

Microfluidic Paper-Based Electrochemical Biosensor

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Abstract—A low-cost paper-based microfluidic device (PAD) for the multiplex electrochemical determination of glucose, uric acid, and dopamine in biological fluids was developed. Using wax printing, PAD containing a central zone, six channels, and six detection zones was fabricated, and the electrodes were printed on detection zones using pre-made electrodes template. For each analyte, two detection zones were used. The carbon working electrode was coated with chitosan-BSA (and enzymes for glucose and uric acid). To detect glucose and uric acid, enzymatic reactions were employed. These reactions involve enzyme-catalyzed redox reactions of the analytes and produce free electrons for electrochemical measurement. Calibration curves were linear ($R^2 > 0.980$) in the range of 0–80 mM for glucose, 0.09–0.9 mM for dopamine, and 0–50 mM for uric acid, respectively. Blood samples were successfully analyzed by the proposed method.

Keywords—Multiplex, microfluidic paper-based electrochemical biosensors, biomarkers, biological fluids.

I. INTRODUCTION

POINT-OF-CARE (POC) has become commonplace in developed nations as well as in developing nations as a way to reduce the number of clinical visits, increase patient satisfaction, increase patient compliance, decrease costs to the healthcare system, improve clinical outcomes, and provide clinical services for people [1]–[3].

Much effort has been dedicated to the development of quantitative paper strip tests, but these devices still required instrumentation and are limited to a single analyte [4], [5]. As an alternative to traditional immune-chromatographic tests, Martinez et al. introduced paper-based microfluidics [6], [7]. This approach, which combines advantages of paper strip tests and microfluidics, holds significant potentials due to its low sample volume, low cost, multianalyte capability, and portability [6]–[9].

The low-cost paper-based microfluidic device (PAD) can be fabricated using wax printing. The wax provides hydrophobic barriers to form hydrophilic areas for detection. Sample can be easily introduced by capillary action through the channel without any additional liquid driving device. PADs have been developed for uric acid, glucose, lactate, protein, and cholesterol detection [6], [9].

Due to its superior analytical performance such as high accuracy and sensitivity, the electrochemical detection is attracting research interests in PAD designs [7], [10]. Electrochemical PADs have lower LODs and higher sensitivity than the PADs using colorimetric detection.

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Furthermore, electrochemical determination is not sensitive to impurities in samples and ambient conditions [4], [11].

Herein, we developed a low-cost and simple PAD for simultaneous determination of glucose, uric acid, and dopamine in biological fluids. The PAD was fabricated through wax printing, and the sample was easily introduced by capillary action through the hydrophobic channel. Enzymatic reactions were used to detect glucose and uric acid. For dopamine detection, dopamine is oxidized to dopamine o-quinone, and the electrons can be used in $\text{Fe}^{3+}/\text{Fe}^{2+}$ reaction. Dynamic ranges for the three analytes were then investigated. The PAD has been successfully applied in multiplex detection of biomarkers in biological fluids.

II. EXPERIMENTAL

A. Materials and Equipment

All chemicals were used as received without further purification. Uric acid, d-(+)-Glucose, glucose oxidase (from *Aspergillus niger*, 215Umg^{-1}), uricase (from *Candida* sp., 2Umg^{-1}), dopamine, BSA, and potassium ferricyanide were purchased from Sigma–Aldrich (St. Louis, MO). Whatman #1 filter paper was obtained from Cole-Parmer (Vernon Hills, IL).

B. Fabrication of Paper Based Microfluidic Biosensors and Electrodes

The flow channels terminating in six detection zones connected to a central sample deposition spot were created as shown in Fig. 1. The diameter of central and detection zones was 1 and 0.5 cm, respectively. The length and width diameters of channels were 1 mm and 0.3 mm, respectively. The pattern of the PAD was drawn by Adobe Illustrator.

To construct the hydrophobic barriers on paper, wax printing was used. In brief, a sheet of Whatman paper was put into a melted wax container. After withdrawing this wax-treated paper from the container and cooling to room temperature, an untreated paper was placed on it. Finally, a pre-heated metal template was put on this complex using hand pressure for one second. The designed pattern was printed on the surface of the untreated paper. This wax printing process could be finalized within a few minutes, and was capable to produce hundreds of copies of PAD.

In each detection zone, three electrode systems were printed using home-made template (Fig. 1). For working and counter electrodes, carbon ink was used. For quasi-reference electrode, silver ink was used. The working electrode has diameter of 0.020 cm^2 .

The carbon working electrodes in glucose detection zones and in uric acid detection zones were coated with chitosan-BSA-glucose oxidase and chitosan-BSA-uricase, respectively.

