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Research Article

Accuracy improvement of electrochemical whole blood ketone sensor based on HCT compensation algorithm

In this manuscript, we demonstrated an electrochemical test strip with HCT (hematocrit) compensation algorithm to improve the accuracy of blood ketone sensor. In the conventional electrochemical sensor, the electrochemical current was directly resolved into the concentration value of the determinant without HCT compensation. For lower or higher HCT blood sample, the measured result was inaccurate. In the proposed design, the blood impedance can be measured to estimate the HCT, which was utilized to compensate the electrochemical current to resolve the more accurate concentration of determinant. The practical blood sample tests demonstrated the proposed design can provide more believable and reliable measured result in clinical point-of-care setting.

Keywords:
Biosensor / Electrochemical sensor / Point-of-care DOI 10.1002/elps.201900472

1 Introduction

In the latest decade, the miniaturized biomedical devices play an indispensable role with increasing importance in the significantly rising healthcare market [1–3]. The application scenarios of point-of-care test vary from the intensive care unit (ICU), chest pain center, emergency department, and even chronic disease management of external hospital patients such as diabetes, gout, hypertension, and hyperlipidemia, etc. Long-term monitoring of the corresponding blood parameters is capable of helping the patients with chronic diseases to obtain an effective cure [4–6]. Therefore, the miniaturized biomedical devices are very important to address the issues at the point-of-care testing. The fundamental requirements of such biomedical devices are summarized. It should be portable, easy handling, cost effective, rapid response, and providing satisfactory measured result, as compared to the bulky clinical lab analyzer such as supporting the fingertip whole blood sample, etc. [7,8].

It is a good strategy to combine the miniaturized biomedical devices together with the smartphone, because the ubiquitous smartphones pave the way for the widely available use of the biomedical devices. For instance, Park et al. presented a smartphone-based microfluidic detection system to characterize the *Salmonella* [9]. Guo et al. reported the utilization of smartphone to implement the ELISA to detect the HIV [10]. Fu et al. demonstrated a smartphone-based photochemical dongle to detect the whole blood creatinine for the proactive renal health management [11]. It is reported that the sensory module can be integrated directly with smartphone to provide the blood test function such as uric acid, glucose, total cholesterol, and triglyceride [12–14]. In the family setting, we reported the usage of TV remote controller as the medical device for diabetes manage of glucose level [15]. In these reported works, the photochemical method can eliminate the effect of hematocrit (HCT) since the blood filtration membrane has filtered out the blood cells. Only the blood plasma can penetrate the membrane to react with the agent. For the typical electrochemical sensor (Fig. 1), there is no membrane to filter out the blood cell which makes the electrochemical sensor susceptible to HCT. When HCT approaches higher or lower level, the measured result by electrochemical sensor is inaccurate. As compared to the photochemical solution, the electrochemical solution is more portable in instrumentation with much lower cost, because of the expensive blood filtration membrane. Therefore, it is rather necessary and crucial to develop an electrochemical sensor with HCT compensation to implement the measurement of determinant.

In this article, we present an electrochemical whole blood ketone sensor with HCT Compensation Algorithm, as illustrated in Fig. 2. One more pair of electrodes (#1 and #5) was utilized to measure the impedance of the whole blood by applying a 1-kHz alternating signal between electrodes #1 and #5 to estimate the HCT. The HCT can induce a coefficient to compensate the electrochemical current generated by the electrochemical reaction which can map more accurate test result from the electrochemical current to determinand concentration. Electrode#2 performed as the working electrode while the electrode#5 as the counting electrode. When the

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Abbreviations: **HCT**, hematocrit; **ICU**, intensive care unit

Color online: See article online to view Figs. 1–4 in color.

