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LOW-COST MICROFLUIDIC ELECTROCHEMICAL PAPER-BASED DEVICE TO DETECT GLUCOSE

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ABSTRACT

Continuous monitoring of the level of glucose is essential to maintain the health of a diabetic patient. The use of invasive methods to obtain blood samples to detect glucose is the current method, but such methods may not be preferred by patients. Non-invasive methods such as the determination of glucose based on saliva or sweat can be preferred by patients for continuous monitoring. A low-cost electrochemical microfluidic paper-based analytical device (µPAD) suitable to monitor glucose levels using sweat and saliva is introduced. This device is extremely low-cost due to the use of a paper matrix and varnish for the fabrication of paper. Pseudo-reference stainless steel is used with a working electrode anodized using sodium potassium tartrate tetrahydrate for the development of a three-electrode system. Cyclic voltammetric-based analysis of glucose with µPAD produced a linear response in the working range of 1-10 mmol dm^{-3} with a limit of detection of 0.058 mol dm^{-3}. The detection of diabetic conditions using saliva and sweat is possible using the device by correlating to the blood glucose levels using prior literature data.

Keywords: Non-invasive, low-cost µPAD, diabetic monitoring, non-enzymatic, environmentally friendly

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INTRODUCTION

Estimating the level of blood glucose using body fluids other than the blood can eliminate the invasive process of blood collection for glucose analysis. Non-invasive collection of saliva and sweat can be conducted easily compared to the invasive method used for the collection of blood. The glucose level in saliva or sweat can be used to estimate the blood glucose levels using the lag time between blood and other biological fluids such as sweat and saliva (Jang and Xu 2018). Unlike invasive devices, non-invasive devices can be used to monitor the body glucose level continuously (Cao *et al.*, 2019). Continuous monitoring allows managing the body glucose level effectively over non-continuous methods. Wearable or tattoo-based electrochemical devices can be used to develop continuous monitoring devices (Bruen *et al.*, 2017).

Paper is the most economical platform material used for the development of point-of-care or wearable electrochemical devices for continuous monitoring (Ataide *et al*., 2020). Microfluidic paper-based analytical devices (µPAD) are economically beneficial and can be used without complicated devices even by a non-technical person to collect scientific information easily. Also, the use of paper for the fabrication of the device is an environmentally friendly approach. The reference electrode of the three-electrode system is typically developed using Ag/AgCl. The use of Ag can make the device expensive and the waste management process is complex due to the toxic effect of Ag. The use of low-cost pseudo-reference electrode material was reported for the development of paper-based devices (Nissanka *et at.* 2022). The use of enzymes for modification is a common method to introduce selectivity to the working electrode. However, an enzyme-based electrode is less stable and the extraction of enzyme for the modification is a complex process (Teymourian *et al*. 2020). Fabrication is the critical step to handle the fluids on paper-based devices, and multiple methods are used for the development of required hydrophobic patterns on the paper. The use of low-cost household industrial-scale varnish paint is reported previously (Nissanka *et at.* 2022). A combination of a low-cost pseudo-reference electrode and simple non-enzyme material used for the modification of working electrodes to develop paper-based devices fabricated with varnish is an economical approach to develop wearable short-term devices. The durability of the paper-based matrix limits the long-term use of these devices (Nissanka *et at.* 2022). These short-term devices can be used to collect real-time glucose levels for a limited period.

The work described in this paper explains the use of low-cost stainless steel as the pseudo-reference electrode material for the development of paper-based devices. The introduced device is suitable for real-time short-term monitoring of the level of glucose in sweat and saliva. The fabrication of the hydrophobic patterns is performed using varnish to reduce the cost.

