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An Enzymatic Amperometric Glucose Sensor on CMOS Chip using Carbon Ink Electrode and Chromatography Paper

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Abstract

An enzymatic amperometric sensor electrodes on CMOS chips using carbon ink and chromatography paper is presented. Carbon ink electrodes formed on a CMOS chip show good electrochemical performance. Such on-chip electrodes in contact with chromatography paper functionalized by glucose oxidase successfully act as a glucose sensor.

1. Introduction

Nowadays, development of low cost and small-sized biosensors has led to many forms of sensor devices, including those based on complementary metal-oxide-semiconductor (CMOS) large-scale integrated circuit (LSI) chips. Such CMOS biosensors have successfully demonstrated potentiometric electrostatic potential and amperometric current sensing [1, 2]. Amperometric sensor electrodes on those CMOS chips are realized by topmost metal layer covered by inert noble metals such as Au or Pt. The sensor electrodes may be functionalized by immobilizing enzymes to form "enzyme electrodes", detecting specific molecules such as glucose [3, 4]. However, the use of noble metals and difficulties in enzyme immobilization are the main obstacles to low-cost fabrication and mass production. In this work, we propose a cost-effective fabrication method of amperometric sensor electrodes and its functionalization by enzymes. We use carbon ink as a substitution for noble metal, and chromatography paper (ChrPr) as enzyme supporting layer attached on top of CMOS chip. Although the use of ChrPr as enzyme supporting layer has been proposed [5], it has not been tested on CMOS platform.

2. Experimental method

The CMOS chips were fabricated with 0.35μm standard process, and three 1.0mm square metal pads were formed on a 5.0mm square chip, (Fig. 1(a)). The working and counter electrodes were defined by dropping and drying carbon ink on two of the metal pads, and the remaining pad was covered by Ag/AgCl ink to form the reference electrode, (Fig. 1(b) and (c)). We first tested electrochemical performance of the carbon ink electrodes by cyclic voltammetry (CV) measurement using an aqueous solution containing $K_4[Fe(CN)_6]$ and Na_2SO_4 (Fig. 2(a)). Next, ChrPr was placed onto the electrodes and fixed by a polyvinyl-chloride (PVC) plate. Chronoamperometry (CA) measurement was then performed with the aqueous solution mentioned above, transferred through the ChrPr by capillary flow (Fig. 2(b)). Finally, ChrPr was dipped into the phosphate buffer solution (PBS) with glucose oxidase (GOD) and $K_3[Fe(CN)_6]$, and dried in refrigerator at 4°C to form enzyme supporting membrane. Such GOD modified ChrPr was then used to detect β-D glucose in PBS (pH7.0) transferred through the ChrPr (Fig. 2(c)). The chemical reaction shown in the bottom right of the next page occurs to give CA current proportional to the glucose concentration. All the electrochemical measurements were performed by ALS/CH Electrochemical Analyzer Model 610DR.

3. Results and discussion

Fig. 3 shows CV measurement results taken under the experimental setup shown in Fig. 2(a), where the aqueous solution with $K_4[Fe(CN)_6]$ is directly dropped onto the on-chip electrodes. The obtained curves show distinct redox current peaks proportional to $K_4[Fe(CN)_6]$ concentration, demonstrating that the carbon ink electrodes work as well-behaving amperometoric electrodes. Fig. 4(a) shows CA measurement results from the setup shown in Fig. 2(b), where the aqueous solution is now transferred onto the on-chip electrodes through the ChrPr. As is demonstrated in Fig. 4(b), the current taken at t = 20s is proportional to $K_4[Fe(CN)_6]$ concentration, indicating that stable electrochemical measurement is possible even with the ChrPr. Fig. 5(a) shows CA measurement results using the glucose solution from the setup shown in Fig. 2(c), where ChrPr now supports GOD and $K_3[Fe(CN)_6]$ as electron mediators. Fig. 5(b) shows measured current at t = 20s as a function of glucose concentration, demonstrating a linear correlation between redox current and glucose concentration relevant for physiological fluids such as human blood.

4. Conclusion

A cost-effective fabrication method of enzymatic amperometric sensor electrodes on CMOS chips using carbon ink and chromatography paper has been presented. Carbon ink electrodes on a CMOS chip have shown good performance, and GOD modified chromatography paper on top of those electrodes successfully acted as glucose sensing membrane. This paves a way for low-cost small-sized smart CMOS biosensors.

References

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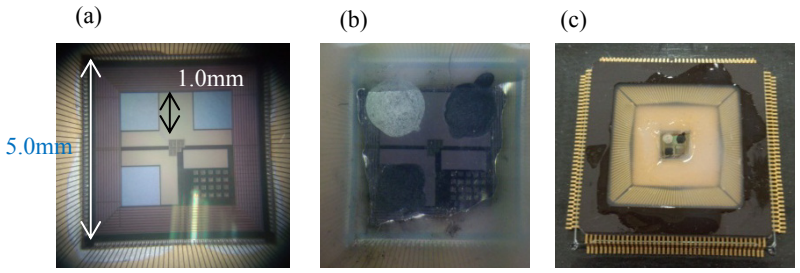


Fig.1 Micrograph of CMOS chip (a) three 1.0mm square metal pads formed on a 5.0mm square chip The CMOS chip. (b) The working and counter electrodes defined by dropping and drying carbon ink , reference electrode defined by Ag/AgCl. (c) The CMOS chip fabricated with 0.35μm standard process.

