV/s. For other monomers, such as AP and ABA, the same procedure was followed for polymerization, but the potential ranged from -0.2 to 0.8 V for AP and from -0.2 to 1.2 V for ABA.

The surface was then thoroughly washed with Milli-Q water and electrochemically cleaned by 25 CV cycles in MES buffer (potential range from -0.2 to 0.8 V, scan rate 50 mV/s) to remove the remaining monomers and/or oligomers.

2.7 Electrochemical, spectroscopic and microscopic characterization

The electrochemical properties of the clean electrodes were checked by observing the oxidation and reduction current peaks with different CV scan rates. The mechanical deformation of the device was studied by bending ($\sim 90^\circ$) the electrode several times and following the redox behaviour at a scan rate of 50 mV/s.

The characterization of the materials on the HP C-PEs was performed by direct analysis of FTIR and Raman spectroscopies without any treatment of the polymeric films. FTIR analysis was performed before and after electropolymerization. In FTIR (from 400 to 4000 cm^{-1}) the number of scans was set to 256, the resolution to 8, Happ-Genzel apodization, Mertz phase correction, and a background gain of 8. In Raman spectroscopy, the average signal-to-noise ratio (peak height/root mean square noise) was measured using 1 mW laser power at sample, 10 min photo bleaching time and 50 μm slit aperture. The surface modification was electrochemically followed up by cyclic voltammetry (CV), square wave voltammetry (SWV), and electrochemical impedance spectroscopy (EIS) to check that polymers had formed on the electrode surface. ESEM and AFM were used to observe the modification of the filter paper by wax.

2.8 Electrochemical measurements

The redox probes in all CV and EIS measurements were 5.0 mmol/L $[\text{Fe}(\text{CN})_6]^{3-}$ and 5.0 mmol/L $[\text{Fe}(\text{CN})_6]^{4-}$ prepared in MES buffer at pH 5.0 unless otherwise stated. In CV assays the potential range was from -0.5 to +0.7 V at 50 mV/s. In EIS, an open circuit potential was set using a sinusoidal potential perturbation of 0.01 V amplitude, and 50 logarithmically distributed frequency values over a frequency range of 0.01 Hz to 100 kHz. In SWV assays the potential range was from -0.4 to +0.6 V.

2.9 Calculation of electroactive surface area

The electroactive area (A_e) was measured with the intensity of the anodic peak current along with the scan rate following the *Randles-Sevcik* equation (eq. 1) for quasi-reversible electron transfer processes [24]. Herein, I_{p_a} is the anodic peak current intensity (A), n is the number of electrons transferred ($n = 1$), A_e is the electroactive surface area (cm^2) of the working electrode, C is the concentration of the redox probe $[\text{Fe}(\text{CN})_6]^{4-}$ (5×10^{-6} mol/ cm^3) in MES buffer containing 0.1M KCl, D is the diffusion coefficient of the redox probe (6.4×10^{-6} cm^2/s [25]) and ν is the scan rate

(V/s). I_{pa} was plotted against the square root of the scan rate and its linear trend was used to calculate A_e [26,27].

$$I_{pa}=(2.65\times 10^5)n^{3/2}A_eCD^{1/2}v^{1/2} \quad (\text{eq.1})$$

2.10 Pseudo reference characteristics of the reference electrode and stability of the working electrode

Pseudo-reference characteristics of the HP C-PEs reference electrode were studied in the two-electrode configuration by conditioning the electrode in HEPES buffer overnight and adding BSA (as a protein model for checking the performance in biochemical assays) in the potentiometric cell. The EMF response of the HP C-PEs reference electrode versus a standard reference electrode (Ag/AgCl 3 M KCl double-junction reference electrode) was recorded. Stability of the HP C-PEs working electrode was checked by recording its EMF response versus the HP C-PEs reference electrode.

