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Amperometric measurements of ethanol on paper with a glucometer

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ABSTRACT

Recent advances in electrochemical analysis on filter paper exemplify the versatility of this substrate for high performance testing. Its low-cost, light-weight, and environmentally friendly properties make it particularly attractive for applications in addressing health and environmental safety needs in low-resource settings and developing countries. However, the main drawback to sensitive electrochemical testing is the use of a potentiostat, a bench-top instrument that is extremely expensive, thereby negating the some of the benefits of paper-based devices. Hence there is a need to develop paper-devices for use with handheld, portable device readers that can extract quantitative readouts. In this study, we developed a method to use micro-paper electrochemical devices, or μPEDs, with a glucose meter, which are used for personal monitoring of blood glucose levels. Ethanol was chosen as a model target analyte due to its importance in the global issue of road safety. μPEDs were simple in design and could be tested with a potentiostat. We observed that inclusion of the stabilizer trehalose was critical to preparing μPEDs for later analysis. In addition, an NAD^+-dependent enzyme was used to impart selectivity to the biosensor, which also represents a class of enzymes with targets relevant to the health and food industry.

1. Introduction

Screen-printed (SP) biosensors are important tools for quick and sensitive detection of analytes important to health, food, and environmental safety [1–3]. Although conventional substrates for SP biosensors include ceramics and plastics, recent advances in high performance testing on filter paper suggest at its potential as a low-cost alternative for sensitive and quantitative electrochemical analysis [4–8]. Filter paper is particularly attractive in applications in global health [9], as it mitigates several barriers to entry in the developing world being that it is environmentally-friendly and extremely cheap. However, electrochemical testing requires a potentiostat in order to perform the necessary analysis, which can be costly, non-portable, and impractical for point-of-care use. In order for paper-based devices to succeed in the field and low-resource settings, there is an urgent need to develop robust and portable solutions that complement current analytical devices built on paper [10].

Examples of portable device readers for electrochemical analysis are limited, but exist in both the research and commercial spheres. In research settings, the CheapStat is an open-source schematic that provides instructions on building a potentiostat for USD $80 in-house [11]. Although the CheapStat allows for flexible and customizable biosensor design, it would be impractical to mass fabricate in the laboratory. Alternatively, commercial glucometers, used by people with diabetes to monitor blood glucose levels, are portable device readers that perform a single electrochemical test: amperometry. Amperometry is a powerful and simple technique that uses a 3-electrode system. A single potential is applied to an electrolytic solution and, in enzymatic-based biosensors, the resulting net redox reactions generate a Faradaic current that is proportional to the target analyte concentration. The current decays with time according to the Cottrell Equation [12].

$$i(t) = \mathrm{nFAC}(D/(\pi t))^{0.5}$$

Where F is Faraday's constant, n is the number of electrons transferred, A is the surface area of the working electrode, D is the diffusion constant, t is time, and C is concentration.

Glucose meters conduct amperometric sensing when a test strip is inserted and spotted with a blood sample. Although glucometers are not capable of the suite of electrochemical analysis techniques as can the CheapStat or a laboratory potentiostat, they are an excellent example of point-of-care testing. Glucometers are affordable (ranging from USD $30 to $100), robust, low-power, and can be used with little training, for personal care or professional medical monitoring. Of note, iBGStar (Sanofi-Aventis, USA) sells a small glucometer that can be connected to a smart phone for additional healthcare management.

The use of these amperometric device readers with paper electrochemical devices in the literature has been limited. The

Abbreviations: ADH, alcohol dehydrogenase; APDMES, 3-aminopropyldimethylsiloxane; NAD^+, beta-nicotinamide adenine dinucleotide; SP, screen-printed; μPED, micro-paper electrochemical devices

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CheapStat was used to measure glucose, lactose, and uric acid in urine with paper-based electrochemical tests [13]. Nie et al. fabricated paper devices that were compatible with a glucose meter (CVS brand), demonstrating detection of glucose, lactose, cholesterol, ethanol [14]. However, in the latter, the authors were required to replicate the complex electrode design of the commercial test strips with a laser cutter. Furthermore, they did not investigate the long-term use of their paper devices. Given that reagents degrade on filter paper [15,16], reagent stability should be evaluated in order to determine the robustness of the platform.

In order to address these challenges, we developed μPEDs, or micro-paper electrochemical devices, for the detection of ethanol using a commercial glucose meter. The devices were simple in design and easy to fabricate. We used one device design that allowed ethanol concentrations to be measured by both a glucometer and a potentiostat. We demonstrated long-term potential with the stabilizer, trehalose. Ethanol, the consumable form of alcohol, was chosen as the model analyte for this platform for its application in assessing the global issues and challenges associated with road safety in developing nations. Furthermore, we used an NAD^+-dependent enzyme to selectively target ethanol, which represents a large class of enzymes used in the food and dairy industry [17], and is largely underrepresented in the literature for paper-based diagnostics.

