Accepted Manuscript

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PII: S0003-2670(18)31100-0

DOI: 10.1016/j.aca.2018.09.029

Reference: ACA 236265

To appear in: *Analytica Chimica Acta*

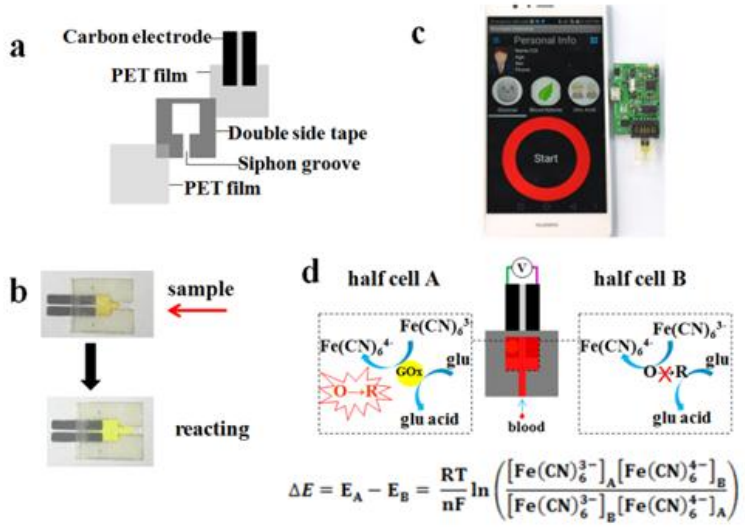
Received Date: 27 June 2018

Revised Date: 11 August 2018

Accepted Date: 13 September 2018

Please cite this article as: M. Sun, Z. Li, Y. Xia, C. Zhao, H. Liu, Concentration Cell-based Potentiometric Analysis for Point-of-Care Testing with Minimum Background, *Analytica Chimica Acta* (2018), doi: https://doi.org/10.1016/j.aca.2018.09.029.

Graphical Abstract



$$\Delta E = E_A - E_B = \frac{RT}{nF} \ln \left(\frac{[Fe(CN)_6^{3-}]_A [Fe(CN)_6^{4-}]_B}{[Fe(CN)_6^{3-}]_B [Fe(CN)_6^{4-}]_A} \right)$$

Concentration Cell-based Potentiometric Analysis for Point-of-Care Testing with Minimum Background

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Keywords: concentration cell; electrochemical; sensor; biosensor; screen print.

Abstract

One of the most critical problems of point-of-care testing is how to reduce the interference of background, especially under resource-limited conditions when sample pretreatment is not available. In this work we report a potentiometric method for point-of-care testing with minimum background. The method is based on the principles of a concentration cell which is a type of galvanic cells. It is an electrochemical cell having two carbon electrodes. The potential of each electrode is determined by ratio of a redox couple (i.e. $Fe(CN)_6^{4-/3-}$) on the electrode surface. On one electrode, the adsorbed enzyme catalyzes the oxidation of analyte by $Fe(CN)_6^{3-}$ which produces $Fe(CN)_6^{4-}$. The shift of the potential was because of the analyte as well as the background. In the other channel, no enzyme was present so that the shift of the potential, if any, is owing to the background. By measuring the potential difference between the two electrodes (i.e. voltage of the concentration cell), analyte can be quantitatively determined with most of the background eliminated. As the proof-of-concept analyte, blood glucose is quantitatively detected using a voltammeter with acceptable selectivity and accuracy. Noble metal electrodes that are indispensable for conventional electrochemical sensing are not required. All these features simplify the fabrication procedure and reduce the cost for the detection. Therefore, we believe it is promising for electrochemical point-of-care testing.

1. Introduction

Chemical analysis have been widely used in various fields such as clinical diagnostics, environmental monitoring and food safety [1-4]. Among all kinds of method, electrochemical method can directly transfer the analyte concentration to electrical signal [5-8]. So they are usually simple, inexpensive, sensitive and intrinsically quantitative, which have been successfully used for all kinds of applications such as point-of-care testing (POCT) [9-12]. For example, electrochemical sensors of glucose have been routinely used by diabetes patients for self-monitoring of blood glucose, and the market of glucose sensors have already accounted for 80% of all chemical sensor market.