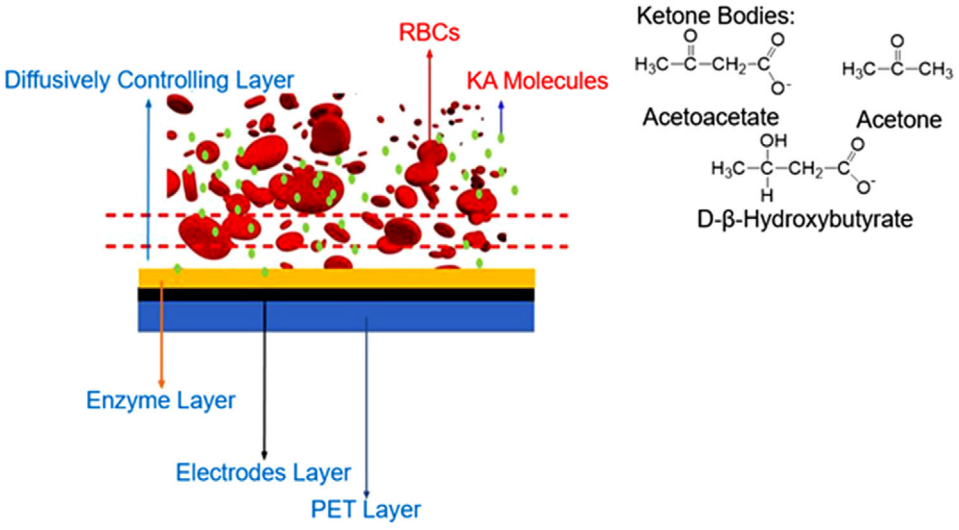

Figure 1. The schematic view of the typical electrochemical ketone sensor. The sensor is composed of the PET layer, printed carbon electrode layer, and enzyme layer.

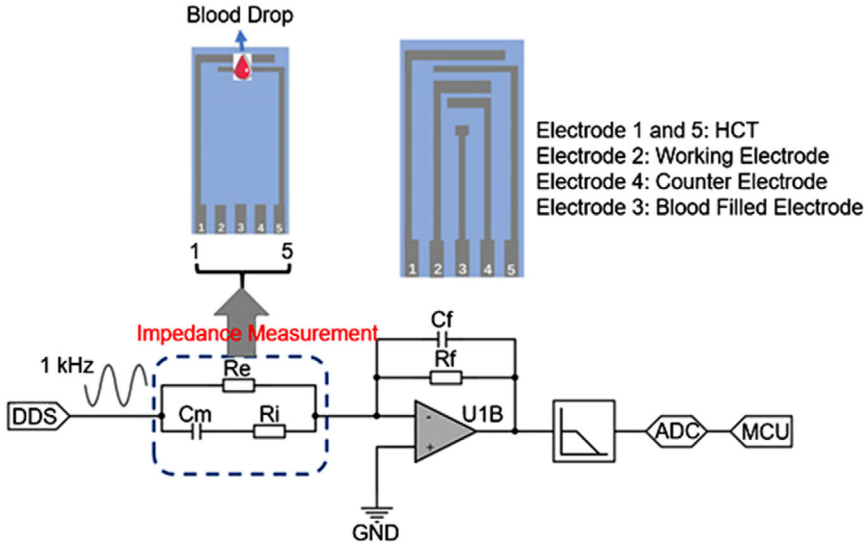

Figure 2. The schematic view of the proposed electrochemical sensor and the circuit diagram of the impedance measurement.

blood flow reaches the electrode#3, the biosensor was activated. A 3.5 μL fingertip whole blood was placed on the strip and the hydrophilic layer can drive the blood flow through the reaction channel and cover the working electrode. More accurate test result with HCT compensation algorithm was obtained as compared to the conventional electrochemical ketone sensor. Therefore, the proposed HCT-based electrochemical sensor is a more promising technique for the point-of-care test in clinical application.

2 Theory and methods

The proposed electrochemical biosensor was based on the kinetics of enzyme-catalyzed reactions. The D-3 hydroxybutyric acid as the major ketone in blood was oxidized into acetoacetic acid with the reduction of NAD to NADH on the presence of paramount D-3 hydroxybutyrate dehydrogenase. The NADH can be oxidized into NAD with the electron transfer at the surface of the working electrode under the electrical voltage of 200 mV. The electron transfer induced a characteristic current generation by the electrochemical reaction. Therefore, the concentration of D-3 hydroxybutyric acid is linearly proportional to the electrochemical current. With the help of the calibration curve, the proposed sensor can directly measure the blood ketone level by the electrochemical method. The working principle of the ketone biosensor was described in the following reaction formula.

$$(1)\,D3\text{hydroxybutyricacid} + \text{NAD} \xrightarrow{D3\text{hydroxybutyratedehydrogenase}} \text{AcetoaceticAcid} + \text{NADH}.$$

$$(2)\text{NADH} \rightarrow \text{NAD} + e^{-}\,\text{under200mV}.$$

A 1 kHz sinusoidal wave was generated and was applied between the electrodes #1 and #5. After the blood droplet was placed, the impedance of the whole blood was measured

