Biosensors and Bioelectronics 94 (2017) 415–419

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

双通道单根试纸上葡萄糖和尿酸的同时监测

Simultaneous monitoring of glucose and uric acid on a single test strip with dual channels

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ARTICLE INFO

Keywords:
Glucose sensor
Uric acid sensor
Electrochemical sensor
Dual channel test strip
Family health management

ABSTRACT

The conventional test strip has usually only one electrochemical reaction channel, which requires two times figure punctures for the self-management of patients suffering from both diabetes and gout. Considering the large number of such patients and for the sake of reducing their pains, we report an enzymatic test strip which can simultaneously monitor glucose and uric acid (UA) with only one fingertip blood droplet. The proposed test strip is composed of dual channels. The glucose in blood is detected in the 1st channel above on the substrate and the UA is characterized in the 2nd channel located at the bottom of the substrate. The proposed design intensively matches the requirement of those patients simultaneously suffering from diabetes and gout. We carried out comparative investigations on the proposed test strip and clinical biochemical analyser, which indicates a good agreement and proved the reliability and accuracy of the proposed test strip, as promising solution for the fast growth of family health management market.

1. Introduction

There is a large number of patients suffering from both diabetes and gout which are chronic diseases that requires long term therapy. For those patients simultaneously suffering diabetes and gout are generally required to take drug treatment as well as regular measurement of blood glucose and UA for a long time. Precise and long-time monitoring of glucose/UA concentration in blood plays a critical role in evaluating the therapy of diabetes and gout patients. Amperometric sensor as one kind of electrochemical method has emerged as a powerful platform in biomedical application due to many significant merits, such as portability, low cost, easy integration, and rapid analysis. Two typically successful applications are the examples of glucose sensor (Wang, 2008; Heller and Feldman, 2008; Gernet et al., 2009; Williams and Korosi, 1970; Wan et al., 2011) and uric acid (UA) sensor (Huang et al., 2008; Zhang et al., 2013; Chen et al., 2005; Ernst and Knoll 2011). The working mechanism of amperometrics-based biochemical sensor is based on enzymatic catalyzed oxidation of the targeting biomolecules with concomitant reduction of $Fe_{(III)}$ to $Fe_{(II)}$ (Williams and Korosi, 1970). The measured concentration of targeting biomolecules is quantified by the amount of transferred electrons which is linearly proportional to the targeting molecules concentration. The conventional enzymatic single channel test strip can usually monitor only one biochemical parameter for each finger blood taken (Lakshmi et al., 2011). Therefore, for those patients suffering from both diabetes and gout, it requires them to puncture their finger twice to take two blood drops for glucose and UA monitoring, which doubles their pain during the self-management of the chronic disease.

Hence there is strong interest and desire in developing highly sensitive and reliable biosensors towards the characterization of multi-analytes from a single drop of human body fluids. Electrochemical multiplexed-assay has emerging as one leading methodology for simultaneously characterizing multi-analytes which is widely applied from biochemical, immune sensor and molecule diagnosis. Multiplexed electrochemical immunoassay for the simultaneous detection of cardiac troponin I (cTnI) and C-reactive protein (CRP) for cardiovascular diseases; interleukin-6 (IL-6) and matrix metallopeptidase-9 (MMP-9) for assessing the index of ischemic stroke were reported (Zhou et al., 2010; Shi et al., 2014; Neumann, 2015). A multiplexing electrochemical immune-sensor was developed for ultrasensitive detection of cancer related protein biomarkers such as prostate specific antigen (PSA) and Interleukin 8 (Wan et al., 2011). Multi-template imprinting incorporating with electrochemical sensor was developed for quantitative recognition of Zn^{2+} and arginine using differential pulse voltammetry

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http://dx.doi.org/10.1016/j.bios.2017.03.026
Received 27 January 2017; Received in revised form 11 March 2017; Accepted 14 March 2017
Available online 15 March 2017
0956-5663/

by synthesizing imprinted polymer mixed with multi-walled carbon nanotubes (Roy et al., 2015). Multiplexed electrochemical molecular diagnostics assay such as DNA sensor and genes sensor by immobilizing highly specific single-nucleotide polymorphism on electrode surfaces has attracted scholars' attention (Wan et al., 2011; Civit et al., 2010). Multiplexed wearable electrochemical sensor on textile weaving for monitoring glucose and hemoglobin also provides a critical guidance for mobile health solution (Choudhary et al., 2015). The above mentioned research paper demonstrates the multiplexed electrochemical sensor in biomedical application with the planar structure in electrochemical reaction channel, which is a promising solution for the rapid development in big-data health market.