For conventional electrochemical detection, a range of detection methods, such as amperometric, coulometric, potentiometric, and impedance methods, have been developed [13-17]. Other than the potentiometric methods, almost all of these methods involve simultaneous measurement of both potential and current, so that a three-electrode system is used to address the problem of uncompensated ohmic drop. The three-electrode system includes a working electrode (WE), on which an electrochemical reaction takes place, a reference electrode (RE), which is used to measure the potential and a counter electrode (CE), which supplies the current required for electrochemical reaction at the WE [18]. The three electrodes are usually made of different electrode materials, including noble metals such as Ag and Pt which are relatively expensive for

disposable test strips. Potentiometric methods do not require the counter electrode but their application is often limited to selective detection of ions.

For tests of samples with complex matrix such as blood or serum, the sample matrix effect may lead to uncertainty of the test results. The selectivity and accuracy of detection is usually improved by the following approaches: (1) modifying the surface of the working electrode with permiselective membrane to exclude the interferences, (2) immobilizing recognition reagents (e.g. enzyme or antibody) to selectively response to the target, and (3) introducing certain electrocatalysts of the analyte to reduce the overpotential to avoid the other redox reactions of the interferents. However, the fabrication and modification of the electrode are trivial and may involve expensive reagents and materials [19-21]. The modification layer on the electrode is fragile which affects the robustness of the test. The immobilized reagents on the electrode may also hinder the electron transfer on the electrode which decreases the detection signal. Furthermore, conventional electrochemical analysis involves sophisticated electrochemical instrumentation which may not be suitable for POCT.

Here we report a new sensing platform that is based on the principles of a concentration cell for POCT. A concentration cell is a type of galvanic cells that has two equivalent half-cells of the same composition differing only in concentrations. It has been used for oxygen sensing for automobile. As shown in **Scheme 1**, the glucose test strip had three layers. The bottom layer

was a transparency film having two screen-printed carbon electrodes. The middle layer was a double-sided adhesive tape patterned using a laser cutter. The top layer was a hydrophilic PET film. After assembly of the three layers, a capillary channel was created for spontaneous sample introduction. The volume of sample introduced into the channel was determined by the volume of the capillary channel which eliminated the requirement of a pipette and ensured reproducible sampling.

2. Experimental Section

2.1. Chemicals and Materials

All the chemical reagents used were of AR level. Glucose oxidase (GOx) from aspergillus niger was purchased from Sigma, carboxymethyl cellulose sodium (CMC) was purchased from Aladdin, $K_3[Fe(CN)_6]$, concentrated phosphate buffered saline (PBS, pH 7.4) were purchased from Sigma-Aldrich. Conductive carbon ink was obtained from Creative Materials, Inc. (Tyngsboro, USA). Transparency film (No. 2190) was obtained from Minnesota Mining and Manufacturing Company (Minnesota , USA). Double faced adhesive tape was purchased from Soken Chemical & Engineering Co.,Ltd (Tokyo, Japan). Laser cutting machine from Huatai Laser Engraving Co., LTD (Shenzhen, China) was used for device fabrication. The electrochemical detection was used with CHI1242b electrochemical workstation. Double-distilled water purified by Milli-Q system was used throughout the experiments. All reagents were used as received without further purification.

2.2. Device fabrication

For the device fabrication, the two carbon electrodes were screen-printed on a hydrophilic treated A4-sized transparency film using conductive carbon ink. Next, the laser engraved double faced adhesive was pasted on the bottom layer. Because each test strip was fabricated using double faced adhesive tape patterned using a laser cutter, so the volume of the blood sample (10 μL) needed to filled with the capillary channel was determined by the hollow area on the adhesive tape. Then, 2.5 μL $K_3[Fe(CN)_6]$ was added to the channel, after completely drying at the temperature of 65 ℃, 0.60 μL GOx / CMC (200 U / mL) was added to the left electrode and 0.60 μL CMC was added to the right electrode. Finally, the hydrophilic treated top layer was attached with the middle layer. The resulting A4-sized multilayer film could be cut to 140 glucose test strips. The GOx / CMC modified carbon electrode was connected to the CHI electrochemical workstation as the working electrode, and the CMC modified carbon electrode was connected to the CHI electrochemical workstation as the reference electrode and counter electrode together. The workstation was used to detect the potential difference between the two carbon electrodes.