In dopamine detection zones, chitosan-BSA was coated on the working electrodes. Sample was added to sample deposition spot and flowed toward the detection zones via capillary forces.

To detect glucose and uric acid, enzymatic reactions were employed. The corresponding enzymes and electron-transfer mediators were incubated in the test zones to react with the target analytes. For dopamine detection, dopamine is oxidized to dopamine o-quinone and the electrons can be used in $\text{Fe}^{3+}/\text{Fe}^{2+}$ reaction.

Each electrochemical cells, on the PAD were characterized using 5 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$ in 0.5 M KCl, through cyclic voltammetry (CV). Using CV, the $\text{Fe}(\text{CN})_6^{3-}$ was reduced at the cathodic electrode and the resultant $\text{Fe}(\text{CN})_6^{4-}$ was oxidized at the anodic electrode (Fig. 2).

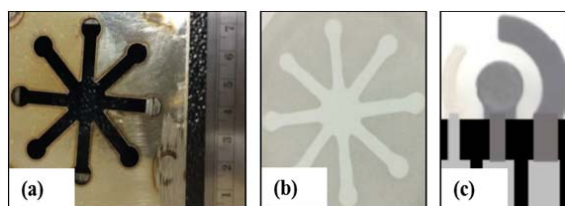


Fig. 1 (a) The stainless steel pattern for PAD fabrication, (b) The PAD, and (c) From left to right, reference, working, and counter electrodes produced by electrochemical pattern

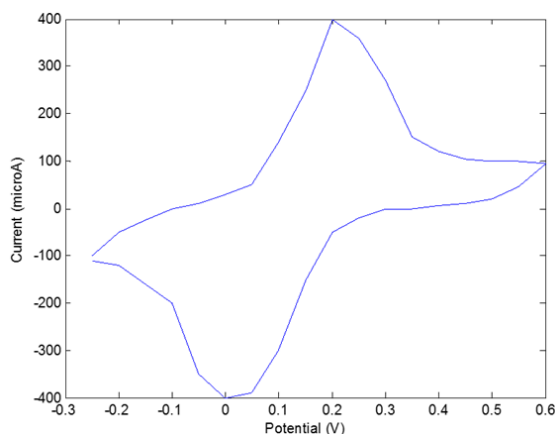


Fig. 2 Electrochemical behavior study of 5 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$ solution in 0.5 M KCl using PAD

C. Assay Procedures

The PAD was used for multiplex detection of the biomarkers in biological fluids. 60 μL of sample was added to central zone and kept in a humid chamber at RT for 5 min.

To detect glucose and uric acid, enzymatic reactions were employed. During the reaction, the enzyme catalyzed the oxidation of corresponding analyte into their oxidized products (glucose to gluconic acid and uric acid to allantoin), with a concomitant reduction of $\text{Fe}(\text{CN})_6^{3-}$ into $\text{Fe}(\text{CN})_6^{4-}$ and the generated electrons were transferred between the

electrodes and were quantified using chronoamperometry. The excel software was employed to perform data analysis.

III. RESULTS AND DISCUSSION

A. Effect of Reagent Volume

First of all, the volume of reagent required for analysis was determined. In commercially paper test strips, the major cost comes from reagents. Hence, the detection zones were designed to be small (0.5 cm diameter) to reduce reagent consumption.

After that, by dropping food dye in the range of 0.5–5 μL into the detection zone, the reagent volume necessary to wet each detection zone was determined. As the results, 0.5 μL of reagent solution cannot completely wet the detection zone whereas 5 μL spread outside the zone. We selected 3 μL of reagent solution for the further experiments.

B. Lifetime of the PAD

Diagnostic devices must remain stable for weeks to be useful in the field. Hence, the performance of PAD was studied after storing the ready-to-use PADs for multiple days at varying temperatures. To test the stability, prepared PADs were dried at ambient condition before storage at either 4 $^{\circ}\text{C}$, room temperature (25 $^{\circ}\text{C}$), or 45 $^{\circ}\text{C}$. Oxidase enzymes can unfold, aggregate, or degrade during dry storage [4]. To stabilize dried proteins during storage, polyols such as mannitol and non-reducing sugars such as trehalose and sucrose can be used [4], [12], [13]. To improve the stability of the enzyme during storage, we added mannitol to solution during PADs preparation. At 14 storage days at room temperature and 45 $^{\circ}\text{C}$, an observable signal decrease was noted. The results showed that the PADs can be kept for 11 days without loss of activity (Fig. 3).

