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Development of an All-Carbon Electrochemical Biosensor on a Flexible Substrate for the Sensitive Detection of Glucose †

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Abstract: Wearable biosensors for the detection of analytes in sweat are an emerging and promising technology with important applications in monitoring a person's physiological state. Sweat, being an easily accessible biofluid, shows great potential as a biological fluid for wearable devices, but also a number of challenges that must be addressed before a sensor can be commercialized. As an example, sensor fabrication on flexible substrates can greatly affect the performance of the device. Herein, the development of an enzymatic electrochemical sensor om a flexible substrate for glucose detection is presented. The sensor's three electrode system were made entirely with carbon-based ink on polyimide substrates and decorated with carbon black. The developed and optimized sensor design exhibited a stable and reproducible performance and was able to detect glucose in concentrations relevant to the ones present in sweat.

Keywords: glucose monitoring; electrochemical biosensor; flexible substrate; carbon black

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1. Introduction

Recently, the increased interest in wearable or implantable sensing devices and platforms has prompted the development of highly sensitive and stable electrochemical sensors on flexible substrates [1,2]. Fabrication of these sensors demands the use of flexible substrate materials that can be bent and mounted on non-flat surfaces, like parts of the human body. Towards this goal, polymer films like polyethylene terephthalate (PET), polyimide (PI), polydimethylsiloxane (PDMS), as well as paper and textiles have been used as substrates for flexible biosensors [3]. Numerous examples of such sensing devices have been published, ranging from simple test strips to patches [4–6] or tattoos [7–11] and smart textiles [12], using different biological fluids like tears [13], sweat [14], urine [15] and saliva [16]. The most common metabolic disease targeted by the majority of the developed sensors is diabetes, due to its prevalence in the population. Diabetes is routinely monitored through the detection of glucose in the bloodstream, a procedure that involves a painful and invasive blood sampling method by finger pricking [17]. Wearable devices, on the other hand, permit non-invasive sampling and continuous glucose monitoring without interrupting the wearer's daily activity [18,19] using sweat as the biological sample [20,21].

Herein, we demonstrate the fabrication of an enzymatic electrochemical sensor that has been developed on flexible substrates. Polyimide (PI) sheets have been chosen as substrates for the drawing of carbon electrodes using conductive ink [22–25]. The electrodes are made using only conductive carbon ink, while carbon black, a cost-effective carbon-based nanomaterial [27], has been applied on the working electrode. The fabricated flexible electrodes exhibit reproducible electrochemical behaviour, while the developed

sensor is able to detect glucose in the concentration range of clinical significance in human sweat.

2. Materials and Methods

Conductive carbon black (Vulcan XC 27R) was kindly provided Cabot Corporation's representatives in Greece (RAWCHEM), having an average particle size of 50 nm and typical bulk density of 6 lbs/ft3. Carbon/graphite conductive ink C2130814D2 was purchased from Sun Chemical (Slough, UK). All other chemicals and reagents were purchased from Sigma Chemical Company (St. Louis, MO, USA). A BioLogic SP-200 potentiostat was used for all electrochemical measurements.

2.1. Fabrication of the Enzymatic Electrochemical Glucose Sensor

PI sheets were used as the flexible substrate onto which electrodes were drawn with the use of a metal mask to imprint the electrode pattern and subsequently fill it up with conductive carbon ink. The working electrodes were then further modified by placing a drop (3μL) f carbon black suspension in an acidified aqueous solution of chitosan (0.05% in 0.05 M HCl) and were left to dry at room temperature. This task was performed twice before moving on to the immobilization of the enzyme. For the latter, a 2 μL drop of glucose oxidase (40 mg/mL) was added and was also left to dry at room temperature. Finally, a 2 μL drop of Nafion (0.1% in H_2O) was placed on top to complete the sensor fabrication process.

2.2. Sensor Characterization

Chronoamperometric detection was used for glucose detection and quantification. Measurements were performed at an applied voltage of 0.6 V and the current was recorded for 180 s so that a stable value was obtained. A range of different concentrations of glucose in PBS 10 mM pH 7.4 was tested by placing a drop on the surface of the sensor so that all three electrodes were covered.