Although there were reports about biosensors capable of detecting multiple targeting biomolecules, specific test strip simultaneously detecting glucose and UA for the self-management of patients suffering from both diabetes and gout has not been report. Taking account of the large number of such patients as well as unnecessary pains due to double punctures for a their daily glucose and UA monitoring, in this paper, we demonstrate an enzymatic test strip with dual channels to simultaneously characterize the glucose and UA concentration by applying single blood drop. The three-dimensional novel structure has two electrochemical reaction channels as illustrated in Fig. 1b, while conventional design of test strip is shown in Fig. 1A-a with only one electrochemical reaction channel. In the novel structure, the channel 1 is designed to perform the electrochemically glucose sensing function. The glucose molecules are catalyzed into gluconic acid by the glucose oxidase (GOD) and in conjugating with concomitant reduction of Fe(III) to Fe(II). The generated $Fe(II)(CN)_6^{-4}$ ions are detected by the amperometric method to evaluate the amount of transfered electrons. With similar working mechanism of channel 1, within the channel 2, the UA molecules are oxidized by uric acid oxidase (UOD) into allantoin in conjugating with concomitant reduction of Fe(III) to Fe(II). The concentration of UA was evaluated by counting the quantity of transferred electrons with the electrochemical analyser in medical smartphone reported in our previous work (Guo, 2016). By applying only one blood drop, the blood can be driven into both channels by the capillary force. The separate channels are able to independently and simultaneously detect the glucose and UA. The proposed enzymatic test strip provides a promising solution for those patients who simultaneously suffer from diabetes and gout by avoiding the two-time finger puncture.

2. Materials and method

The proposed test strip with dual channels was fabricated by a semi-automatic screen-printer, which was applied to print carbon as working and counter electrodes. The whole fabrication procedure was illustrated in Fig. 1B. Polyethylene terephthalate (PET, label 1) was chosen as the substrate. Initially, a carbon ink was performed to work as the working electrode. A silver layer was first pre-coated before printing the carbon ink (Acheron, Japan) to enhance the conductivity of the screen printed carbon electrodes on a flexible polypropylene film. Subsequently, the strip was baked in specifically dry box at 75 °C for 40 min (Guo, 2016). After drying step, an insulator layer (label 3) was finally affixed over the electrode layer (label 2) leaving the working area (label 4) for immobilization of enzyme (GOD). As the next step, reversing the substrate as indicated Fig. 1B-d, the second electrode layer (label 5) was printed over the other side of the PET substrate as the first layer electrode fabrication process. In the same way, the unit was baked again in a drying box at 75 °C for 30 min. After the second drying step, another insulator layer (label 6) was affixed over the second electrode layer, leaving the working area (label 7) for immobilization of enzyme (UOD). During the immobilization of GOD, the GOD solution was dropped within working area (label 4). After baking at 40 °C for 60 min, the first cover slip was affixed on the first insulator layer (label 3). As following step, UOD solution was dropped within working area (label 7). After the same baking condition, the second cover slip was affixed on the second insulator layer (label 6). Finally, the dual channels were constructed.

3. Results and discussion

Investigation of the accuracy and reliability of the proposed test strip with dual channels was conducted by assaying specific solutions of the mixture of glucose and UA in phosphate buffered saline (PBS, pH=7.4). Six groups of mixture of glucose and UA in PBS were prepared to test the strip as shown in Table S1 in the Supporting Information (SI).

Chronoamperometry (CA) method was used to characterize the proposed test strip since it is rapid, accurate, and sensitive to analyse the electrochemical reaction (Wang, 2008; Heller and Feldman, 2008; Gernet et al., 2009). Smartphone-based portable electrochemical analyser was utilized to provide working potential (@390 mV for GOD electrode and @300 mV for UOD electrode) and record the electrochemical current (Guo, 2016). The typical chronoamperometric curves obtained by the electrochemical analyser with response to the mixture (group 1–6) of glucose and UA of various concentrations in PBS under the applied electrical potential are given in Fig. 1B, respectively. Fig. 2a (glucose) and Fig. 2c (UA) illustrate the recorded electrochemical current profiles after applying the mixture solution. At the beginning stage when the electrical potential was applied to the WE (working electrode), the quantity of the transferred electrons significantly increased with the electrochemical reactions enzymatically catalyzed by excessive quantity of GOD (for the 1st channel) and UOD (for 2th channel). Rapid oxidation of glucose/UA molecules on the surface of WE leads to a peak amplitude of the current within a very short moment, which is observed from Fig. 2a and c. Subsequently, the electrochemical current undergoes a rapid attenuation resulting from the exhaustion of the glucose and UA molecules on the surface area of WE. At last, the electrochemical equilibrium was reached. The current profile remained steady value which can be explained that the determin and transferring from bulky solution to surface of WE governed the electrochemical reaction.

