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Paper-based enzymatic reactors for batch injection analysis of glucose on 3D printed cell coupled with amperometric detection

Anderson A. Dias[a], Thiago M.G. Cardoso[a], Rafael M. Cardoso[b], Lucas C. Duarte[a], Rodrigo A.A. Muñoz[b], Eduardo M. Richter[b], Wendell K.T. Coltro[a,*]

[a] Instituto de Química, Universidade Federal de Goiás, Goiânia 74690-900, GO, Brazil
[b] Instituto de Química, Universidade Federal de Uberlândia, Uberlândia 38408-100, MG, Brazil

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ABSTRACT

This report describes for the first time the development of paper-based enzymatic reactors (PERs) for the detection of glucose (Glu) in artificial serum sample using a 3D printed batch injection analysis (BIA) cell coupled with electrochemical detection. The fabrication of the PERs involved firstly the oxidation of the paper surface with a sodium periodate solution. The oxidized paper was then perforated with a paper punch to create microdisks and activated with a solution containing *N*-hydroxysuccinimide (NHS) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC). Glucose oxidase (GOx) enzyme was then covalently immobilized on paper surface to promote the enzymatic assay for the detection of Glu in serum sample. After the addition of Glu on the PER surface placed inside a plastic syringe, the analyte penetrated through the paper surface under vertical flow promoting the enzymatic assay. The reaction product (H_2O_2) was collected with an electronic micropipette in a microtube and analyzed in the 3D BIA cell coupled with screen-printed electrodes (SPEs). The overall preparation time and the cost estimated per PER were 2.5 h and $0.02, respectively. Likewise the PERs, the use of a 3D printer allowed the fabrication of a BIA cell within 4 h at cost of $5. The coupling of SPE with the 3D printed cell exhibited great analytical performance including repeatability and reproducibility lower than 2% as well as high sampling rate (30 injections h^{-1}) under low injection volume (10 μL). The limit of detection (LD) and linear range achieved with the proposed approach was 0.11 mmol L^{-1} and 1–10 mmol L^{-1}, respectively. Lastly, the glucose concentration level was successfully determined using the proposed method and the values found were not statistically different from the data achieved by a reference method at confidence level of 95%.

1. Introduction

Since the first reports published by Whitesides group [1,2], paper has emerged as a powerful, promising and disposable platform for sensing and biosensing applications [3–8]. This substrate provides numerous advantages including very low cost, biocompatibility and, most importantly, global affordability [1–10]. Paper is a cellulosic material which can be chemically modified through its hydroxyl groups and then offer catalytic functions desirable for biosensing purposes [9]. Furthermore, the porous structure of paper substrate facilitates chemical assays based on lateral and vertical flow [1,2,10], chromatographic separations [11,12] as well as other applications involving, for example, the use of microzone plates [13,14] and flow injection analysis (FIA) [15,16].

The chemical modification of the paper surface may led to the development of microstructured catalytic reactors or bioreactors [9,10]. This process can be performed through different ways including the silane coupling technique [9,17], layer-by-layer assembly [18], chemical oxidation with sodium periodate [19,20], microencapsulation [21,22] as well as the incorporation of nanomaterials [23–25]. The incorporation of enzymes in paper surface exhibits great promise as simple, inexpensive and portable biosensors with high potential to be used in clinical assays [3]. Regarding the immobilization process, the major drawback is to ensure the covalent attaching of enzymes in paper surface and thus to avoid problems promoted by the washing away effect through lateral flow assays [10]. This phenomenon has negatively affected the analytical performance of assays based on digital image analysis [20,26]. However, the analytical reproducibility of experiments

* Corresponding author. Fax: +55 62 3521 1127.
E-mail address: wendell@ufg.br (W.K.T. Coltro).

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based on electrical signal may be also compromised, especially when lateral or vertical flow assays are employed.

Batch injection analysis (BIA) was proposed around 25 years ago by Wang and Taha [27] as an alternative technique to perform rapid assays [27,28]. In comparison with FIA systems, one of the highlighted advantages refers to the absence of pumps and valves [27–29]. The coupling of BIA with electrochemical detectors provides advantages for applications in analytical chemistry field including adjustable selectivity, high sensitivity, high sampling rates, accuracy and precision [27,28,30,31]. Among the electrochemical methods, amperometric detection (AD) has been the most popular detector used in BIA systems. The operating principle of a BIA-AD system involves basically the injection of a sample plug through an electronic micropipette directly on the working electrode surface designed in a wall-jet arrangement [28,31], in which the electrochemical cell is kept immersed in a large volume of electrolyte solution [28]. Most of the reports found in the literature make use of conventional electrodes arranged in a 3-electrode cell [27–34]. In 2014, Tormin and co-workers proposed for the first time the use of screen-printed electrodes (SPEs) in a BIA cell [35]. The approach described by the authors revealed a simple, robust, high-throughput and portable system for on-site analysis. Most recently, Caramit et al. [36] also reported a BIA cell with SPE for on-site determination of carbendanzim, catechol and hydroquinone in tap water. BIA cells have been commonly fabricated in glass [27,37–39] or polymeric materials like polypropilene [34,35,40,41] with volume capacity between 40 and 200 mL. However, the production of these BIA cells involves laborious steps and requires sophisticated instrumentation as well as specialized technical services.

