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A paper-based amperometric glucose biosensor developed with Prussian Blue-modified screen-printed electrodes

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ABSTRACT

This paper describes a simple inexpensive paper-based amperometric glucose biosensor developed based on Prussian Blue (PB)-modified screen-printed carbon electrodes (SPCEs). The use of cellulose paper proved to be a simple, "ideal" and green biocompatible immobilization matrix for glucose oxidase (GOx) as it was successfully embedded within the fibre matrix of paper via physical adsorption. The glucose biosensor allowed a small amount (0.5 μL) of sample solution for glucose analysis. The biosensor had a linear calibration range between 0.25 mM and 2.00 (R^2 = 0.987) and a detection limit of 0.01 mM glucose (S/N = 3). Interference study of selected potential interfering compounds on the biosensor response was investigated. Its analytical performance was demonstrated in the analysis of selected commercial glucose beverages. Despite the simplicity of the immobilization method, the biosensor retained ca. 72% of its activity after a storage period of 45 days.

1. Introduction

New strategies intended for rapid detection of analytes that are of clinical and environmental importance without requiring sophisticated instrumentation is currently in high demand [1]. Lately, paper has drawn much interest as a potential material for biosensors in analytical and clinical chemistry due to it being versatile, highly abundant and inexpensive [2]. Upon varying its pulp processing, paper can be made thin, lightweight and flexible to suit a specific application [3]. Cellulose fibres, being the key component of paper, allow liquid to infiltrate its hydrophilic fibre matrix with no active pumps or external sources required [4]. Hence, this makes paper a possible simple and effective matrix for enzyme immobilization in paper-based biosensors via physical adsorption. In fact, antibodies [5], DNA aptamers [6], phages [7] and cells [8] have also been applied in the development of viable paper-based biosensors apart from enzymes [9]. Many paper-based analytical devices are used as low-cost substitutes for medical point-of-care diagnostics due to the necessity of fast, reliable and affordable diagnostic tools in poverty-stricken developing countries [10]. Lately, Whitesides' group has developed techniques for creating microfluidic devices from patterned paper and demonstrated the use of paper-based microfluidic devices for colorimetric detection of glucose and protein in artificial urine [11–14].

Glucose oxidase (GOx) is widely used in practical applications for quick and precise glucose analysis. To date, 85% of the world biosensor market is currently dominated by electrochemical glucose biosensors [15,16] based on GOx-modified screen-printed carbon electrodes (SPCEs). As shown in Eq. (1), GOx catalyzes glucose oxidation in the presence of molecular oxygen (O_2) producing gluconic acid and hydrogen peroxide (H_2O_2).

$$\text{Glucose} + O_2 \xrightarrow{\text{GOx}} \text{Gluconic acid} + H_2O_2 \quad (1)$$

Monitoring of glucose levels can be based on either O_2 consumption or H_2O_2 generation. Traditionally, most electrochemical glucose biosensors were based on O_2 detection but the detection of H_2O_2 generation was found to be more sensitive [17]. At conventional electrodes, H_2O_2 detection occurs at relatively high potential ca. +0.60 V vs Ag/AgCl which leads to serious interference [18]. Thus, suitable electrocatalysts are employed to resolve this issue [17].

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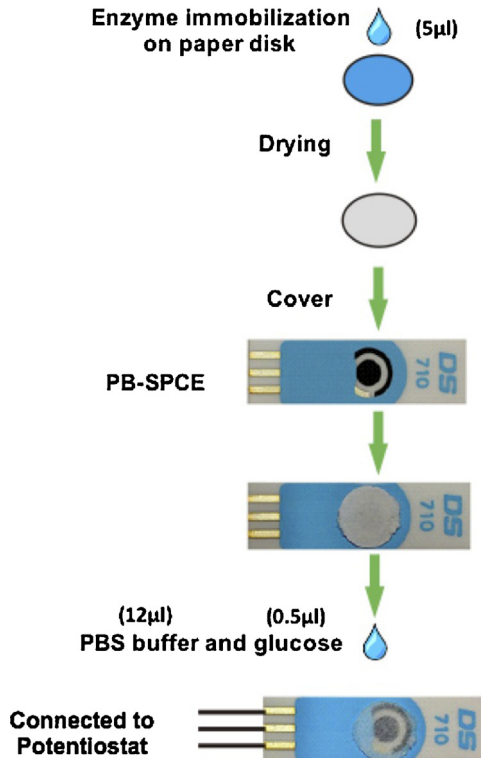

Fig. 1. Schematic illustration of GOx paper disc preparation and integration with the PB-SPCE. 5 μL GOx solution was dropped on the surface of the paper disc with subsequent drying in air at room temperature; the paper disc with immobilized GOx was placed on top of the PB-SPCE surface for complete coverage of working, counter and reference electrodes; 12 μL PBS buffer solution with 0.5 μL glucose solution was dropped on the paper disc for electrochemical detection.