2.3. Experimental procedures

To initiate the test, the sample was introduced into the hydrophilic channel by capillary action. The GOx immobilized on the electrode catalyzed the oxidation of glucose and simultaneous reduction of $Fe(CN)_6^{3-}$ to $Fe(CN)_6^{4-}$. After 5 seconds, the potential was measured using the electrochemical workstation. After 40 seconds, the reaction was completed. All of the experiments previously discussed were carried out at a room temperature of 25 ℃ unless specially indicated.

In this work, glucose in human blood was analyzed as the proof-of-concept target, so glucose oxidase (GOx) was absorbed on one of the electrodes for catalyzing the oxidation of glucose in the sample by $Fe(CN)_6^{3-}$ that was preloaded in the capillary channel. The catalytic reaction led to conversion of $Fe(CN)_6^{3-}$ to $Fe(CN)_6^{4-}$ and thus potential shift of the electrode to negative direction. On the other electrode, no GOx was present so that the potential shift, if any, was due to the effect of sample matrix. The potential difference between the two electrodes was measured for quantitative glucose determination [22].

3. Result and discussion

3.1. Optimization of reaction time

As shown in **Figure 1**, the potential first shifted rapidly to negative direction owing to the reaction between the $Fe(CN)_6^{3-}$ and glucose, and then approached a steady state at about 40 s after sample introduction. The steady state indicated that the reagents preloaded in the channel was dissolved. The reagents were in large excess for the detection reaction, which was almost completed at 40 s. For reproducible detection, a reaction time of 40 s was chosen for further experiments. With increasing concentration of glucose in the sample, more $Fe(CN)_6^{3-}$ was converted to $Fe(CN)_6^{4-}$ so that more negative potential was obtained. Note that for the blank, the potential measured was about zero, and didn't change with time, which means most of the background for the sample matrix can be eliminated by this method.

3.2. Optimization of $Fe(CN)_6^{3-}$ and GOx concentration

Because the potential of the electrodes was governed by the ratio of $Fe(CN)_6^{3-}$ to $Fe(CN)_6^{4-}$, so we studied the influence of the $Fe(CN)_6^{3-}$ concentration in the preloaded reagent mixture on the potential signal. We fabricated a series of test strips preloaded with the same reagent solution but containing $Fe(CN)_6^{3-}$ at different concentrations. After complete drying of the reagent, these test strips were used to analyze a sample containing glucose at 15 mM. As shown in **Figure. 2a**, the potential difference (ΔE) between the two electrodes increased with increasing concentration of $Fe(CN)_6^{3-}$. However, we found that beyond 500 mM the $Fe(CN)_6^{3-}$ may have solubility problem in blood sample, and they may not be completely dissolved during the test which affect the reproducibility of the detection. So for further experiments, 500 mM was used as the optimal concentration of $Fe(CN)_6^{3-}$.

Similar to $Fe(CN)_6^{3-}$, the GOx should be in large excess so that a wide linear range for glucose analysis can be obtained. The concentration of GOx instead of glucose would determine the reaction rate at high glucose concentration, and the potential signal would not change with glucose concentration. We investigated the concentration of GOx on the detection signal. We fabricated a series of test strips preloaded with the same reagent solution but containing GOx at different concentrations. After complete drying of the reagents, these test strips were used to analyze a sample containing glucose at 15 mM. As shown in **Figure. 2b**, the potential signal measured at 40 s increased with increasing concentration of GOx in the preloaded reagent. The potential signal

reached a plateau at 200 U / mL. Since the stock solution we obtained from the supplier was 200 U / mL, so we chose the 200 U / mL as the optimal concentration of GOx for further experiments.

3.3. Sensitivity and selectivity

To evaluate the applicability of the method for POCT, a human blood sample with known glucose concentration (3.5 mM) was obtained. Additional glucose was added into the sample so that a series of samples with glucose at different concentrations ranging from 3.5 mM to 15 mM was obtained. The concentrations of glucose in these samples were analyzed using the test strip. As shown in **Figure.3a**, the potential was linearly correlated to the concentration of glucose in the sample from 3.5 mM to 15 mM. The limit-of-detection, which was calculated as three times the standard deviation of the blank divided by the slope of the calibration curve, was 1.3 mM.

