

## Potentiometric Glucose Detection by Paper-based Electrochemical Sensor on CMOS Chip

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### Abstract

*This paper presents a low cost portable medical device for biochemical sensor using CMOS chip and paper-based fluidic channel. We measured a potential produced by enzyme activity of glucose between the working and reference electrode on CMOS chip. A liquid sample is transported by paper-based fluidic channel, which is made of chromatography paper and silicone resin, and consists of the area for filtering a sample (filter layer) and that for reacting enzyme (enzyme layer). The paper-based fluidic channel is used by combining CMOS chip, and the solution with glucose is dropped from top of the paper-based fluidic channel. The concentrations of glucose are detected by potentiometry (open circuit potential time). The experimental results show that the glucose concentration is measured by CMOS chip and paper-based fluidic channel.*

**Keywords:** CMOS LSI chip, paper-based fluidic channel, electrochemical detection, glucose, open circuit potential time

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### 1. Introduction

In recent years, population of elderly is increasing at developed countries. In particular, 40% of Japanese population is expected to be over 65 years old [1]. As elder people tend to have difficulty in moving a long distance, the demand for medical checkup at home is increasing. Meanwhile, low cost portable medical devices would improve healthcare environment in developing countries as well. Thus, an inexpensive and portable medical testing equipments are required in most of the countries. These medical testing equipments include a glucose sensor and an alcohol sensor.

The sensor developments have been carried out by using a CMOS chip and electrochemical measurement [2]. The CMOS chip miniaturize to the mm size and integrate electronic circuits. Also, electrochemical measurement is possible to quantify the measurement results. The research is conducted by combining potentiometry as one of the electrochemical measurement method with CMOS chip [3]. The research detected enzyme activity, and the potential is measured by using the circuits [4]. The bottom of microwells and outlet of the solution were formed by an epoxy resin (SU-8), and the pump is used to deliver the solution from the outlet [4, 5]. However, this method requires the external pump in addition to the CMOS chip. It is sufficient for use in the laboratory, but it is not suitable as the small medical testing equipments [6].

In this work, we focused on capillary of chromatography paper (CHRPR) and silicone resin. The capillary of CHRPR enables to deliver a solution, and CHRPR is possible to process miniaturization [7]. In addition, silicone resin do not contaminate the solution [8]. We conducted detection of glucose by potentiometry using CMOS chip and fluidic channel made of CHRPR and silicone resin.

### 2. Research Method

We used the open circuit potential time (OCPT) as the measurement method. The OCPT is theoretically represented by the Nernst equation:

$$E = E^{\circ} + \frac{RT}{nF} \ln \left( \frac{a_{\text{Ox}}}{a_{\text{Red}}} \right), \quad (1)$$

where  $E$  is the electrode potential [V],  $E^\circ$  is the standard electrode potential [V],  $R$  is the gas constant [ $\text{JK}^{-1}\text{mol}^{-1}$ ],  $T$  is the temperature [K],  $n$  is the number electron,  $F$  is the Faraday constant [ $\text{C/mol}$ ],  $a_{\text{Ox}}$  is the oxidant concentration [M],  $a_{\text{Red}}$  is the reductant concentration [M]. The electrode potential is determined by the standard electrode potential and the ratio of the oxidant concentration and reductant concentration in the solution [9].

In the measurement of glucose, the oxidant is ferricyanide (FERRI,  $[\text{Fe}(\text{CN})_6]^{3-}$ ) and the reductant is ferrocyanide (FERRO,  $[\text{Fe}(\text{CN})_6]^{4-}$ ). FERRO are produced by reducing FERRI, after the glucose is oxidized by the glucose oxidase (GOD) (Figure 1).

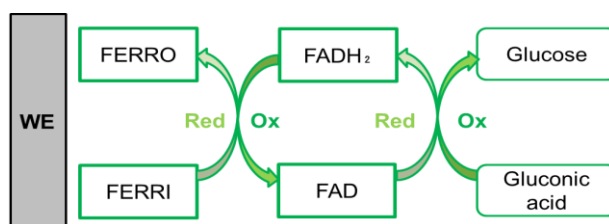


Figure 1. The enzyme reaction of glucose

The electrode potential is determined by the concentration ratio of the FERRO and FERRI. Increasing the concentration of glucose decreases the electrode potential because increasing the concentration of glucose produces the FERRO. We used an electrode that was fabricated on the CMOS chip to measure the glucose (Figure 2).