METHODOLOGY

Whatman No.1 filter paper was acquired from GE Healthcare UK Ltd, Buckinghamshire, UK. Stainless steel and copper wire were purchased from a local vendor in Colombo, Sri Lanka. Polyurethane varnish was purchased from Nippon Paint Lanka (Pvt) Ltd, Rajagiriya, Sri Lanka. HB #2 pencil was acquired from Printxcel Pvt. LTD, Athurugiriya, Sri Lanka. Super Glue was purchased from ALTECO Group of Companies, Japan. Cu wire (30 mm length and 1 mm diameter) was cleaned by dipping in a 50 mmol dm^{-3} KOH solution with 25% (v/v) H_2O_2 for 10 min. Cleaned Cu wire was anodized using 5 scans of cyclic voltammetric (CV) from -1 V to +1 V at a scan rate of 100 mV s^{-1} in 0.5 mol dm^{-3} sodium potassium tartrate tetrahydrate. Whatman No.1 filter paper was used as the platform for the paper-based device. The hydrophobic area of an inner diameter of 12 mm and outer diameter of 20 mm was fabricated using polyurethane varnish. Super Glue was used to attach a 30 mm HB#2 pencil graphite rod as the counter electrode (CE), 30 mm modified Cu wire working electrode (WE) and 30 mm stainless-steel sheet pseudo-reference electrode (RE) onto the paper platform. A piece of packing tape was used to seal the bottom of the sample well to prevent leaking (Figure 01).

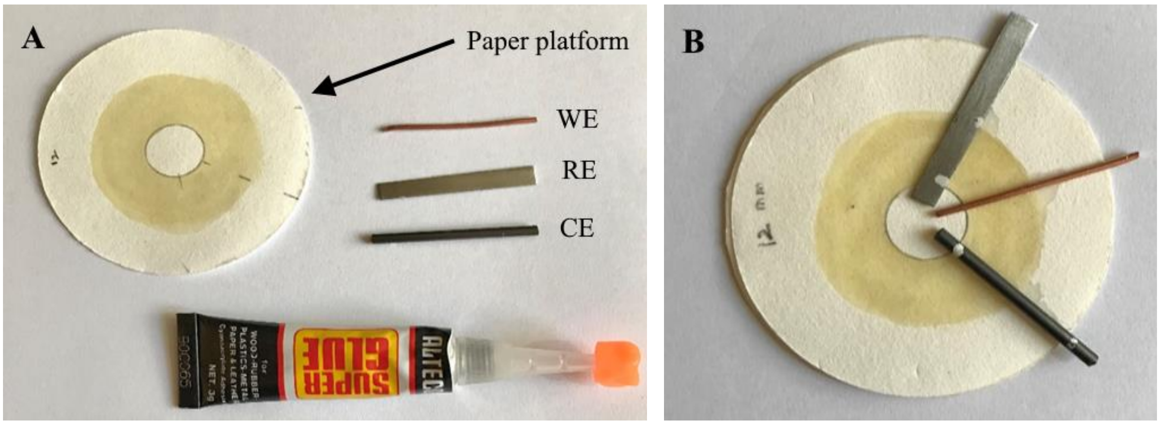

Figure 1: A. Paper platform with polyurethane varnish-based hydrophobic, WE - Cu working electrode, RE - stainless steel pseudo reference electrode, and CE - HB #2 pencil graphite rod counter electrode. B. Finalized three-electrode paper-based µPAD.

The developed paper-based device was tested using a volume of 100 µL 10 mmol dm^{-3} $K_4Fe(CN)_6$ / $K_3Fe(CN)_6$ in 0.1 M KCl by performing a CV scan from -0.8 V to 0.8 V at a scan rate of 25 mV s^{-1}. The use of a stainless steel pseudo-reference electrode was validated against the standard Ag/AgCl reference electrode.

A concentration series of glucose (2, 3, 4, 5, 6, 7, 8, 9, 10 mmol dm^{-3}) was prepared by mixing respective volumes of a glucose stock solution (50 mmol dm^{-3}) with 0.1 mol dm^{-3} NaOH. The same paper-based electroanalytical device fabricated with the modified Cu electrode was used to collect three responses of each glucose concentration to develop the calibration plot. The CV was carried out for the concentration series of glucose in the potential range of -0.3 V to 1 V at a scan rate of 25 mV s^{-1}.