Human blood contains various electrochemically active compounds (e.g. ascorbic acid, lactic acid and uric acid) that can cause interference to electrochemical sensing. So the selectivity of the method was evaluated. Four samples containing glucose at 5 mM was prepared. Three of them also contained ascorbic acid (AA) at 2.0 mM, lactic acid (LA) at 30 mM and uric acid (UA) at 1.0 mM, respectively. As shown in **Figure. 3b**, these three major interfering substances in human blood for electrochemical sensing didn't cause significant change of the potential signal. The relative deviation for the signal was within 4.3 %, so the selectivity of the method was acceptable.

3.4. This method compared with standard method

The test results obtained using this method for human blood glucose analysis were also compared with that obtained using a standard method in a hospital. The standard method was based on a colorimetric method which is automatically carried out by a biochemical analyzer [23]. In this hospital, Beckman Kurt AU68 automated biochemical analyzer was used as the standard method for determination of blood glucose, and the test results were compared with the test results obtained using our method. As shown in **Figure 4**, the results by our methods were in agreement with that obtained using the standard method. The maximum relative variation was 10%, which demonstrated the accuracy of the method for POCT.

4. Conclusion

In conclusion, we have reported a potentiometric method based on the principles of concentration cell for POCT of human blood glucose. The method can eliminate the background because of sample matrix which can affect the accuracy for POCT under resource-limited conditions. It only relies on two screen-printed carbon electrodes for quantitative detection with acceptable selectivity and accuracy, which dramatically simplifies the fabrication procedure and reduces the cost. It can also be used in the future for analysis of targets other than glucose. So, we believe it is a promising sensing platform for POCT.

Acknowledgments

We gratefully acknowledge financial support from Chinese Recruitment Program of Global Experts, Innovative and Entrepreneurial Talent Recruitment Program of Jiangsu Province, State Key Project of Research and Development (2016YFF0100802), the Fundamental Research Funds for the Central Universities (2242018K41023). This work was supported by the Key Project and Open Research Fund of State Key Laboratory of Bioelectronics, Southeast University.

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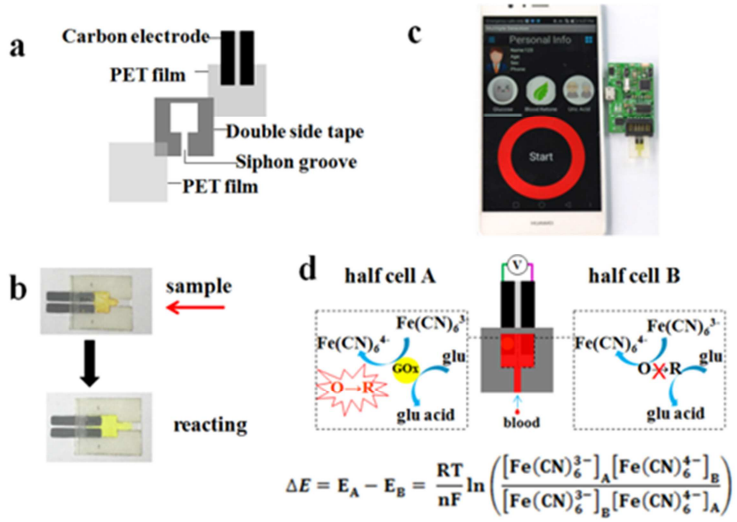


Scheme 1. a) The layers for fabrication of the test strip. b) Photographs of the test strip before and after the sample introduction. c) Photographs of the sensing system including the test strip, the miniaturized voltammeter which was connected to the smartphone App via Bluetooth. d) The principle for the concentration cell-based potentiometric analysis.