3. Results and discussion

3.1 Fabrication of the paper-based electrodes

The fabrication of the homemade paper-based electrodes using a filter paper as substrate relies on a simple concept: the printed electrodes must be electrically independent and contain minimum conductivity features to allow its subsequent use in electrical-based sensing. Thus, two stages are included herein: hydrophobization and electrode printing.

Hydrophobization was made by casting of a wax layer. This layer converted the original surface of the filter paper (Figure S1A and S1C, Supplementary Information), which evidenced long fibres (and thereby high porosity), into an more homogeneous and less porous support (Figure S1B and S1D, Supplementary Information). The hydrophobic character of the modified paper was followed by casting a drop of redox probe aqueous solution on top of the cellulose/wax. The observed contact angle (Figure S1E, Supplementary Information) was $\sim 90^\circ$, thereby confirming the limited water permeation.

Electrodes were printed manually, on top of the hydrophobic substrates, using a carbon-ink. A single layer of this ink was enough to generate good electrochemical features. As can be seen in Figure 1, the shape of the final electrodes was not well defined. This happened because the ink

was casted with the help of a rolling-ball pen (the ink was not inside the pen, but instead in the external side of the sharp end of the tip, used to carry the ink as in a spatula). Of course, the ink could be applied with the help of a filled ink-pen support, allowing a much better control of the electrode shape, but this had two disadvantages. First, an empty pen support capable of ink filling is not promptly available. Second, this would be more expensive for requiring much more ink. Moreover, this roughly defined electrode format was important to keep, as this relate to the most simple strategy for ink application that may be tried out around a chemistry lab, and it was important to show that successful electrochemical systems may also be produced, even under these very simple conditions. Finally, it is important to note that the use of a single layer of ink was sufficient to design successful electrochemical systems. Our previous experience shows that at least 4 layers are necessary [28] for which having a single layer largely simplifies the overall fabrication process.

We also tried to use glossy paper or plastic, instead of paper with a layer of wax, but when we tried to print the ink over these surfaces, the ink spread and it was not adsorbed. So it was not possible to properly print the electrodes. In contrast, wax was sticky and ink highly adsorbs over wax, so the electrodes can be correctly printed.

In terms of configuration, conventional formats of 2- or 3-electrodes were set, to explore this fabrication procedure for several applications. The 2-electrode systems are widely use in potentiometric readings (combining indicator and reference electrodes), while the 3-electrode systems are common in electrochemical devices (combining working, counter and reference electrodes). The reference electrode was further modified by depositing a Ag/AgCl ink, as conventional pseudo-reference electrodes are typically made of Ag/AgCl.

The overall cost of these electrodes is about 6 cents per electrode.

3.2 Electrochemical cleaning and conditioning

Figure 2 shows the electrochemical properties of the 3-electrode HP C-PEs, before and after cleaning. The CV of the uncleaned system shows that the anodic and cathodic peak separation ($E_{p_a}-E_{p_c}$) is 0.40V, and the difference between the anodic and the cathodic current peak intensity ($I_{p_a}-I_{p_c}$) is 121 μ A, indicating that the interfacial redox process was evident. When the HP C-PEs

were electrochemically cleaned, there was a significant improvement in the electrical properties observed. The peak separation was lower ($E_{p_a} - E_{p_c} = 0.19$ V) and the current peak difference higher ($I_{p_a} - I_{p_c} = 233$ μ A). Overall, the pre-treatment increased the active area of the printed electrodes [29], in a way that peak current intensities reached higher values than those reported in other paper electrodes using carbonaceous materials [30,31], in paper electrodes using gold nanoparticles [32] or in paper electrodes using carbonaceous materials and gold nanoparticles [33]. This result was supported by SWV and EIS. In the case of SWV, the current peak increased by 230% (213 μ A for the as-prepared non-cleaned electrodes and 726 μ A after cleaning and conditioning), and for EIS the charge transfer resistance (R_{ct}) decreased by 930% (R_{ct} of 3048 Ω for the non-cleaned electrodes and 317 Ω after cleaning and conditioning). The most interesting and noteworthy result was seen in CV assays, where the ratio of the cathodic and the anodic current (I_{p_c}/I_{p_a}) is 0.94 (after cleaning and conditioning the electrodes), which is a characteristic of the quasi-reversible performance of the fabricated device and that is even better than the typical behaviour of commercial electrodes ($I_{p_c}/I_{p_a} = 0.84$) [34].