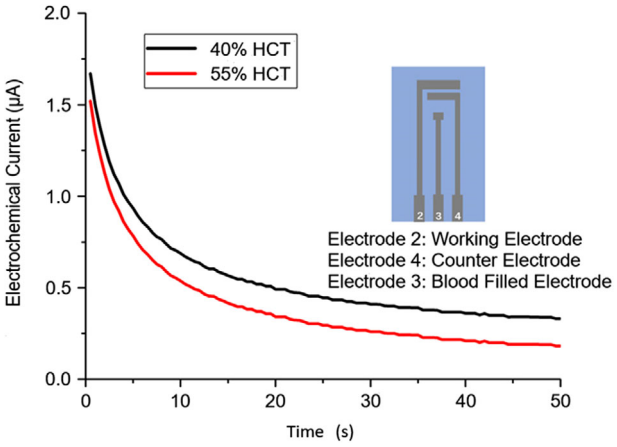

Figure 3. The typical I-t curve of the electrochemical sensor by applying the same concentration of blood ketone with 40% and 55% HCT, respectively.

which can reveal the HCT information. The electrochemical current induced by the dehydrogenase catalyzed reaction was recorded by the electrometer, simultaneously. During the process of resolving current to the measured concentration of the determinand, the HCT correctified coefficient makes a compensation on the electrochemical current and results in a more accurate resolved concentration of the determinand (the blood ketone molecules in this letter).

The proposed electrochemical test strip was fabricated by the standard screen printing process. The enzyme solution was loaded within the reaction channel and fully covered on the working electrode surface. As the following step, the enzyme solution was immobilized through the stove tunnel at 37°C for 18 min. The hydrophilic layer was pasted on the test strip to form the final test strip.

3 Results and discussion

For the sake of estimating the blood HCT effect on the sensor accuracy of the proposed electrochemical test strip, we investigated the HCT effect on the electrochemical current. Fingertip whole blood was obtained by a disposable lancet punching the finger and collected by the EDTA anticoagulant tube. The blood plasma was separated by the centrifugal machine with the rotation speed at the 3000 rpm for 10 min. The micropipette was utilized to prepare the blood sample with different concentrations of red blood cells, which resulted in different HCTs. As the Fig. 3 indicated, the typical I-t profile of electrochemical sensor shows different electrochemical current under the same blood ketone level but different HCTs. A total of 40% HCT with the same concentration of blood ketone has a higher current amplitude as compared to the 55% HCT blood sample. As mentioned before, this phenomenon happed due to the fact that higher HCT means more red blood cells exist in the blood sample. Red blood cells can impede the ketone molecules diffuse or transfer from the bulky blood solution to the surface of the working electrode. Less molecules approach to the electrode surface results in weaker electrochemical reaction. That means the smaller electrochemical current is generated. Therefore, if there is no HCT compensation, the measured concentration of the target blood ketone molecules was lower than the practical ketone concentration in the blood sample.

In order to compensate the electrochemical sensor, the HCT needs to be estimated. In the proposed sensor, 1 kHz signal was generated and applied to the impedance measurement electrodes 1 and 5. Different red blood cells were spiked in different blood plasma samples with the same blood ketone level (but different HCTs), which were applied to the test strips. Figure 4 indicated the measured impedance between electrodes 1 and 5 as a function of HCT. The quantitative relationship between the measured impedance and the HCT was obtained by the parabolic curve fitting. In the Fig. 4B, the decrease in electrochemical current due to the HCT effect was quantitatively measured. The decrease in electrochemical current was oppositely linear proportional to the HCT. Therefore, once the blood impedance was measured, the HCT can be estimated by the quantitative curve. We can speculate the decrease in electrochemical current with the HCT parameter. When resolving the current signal to the concentration of determinant, the compensation was made in order to obtain a more accurate measured value.

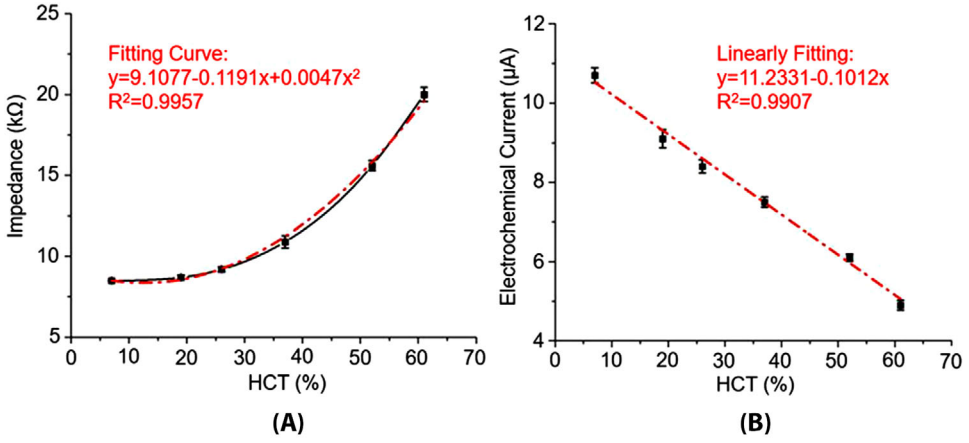

Figure 4. (A) The measured impedance under 1 kHz signal as a function of different HCT; (B) the decrease in the measured electrochemical current with the HCT increased.