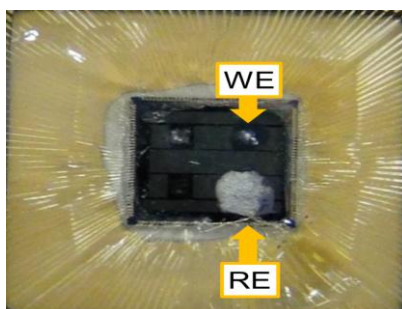


Figure 2. CMOS chip electrode

The CMOS chip (Rohm 0.18 $\mu\text{m}$  1P5M standard CMOS process) was designed in our laboratory. The four metal pads on the chip are arranged, and they are wired to the outside of chip. We made working electrode (WE) and reference electrode (RE) on these metal pads. The WE was made by graphene ink (Sigma–Aldrich, 798983), the metal pad is covered with graphene ink, and the CMOS chip is heated for 60 minutes at 300 °C on a hot plate. In a method for fabricating the RE, the metal pad is covered with the silver-silver chloride ink for the reference electrode (Ag/AgCl, BAS, 011464), and then naturally dried at room temperature for 12 hours (humidity is 60%, temperature is 25°C). After electrodes are formed, the bonding wires are coated by the silicone resin (Momentive Performance Materials Japan LLC, TSE399–C) in order to prevent from the leakage of the solution and disconnection of wiring.

The fabricated electrodes are combined with a fluidic channel made from CHRPR (CHRPR fluidic channel) (Figure 3) [10].

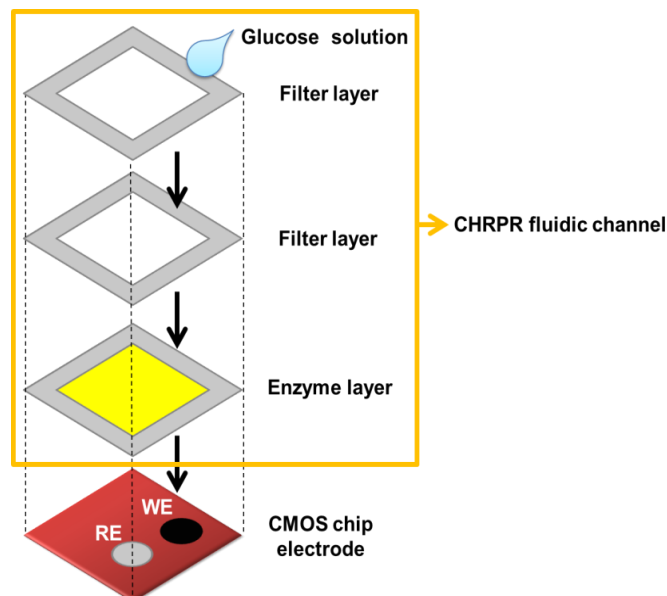


Figure 3. Bird's-eye view of a combination of CMOS chip electrode and CHRPR fluidic channel

The CHRPR fluidic channel consists of two pieces of paper. The first sheet filters the samples (filter layer) and the second sheet contains an enzyme and mediator (enzyme layer). The CHRPR fluidic channel was made of CHRPR (Whatman 1CHR) and silicone resin (TSE3996-C). The fabrication of the CHRPR fluidic channel are used for a robot arm (DESKTOP ROBOT MUSASHI ENGINEERING, INC, SM200SX-3A) and the dispenser (Precision dispensing machine, Musashi engineering, ML-5000X II). We fabricated the patterns of the fluidic channels using the software which controls the robot arm. The robot arm dropped a silicone resin to CHRPR in the syringe, and the silicone solidifies by humidity (humidity is 60%, temperature is 25°C) for one day. The patterns of the CHRPR fluidic channel are shown in Figure 4.