The calculated electroactive surface area (A_e) of the WE of the cleaned HP C-PE was 0.135 cm² (13.5 mm²) but this value for the non-cleaned electrode was 8.9 mm². The process of cleaning reduces surface passivation and therefore increases the available surface area for electron transfer. Since the geometric surface area (A_g) of the WE was 9.6 mm², the calculation of A_e means an increase of about 41% from A_g , which indicates the catalytic properties of the electrode materials and the roughness of the surface. The activity ratio (A_e/A_g) was 1.41. These values of A_e and activity ratio are better than values reported for other paper-based electrodes [35].

The current density of our cleaned HP C-PE was compared with a commercial electrode (SWCNTs-SPE, DropSens). Interestingly, it was found that the anodic peak current density of the homemade electrode was 135 μ A/cm² higher than the commercial one and a similar trend was observed for the cathodic peak current density (Figure S2, Supplementary Information), indicating the good electrochemical properties of our homemade HP C-PE.

Overall, the electrochemical features of the HP C-PEs electrodes are of great quality and may be favourably compared to other devices, especially considering the great simplicity and the very low-cost involved in the construction of these electrodes, which may be developed in any laboratory.

3.3 Electrochemical characterization

To characterize the electrochemical performance of the electrodes, CV assays were recorded with different scan-rates, using the same redox probe concentration. In these assays, the peak separation (oxidation and reduction) increased with increasing scan-rate (Figure 3), which is indicative of slow electron-transfer kinetics associated with the use of nonconductive binder material on the surface of the carbon ink electrodes [36]. Interestingly, the peak current as a function of the square root of the scan-rate was linear (R^2 were 0.9977 and 0.9962, respectively, for oxidation and reduction), evidencing a diffusion-controlled behaviour of redox processes at the electrode surface [34]. Overall, this behaviour was as expected for a 3-electrode system.

3.4 Mechanical deformation

HP C-PEs are rigid enough to be easily connected to the portable switch box (Figures 1I and 1J) but they are also soft enough to bend (Figure 4A) without breaking. The bending properties have been observed by bending the cleaned and conditioned HP C-PEs several times and then observing the electrochemical properties by CV. The HP C-PEs can be bent at least 10 times without any significant change in the shape of the CV voltammogram (Figure 4B).

In general, these results confirmed the good stability of the ink layer printed on the cellulose substrates, which maintains its integrity after consecutive bending. Moreover, it also confirmed the great stability of the electrodes in the presence of aqueous solutions, as these readings were made consecutively.

3.5 Electropolymerization

The possibility of modifying the electrode surface by electropolymerization was also explored herein. This is amongst the common approaches of electrode modification towards analytical applications. The monomers used for this purpose were phenol, AP and ABA, tested on HP C-PEs of 3-electrode system. The CV voltammograms obtained in this study are shown in Figure 5.

Figure 5A shows the CV curves (1–5 cycles) for the electropolymerization of phenol on HP C-PEs. During the anodic scan of the first cycle, a well-defined oxidative peak around 0.75 V was observed, but no reductive peak was observed during the cathodic scanning. This indicated an irreversible polymerization reaction, as expected. The peak current was around 65 μA for the first anodic scan, which decreased to around 15 μA after the second anodic scan. No oxidative peak

was observed after the successive 4th and 5th anodic scans except the charging current which is highly expected because the growth of polymers on the electrode surface produces a resistive and passive surface. The resistive and passive surface is limited by the inability of electron transfer. The polymerization of other monomers (AP, ABA) was similar and characteristic peaks were observed around 0.6 V for AP and 0.75 V for ABA (Figures 5B and 5C).