Prussian Blue (PB) or ferric ferrocyanide ($Fe^{III}_4[Fe^{II}(CN)_6]_3$) has been used in the development of amperometric glucose biosensors because of its excellent electrocatalytic properties for H_2O_2 reduction [17,19–21] at low applied potential. The reduction current of H_2O_2 on PB-modified electrodes is two orders of magnitude higher than that of O_2 reduction for glucose analysis [22]. Moreover, the high catalytic activity and selectivity of PB lowers the operating potential to avoid or greatly reduce the contribution from potential interfering compounds, e.g. ascorbic acid [18,20]. Previous literature has reported applied potentials ranging from +0.18 to −0.40 V vs Ag/AgCl for PB-modified glucose biosensors [23–26]. Furthermore, an oxidized state of PB, Berlin Green, also allows glucose detection but it is based on electro-oxidation of H_2O_2 at PB-modified electrodes [17]. So far, PB-modified platinum [27], carbon paste [28], graphite [29], glassy carbon [21] and SPEs [30] have been studied for glucose detection.

Previously, we reported on the integration of paper disc with SPCEs for the development of a ferrocene carboxylic acid mediated glucose biosensor [31]. As the mediator was incorporated in the paper disc, it reached saturation and leached out easily. Saturation of the mediator on the paper disc could be a limiting factor for the linearity of the biosensor response. The applied potential of the above biosensor was in the positive range, ca. 0.25 V, a relatively high operating potential which could lead to interference from other oxidizable compounds, e.g. ascorbic acid. In this study, we would like to overcome the above limitations of the paper disc mediated glucose biosensor [31] and explore the development of glucose biosensor by immobilizing GOx within porous structure of paper discs placed on top of PB-modified SPCEs, as shown in Fig. 1. With this biosensor, only 0.5 μL of analyte was required for the detection of glucose. This results in cost saving due to a reduction in reagent consumption and prevents incessant exposure to bulk solution, as in a typical mediated glucose biosensor [31]. Therefore, leaching of PB from the SPCE, resulting in loss of catalytic activity, is significantly reduced. The various parameters for the optimization of the biosensor development such as applied potential, pH and GOx loading were investigated.

2. Experimental

2.1. Reagents and instrumentation

All reagents used were of analytical grade. GOx from *Aspergillus niger* (type X-S, EC 232-601-0; 234,900 U g^{-1} solid) were purchased from Sigma–Aldrich (St. Louis, MO, USA). β-D-Glucose was purchased from Nacalai Tesque (Kyoto, Japan). For validation studies, glucolin glucose powder (420 g) and hydralyte were purchased from a local supermarket and pharmacy store respectively. All solutions were prepared with 18 mΩ ultrapure water obtained from Millipore Alpha-Q water system (Bedford, MA, USA). All electrochemical characterizations and measurements were performed using a four-channel system (eDAQ QuadStat, e-Corder 8 and Echem software, eDAQ Europe, Poland).

PB-SPCEs (DRP-710) and the boxed connector for SPEs (DRP-DSC) were purchased from DropSens (Asturias, Spain). The working electrode (4 mm diameter) consisted of carbon/Prussian Blue, while Ag/AgCl and a carbon ring were the reference and counter electrodes, respectively. Paper discs were cut from Grade 1 filter papers (Whatman Asia Pacific Pte Ltd., Singapore). Data points were plotted using Microsoft Excel (USA) and ORIGIN (Northampton, MA, USA).

2.2. Preparation of GOx discs and electrochemical characterization

Grade 1 filter paper was cut into round discs with ca. 9 mm diameter using a paper punch. Then, 5 μL of GOx solution (150 U mL^{-1} in 0.1 M PBS, pH 7.0) was carefully added to each paper disc and allowed to dry at room temperature (25 °C). These paper discs laden with GOx were used for glucose analyses and stored at 4 °C for stability study of the biosensor.

Before conducting any electrochemical measurements, each new PB-SPCE was pre-conditioned by successive cyclic scanning at 25 mV s^{-1} from +0.30 V to −0.50 V for 5 cycles. The PB-SPCE was slotted into the boxed connector that was connected to the potentiostat. Typically, the paper disc with immobilized GOx was placed on top of the PB-SPCE to completely cover the working, counter and reference electrodes before each measurement.

As shown in Fig. 2, 12 μL of 0.1 M pH 7.0 PBS buffer solution was added onto the paper disc and good contact was formed with the PB-SPCE with this volume of buffer. Amperometric experiments were carried out at a potential of −0.30 V while cyclic voltammetry scans were performed from +0.30 V to −0.50 V at a scan rate of 10 mV s^{-1}. Unless otherwise indicated, all measurements were performed in triplicates on paper discs prepared using 5 μL from a 150 U mL^{-1} GOx solution (0.75 U $disc^{-1}$), unless stated otherwise, and glucose detection was performed using 0.5 μL from a 1 mM glucose solution. Furthermore, all different concentrations of GOx and glucose solutions were prepared using 0.1 M pH 7.0 PBS buffer solution, unless stated otherwise.

3. Results and discussion

3.1. Cyclic voltammetric investigation and electrocatalytic properties of the PB-SPCE

Typically, there are two major groups of peaks observed in cyclic voltammograms for PB-SPCEs. The cathodic group