We fabricated an enzyme layer on one of the fluidic channels. The enzyme layer was functionalized by glucose oxidase (GOD, Wako 190units/ g, 074-02401), potassium ferricyanide (FERRI, Wako, 161-03725) and phosphate-buffered saline (0.01 mol/L, PBS, Wako, 164-18541).

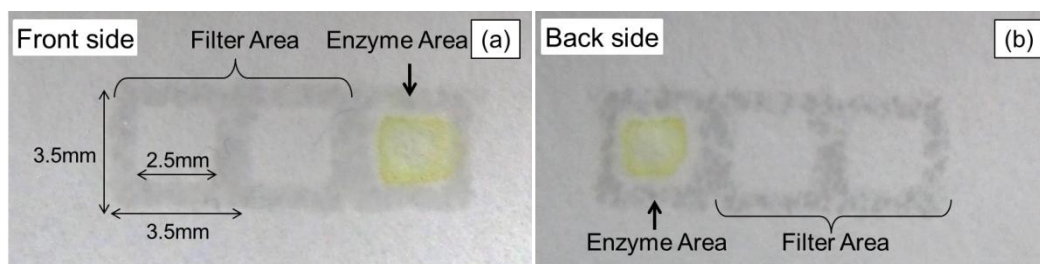


Figure 4. The patterns of the fluidic channels, (a) Front side, (b) Back side

The enzyme solution contains the GOD and Ferri in the PBS, and was dropped into the channel, and dried in the constant temperature dryer (As-one ONW-300S) for 5 minutes (60°C). After heating, the filter layer and enzyme layer are cut (3.5mm square) and laminated. The circumference of the filter layer and the enzyme layer are painted by silicone resin (TSE397-C). The silicone is fixed by humidity (humidity 60%, temperature 25 degrees) for 5 minutes (Figure 5).

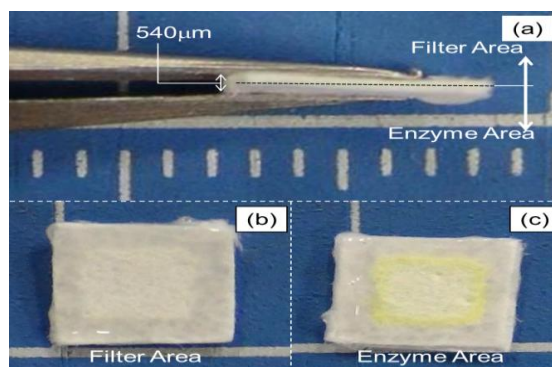


Figure 5. CHRPR fluidic channel, (a) aspects, (b) filter layer, (c) enzyme layer

In the measurement, the CHRPR fluidic channel was placed on the CMOS chip electrode. The CMOS chip electrode was connected to the electrochemical measurement analyzer (ALS, ALS/CH Instruments Electrochemical Analyzer Model 610DR) (Figure 6).

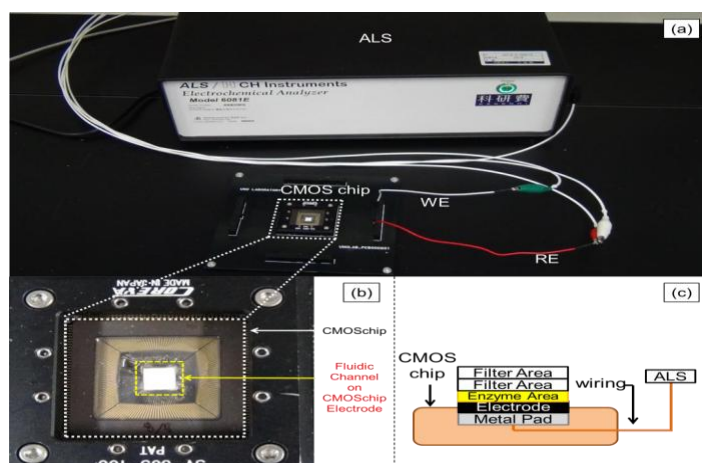


Figure 6. (a) Connecting the CMOS chip and the electrochemical measurement analyzer, (b) Placing CHRPR fluidic channel on the CMOS chip electrode, (c) The schematic view of the CMOS chip, CHRPR fluidic channel and electrochemical measurement analyzer,

### 3. Results and Analysis

The glucose solution was dropped on top of the filter layer. The concentrations of glucose in the solution are 0.5mM, 1.0mM, 2.0mM, 5.0mM and 10mM. The glucose solutions contain glucose (MP Biomedicals, 492-61-5) in the PBS. The sample volume was 20 $\mu$ L and the measurement time was 150s.