When compared to similar commercial 3-electrode systems [37], it was interesting to note that the peak potential of AP oxidation (in the first CV cycle) was similar (at around +0.6V) but the homemade electrode gives more sharper behaviour. This indicated that the catalytic properties of these homemade electrodes are more effective than commercial devices of analogous nature. In a whole, this confirmed to suitability of HP C-PEs for a prompt modification with nanomaterials of interest.

3.5.1 Electrochemical follow-up

The formation of polymers on the electrode surface was further confirmed by comparing the surface blocking behaviour with respect to the clean blank electrode, using CV and SWV (Figure 6). Figure 6 shows the redox peaks (in CV) and a high current of around 75 μA (in SWV) in the blank electrode, whereas after polymerization the surface of the electrodes is electrically “blocked” as the CV redox peaks significantly decreased and the intensity of SWV current is significantly lower.

3.5.2 FTIR and Raman spectroscopies follow-up

The existence of polymers on the surface of the working electrode of HP C-PEs was also checked by FTIR and Raman spectroscopies. Here, the characterization of the phenol polymerization is presented as a representative example. The results for poly(AP) and poly(ABA) were similar.

The characteristic functional group of phenol is hydroxyl ($-\text{OH}$) [38], but its presence in poly(phenol) is expected to be small accounting the chemical change promoted by polymerization. This was indeed confirmed in the FTIR spectra (Figure S3, Supplementary Information). While $-\text{OH}$ is a highly absorbing group in wavenumbers above 3000 cm^{-1} , its presence in the polymeric structure was noted by a small and broad peak within 3200 to 3400 cm^{-1} . Moreover, this broad peak was a new peak compared to the blank electrode, thereby confirming the presence of an additional layer with a different chemistry. It is also important to note that the peak at about 1020

cm^{-1} increased significantly when compared to the blank electrode, and it was likely assigned to C–O stretching, as expected in the final poly(phenol) structure.

Raman spectroscopy supports the FTIR results and shows that the I_D/I_G ratio (0.98) after polymerization, is higher than the ratio of the clean electrode ($I_D/I_G = 0.85$) (Figure S4, Supplementary Information), mostly accounting the increase in intensity of the D peak. This result is expected because the prevailing ordered structure of a carbon matrix is changed by the addition of the polymeric poly(phenol) structure, which is more disordered and contains additional sp^3 hybridization.

Above all, the electrodes were successfully modified by electropolymerization, which is particularly useful for the preparation of modified electrochemical biosensors, including molecularly-imprinting polymer based bio-receptors, as proven in the detection of bacterial flagellar filaments [21].

3.6 The 2-electrode systems.

HP C-PEs with 2-electrodes system contained an Ag/AgCl reference electrode and a carbon working electrode. The pseudo-reference characteristics of this reference electrode were studied using potentiometric measurements with respect to a standard reference electrode (Ag/AgCl 3 M KCl double-junction reference electrode). The HP C-PE reference electrode was first stabilized and conditioned in HEPES buffer overnight, and then the electromotive force (EMF) response was monitored against the commercial reference electrode. The results showed that the HP C-PE Ag/AgCl electrode was very stable throughout time. However, these electrodes should also be stable in the presence of other molecules that may be present in the solution. This was tested by consecutive addition of a model protein, BSA, into the solution, ranging from 125 ng/mL to 9.70 $\mu\text{g/mL}$. It was very important to note that no changes in the EMF were observed (Figure S5A, Supplementary Information), which is an excellent result, especially considering that this was a pseudo-reference electrode. In contrast to commercial devices, here there were no external solutions to protect the electrode against poisoning, which would lead to a constant drift, something that must be avoided.

Moreover, the stability of the working electrode of HP C-PE (two-electrode system) was checked with respect to the pseudo-reference electrode in HEPES buffer. In general, EMF readings were very stable and did not evidence constant drifting (Figure S5B, Supplementary Information),

advising that the electrode may undergo suitable modifications to proceed further with potentiometric readings.