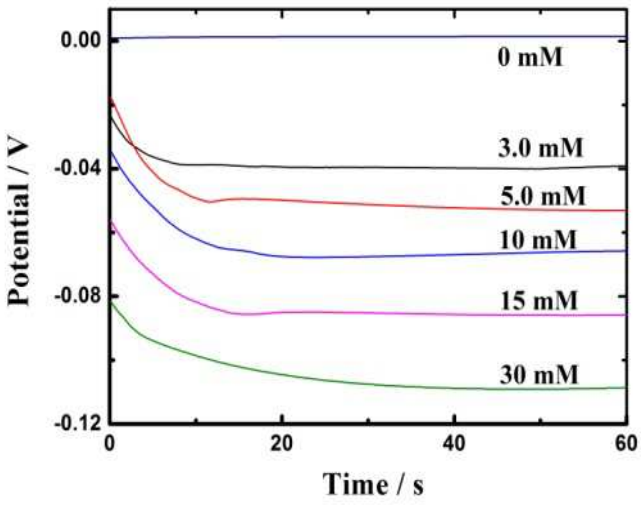


Figure 1. Open circuit potential measured as a function of time for testing of 0.010 M PBS (pH 7.4) samples containing glucose at different concentrations. The concentration of glucose in the sample is indicated. The test strips were preloaded with 2.5 µL of 0.010 M PBS solution (pH 7.4) containing 500 mM $Fe(CN)_6^{3-}$ as well as 0.60 µL GOx/carboxymethylcellulose (CMC) (200 U / mL) on one electrode and 0.60 µL CMC on the other electrode, respectively. The preloaded reagents were allowed to completely dry. The potential in this figure was monitored in real time by an electrochemical instrument.

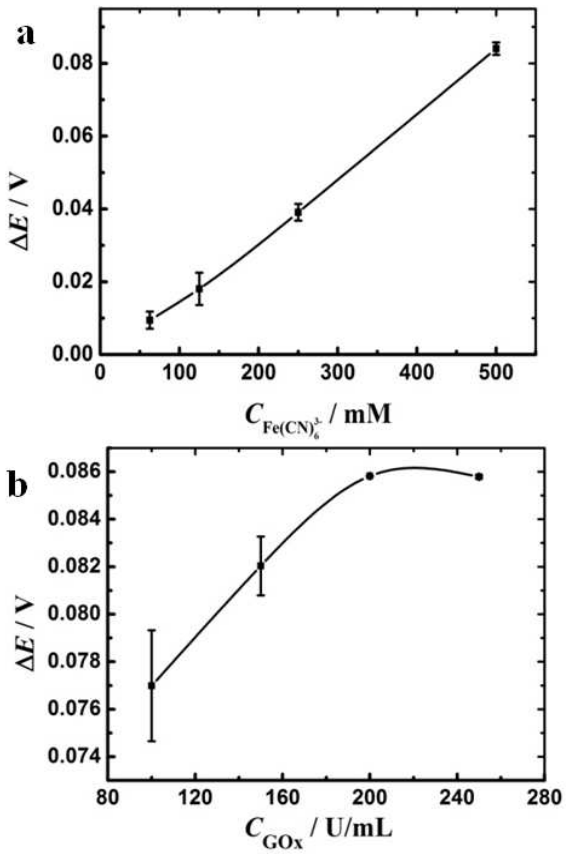


Figure 2. a) Potential difference between two carbon electrodes measured as a function of the concentration of $Fe(CN)_6^{3-}$. The test strips were preloaded with 2.5 μL of 0.010 M PBS solution (pH 7.4) containing 0.60 μL GOx/CMC (200 U/ mL) on one electrode and 0.60 μL CMC on the other electrode as well as $Fe(CN)_6^{3-}$ at different concentrations. The reagents were allowed to completely dry before use. A sample containing glucose at 15 mM was analyzed using the test strips. The error bars represent standard deviation for three replicated tests. b) Potential difference between two carbon electrodes measured as a function of the concentration of preloaded GOx. The test strips were preloaded with 2.5 μL of 0.010 M PBS solution (pH 7.4) containing 0.60 μL GOx/CMC（200 U / mL）on one electrode and 0.60 μL CMC on the other electrode as well as

$Fe(CN)_6^{3-}$ at different concentrations. The reagents were allowed to completely dry before use. A sample containing glucose at 15 mM was analyzed using the test strips. The error bars represent standard deviation for three replicated tests.

