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Electrochemical detection of glucose from whole blood using paper-based microfluidic devices

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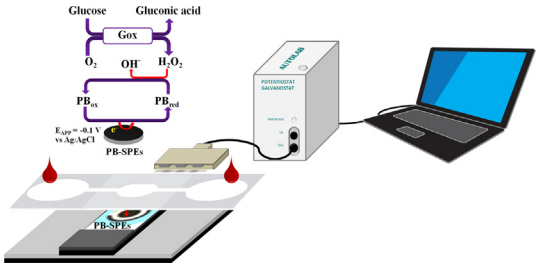
HIGHLIGHTS

- A novel platform of paper-based microfluidic devices for glucose assay from whole blood sample was established that incorporates plasma isolation methods.
- Dumbbell shaped μPADs was fabricated to obtain the uniform plasma separation and reproducible electrochemical signals.
- Two pieces of VF2 membranes combined with Whatman No. 1 paper using wax-dipping fabrication method to form a dumbbell shaped μPAD.
- Conventional Prussian blue-modified screen printed electrode (PB-SPEs) was placed underneath the middle part of a dumbbell shaped μPAD.

GRAPHICAL ABSTRACT



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ABSTRACT

Electrochemical paper-based analytical devices (ePADs) with integrated plasma isolation for determination of glucose from whole blood samples have been developed. A dumbbell shaped ePAD containing two blood separation zones (VF2 membranes) with a middle detection zone was fabricated using the wax dipping method. The dumbbell shaped device was designed to separate plasma while generating homogeneous flow to the middle detection zone of the ePAD. The proposed ePADs work with whole blood samples with 24–60% hematocrit without dilution, and the plasma was completely separated within 4 min. Glucose in isolated plasma separated was detected using glucose oxidase immobilized on the middle of the paper device. The hydrogen peroxide generated from the reaction between glucose and the enzyme pass through to a Prussian blue modified screen printed electrode (PB-SPEs). The currents measured using chronoamperometry at the optimal detection potential for H_2O_2 (−0.1 V versus Ag/AgCl reference electrode) were proportional to glucose concentrations in the whole blood. The linear range for glucose assay was in the range 0–33.1 mM (r^2 = 0.987). The coefficients of variation (CVs) of currents

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were 6.5%, 9.0% and 8.0% when assay whole blood sample containing glucose concentration at 3.4, 6.3, and 15.6 mM, respectively. Because each sample displayed intra-individual variation of electrochemical signal, glucose assay in whole blood samples were measured using the standard addition method. Results demonstrate that the ePAD glucose assay was not significantly different from the spectrophotometric method ($p = 0.376$, paired sample t-test, $n = 10$).

1. Introduction

Paper-based analytical devices, in the form of paper-based indicators and dipstick assays, have long been used in medical laboratories for rapid disease screening and diagnosis [1]. Generally, the utilities of paper devices were used for testing in emergency cases, self-monitor by patients and semi-quantitative screening applications. Recently, microfluidic paper-based analytical device (μPAD) was reported by Whiteside's group as alternative devices for point-of-care testing for developing countries [2]. μPADs provide simultaneous detection of multiple analytes on the same device, in which the platform is inexpensive, easy-to-use, and portable. μPADs can be fabricated using variety of methods, including photolithography [2–4], polydimethysiloxane (PDMS) plotting [5], plasma treatment [6], inkjet printing [7], paper cutting [8], wax printing [9], wax screen printing [10] and wax dipping [11]. The μPAD made from these techniques samples were transported through the hydrophilic flow channel of paper based on capillary forces without an assistance of any external power supplies such as syringes pump or high voltage power supply [12]. μPADs have been used for a variety of analytes including biochemical molecules [2,3,11,13–15] and environmental samples [16,17]. Moreover, the bioactive paper based devices have been extensively studied for blood group testing based on the principle of hemagglutination reaction [18–22]. There are different ways to detect analytes on paper based platforms, including colorimetry [2,3,11,23], electrochemistry [16,17,24], chemiluminescence [25], electrochemiluminescence [26] fluorescence [27] and surface enhanced Raman spectroscopy [28]. The most common detection method is colorimetry, which uses either an enzymatic or chemical reaction [12] on the hydrophilic area of paper to produce a color change. The resulting color intensity is proportional to the analyte's concentration. To quantify the analytes, a digital camera, cell phone, or scanner is typically used to record the color intensity [29]. However, errors can occur when recording the intensities from colorimetric assay on paper due to variations in the background color of paper, the lighting, and the manufacturing process [30]. Furthermore, the mobility of the fluid in porous network can cause uneven color development in the detection area. This is particularly problematic when the colored product concentrates at the edge of the detection zone. To avoid these problems, electrochemistry has been used as an alternative detection method with μPADs.