4. Conclusions

Paper-based electrode fabrication is a promising technology but it is still limited in terms of the rigidity, flexibility and design of the electrode. Herein, we have shown that manually homemade carbon-printed electrodes on paper-based substrates can be successfully fabricated with a simple, easy and cost-effective procedure, available in any chemistry lab and obtaining very good electrochemical characteristics. The fabricated electrodes are portable and can be used as disposable devices, offering therefore promising possibilities of worldwide use in times of pandemics, if a suitable biorecognition element is added into it. We also show that the fabricated paper-based electrodes have special mechanical properties such as rigidity so that they can be easily and quickly connected to portable connector boxes and flexibility so that they can be bent.

Moreover, the applicability of these electrodes has already been proven, following our previous work [21], about which the details of fabrication, mechanical deformation and characterisation of the electrodes have been thoroughly presented herein.

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Figure captions

Figure 1. A) Fabricated HP C-PEs (three-electrode design). RE: reference electrode, WE: working electrode, CE: counter electrode. The smaller picture on top shows the two-electrode design. Schematic presentation of the process and fabrication of the device and its connection with the portable switch box: (B) filter paper; (C) hydrophobic filter paper; (D) mask placed on the hydrophobic filter paper; (E) sketch of the electrodes; (F) carbon ink-printed electrode; (G) reference electrode (left) with Ag/AgCl ink; (H) final device partly covered by the plastic tape; (I) three-electrode and (J) two-electrode based devices connected to a portable switch box.

Figure 2. Electrochemical performance of the fabricated HP C-PE, by (A) CV, (B) SWV and (C) EIS (Nyquist plots) assays, in 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-}$ and 5.0 mM $[\text{Fe}(\text{CN})_6]^{4-}$, prepared in MES buffer pH 5.0, before (orange) and after (blue) electrochemical cleaning.

Figure 3. Behaviour of the cleaned electrodes in 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-}$ and 5.0 mM $[\text{Fe}(\text{CN})_6]^{4-}$, in MES buffer pH 5.0, (A) under increasing CV scan-rate, and (B) the corresponding oxidation/reduction current plotted with the square root of scan rate (mV/s).

Figure 4. Bending of an HP C-PE electrode (A); and the corresponding cyclic voltammograms after consecutive bending (B), in a solution of 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-}$ and 5.0 mM $[\text{Fe}(\text{CN})_6]^{4-}$, prepared in MES buffer pH 5.0.

Figure 5. Electropolymerization on the HP C-PE electrodes, of (A) Phenol, (B) AP and (C) ABA, using CV in sodium acetate buffer pH 5.0. Scan rate 50 mV/s.

Figure 6. Follow up of surface modification of the HP C-PE electrodes due to electropolymerization, by (A) CV and (B) SWV, in a solution of 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-}$ and 5.0 mM $[\text{Fe}(\text{CN})_6]^{4-}$, in MES buffer pH 5.0.

