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以智能手机为电化学分析仪的尿酸监测

Uric Acid Monitoring with a Smartphone as the Electrochemical Analyzer

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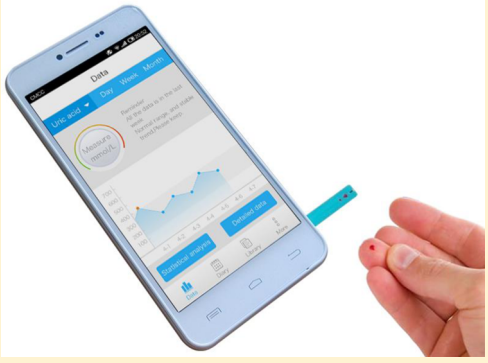
ABSTRACT: We report the world's first medical smartphone as an electrochemical analyzer, which is incorporated with an enzymatic test strip for rapid characterization of UA (uric acid) in peripheral whole blood. A disposable electrochemical uric acid test strip was connected to the electrochemical module integrated with the smartphone through a specific interface, a slot around the edge of smartphone. A 3 μL human finger whole blood drop is applied on the strip for UA evaluation and compared to the clinical biochemical analyzer with satisfactory agreement. The measured data was saved and uploaded into a personal health management center through the mobile Internet.



Uric acid (UA) is a purine metabolic product and is related to many clinical diseases, such as gout, kidney disease, and heart disease, which can result in high UA in the blood.[1−3] Many medical investigations have indicated that the rise of blood serum UA can cause cardiovascular disease.[4] Therefore, the monitoring of UA in blood is a critical point for evaluating the therapy of gout patients over a long time. In the clinical laboratory, there are two main screening methods for measuring the UA level in blood serum including the nonenzymatic and enzymatic methods. In the nonenzymatic solution, the blood serum was mixed with phosphotungstic acid under alkaline conditions and the absorbance at λ = 660 nm of the reaction product of phosphotungstic acid and UA has a linear relationship with the UA concentration in the sample. In the enzymatic solution, the basis of the working principle is that an excess amount of uricase oxidase (UOD) enzyme is utilized to catalyze blood UA decomposing into allantoin, and then, the difference in absorbance at λ = 290 nm of the enzyme catalyzed product is linearly enhanced with the increase of UA concentration.[5] However, the optics-based method requires bulky equipment and complicated blood sample preprocessing procedures such as centrifugation, plasma separation, serum extraction, a quality control process, etc. Therefore, the development of a portable, cost-effective, fast platform to provide a point of care (POC) solution for UA monitoring is highly appreciated.

In recent years, the smartphone-based biomedical device has attracted many research groups involved in this area due to the large number of smartphone users. The biomedical device constructed with a smartphone is capable of being widely applied in family-based smart health management. Park et al. demonstrated smartphone-based detection of *Salmonella* on paper microfluidics by evaluating Mie scattering from the digital images taken at an optimized angle and distance with a smartphone.[6] Laksanasopin et al. demonstrated a smartphone dongle for diagnosis of HIV by laboratory-based enzyme-linked immunosorbent assay (ELISA).[7] Barbosa et al. presented a novel, power-free, and flexible detection system for portable colorimetric and fluorescence immunoassay detection of prostate specific antigen (PSA) based on the smartphone.[8] Liao et al. reported a cost-effective, minimally instrumented, smart cup platform for rapid, quantitative molecular diagnostics of pathogens at the point of care.[9] These research groups have demonstrated the smartphone-based biomedical device for implementing biomolecules detection covering biochemical, immune, and molecular levels. However, in these published papers, it is still necessary to utilize a smartphone accessory or dongle to convert the biomedical information to a signal which can be directly recognized by the smartphone. On the other hand, electrochemical methods have been widely utilized in biomedical application because of their many merits, such as portability, low cost, easy integration, and rapid analysis; one typical example is the glucometer.[10−14] It is a very promising solution to integrate the smartphone with the electrochemical analyzer for point of care monitoring of UA.