With ten independent blank samples, the limit of detection (LoD) and limit of quantification (LoQ) was calculated using LoD = $\bar{x}$ + 3SD and LoQ = $\bar{x}$ + 10SD, respectively. Repeatability, reproducibility, and precision were determined using 10 independently prepared samples of 5.5 mmol dm^{-3} glucose in 0.1 mol dm^{-3} NaOH. The interference was due to uric acid was studied. A synthetic sweat solution with the concentration of 6 mmol dm^{-3} glucose in 0.1 mol dm^{-3} NaOH was spiked with 6 mmol dm^{-3} uric acid was tested using the same CV parameters used for the analysis of glucose to determine the interference.

RESULTS AND DISCUSSION

Whatman No.1 filter paper was selected as the platform for the development of a low-cost, biodegradable, fast and portable paper-based device for the detection of glucose. The Whatman No.1 filter paper was specifically used in the developed µPAD due to its retention properties, ease of modification and lightweight. The material cost for the development of a single device is roughly 0.1 United States Dollars. Low-cost polyurethane varnish was used to fabricate the hydrophobic barrier due to the ease of producing patterns (Nissanka *et at.* 2022). Polyurethane varnish diffuses throughout the paper easily and acts as an excellent electric insulator. A circular sample introducing zone with an inner diameter of 12 mm was fabricated. Electrodes are placed with similar spacing between them by allowing a uniform flow of the sample towards all electrodes.

The cleaning step (dipping in a 50 mmol dm^{-3} KOH solution with 25% (v/v) H_2O_2 for 10 min) enhances the effectiveness of the modification process of the WE. Low-cost, low resistance and workability are the other advantages of using Cu for the development of working electrodes. The surface of the copper electrode was modified to create CuO by electrochemical anodization with a solution of 0.5 mol dm^{-3} sodium potassium tartrate tetrahydrate (Keqing Han *et al*., 2017). The HB #2 pencil carbon rod was used due to the wider availability and low cost. A thin stainless-steel plate was used as a pseudo-reference electrode due to its low cost and applicability. Based on the CV with a mixture of 10 mmol dm^{-3} $K_4Fe(CN)_6$ / $K_3Fe(CN)_6$ in 0.1 mol dm^{-3} KCl, the anodic peak potentials were 0.43 V and 0.30 V with standard Ag/AgCl reference and stainless-steel pseudo reference electrodes, respectively as shown in Figure 02. A potential shift of ~0.13 V was observed due to the defined potential of the pseudo reference electrode. Defined current peaks observed with stainless steel pseudo reference electrode for 10 mmol dm^{-3} $K_4Fe(CN)_6$ / $K_3Fe(CN)_6$ in 0.1 mol dm^{-3} KCl confirms the suitability of stainless steel as the reference electrode.

