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Development and characterization of carbon based electrodes from pyrolyzed paper for biosensing applications

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ABSTRACT

This article details the study of electrochemical behavior of new carbon electrodes based on pyrolysis of different paper sources to be used in biosensor applications. The resistivity of the pyrolyzed papers was initially used as screening parameters to select the best three paper samples (imaging card paper, multipurpose printing paper, and 3MM chromatography paper) and assemble working electrodes that were further characterized by a combination of microscopy, electrochemistry, and spectroscopy. Although slight differences in performance were observed, all carbon substrates fabricated from pyrolysis of paper allowed the development of competitive biosensors for uric acid. The presented results demonstrate the potential of these electrodes for sensing applications and highlight the potential advantages of 3MM chromatography paper as a substrate to fabricate electrodes by pyrolysis.

1. Introduction

Since the first report in 1962 [1], the field of biosensors has made astounding progress. Doctors and patients are increasingly relying on simple, fast, and cost-effective biosensors to monitor a variety of analytes [2]. Although a variety of biosensors are currently available, the versatility of electrochemical biosensors clearly stands out in the move towards simplified testing [3]. Electrochemical biosensors have now moved beyond the classic examples involving glucose oxidase and have been adapted to quantify a wide range of analytes, feature adequate sensitivity for clinical applications, offer independence from optical interferences, require minimal sample and power consumption, and are highly compatible with modern microfabrication techniques [4–7]. Moreover, coupling the simplicity of the instrumental requirements of electrochemical detectors with the increased availability and computing capabilities of mobile phones, several groups have developed portable electrochemical platforms that could soon be adapted to biosensing applications [8–10]. It is also clear that along with the growth in the use of these devices, demands for cost and time-effective ways to produce biosensors are also increasing. In this regard, one of the most important points to consider is the selection of the transducer. While recent advances in nanotechnology have allowed the development of improved sensors based on modified metallic surfaces, the vast majority of the community has opted for carbon-based material [11–13]. Aside from traditional examples such as glassy carbon [14] and mesoporous carbon [15], carbon-based nanomaterials (nanodiamonds, nanofibers, nanorings, and nanotubes) [16,17] share a common structure composed of sp^2-bonded atoms that supports electrical conductivity [16], the capacity to form charge-transfer complexes [18], unique optical properties [19], chemical reactivity [20,21], and the possibility to interact with a variety of biorecognition elements [22].

Because most of these materials also share the high costs and limited accessibility linked to the fabrication methods (such as chemical vapor deposition or screen-printed electrodes) [23–25], many researchers have focused their attention on the development of platforms that are more suitable for the needs in low-income communities. Among them, it is important to highlight the possibility to fabricate electrodes from carbon-ink or pyrolysis of organic precursors. The former electrodes have been micromolded using PDMS [26], painted over Au wires [27], and more recently adapted to perform detection in paper-based microfluidic devices [28,29]. The latter electrodes can be fabricated by pyrolysis from a variety of organic precursors including photoresist [30–35], parylene C [36], proteins [37] or even insects [38]. While all these materials have the potential to enable operational electrochemical sensors, most of them offer limited surface area and their application for

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the development of electrochemical biosensors is restricted by the resistivity of the material, which results in quasi-reversible [39,40] or irreversible electrochemical behavior [37,41].

To address these deficiencies, this report describes the possibility to prepare electrodes by pyrolysis of paper. The resulting electrodes, which can be fabricated from a variety of substrates, feature low resistivity, large surface area, and uniform thickness. More importantly, the electrodes can be used for the development of biosensors by simply immersing them in a solution containing a selected enzyme. Herein we present results related to the selection of the substrate, the characterization performed (microscopy and electrochemical) as well as their potential for the development of an electrochemical biosensor for uric acid.

2. Materials and methods

2.1. Reagents

Recombinant uricase (also known as urate oxidase) from *Candida* sp. expressed in *Escherichia coli* and potassium hexacyanoferrate (III) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Uric acid, 99% was obtained from Alfa Aesar (Ward Hill, MA, USA). Sulfuric acid (ACS/FCC, BDH ARISTAR, 95.0–98.0%) was acquired from VWR (Pittsburgh, PA, USA). All aqueous solutions were prepared using 18 $M\Omega\cdot cm$ water (NANOpure Diamond, Barnstead; Dubuque, IA) and analytical reagent grade chemicals. Phosphate buffer solution was prepared by dissolving anhydrous Na_2HPO_4 (Fisher Scientific; Fair Lawn, NJ, USA) in ultrapure water. The pH of the solutions was measured using a glass electrode connected to a digital pH meter (Orion 420A+, Thermo; Waltham, MA, USA) and adjusted with 1 $mol\cdot L^{-1}$ solutions of either NaOH or HCl. Standard uric acid solution was freshly prepared in phosphate buffer solution (10 $mmol\cdot L^{-1}$, $pH = 8.5$) before each experiment.