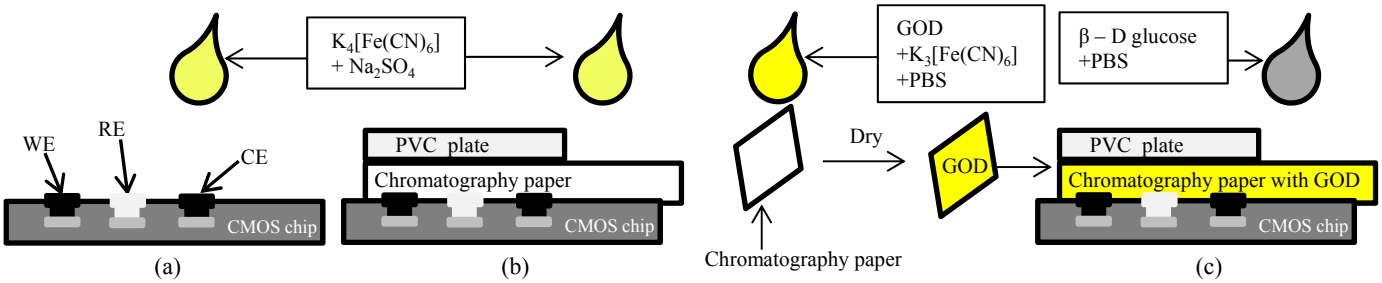


Fig.2 Schematic illustrations of experimental setups (a) directly dropping an aqueous solution on electrodes. (b) Chromatography paper placed onto the electrodes and fixed by a polyvinyl-chloride plate. (c) Chromatography is dipped into the phosphate buffer solution (PBS) with glucose oxidase (GOD) and $K_3[Fe(CN)_6]$, and dried in refrigerator at 4°C, then used to detect β-D-Glucose in PBS (pH7.0) transferred through the Chromatography.

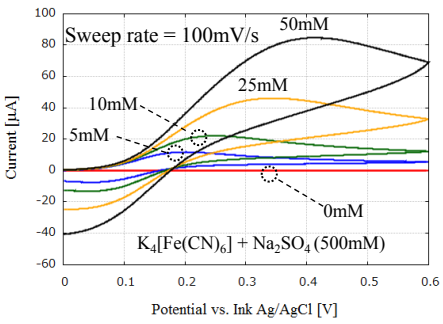


Fig.3 Cyclic voltammograms for various $K_4[Fe(CN)_6]$ concentrations : 0, 5, 10, 25 and 50mM under the experimental setup shown in Fig. 2(a).

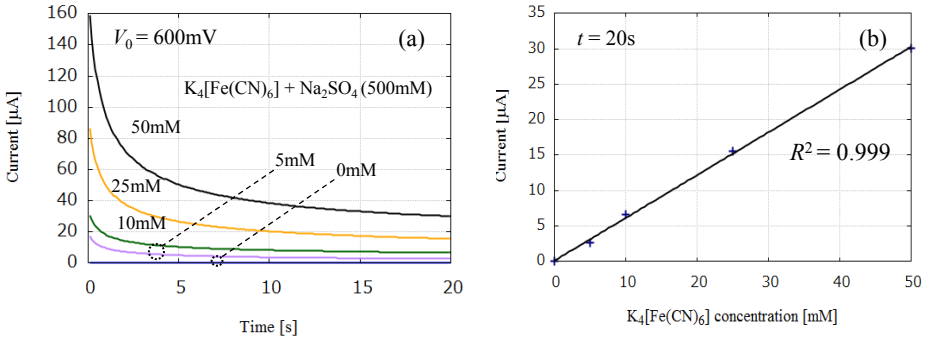
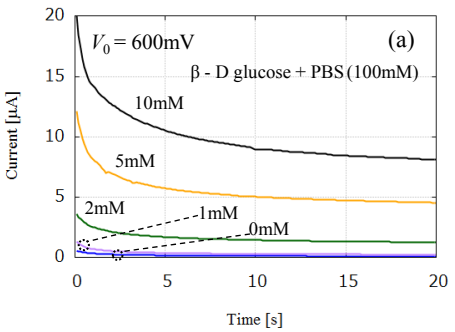
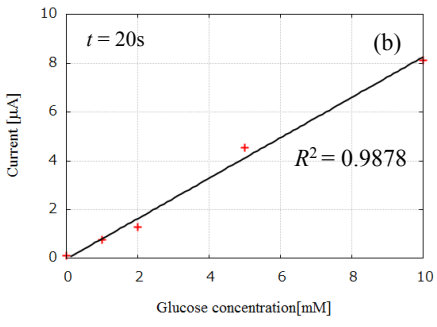


Fig.4 (a) Chronoamperometric curves for various $K_4[Fe(CN)_6]$ concentrations : 0, 5, 10, 25 and 50mM from the setup shown in Fig. 2(b). (b) The current taken at t = 20s as function of $K_4[Fe(CN)_6]$ concentration.





Glucose + $2[Fe(CN)_6]^{3-}$
→ Gluconic acid + $2[Fe(CN)_6]^{4-}$

$[Fe(CN)_6]^{4-} \rightarrow [Fe(CN)_6]^{3-} + e^-$

Fig.5 (a) Chronoamperometric curves for various glucose concentrations :0, 1, 2, 5 and 10mM from the setup shown in Fig. 2(c). (b) Calibration plot of current at t = 20s as a function of glucose concentration.