In this paper, we demonstrate the world's first medical smartphone as an electrochemical analyzer, which is incorporated with an enzymatic test strip for point of care characterization of UA in peripheral whole blood. The overall cost of the proposed medical smartphone is less than 60 US

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dollars. As Figure 1a illustrated, a disposable electrochemical uric acid test strip was connected to the electrochemical

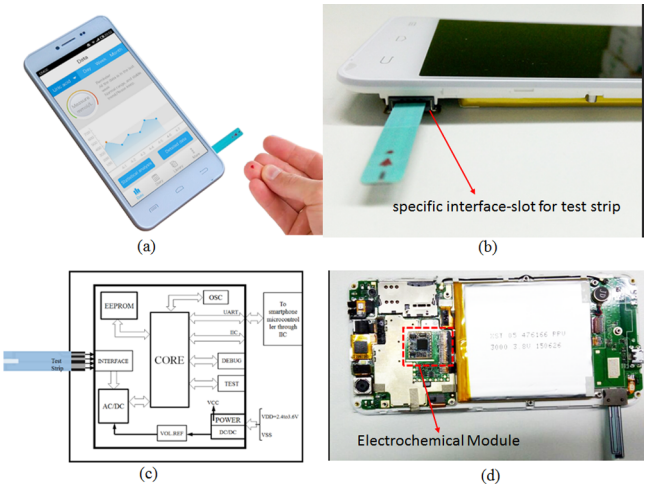

Figure 1. (a) Photograph of the proposed medical smartphone in which an electrometer has been preburied. (b) Side photograph of the proposed medical smartphone: slot is the interface for insertion of UA test strip. (c) Schematic structure view of the electrometer; a micro controller for resolving the electrochemical current formed in the test strip. (d) Top view of the layout of the medical smartphone: the electrochemical module is integrated with the main print circuit board of the smartphone.

module (Figure 1c,d) integrated with the smartphone through the specific interface, a slot (Figure 1b) around the edge of the smartphone. A 3 μL human peripheral whole blood drop is applied on the strip for UA characterization and compared to the clinical biochemical analyzer with satisfactory agreement. The proposed medical smartphone provides a mobile screening electrochemical station for point of care testing of many biochemical parameters of human blood under a flexible spot, which is a promising technology for meeting the urgent need of the mobile health application.

THEORY

During the enzyme catalyzing process, uric acid oxidase was catalyzed by the oxidation of uric acid to allantoin with concomitant reduction of Fe(III) to Fe(II). The Fe(II) $(CN)_6^{-4}$ ions generated were characterized.[15] Equation 1 illustrates the working principle of enzymatic analysis of uric acid. The electron transfer mediator ferricyanide plays an indispensable role in the procedure of fast oxidization of the product with concomitant reduction of Fe(III) to Fe(II); the Fe(II) $(CN)_6^{-4}$ ions were amperometrically analyzed by monitoring current change between working electrode and counter electrode through the electrochemical analyzer buried in the smartphone. The buried electrochemical analyzer is a reader that translates the current signal into the concentration of uric acid which is able to be easily displayed and stored in the smartphone.

$$K^+ + \text{uric acid} + K_3Fe(CN)_6 \xrightarrow{\text{enzyme}} \text{allantoin} + K_3Fe(CN)_6$$

$$K_3Fe(CN)_6 \rightarrow K^+ + e^- + K_2Fe(CN)_6 \quad (1)$$

EXPERIMENT

Fabrication of the Electrochemical Test Strip. A disposable test strip was fabricated by a screen-printer with the printed electrodes which were composed of carbon compound as the working and counter electrodes. A carbon ink was performed to work as the working electrode. A silver layer was first precoated before printing the carbon ink (Acheron, Japan) to increase the conductivity of the screen printed carbon electrodes on a flexible polypropylene film (50 mm × 70 mm). Subsequently, the strip was baked in a specific drybox at 75 °C for 40 min. After the baking procedure, an insulator stick layer was attached above the electrode layer with working area of 0.065 cm^2. The average value of measured resistance for the 50 strips using a commercial multimeter is 75 ± 2 ohm·cm^{-1}.

RESULTS AND DISCUSSION

The performance of the above-mentioned electrochemical test strips was evaluated by assaying standard solutions of UA (200−1000 μmol/L). Blood usually contains from 200 to 425 μmol/L UA, with higher levels indicating health problems such as gout. Two μL of enzyme solution was applied on the working electrodes above the strips; then, the device was dried in air to enable the enzyme to be uniformly immobilized within the working area. It has been found that the drying time of 20−60 min could result in a relatively strong electrochemical signal. Thus, 20 min was selected as the optical drying time for subsequent experiments. A commercial electrochemical workstation (Electrochemical Workstation CHI 660A) at 300 mV (versus screen-printed carbon electrode) was used to perform the electrochemical characterization of the UA test strip with response to various concentrations of UA in PBS.