3. Results and Discussion

3.1. Flexible Electrodes Fabrication and Performance

Polyimide (PI), polyethylene terephthalate (PET), cyclic olefin copolymer (COC) and polydimethylsiloxane (PDMS) were tested as substrate materials for the fabrication of the glucose sensor due to their flexibility. Electrodes fabricated on PI sheets showed the best adhesion to the substrate and this is why PI was chosen for the fabrication of the sensor. A three-electrode system was hand-drawn on the PI substrate using a carbon/graphite ink. To achieve the same pattern each time, a stainless-steel mask was fabricated with the electrode design. The mask was used to carve the design on the PI sheets before drawing the electrodes. The hand-drawn electrodes were reproducibly fabricated with very small performance variation This was validated by cyclic voltammetry measurements (Figure 1). To increase the sensitivity of the working electrode, carbon black was used, as a cost-efficient alternative to other carbon-based nanomaterials, such as carbon nanotubes and grapheme oxide, which have been used for the fabrication of electrochemical glucose sensors [27–29]. Carbon black exhibits excellent electrical conductivity and offers fast electron kinetics due to its numerous defect sites, thus making it an ideal candidate for the development of low-cost biosensors [26].

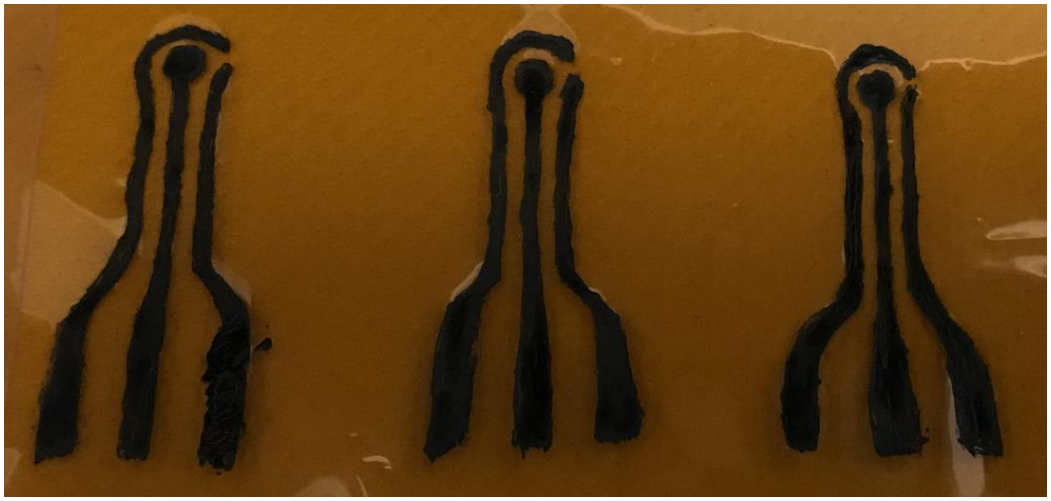


Figure 1. Hand-drawn all-carbon three electrode system on PI sheets.

3.2. Enzymatic Glucose Sensor Performance

The working electrode was further modified with glucose oxidase which was adsorbed on top of a chitosan layer and stabilized with the use of Nafion. Glucose oxidase entrapment allowed the enzyme to maintain its activity, whereas crosslinking the enzyme with glutaraldehyde had an impact on the enzyme's active site and its turnover number and thus decreased the linear dynamic range over which glucose could be detected and also increased the limit of detection (results not shown). A one-step modification was also tested by mixing the enzyme with the carbon black/chitosan dispersion and depositing it on the surface of the working electrode. Once again, the performance of the sensor was inferior to that of the sensor where the enzyme was adsorbed on the chitosan matrix and stabilized by Nafion. The obtained results from the chronoamperometric interrogation of the sensor (Figure 2) demonstrate that the sensor was able to detect glucose at concentrations ranging from 0.05 mM–2 mM (Figure 3). Despite, therefore, the simple design of the sensor, glucose could be detected at concentrations relevant to sweat and within the range of clinical significance [14,31,32]. Lower limits of detection have been achieved in other published work, albeit with the use of mediator-modified electrodes such as Prussian blue [5].or with much more complex designs involving numerous steps for the biomodification of the working electrode.