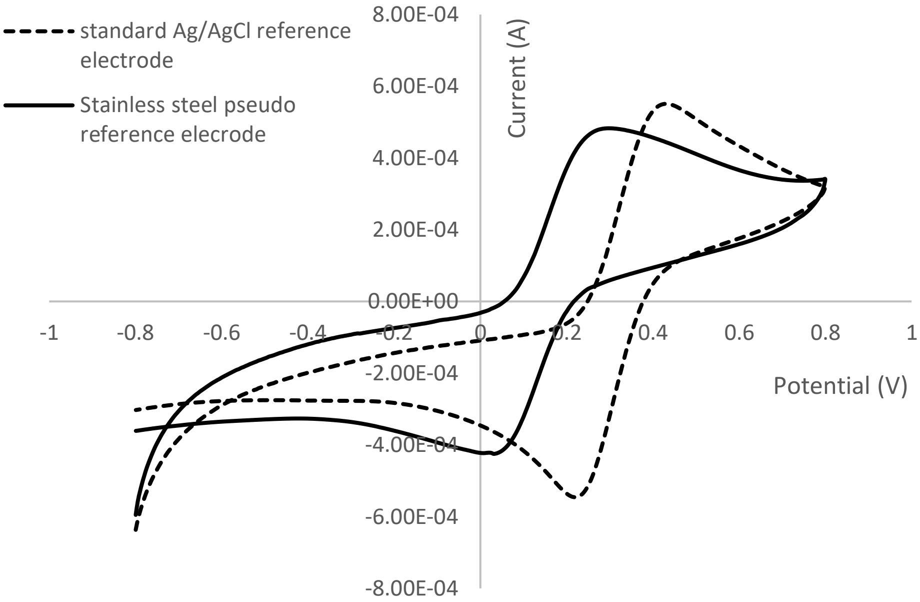

Figure 2: Cyclic voltammograms collect for 10 mM $K_4Fe(CN)_6$ / $K_3Fe(CN)_6$ in 0.1 mol dm^{-3} KCl with standard Ag/AgCl reference electrode and stainless steel pseudo reference electrode based µPAD at a scan rate of 25 mV s^{-1} for -0.75 to +0.75 V.

The cyclic voltammograms collected for the modified working electrodes under the optimized parameters using the µPAD in the absence and presence of 5 mM glucose in 0.1 M NaOH are shown in Figure 03. Based on the cyclic voltammograms, bare copper surface and anodized copper electrochemically react with glucose in alkaline media. Metallic copper catalyzes the oxidation of glucose in alkaline media. The high current response of the modified electrode shows the increase in sensitivity of the anodized copper for glucose determination.

The voltammograms collected using the developed µPAD at a scan rate of 25 mV s^{-1} for -0.75 to +0.75 V for the glucose solutions in 0.1 mol dm^{-3} are shown in Figure 04. Peak current at 0.7 V at each glucose concentration ranging from 2 to 10 mmol dm^{-3} collected at a scan rate of 25 mV/s is shown in Figure 05. A linear relation between the current and the glucose concentration was observed in the working range of 2 – 10 mmol dm^{-3} glucose. The linear regression equation and the correlation coefficient for the constructed calibration plot are $y = 3\times10^{-5}x + 2\times10^{-5}$ and $R^2 = 0.9764$, respectively.

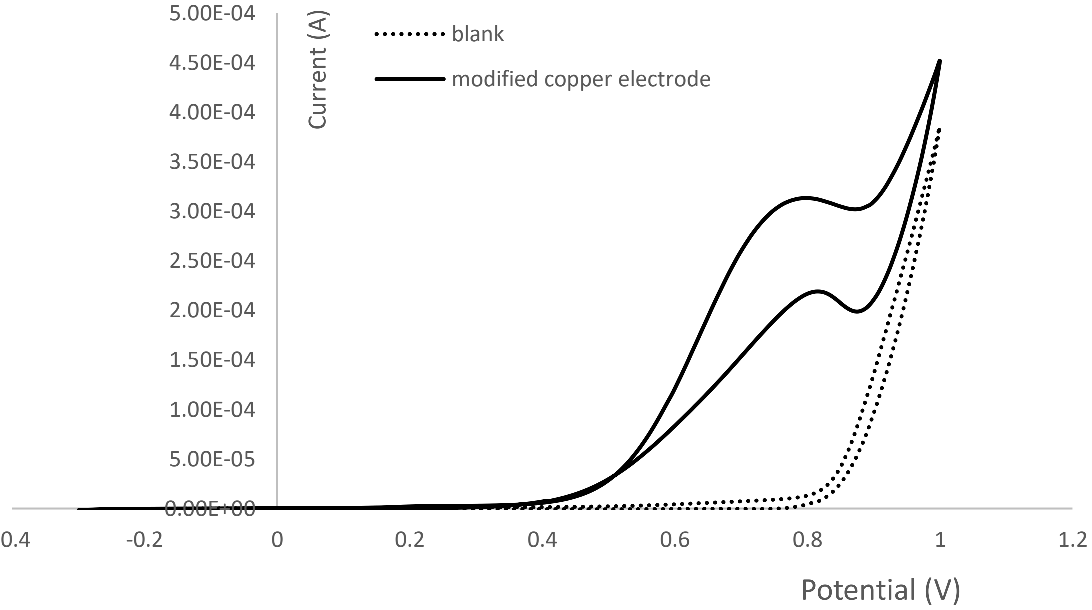

Figure 3: Cyclic voltammograms collected at a scan rate of 25 mV s^{-1} using the modified copper electrode in 0.1 M NaOH and 5 mM glucose in 0.1 M NaOH.