The chronoamperometry (CA) method was utilized for the characterization of the proposed UA test strip because it is accurate, highly sensitive, and portable; also, it can be performed in an electrochemical (EC) reader. The typical chronoamperometric response as a result of various concentrations of UA with a working potential at 300 mV is given in Figure 2a. In the early state when applying electrical potential on the working electrode, the concentration of electron increased within the SPE (screen printed electrode) with the help of the enzymatic catalyzed reaction as given in eq 1. Therefore, the higher electrochemical currents resulting from the anodic oxidation of UA molecules in the surface area of SPE were acquired at the initial stage of applying 300 mV to the working electrode, which was observed beginning at t = 0 in Figure 2a. Subsequently, there was a phenomena that exponential decay in the electrochemical current was shown in the curve because of the exhaustion of the concentration of UA around the surface of the working electrode. Finally, the electrochemical current reached the steady stage due to the fact that the mass transfer of UA molecules from bulky solution to the surface of working electrode controlled the electrochemical current. From the result in Figure 2a, the electrolysis time was less than 40 s when the steady electrochemical currents were acquired. Therefore, it is suitable to choose 40−41 s for the current sampling time. In the proposed medical smartphone, the current recording time interval is set between 40 and 41 s. During this experiment, 5 UA test strips were used for the three samples of UA concentration: 0.2, 0.5, and 1 mmol/L, respectively. The correlation curve between the steady electrochemical UA concentration was obtained as shown in

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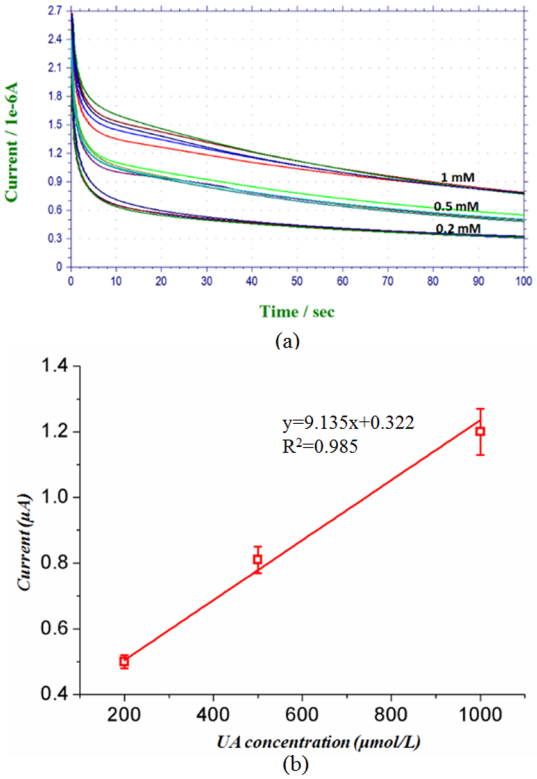

Figure 2. Electrochemical characterization of the test strips: (a) typical chronoamperometric curves for UA at different concentrations: 0.2, 0.5, and 1 mmol/L (5 test strips used for the same concentration); (b) the linear fitting correlation curve between the steady electrochemical UA concentration.

Figure 2b with the slope of 9.135 (intercept, 0.322; correlation coefficient, 0.982). The maximum standard deviation indicated less than 5.8% for 5 test events for each solution, which demonstrated the proposed test strip was quite acceptable for reproducibility.

In order to evaluate the accuracy comparison of the commercial biochemical with the proposed medical smartphone, we plotted a calibration curve for the result comparison between UA in human serum (obtained from the biochemical analyzer result) and finger whole blood (obtained from the proposed medical smartphone) as shown in Figure 3. 153 samples from 153 patients of clinical trials including fingertip

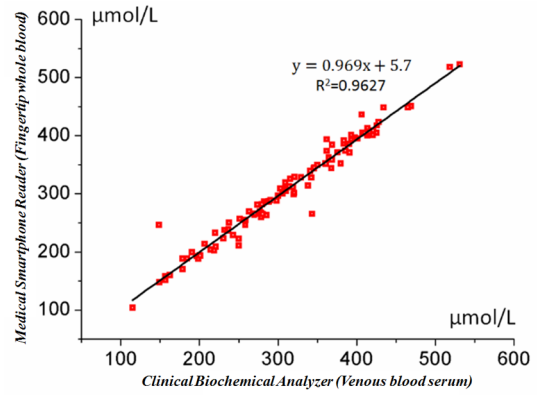

Figure 3. Measured UA concentration comparison between the medical smartphone reader and the clinical biochemical analyzer.