2. Methods

2.1. Materials

A commercial glucometer was purchased from a local pharmacy for approximately USD$40 (OneTouch, Lifescan, Inc., USA). Alcohol Dehydrogenase from *Saccharomyces cerevisiae*, beta-nicotinamide dinucleotide (NAD^+), potassium ferricyanide, ethanol, phosphate buffer (PB) was purchased from Sigma. Whatman Grade-1 filter paper was purchased from Fisher Scientific. Trehalose was donated from SriTechnologies (GA, USA). The pH of the phosphate buffer (PB) was adjusted with sodium hydroxide. 3-aminopropyldimethylsiloxane (APDMES) was purchased from Gelest, Inc (PA, USA). Graphite ink was purchased from Ercon, Inc. (MA, USA).

2.2. μPED fabrication

Reagent preparation and fabrication of the μPEDs were based on previously described methods [8,14]. Briefly, circular hydrophobic barriers were patterned onto filter paper (Whatman Grade-1) using a commercial wax printer. The wax-patterned papers were melted on a hot plate for 3 min at 100 °C. Then, graphite was screen-printed using a homemade stencil. The stencil pattern was designed in AutoCad and cut into cellulose acetate film, 0.05 mm thick, with a cutter plotter (Graphtec Craft ROBO Pro, Graphtec America, CA, USA). The patterned sheets were dried on a hot plate for 20 min at 65 °C, then cooled at room temperature for 1 min. An example of resulting μPEDs are shown in Fig. 1D.

2.3. μPED optimization and preparation

In order for ethanol to be successfully detected on μPEDs with a glucose meter, multiple iterations of optimization were performed. μPED designs varied, including by electrode dimensions, reference electrode material, and working electrode surface area. Reagent optimization involved varying the concentration, volume, and ratio (v/v) of the sample solution to the detection reagent solution (which contained ADH, NAD^+, and KCN). The sample volume and time to allow the sample to wet the μPED was also determined to be relevant optimization parameters. Due to the narrow range of currents detectable by the glucose meter, successful optimization of μPEDs was determined if the glucose meter displayed a numerical value within 10 insertions of the same μPED (see Section 2.5).

After optimization, each μPED was spotted twice with 4 μl of 2% wt 3-aminopropyldimethylsiloxane (APDMES), with 15 min to dry between each spotting. To test the response of the tests using a glucose meter, the following stock solution of detection reagent was prepared: 160 Units/ml ADH, 5 mmol L^{-1} NAD^+, 500 mmol L^{-1} KCN (0.1 mol L^{-1} PB, pH 8). Due to the light-sensitive nature of NAD^+ and KCN, these reagents were prepared in the dark. As necessary, trehalose was added to the reagent stock solution to a final concentration of 5% (w/v). Sample solutions of ethanol were prepared in glass vials and diluted in 0.1 mol L^{-1} PB, pH 8.

2.4. Relevant glucometer circuitry

Although the circuitry of a commercial glucometer was proprietary, we gained useful information through visual and electrical analysis using a multimeter. First, the insertion port for the test strip contained five pins (Fig. 1). Pins 1, 2, and 3 connected to the working, counter, and reference electrode of the test strip. Pins 4 and 5 also connected to the test strip and when short-circuited, turned on the glucometer. These latter pins were only necessary for turning on the glucometer and had no electrical effect on the electrodes. When turned on, two pins maintained a constant potential difference of approximately +0.4 V. We screen-printed a strip of carbon paste onto a 0.03 mm acetate film that could short-circuit Pins 4 and 5 (Fig. 1). The short-circuit strip was thin enough to allow insertion of the μPED later.

For each measurement in our study, the glucometer was turned on by short-circuiting Pins 4 and 5. A μPED was then inserted. Upon introduction of a sample, the glucometer immediately began a 5 s countdown. There were four general outputs by the meter: “Lo”, “Hi”, “Er” for error, and a numerical value between 0 and 600 (calibrated to a concentration of glucose).

2.5. Ethanol analysis using a glucometer

After optimization, μPEDs were tested with (1) freshly spotted reagents and (2) dried reagents. For the former, the glucometer was manually turned on by short-circuiting Pins 1 and 2. A μPED was then inserted, followed by spotting a 1:1 mixture of the reagent stock solution and sample (6 μl total). The meter output was recorded. Multiple readings were taken of the same μPED by re-shortcircuiting Pins 4 and 5, then reinserting the μPED. Results were recorded until an “error” message was obtained twice in a row.

For the latter, 5 μl of detection reagent solution was spotted onto each μPED and allowed to dry for at least 1 h at room temperature. To test these μPEDs, the glucometer was first turned on. Sample solution (8 μl) was spotted onto the μPED, left to incubate for 5 s, then inserted into the glucometer, and the results recorded as described.

2.6. Electrochemical testing

The potentiostat (CH Instruments 440) was used to perform amperometry at +0.4 V versus carbon. To mimic the glucometer, amperometry was performed using the potentiostat, but alternating the potential between open circuit potential (0 V vs carbon) and +0.4 V versus carbon every 5 s. The μPEDs and reagents were of the same design and concentrations, respectively.