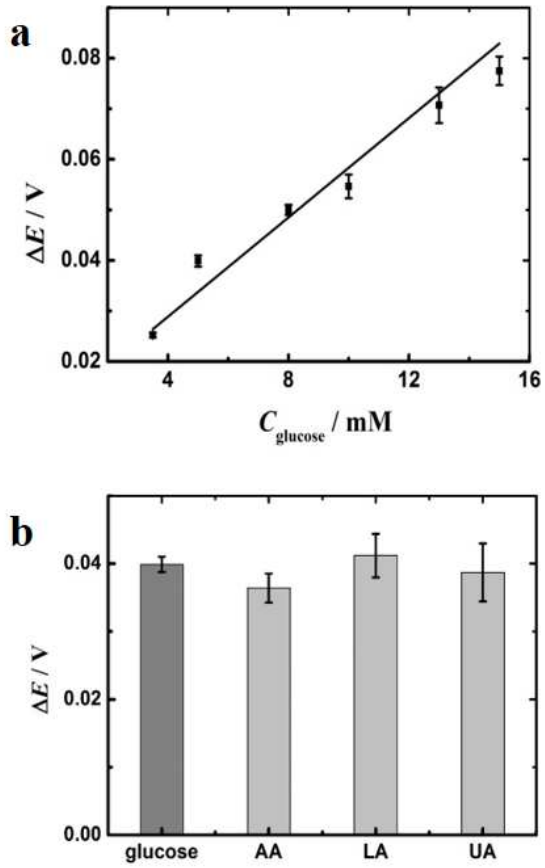


Figure 3. a) Potential difference between two carbon electrodes measured as a function of the concentration of glucose in a blood sample. The error bar represents standard deviation for three replicated tests. b) Potential difference between two carbon electrodes measured for testing of four samples to evaluate the selectivity of the method. All of the samples contained glucose at 5 mM. Three samples also contained ascorbic acid (AA) at 2.0 mM, lactic acid(LA) at 30 mM and uric acid(UA) at 1.0 mM, respectively. The error bars represent standard deviation for three replicated tests.

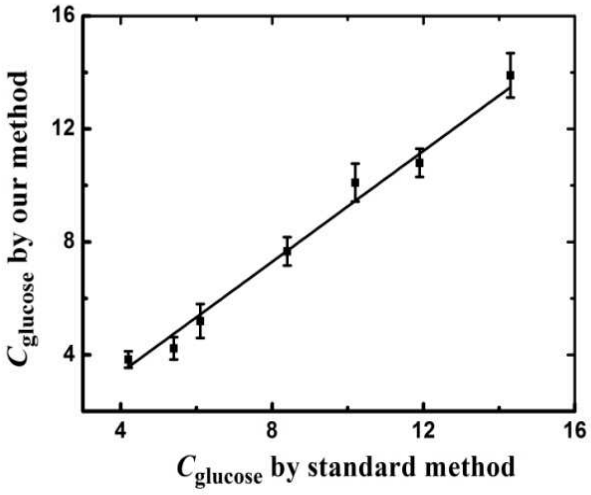
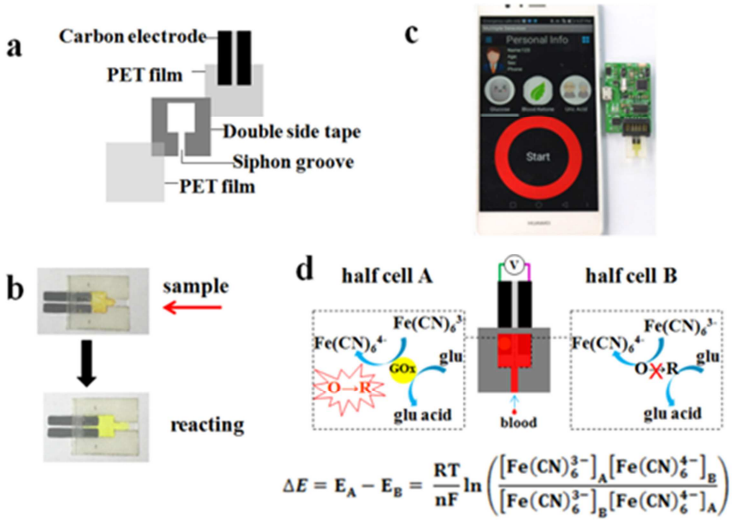


Figure 4. This test results obtained by the proposed method compared with that obtained by the standard method in a hospital.

Graphical Abstract



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Highlights

1. A potentiometric sensing method was developed based on the principles of a concentration cell for bioanalysis.
2. The method can eliminate the background interference from sample matrix which improves accuracy for point-of-care testing.
3. The electrochemical sensor is based two identical screen printed carbon electrode, which is simpler and more cost-effective than conventional glucose test strip.