Table 1. The accuracy comparison with/without HCT correction, summarized in three test groups

Deviation $= \left| \frac{Y\text{measured} - Y\text{truth}}{Y\text{truth}} \right| \times 100\%$ (1)

KA Blood samples (mmol/L)	Without HCT compensation (%)	With HCT compensation (%)
0.71	<25	<12
1.96	<25	<12
3.38	<20	<8

Each group contain six blood samples with different HCTs from 20% to 70%.

Three blood samples from three patients are divided into three groups with the blood ketone level at 0.71, 1.96, and 3.38 mol/L, respectively. Each blood samples were mixed with different concentrations of red blood cells. Finally, six blood samples from each group were applied to the proposed electrochemical test strip with HCT at 20%, 30%, 40%, 50%, 60%, 70%, respectively. For each group, the HCT correctified measurement result was compared to the measured result without HCT correction. The accuracy comparison with/without HCT correction of the three group was summarized in Table 1. It demonstrated the HCT corrected algorithm can significantly improve the accuracy as compared to the conventional electrochemical test strip.

4 Concluding remarks

In this manuscript, we have presented an accuracy improved electrochemical ketone test strip with HCT compensation algorithm. As compared to the conventional electrochemical ketone sensor, the proposed ketone sensor can have a good tolerance with respect to the wide range of blood cell, which is very useful to improve the measured accuracy of point-of-care test field.

The authors have declared no conflict of interest.

References

[1] Sun, J., Xianyu, Y., Jiang, X., *Chem. Soc. Rev.* 2014, *43*, 6239–6253.

[2] Guo, J., Zeng, F., Guo, J., Ma, X., *J. Mater. Sci. Technol.* 2020, *37*, 96–103.

[3] Zhang, L., Ding, B., Chen, Q., Feng, Q., Lin, L., Sun, J., *TrAC Trends Anal. Chem.* 2017, *94*, 106–116.

[4] Guo, J., *Anal. Chem.* 2016, *88*, 11986–11989.

[5] Guo, J., Ma, X., *Biosens. Bioelectron.* 2017, *94*, 415–419.

[6] Guo, J., *Anal. Chem.* 2017, *89*, 8609–8613.

[7] Xu, D., Huang, X., Guo, J., Ma, X., *Biosens. Bioelectron.* 2018, *110*, 78–88.

[8] Huang, X., Xu, D., Chen, J., Liu, J., Li, Y., Song, J., Ma, X., Guo, J., *Analyst* 2018, *143*, 5339–5351.

[9] Park, T. S., Li, W., Mccracken, K. E., *Lab Chip* 2013, *13*, 4832–4840.

[10] Guo, T., Patnaik, R., Kuhlmann, K., Rai, A. J., Sia, S. K., *Lab Chip* 2015, *15*, 3514–3520.

[11] Fu, Y., Tan, H., Wu, X., Wu, X., Yang, Y., Gao, Y., Liu, R., Qi, M., Chen, X., Ning, Y., Sun, W., Chang, N., Ma, J., Cheng, K., Yang, H., Li, Q., Wang, P., Wu, C., Xian, H., Wang, L., *Electrophoresis* 2020, *41*, https://doi.org/10.1002/elps.201900136.

[12] Wang, J., Huang, X., Tang, S. Y., Shi, G. M., Ma, X., Guo, J., *IEEE J. Biomed. Health* 2018, *23*, 66–71.

[13] Fu, Y., Guo, J., *IEEE Trans. Biomed. Circ. Syst.* 2018, *12*,784–790.

[14] Xu, J., Wang, L., Shen, Y., Yuan, K., Nie, Y., Tian, Y., Jian, X., Ma, X., Guo, J., *IEEE Trans. Ind. Inform.* 2018, *15*, 2321–2329.

[15] Guo, J., *IEEE Trans. Ind. Inform.* 2017, *14*, 2592–2597.