According to the electrochemical theory, the concentration of determin and molecule is linearly proportional to the steady current value (Williams and Korosi, 1970). Fig. 2a indicates that the electrochemical current as a response of glucose remains steady after 3 s and Fig. 2c show that the electrochemical current as a response of UA remains steady after 4 s. The optimal time interval of current acquisition was set between 3 s and 4 s for glucose and between 4 s and 5 s for UA. By statistical analysis on the current profile in this interval, the plot of steady current as a function of glucose/UA concentration was drawn in Fig. 2b and d within the range from 2.25 mmol/L to 30 mmol/L for glucose and 400–930 μmol/L for UA, respectively. The linear correlation curves between the current and concentration were added in Fig. 2b for glucose (the slope, 0.69; intercept, 0.91; correlation coefficient, 0.98) and Fig. 2d for UA (the slope, 0.0087; intercept, −2.67; correlation coefficient, 0.997). The good linear relationship between steady current value and concentration of determinand indicate good agreement with the theoretical prediction. Table 1 summarizes the reproducibility experiment result. Three mixture samples of glucose and UA of specific concentration were used to test the proposed test strip, and each sample was tested 10 times. The measured coefficient of variation (CV %) of the mixture sample are less than 3% for glucose and less than 4% for the UA, which demonstrate the proposed test strip is highly reliable and reproducible.

In order to evaluate the accuracy and reliability for screening the patients simultaneously suffering from diabetes and gout in clinical use, comparative investigation between the proposed test strip with the clinical biochemical analyser was performed. Fingertip peripheral blood from 103 patients were collected by continuous steps: professionally puncture fingertip with a disposable needle and collect