C. Sensitive and Selective Detection of the Biomarkers

To demonstrate the efficacy of the system, the dynamic ranges of glucose, dopamine, and uric acid were studied. Under optimized conditions, various concentrations of the biomarkers were tested on the PAD. Furthermore, the control (blank) tests were used. The calibration curve for each biomarker was established by plotting current intensities against different concentrations. The calibration curve of the glucose, dopamine, and uric acid were in the ranges of 0–80 mM ($R^2 = 0.990$), 0.09–0.9 mM ($R^2 = 0.980$), and 0–50 mM ($R^2 = 0.986$), respectively. Based on $3\sigma/(\text{slope})$ method, the limits of detection for glucose, dopamine, and uric acid were 0.03 mM, 0.01 mM and 0.09 mM, respectively (Table I).

To investigate the selectivity of the proposed PAD for glucose, dopamine, and uric acid detection, solutions of the three analytes were prepared separately. In each experiment, one of the solutions was inserted in the central zone. For example, when we inserted the glucose solution, only the signals of glucose detection zones were changed and the signals for dopamine and uric acid did not change apparently. The results indicate excellent specificity toward the analytes.

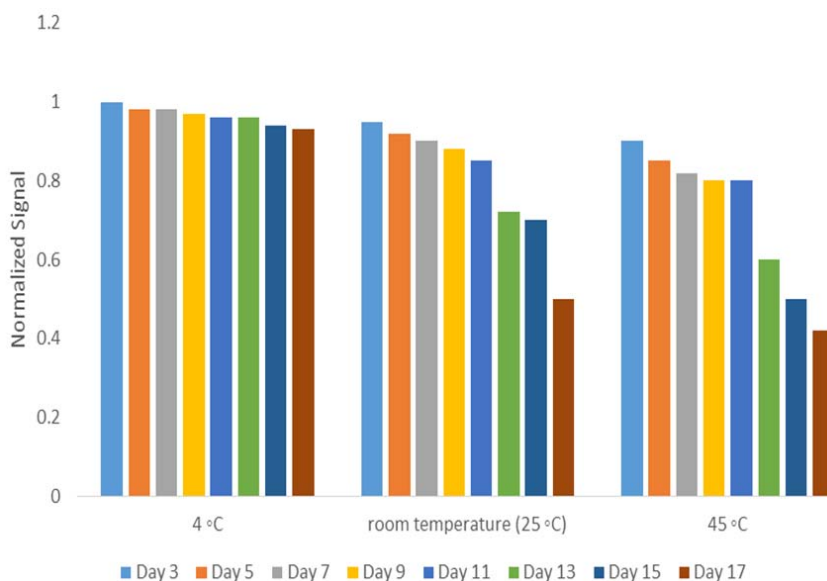


Fig. 3 Lifetime study of the PAD in 3 different temperatures

TABLE I
LINEAR DYNAMIC RANGE AND LIMIT OF DETECTION (MM) OF THE PROPOSED METHOD

Analyte	Linear Dynamic Range	LOD	R ²
Glucose	0-80	0.03	0.990
Dopamine	0.09-0.9	0.01	0.980
Uric acid	0-50	0.09	0.986

D. Sample Detection

The PAD device was next evaluated for glucose, dopamine, and uric acid analysis in real samples.

Human blood samples were selected as real samples for analysis by the PAD device, and the results are presented in Table II. This method revealed good average recovery rates from 95% to 102%. These values are in accordance with the required accuracy in trace analysis from complex matrix samples. The results showed that our PAD can successfully be applied for glucose, dopamine, and uric acid screening tests.

IV. CONCLUSION

As a proof of concept, the PAD for multiplexed detection of three biomarkers including glucose, dopamine, and uric acid was used.

Our PADs were successfully applied to simultaneous analysis of glucose, dopamine, and uric acid in human blood samples.

TABLE II
DETERMINATION (MM) OF GLUCOSE, DOPAMINE, AND URIC ACID IN BLOOD SAMPLES (N=3)

Samples	Analytes	Detected	Added	Found	RSD (%)
Blood 1	Glucose	4.2	10.0	14.1	2.1
	Dopamine	-	0.20	0.19	5.3
	Uric acid	0.9	5.0	6.0	1.8
Blood 2	Glucose	4.8	20.0	25.0	3.5
	Dopamine	-	0.10	0.11	8.7
	Uric acid	0.5	10.0	10.5	1.1

The results indicated the feasibility of multiplex detection of analytes by the PADs and showed that our PAD provides sufficient analytical performance (dynamic linear range and LOD) for clinical assays of all the three biomarkers. Our home-made PADs are sensitive, selective, easy-to-use, inexpensive, and portable. It is easy to successfully extend this method for the simultaneous detection of other biomarkers and analytes. This PAD platform will have promising applications in on-site environmental and POC diagnosis tests.

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