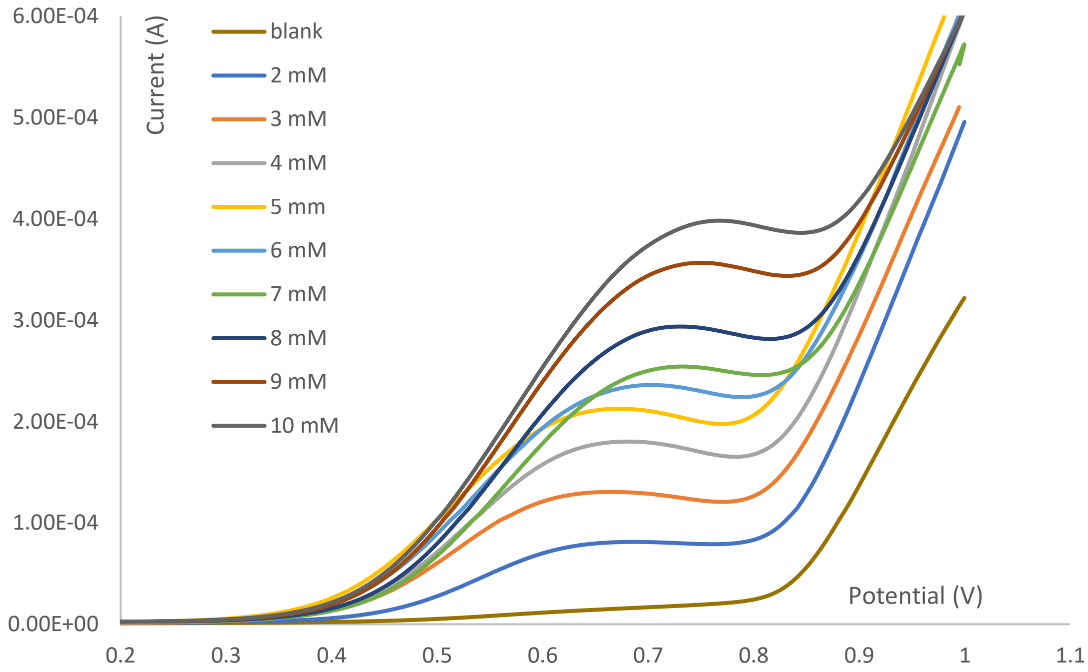

Figure 4: Collected cyclic voltammograms using the developed μPAD at a scan rate of 25 mV s^{-1} for glucose solutions of 2, 3, 4, 5, 6, 7, 8, 9, 10 mmol dm^{-3} in 0.1 mol dm^{-3}