peripheral blood and venous blood can be used for the test. Both kinds of blood were sampled in fasting state, with the subjects sitting for 5 min before blood collection. Peripheral blood was collected by a professional puncturing the fingertip with a disposable needle and collecting blood for reagents detection. Either ring finger or middle finger is preferred, and blood is collected on both sides of the fingertip. When the blood volume is not enough for a single finger, the professionals use the blood from two fingertips. Patients with a history of paronychia, swelling or skin diseases, should be treated separately. Venous blood samples were taken by health care professionals (using EDTA anticoagulant) with an average volume of about 3 mL. After centrifugation at 4 °C, 3000 rpm for 30 min, the blood serum was extracted by taking the supernatant in the tube for comparative study between the automatic biochemical analyzer and the smartphone reader with the test strip (fingertip whole blood). The correlation curve between the clinical biochemical analyzer and medical smartphone obtained from 153 patients has a slope of 0.969 (intercept, 5.7; correlation coefficient, 0.9627) varying from 100 to 600 μM/L, which demonstrated that the reliability and accuracy are highly acceptable. The main difference between the medical smartphone reader and the clinical biochemical analyzer resulted from the different blood samples. For the electrochemical detection method, the presence of blood cells can have an influence on the measurement result by affecting the mass transfer of target molecules from the bulky solution to the surface of the working electrode.[16,17] It has been summarized that there are several possible mechanisms to describe the cells or other nontarget molecules effect on the enzyme catalyzing process. Erythrocytes of higher concentration in the fingertip blood sample can mechanically retard the plasma diffusing into the electrochemical reaction layer.[18] The blood cells or molecules can affect the hematocrit of the blood sample. The changes in hematocrit be significant for blood viscosity. A higher value of viscosity results in lower fluid permeability, therefore leading to slower diffusion.[19] Other factors such as microclot formation in the blood samples or on the test strips, hemolysis, protein deposition, fibrin aggregation, and platelet can have a significant effect on the test result.[20] These are the main reasons why the national standard allows inaccuracy (taking glucose measurement, for example, less than 15%) of a measured result within a certain tolerance between the POCT device and the biochemical analyzer in the clinical laboratory.[21] In the point of care test application, in the case of abnormal hematocrit, such as infant glucose measurement, a patient of hemodialysis, the hematocrit can result in significant error in the test result. Future works will be focused on the solution to compensate the test result such as roughly evaluating the hematocrit through the viscosity or electrical conductivity method.

Three different concentrations (low, middle, and high) of blood samples are used for the test as compared with the biochemical analyzer. The blood sample was tested by three groups of UA test strips, and the each group test was repeated 10 times in three smartphone readers, respectively, which then generated 900 test results. UA test strips for manufacturer differences are statistically evaluated, respectively. Samples are randomly selected in three blood UA concentrations, namely, low concentration range (<200 μmol/L), median range (201−435 μmol/L), and high range (>436 μmol/L). The test results are summarized in Table 1.

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Table 1. Three Different Concentrations of Blood Samples Are Used for the Test[a]

UA concentration	test strip #1			test strip #2			test strip #3		
	sample number	test result (μmol/L)	CV (%)	sample number	test result (μmol/L)	CV (%)	sample number	test result (μmol/L)	CV (%)
UA (low concentration)	30	229.43 ± 10.47	4.56	30	237.63 ± 9.16	3.85	30	232.73 ± 9.06	3.89
UA (mid concentration)	30	455.77 ± 10.97	2.41	30	472.90 ± 11.09	2.35	30	460.6 ± 12.26	2.66
UA (high concentration)	30	749.57 ± 11.86	1.58	30	761.60 ± 13.95	1.83	30	747.23 ± 16.44	2.20

[a]For each blood sample, three batches of the UA dipstick test were repeated 10 times in three multi-function testers, respectively, and then 900 test results were obtained. Inter- and intrabatch differences of UA test strips are calculated, respectively. Samples are randomly selected in three blood UA concentrations, namely, low concentration range (<200 μmol/L), median range (201−435 μmol/L), and high range (>436 μmol/L).

CONCLUSIONS

In order to improve national self-management of gout, the products ensure not only the accuracy and precision to meet the requirements of in vitro diagnostic medical devices but also mobility, health, and safety. We redefine the smartphone and enable it to monitor UA as an electrochemical reader. Patients equipped with such a medical smartphone can upload their blood UA level and can consult an online doctor for better recovery.

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Notes

The author declares no competing financial interest.

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