Figure 1

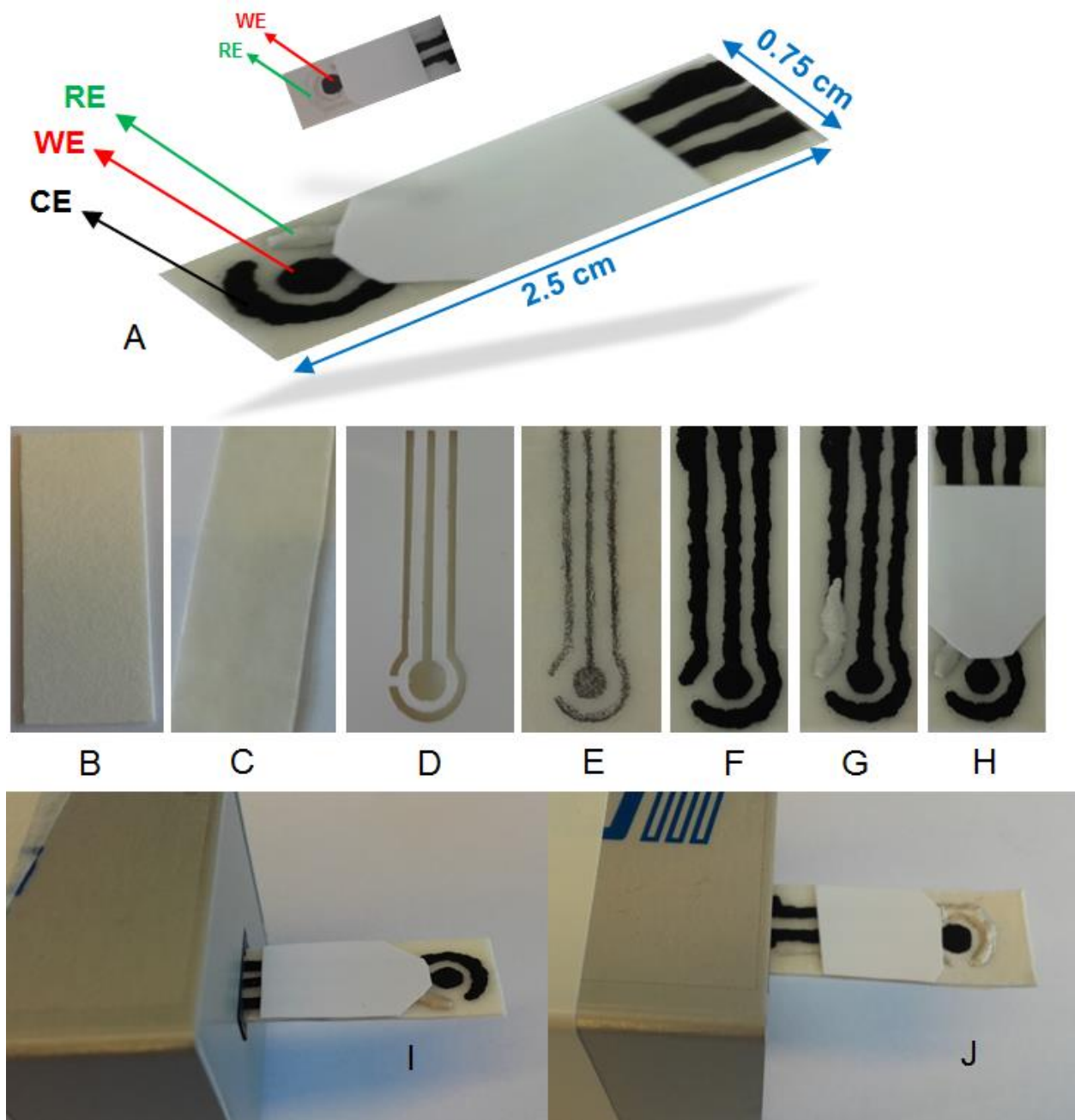


Figure 2

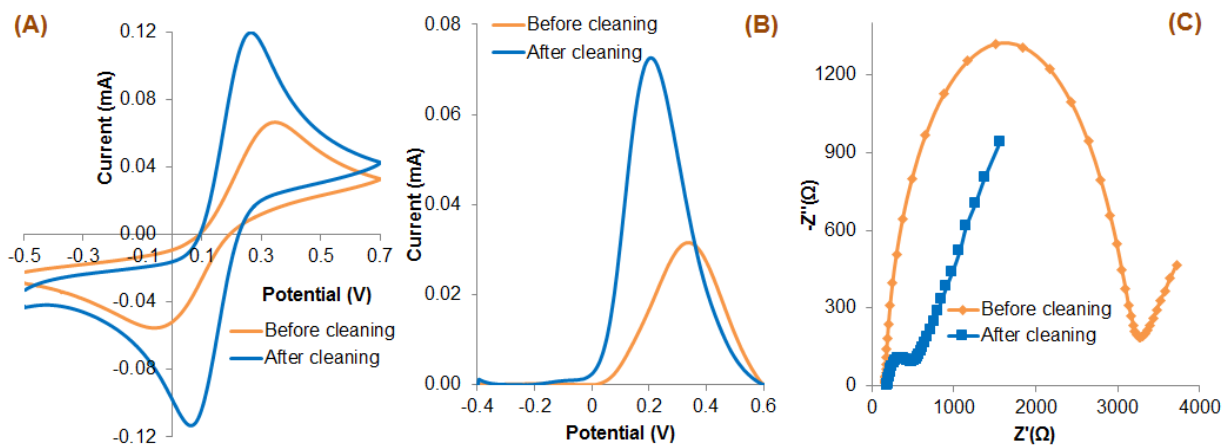