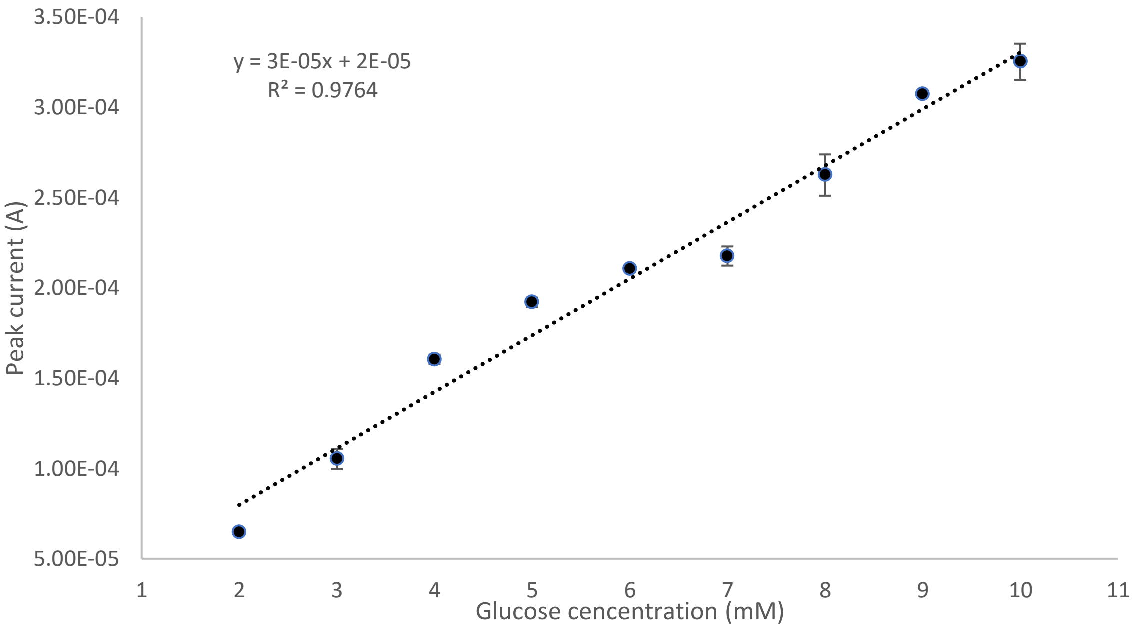

Figure 5: The calibration plot of peak current collected at 0.7 V of the voltammograms obtained at a scan rate of 25 mV s^{-1} for -0.75 to +0.75 V using glucose concentrations of 2, 3, 4, 5, 6, 7, 8, 9, 10 mmol dm^{-3} in 0.1 mol dm^{-3} NaOH with the developed μPAD.

Limit of detection (LoD) and limit of quantification (LoQ) of the glucose detection method based on the developed µPAD are 0.05 and 0.48 mmol dm^{-3}, respectively. LoD values of similar methods are given in Table 01. LoD of some similar, previously reported methods is lower than that of the method introduced. However, those methods are based on enzymes and, they are much more complicated and expensive than the µPAD method described in this paper. Purification of enzyme and immobilization of it on the platform are the two major disadvantages of the use of enzymes in the detection of glucose (Teymourian *et al*. 2020). The lower stability of enzyme-based devices makes them less useful as point-of-care devices. As a point-of-care device for the detection of glucose, the work reported in this paper if free of heavy metals that can produce harmful effects, and this is one major advantage over the other similar non-enzymatic devices. Additionally, the use of smaller volumes for a single test allows this device suitable for the detection of glucose in biological fluids.

Table 1: Comparison of the limit of detection (LoD) of paper-based devices for glucose detection.

Device	LoD	Detection Method	Reference
Enzymatic GOx based µPAD	0.3 mM	Colorimetric	Beak *et al.*
Enzymatic GOx based µPAD	0.005 mM	Electrochemical	Cao *et al.*
Non-enzymatic Co based µPAD	0.15 mM	Electrochemical	Wei *et al.*
Non-enzymatic Au based µPAD	0.65 mM	Colorimetric	Pinheiro *et al.*
Non-enzymatic Ni based	0.001 mM	Electrochemical	Ahmad *et al.*
Non-enzymatic µPAD (free of harmful heavy metals)	0.05 mM	Electrochemical	This work

A summary of performance parameters determined for this proposed device is given in Table 02. The availability of oxidizable compounds in sweat (Chen *et al*. 2001) and saliva (Humphrey *et al*. 2001) are negligible to the amount of glucose of diabetic patients. However, due to the availability of urea in sweat, the interferences due to uric acids were studied. The voltammograms for the blank, 6 mmol dm^{-3} glucose, and 6 mmol dm^{-3} glucose solutions in the presence of 6 mmol dm^{-3} uric are shown in Figure 06. The current at 0.7 V for both voltammograms collected for 6 mmol dm^{-3} glucose and 6 mmol dm^{-}3 glucose solutions in the presence of 6 mmol dm^{-3} uric produced a similar current at 0.7 V.

Table 2: Estimated performance parameters of the proposed non-enzymatic µPAD (free of harmful heavy metals) for the detection of glucose.