2.2. Carbon electrode fabrication

Electrodes described in this manuscript were fabricated by pyrolysis of commercially available paper samples and used as received. In order to probe a range of potential substrates, multipurpose printing paper (75 $g\cdot m^{-2}$, Spectrum; El Paso, TX), JP40 filter paper (80 $g\cdot m^{-2}$, Quanty; J-Prolab, Brazil), Gilbert 100% Cotton Linen Paper (90 $g\cdot m^{-2}$, Neenah Paper; Neenah, WI), Whatman filter paper #4 (96 $g\cdot m^{-2}$, GE Healthcare; Pittsburgh, PA), drawing paper (130 $g\cdot m^{-2}$, Strathmore; Appleton, WI), imaging card paper (138 $g\cdot m^{-2}$; JetPrint; Memphis, TN), Whatman 3MM Chromatography Paper (189 $g\cdot m^{-2}$, GE Healthcare; Pittsburgh, PA), and Wausau Astroparche (220 $g\cdot m^{-2}$, Neenah Paper; Neenah, WI) were selected. The selection was made based on availability and not by the composition of the paper, which is proprietary information.

The electrodes were fabricated using a procedure adapted from a previous publication from our group [37,41]. Briefly, 2.5 cm × 5 cm pieces of paper were cut and placed in a tube furnace (Type F21100, Barnstead–Thermolyne; Dubuque, IA, USA). The tube furnace was then flushed for 5 min with Ar (to remove the O_2 from the quartz tube and avoid oxidation reactions) and turned on. Once the furnace reached 1000 °C (typically within 30 min, see Supplementary Information), 5% H_2 was added to the Ar flow to create a reducing environment and flowed in the tube for 1 h. Next, the hydrogen was turned off and the tube furnace was allowed to cool down to room temperature under 100% Ar flow (typically about 1 h). The samples were then removed from the furnace and affixed to a Plexiglas substrate using double sided tape. In order to define a uniform electrode size, the electrodes were then patterned using a commercial CO_2 laser engraver (Mini24, Epilog Laser Systems; Golden, CO, USA) [42–44], defining electrodes like the one shown in Fig. 1. To keep water from wicking up the stem of the electrode (and modifying the electrode area), paraffin paper was wrapped around the base of the stem (between the circular pad and the contact area) and melted in place using a hot air gun. Finally, silver paint (SPI Supplies; West Chester, PA, USA) was applied to the tip of the stem to define a contact area for the potentiostat. The resistance of the electrical contact (<60 mΩ/sq./mil) was considered negligible with respect to that of the carbon electrode.

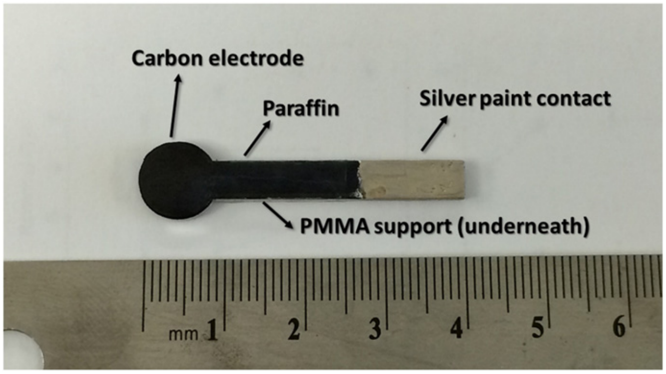

Fig. 1. Photography of one electrode fabricated using pyrolyzed paper. Figure also shows the section coated with paraffin and the section coated with silver paint.

2.3. Electrochemical techniques

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were employed to investigate the electrochemical performance of the carbon electrodes produced from paper. The experiments were performed using 100 $mmol\cdot L^{-1}$ H_2SO_4, as supporting electrolyte, containing 1.0 $mmol\cdot L^{-1}$ $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$, as the redox couple. In all cases, a standard three-electrode cell comprised of the paper-derived electrodes, a silver/silver chloride ($Ag|AgCl|KCl_{sat}$), and a platinum wire were used as working, reference, and counter electrode, respectively. CV experiments were carried out using a CHI812 Electrochemical Analyzer (CH Instruments, Inc.; Austin, TX). EIS data were obtained using a PC-controlled CHI 660A potentiostat by scanning from 10^{-4} Hz to 10^5 Hz at a 5 mV amplitude, with 10 data points per frequency decade. The impedance spectra were then analyzed with the simulation software *Zview*-Impedance® (version 2.4a) by fitting the spectra with a Randles-type equivalent circuit.

2.4. Thermogravimetric analysis

The pyrolysis of the paper samples was studied by measuring the weight loss of the selected samples while the temperature was increased at 5 $°C\cdot min^{-1}$ from 21 °C to 800 °C using a TGA-50 Thermogravimetric Analyzer (Shimadzu Corp.; Tokyo, Japan). TGA experiments were performed in the presence of pure N_2 as an inert purge gas to prevent undesirable reactions between the paper samples and air. The N_2 was also used to remove the gaseous and condensable products evolved during the paper pyrolysis. As a negative control, silica nanopowder was used.

2.5. Raman spectroscopy

A confocal Raman automated imaging spectrometer (iHR320, Horiba Jobin Yvon) was used to examine the composition of the carbon electrodes from pyrolyzed paper. The Raman spectrometer was equipped with an excitation laser of 488 nm wavelength and a Synapse CCD detector. The Raman spectra were collected in the 1000–1800 cm^{-1} range, with an exposure time of 2 s, an accumulation factor of 2, and with a 100× objective.

2.6. Uric acid biosensor

To obtain an amperometric biosensor for uric acid, the selected carbon electrodes were immersed in a phosphate buffer solution