The experimental results shown in Figure 7 indicate that the glucose concentration is measured using the CMOS electrodes and the CHRPR fluidic channel. The decreases of the potential with increasing the concentration of glucose were confirmed by the results at 150s. The decrease is attributed to the fact that FERRO was produced by enzyme reaction of glucose and the concentration of FERRO was higher than FERRI. In this way, Potential takes negative value according to the Nernst equation Equation (1), and the decrease of potential was confirmed. In addition, the reproducibility was represented by 1.0mM, 2.0mM, 5.0mM and 10mM.

The calibration curve of average potential and errorbars in 150s are shown in Figure 8. The slope of the calibration curve was 45mV, while the theoretical value is 26mV. One possible explanation for the discrepancy between the theoretical and experimental slopes is the

detection accuracy of 0.5mM. The experimental slope approaches theoretical one by improving detection accuracy of 0.5mM. The  $R^2$  of the experimental results was 0.929, so reproducibility is gained by the experiment because the  $R^2$  is close to unity. The minimum glucose concentration of detection by the experiment was 1mM. The concentration of glucose for diagnostics of diabetes is 11mM. Thus, the results of the experiment suggest the ability to apply for medical check-up.

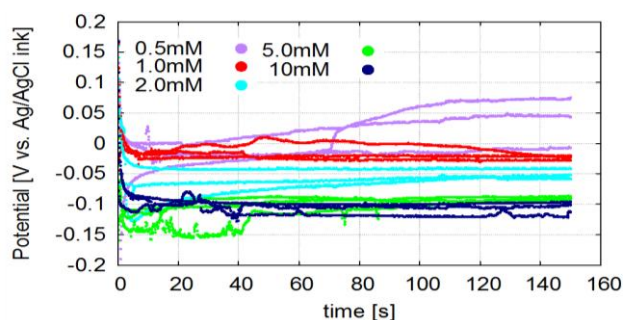


Figure 7. OCPT for three concentrations of glucose (Horizontal axis is time [s] and vertical axis is potential [V])

Table 1. Average potential in 150s

Concentrations of glucose [mM]	Average potential [V]
0.5	0.03738
1.0	-0.02339
2.0	-0.05054
5.0	-0.08991
10	-0.10280

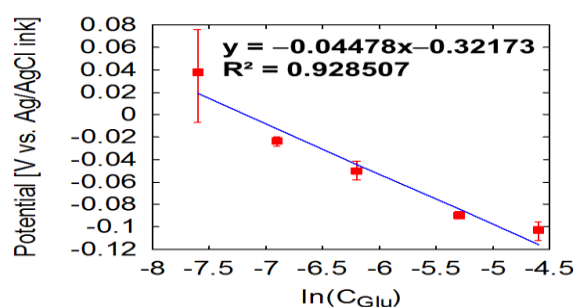


Figure 8. Calibration curve of average potential and errorbars in 150s (Horizontal axis is logarithm of glucose concentrations [M] and vertical axis is potential [V])

#### 4. Conclusion

In this paper, we measured the concentrations of glucose (0.5mM, 1mM, 2mM, 5mM and 10mM) using CMOS chip electrode and CHRPR fluidic channel by electrochemical measurement. The glucose concentration and the reproducibility were represented by the measurement (the slope is 0.045,  $R^2$  is 0.929). It was confirmed with a combination of CHRPR fluidic channel, which have conventional equivalent function, and a CMOS chip was possible to measure glucose concentration. Future prospects are to develop biosensing device based on the CMOS chip electrode and the CHRPR fluidic channel for a blood test, a gas test and a urine test.

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