Performance parameter	**Calculated value**
LOD	0.058 mM
LOQ	0.489 mM
Sensitivity	3.13 ×10^{-5} A/mM
Precision	15.59%
Repeatability	1.58 ×10^{-4} A
Bias	5.53 mM

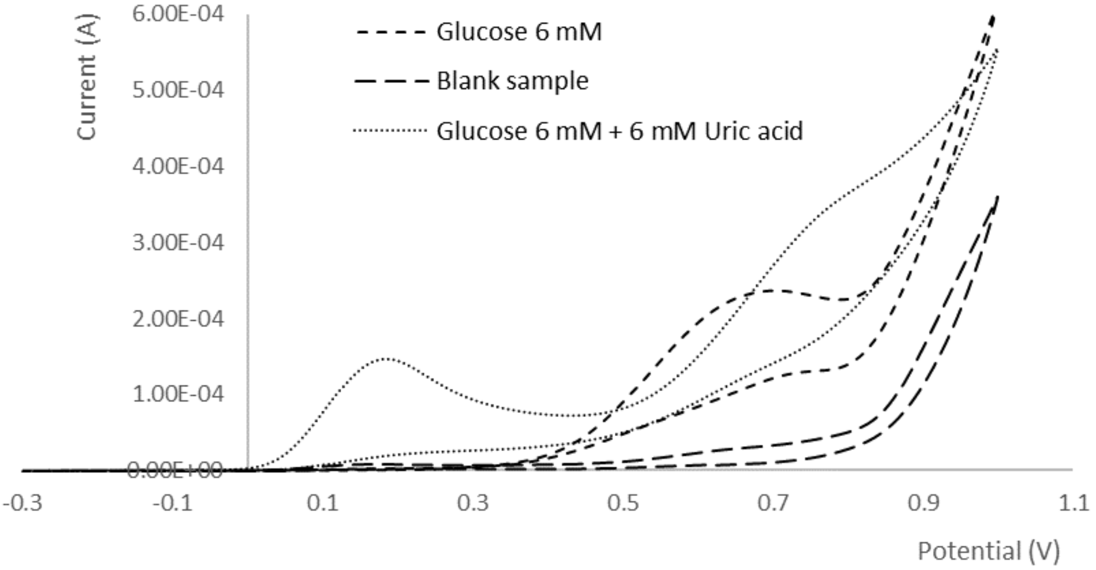

Figure 6: Cyclic voltammograms collected for the blank, 6 mmol dm^{-3} glucose, and 6 mol dm^{-3} glucose solutions in the presence of 6 mmol dm^{-3} uric acid in 0.1 mol dm^{-3} NaOH at 25 mV/s scan rate using the developed µPAD.

Typical levels of glucose in various body fluids are given in Table 03 (Bruen *et al.* 2017). Based on the information in Table 03, the proposed device is suitable to detect diabetic conditions using sweat, saliva, or urine. Using the time lag, the glucose level in the blood can be back-calculated using the glucose level in sweat and saliva.

Table 3: Summary of glucose levels in blood, urine, sweat and saliva of a healthy and diabetic person with the lag time to the blood glucose level.

Body Fluid	Glucose level for a healthy person	Glucose level under diabetic condition	Time lag
Blood	4.9 – 6.9 mM	2 – 40 mM	-
Urine	2.78 – 5.55 mM	>5.55 mM	~ 20 mins
Sweat	0.06 – 0.11 mM	0.01 – 1 mM	~ 20 mins
Saliva	0.23 – 0.38 mM	0.55 – 1.77 mM	~ 15 mins

CONCLUSIONS

The introduced low-cost µPAD fabricated with varnish is suitable for the detection of glucose in sweat. The anodization of the Cu working electrode facilitates the detection of glucose. The stainless-steel pseudo-reference electrode maintains sufficient stability within the voltage range used in this study for the detection of glucose. The peak current at 0.7 V maintained a linear relationship with the working range glucose concentration of 2 – 10 mM. The detection method is validated, and the µPAD can detect glucose concentrations low as 0.058 mM.

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