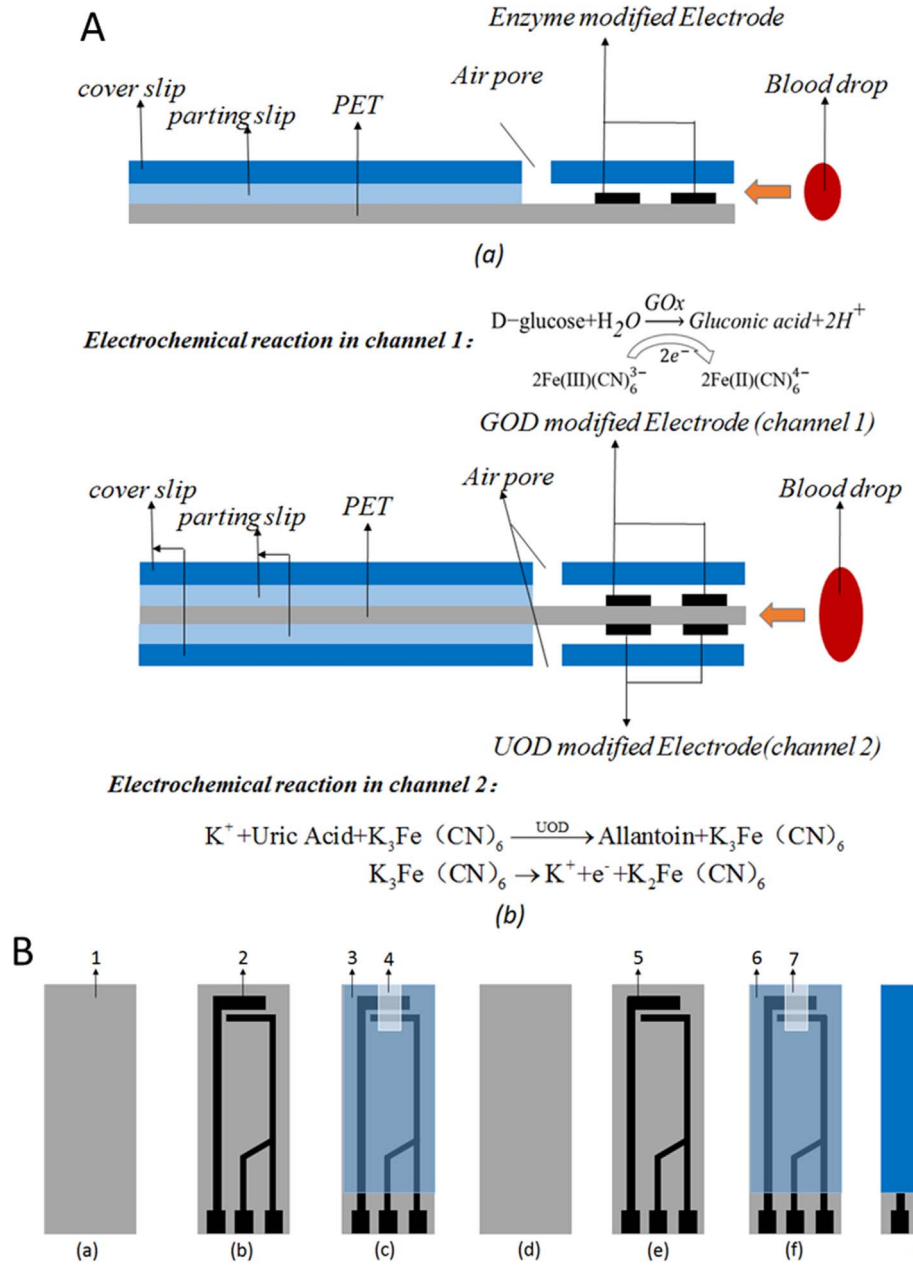

Fig. 1. A. (a) the structure view of the conventional enzymatic test strip with only one electrochemical reaction channel and (b) the novel structure of the proposed enzymatic test strip with dual channels for two separately electrochemical reaction; B. (a) the PET substrate; (b) the profile after carbon electrodes were printed; (c) an insulator as parting slip was affixed on the electrode layer; (d) the view of the reverse side of the PET; (e) the view of the other side of PET after second electrode layer printed; (f) profile after second insulator layer was affixed; and (g) the single side view of the proposed test strip.

fingertip blood for detection. The whole finger blood was directly applied to the proposed test strip and analysed by the smartphone-based electrochemical analyser. The blood sample was pre-processed before applying to the clinical biochemical analyser. Whole finger blood samples were taken by health care professionals (using EDTA anticoagulant) with an average volume of about 1 mL. After centrifugation at 4 ℃, 3000 rpm for 30 min, the blood serum was collected by taking the supernatant of the tube and applied to automatic biochemical analyser. The threshold was set with grid line by low concentration range (< 6 mmol/L), median range (6–11 mmol/L), and high range (> 11 mmol/L) for glucose and low concentration range (< 200 μmol/L), median range (201–435 μmol/L), and high range (> 436 μmol/L) for UA, as depicted in SI (supplied materials). It indicates the distribution of patient's medical data divided by the set threshold from proposed test strip. The result in SI (supplied materials) reveals that the proposed test strip screened out that there were five patients simultaneously suffering from high glucose and UA level, which was as well verified by the clinical result from biochemical analyser. The comparative study demonstrates the reliability and accuracy of the proposed test strip with dual channels for screening out the patients with high level of both blood glucose and UA.

For the sake of further evaluation on the repeatability of the proposed test strip for both glucose and UA evaluation, the five patients screened from the above mentioned investigation were involved in the repeatable experiment. Whole finger blood from each patient was test 10 times with the proposed test strip. As illustrated in Table S2 in the SI, glucose/UA concentrations measured by the proposed test strips were statistically analysed. It indicates no significant difference among the five patients of proposed test strips when applying the same target. These results indicate that the proposed test strip is highly reprodu-