Figure 3

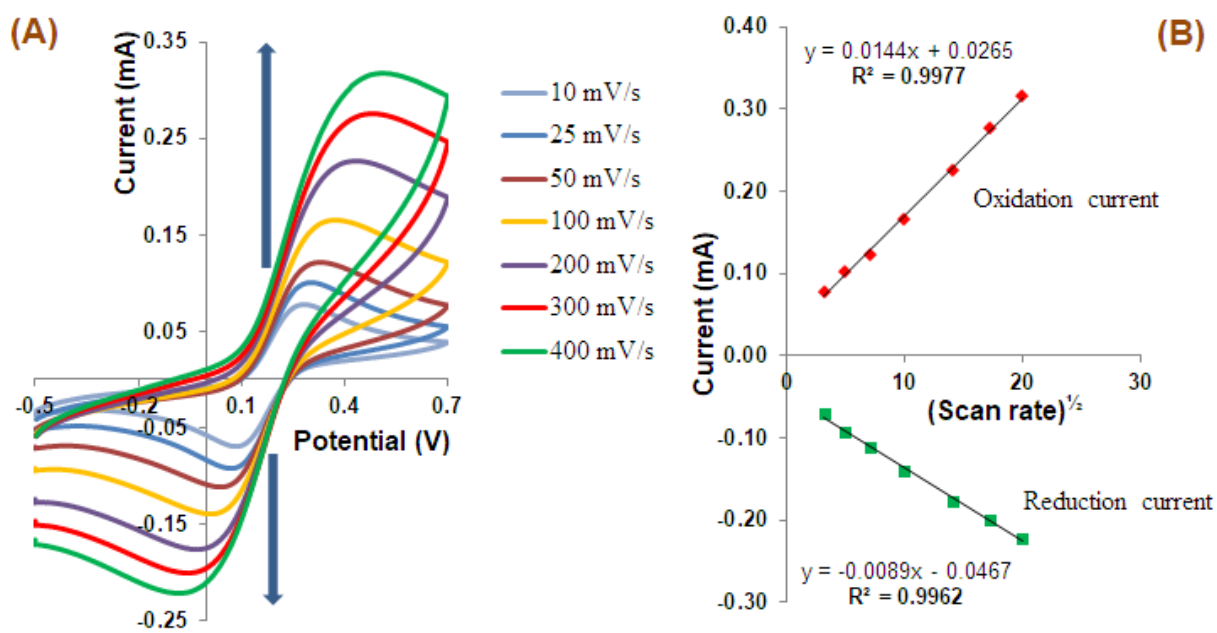


Figure 4