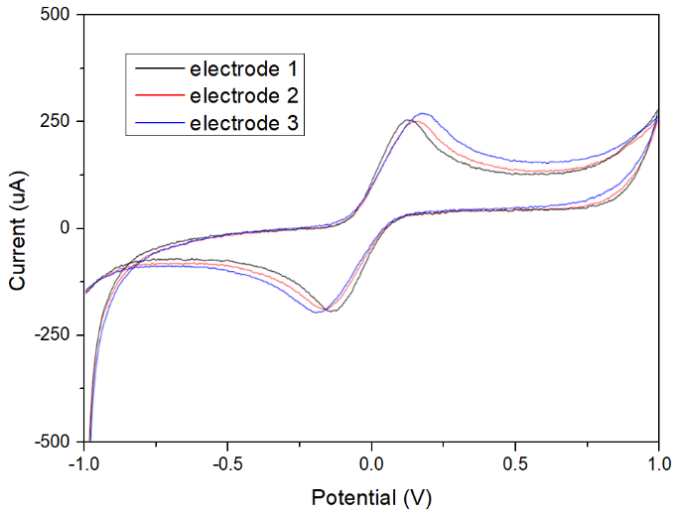
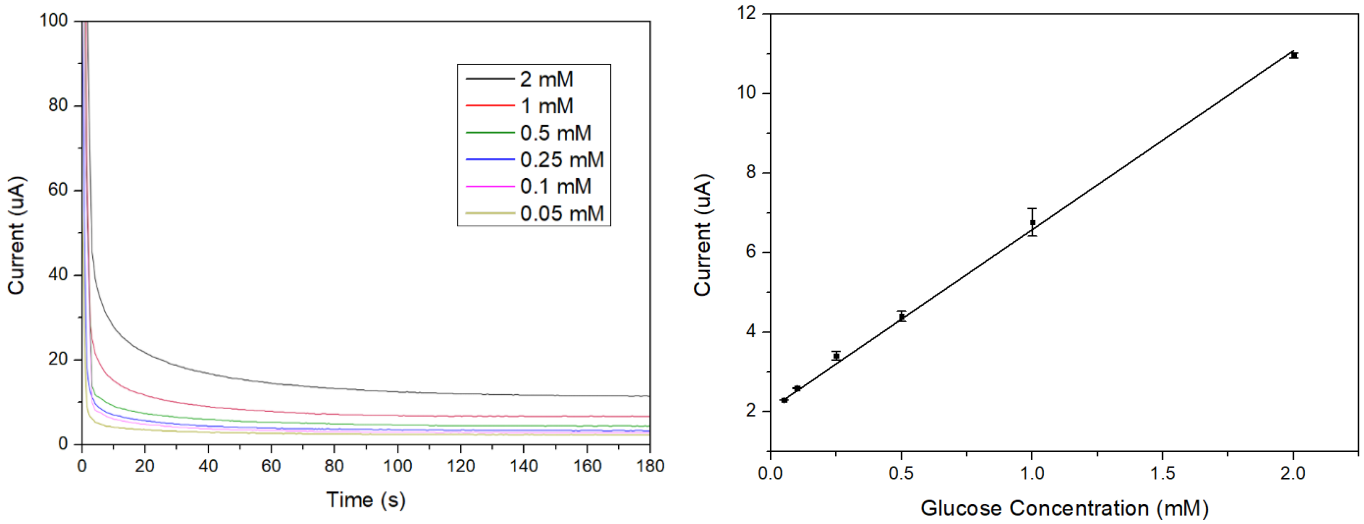


Figure 2. Cyclic voltammogram of 10 mM ferrocyanide/ferrocyanide redox couple in PBS 1x pH 7.4.



(**a**) (**b**)

Figure 3. (**a**) Chronoamperometric response of the biosensor to increasing glucose concentrations from 0 to 2 mmol L^{-1} in PBS 1x pH 7.4. (**b**) Corresponding calibration curve. The points in the plot are the mean value ± SD (n = 3).

4. Conclusions

Flexible electrochemical sensors are the key towards the development of wearable devices that could offer continuous monitoring of biomarkers in relevant biological fluids. Herein, we have demonstrated the sensitive and reproducible detection of glucose with the use of a hand-drawn three electrode system fabricated entirely with the use of carbon ink. Furthermore, utilization of carbon black allowed us to achieve a very low limit of detection and a linear dynamic range at glucose concentrations relevant to human sweat. The sensor could be further integrated with a microfluidic network to develop a wearable patch for glucose monitoring or could be used as the basis for development of sensors for other biomarkers of clinical or metabolic significance in sweat.

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Conflicts of Interest:

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