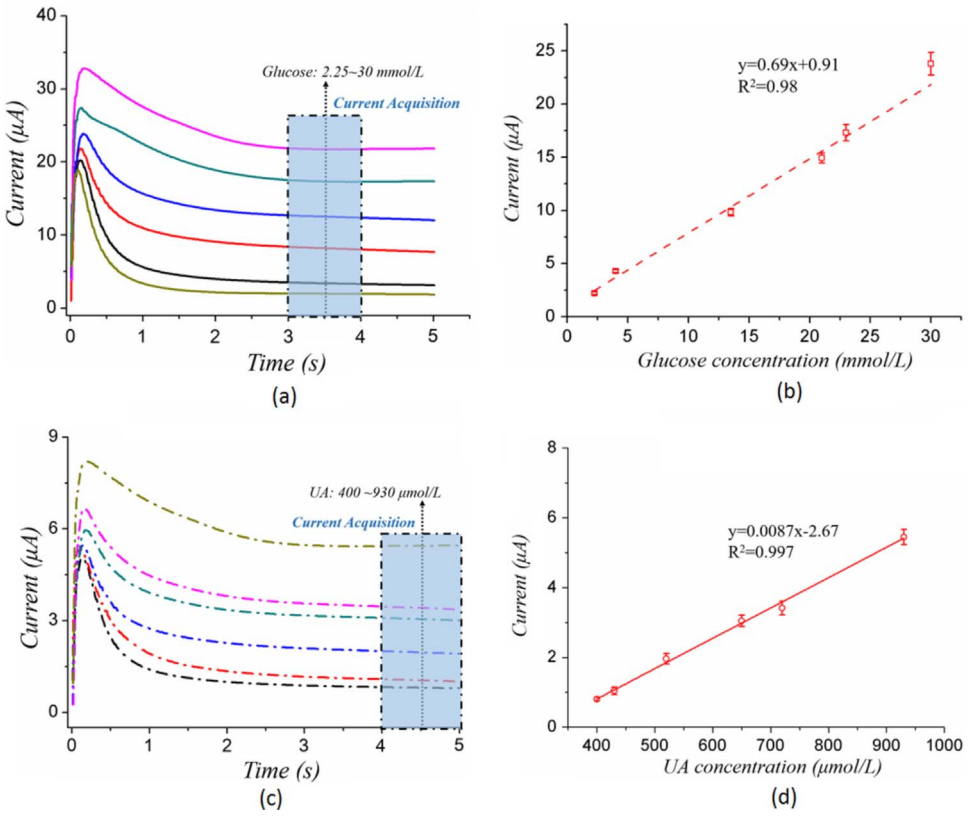

Fig. 2. a) The chronoamperometric curves as a response of glucose with concentration ranging from 2.25 mmol/L to 30 mmol/L; b) the linear correlation between glucose concentration and the statistical value of steady current value; c) the current profile as a response of UA with concentration ranging from 400 μmol/L to 930 μmol/L; and d) the linear correlation between UA concentration and the statistical value of steady current value.

Table 1
Three mixture solutions of glucose and UA groups were used to test the proposed test strip, and each sample was tested 10 times. The measured concentration of glucose and UA, and coefficient of variation (CV %) information are summarized in the table.

Glucose			UA		
Samples	Average measured value (mmol/L)	CV (%)	Samples	Average measured value (μmol/L)	CV (%)
1	4.08	2.31	1	409	3.27
2	13.1	1.82	2	532	2.82
3	31.21	2.78	3	915	3.62

cible and reliable for simultaneous blood glucose and UA monitoring, suggesting that it is feasible for family health management application.

In order to further evaluate the stability and reproducibility in clinical use, real whole blood samples from three groups A, B and C (divided according to the glucose concentration of patients) were applied to test the proposed test strips. For low glucose level interval, the measured maximum CV is less than 5% (Table S3 in the SI summarizes the detail test result information for glucose); in the middle glucose interval, the maximum CV is less than 5.2% and for high glucose level interval, the CV is less than 5%. In the UA test experiment, the maximum CV is 12.64%, 10.2% and 9.73%, for low, middle and high UA concentration solution, respectively (Table S4 in the SI summarizes the detail information for UA). Those clinical experiments demonstrates the proposed test strip can be commercially applied in the clinical use for long-term monitoring glucose and UA with the whole blood sample.

4. Conclusions

In summary, we successfully prepared a new type of a dual channel enzymatic test strip which is capable of detecting glucose and UA simultaneously by using only one blood drop sample. The working mechanism of the presented test strip is based on electrochemical method by enzymatically catalysis of targeting molecules. The proposed test strip aims at alleviating the pain of those patients who simultaneously suffers from diabetes and gout in their self-health management.. It can significantly reduce the pain of these patients by only pricking the finger once, instead of pricking the fingers twice, for both glucose and UA measurement respectively. Comparative study on the accuracy and reliability of the proposed test strip indicate that the test strip is consistent with the clinical biochemical analyser, which demonstrate its great potential as a new method solution for family health management. Future research should be carried out to integrate the test strip with portable monitoring devices, which will lead to a complete set of family healthcare products.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2017.03.026.

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