In this way, this report describes for the first time the fabrication and use of paper-based enzymatic reactors (PERs) for detection of glucose in artificial serum sample using a 3D printed BIA cell coupled with electrochemical detection. A 3D printer has been chosen to produce a BIA cell due to its capability of one-step manufacturing in relatively short processing time [42,43]. The parameters associated with the enzymatic reactions as well as the electrochemical measurements were successfully optimized prior to the quantitative analysis of glucose in artificial serum sample. The data achieved through the proposed devices were compared to the values provided by a reference analytical method.

2. Material and methods

2.1. Chemicals, materials and samples

Potassium chloride, monobasic and dibasic sodium phosphate salts, D-(+)-glucose, *N*-hydroxysuccinimide (NHS), sodium periodate, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), hydrogen peroxide aqueous solution (30%, w/w), glucose oxidase (GOx) (from Aspergigillus niger, 138.8 kU/g) were aquired from Sigma Aldrich Co. (Saint Louis, MO, USA). Filter paper (model JP 40, 12.5 cm diameter and 25 μm porous) was purchased from JProlab (São José dos Pinhais, PR, Brazil). The polymer composed of a mixture containing acrylonitrile butadiene styrene (ABS) was received from MovtecH Comercial Tecnologia LTDA-ME (São Bernardo do Campo, Brazil) to prepare the 3D electrochemical cell. All chemicals were of analytical grade and used as received, without further purification. Analytical solutions were prepared using ultrapure water processed through a water purification system (Direct-Q® 3, Milipore, Darmstadt, Germany) with resistivity equal or higher than 18.2 MΩ cm. A solution composed of 20 mmol L^{-1} phosphate buffered saline (PBS) and 0.1 mol L^{-1} KCl (pH 6.0) was used as supporting electrolyte. The glucose solutions were prepared in 200 mmol L^{-1} PBS solution. An artificial serum sample (lot number #SCNO 13041) was acquired from Doles Ltda. (Goiania, GO, Brazil).

2.2. Fabrication of paper-based reactors

The fabrication of PERs was performed in three sequential steps. First, the paper substrate was exposed to a 0.5 mol L^{-1} sodium periodate solution during 30 min at room temperature in the absence of light to convert hydroxyl into aldehyde groups, as previously demonstrated [19,20]. After the oxidation, filter paper sheets were immersed into a glass beaker containing 400 mL of ultrapure water and washed in an ultrasonic bath during 10 min. After washing step, paper sheets were dried at 60 °C during 30 min to remove the excess of water. Then, the paper platform was perforated with a paper punch to create microdisks with 1.25 cm diameter. Lastly, the paper surface was activated by adding 7.5 μL of a solution containing EDC/NHS (0.1 mol L^{-1} each) with subsequent drying at room temperature during 10 min. After the chemical activation step, an aliquot of 7.5 μL of GOx (138 U mL^{-1}) was added to the paper surface to be covalently immobilized. Prior to enzymatic assays, the paper platform was thoroughly washed in an ultrasonic bath with supporting electrolyte to remove the non-immobilized enzymes from its surface. During this procedure, five paper-reactors were placed inside a glass beaker containing 10 mL of supporting electrolyte solution and then washed during 10 min. Afterwards, they were kept at room temperature during 20 min for a complete drying.

2.2.1. Enzymatic assays

The feasibility of the PERs was demonstrated through the enzymatic assay for glucose detection, as schematically represented in Fig. 1. A single PER was firstly inserted into a plastic syringe and connected to a disposable microtube with volume capacity of 1 mL (Fig. 1a). Then, a 50-μL-aliquot of the glucose standard or artificial serum solutions were added on the reactor surface and allowed to react during 10 min (Fig. 1b). Afterwards, the PER was washed with 450 μL of a KCl solution prepared at concentration of 0.11 mol L^{-1} (Fig. 1c). The washing was carried out inside the syringe by pressuring the plunger. After the washing step, the product of the reaction was collected in the plastic microtube with an electronic micropipette (Fig. 1d) and analyzed through a 3D-printed BIA cell coupled with amperometric detection without any pre-treatment step.

2.3. Construction of 3D-printed BIA cell

The BIA cell was fabricated by using a 3D printer model RepRap from Prusa Movtech (MovtecH Comercial Tecnologia LTDA-ME, São Bernardo do Campo, Brazil) by fused deposition modeling (FDM) method. The desirable configuration was first designed in a three-dimensional model through the computer aided design (CAD) system in the graphic software AutoCAD® 2014 Free Student version (Autodesk Inc., San Rafael, USA). The CAD file was then converted in computer aided manufacturing (CAM) format and sent to the 3D printer in order to deposit layer-by-layer the ABS polymer according to the 3D layout. The dimensions of the 3D-printed cell were 9.0 cm × 9.0 cm × 3.5 cm, as shown in Fig. 2a. The electrolyte volume added on the 3D-printed cell was 100 mL. A rectangular cutting (1.0 cm width × 1.5 cm height × 1.5 cm length) was designed to ensure the coupling of electronic micropipette and SPE. The micropipette tip (100 μL Combitip®) was positioned around 2 mm from the center of the working electrode (Fig. 2b).