μPADs coupled with electrochemical detection or electrochemical paper-based analytical devices (ePADs) are capable of selective detection analytes at low levels (nM) [24]. Currently, there are two techniques reported for fabricating ePADs: (i) direct printing of electrodes onto paper [17,24,31] and (ii) by placing a μPAD onto a screen-printed electrode [16,32]. Dungchai et al. successfully fabricated the μPEDs for simultaneous detection of glucose, lactate and uric acid in biological samples [24]. Kubota et al. have reported a new approach for determination of glucose on paper using graphite pencil electrodes [33]. ePADs have found use for measurements in clinical [10,24,32,34] and environmental samples [16,17].

To work with whole blood samples, the process of separating plasma or serum is a frequently required. Centrifugation is the most common method for this separation [35]. As an alternative, several research groups attempted to directly separate plasma from whole blood using μPADs. Two general approaches for blood separation on μPADs have appeared. One approach is to use heamagglutination between immobilized antibodies and red blood cell antigen. The resulting plasma can be separated and flows through the hydrophilic paper [15]. The other approach uses different types of paper, e.g. blood separation membranes, connected with Whatman No.1 in either vertical [14] or lateral platforms [13]. Currently, paper based microfluidics for blood separation has been used for determining glucose [15], total protein, [13] and liver function [14], primarily using colorimetric detection. However, analysis of biological markers from whole blood sample based on ePADs has not been reported.

In this study, we fabricated a new μPAD platform for electrochemical detection of glucose from whole blood samples following plasma isolation. Effective separation of the blood cells from the plasma fraction is essential for reliable analysis of glucose. First, glucose levels are reduced when plasma specimens remain in prolonged contact with red blood cells [36]. In addition, hemoglobin interferes with the glucose assay [37].

Here, the wax dipping technique [11] was used to create the microfluidic patterns on the paper because it allows for two different types of paper to be joined together. The final device was composed of two blood separation zones that directed isolated plasma to a middle detection zone. The resulting flow generated a uniform gathering of plasma at the electrode and much better reproducibility of electrochemical signals. Selectivity of the method can be achieved by the proper choice of redox mediator and also detection potential. Commercially available Prussian blue modified screen-printed electrodes (PB-SPEs) selective catalyst for the hydrogen peroxide generated from the enzymatic reaction between glucose and glucose oxidase were attached underneath the paper detection zone. Combining electrochemical detection with plasma isolation μPADs is an alternative approach for point of care testing because it provides an inexpensive, easy-to-use, portable, and decrease time for pre and post analytical process.

2. Experimental

2.1. Materials, chemicals and equipments

All chemicals used were analytical grade. D-(+)-Glucose, glucose oxidase (*Aspergillus niger*–TypeII, 17,300 U mg^{-1}) and hemoglobin were purchased from Sigma (St. Louis, USA). Hydrogen peroxide (30%), potassium dihydrogen phosphate and dipotassium hydrogen phosphate were purchased from Merck (Darmstadt, Germany). Glucose assay reagent (Glucose liquicolor) and human control serum (HumatrolN, HumatrolP) were obtained from HUMAN (Wiesbaden, Germany). The stock solution of D-(+)-glucose was prepared in a potassium phosphate buffer (pH 6.0) and allowed to equilibrate overnight before use. Whatman No. 1 filter paper and blood separation paper, VF1 and VF2, were purchased from Whatman International Ltd. (Maidstone, England). Glass slides were obtained from Sail Brand (Jiansu, China). Iron molds were made-to-order by a laser cutting shop in Bangkok, Thailand. White pellets of beeswax and permanent magnets were purchased in Bangkok, Thailand. A hotplate (model C-MAG HS7) and thermometer (model ETS-DS) from IKA Company (Wilmington, USA) were used to