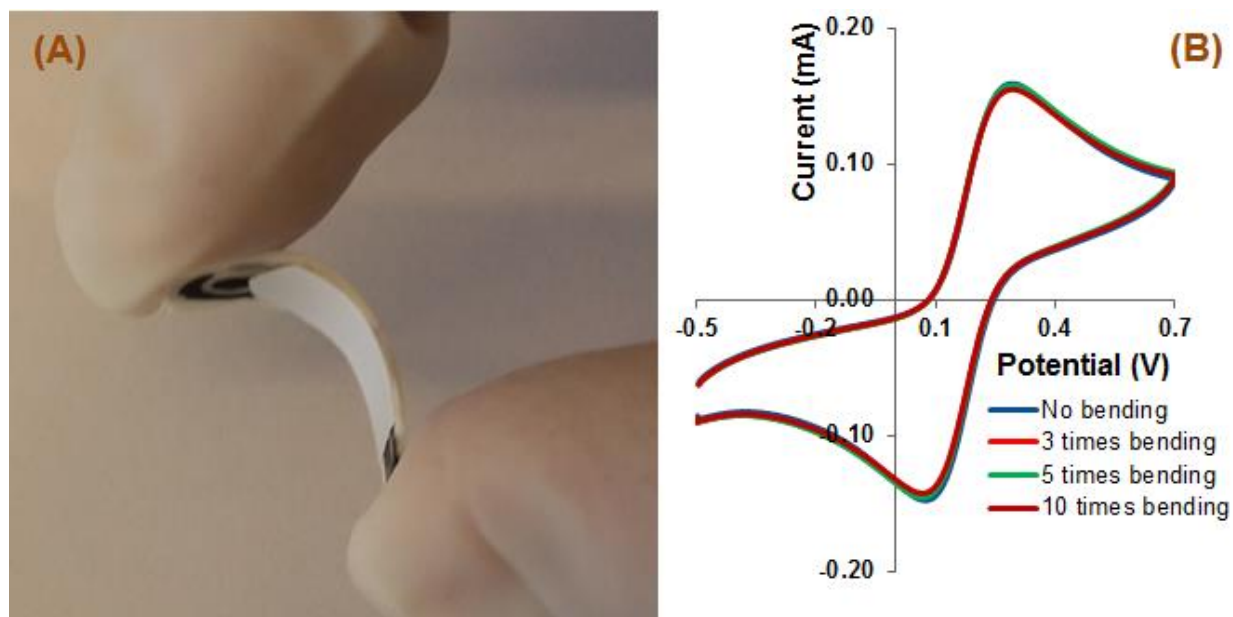


Figure 5

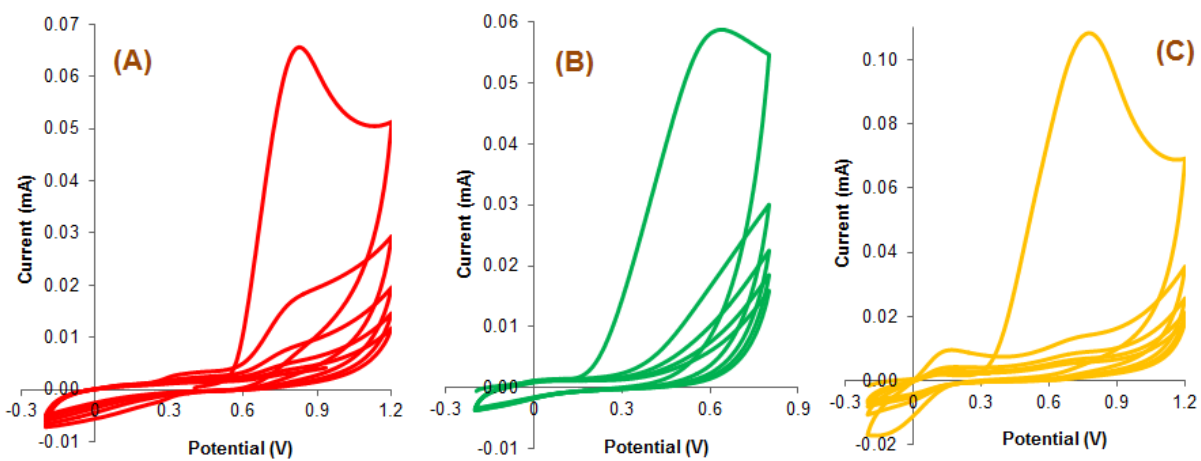


Figure 6

