An amperometric on-chip glucose biosensor based on enzyme entrapment with pre-reaction to lower interference in a flow injection system

Po-Chin Nien[a], Meng-Chi Huang[b], Fuh-Yu Chang[b] and Kuo-Chuan Ho[a,c,*]

[a]Department of Chemical Engineering and [c]Institute of Polymer Science and Engineering, National Taiwan University, Taipei 10617, Taiwan

[b]Mechanical Industry Research Laboratories, Industrial Technology Research Institute, Hsinchu 310, Taiwan

*Corresponding E-mail: kcho@ntu.edu.tw

ABSTRACT

In this study, the flow injection analysis was applied to the enzyme-entrapped electrode on a chip for sensing glucose. The on-chip microelectrode was fabricated by the standard photolithography in clean-room environment and the microfluidic channel height of 100 μm on the chip was formed by poly(dimethylsiloxane). The conducting polymer, poly(3,4-ethylenedioxythiophene), PEDOT, was electropolymerized to entrap enzyme by cyclic voltammetry. At 10 ml/hr, the enzyme electrode was set at 0.7 V (*vs.* Ag) and sensing of H_2O_2 was done by injecting various concentrations of glucose (Glu) samples. The linear relationship between the sensing current and the glucose concentration, ranging from 0.1 to 20 mM, was obtained with a sensitivity of 8 nA mm^{-2} mM^{-1}. Besides, the response time and the recovery time were about 30 and 230 s, respectively. For a single-potential test, both 0.08 mM AA and a blend of 0.08 mM AA plus 10 mM Glu samples reached 31.3 % and 145.5 % of the current for 10 mM Glu, respectively. In contrast, the current for the blend sample reached 99.6 % that of 10 mM Glu in a bi-potential test.

Keywords: biosensor, enzyme, glucose, interference, microfluidic, on-chip.

1 INTRODUCTION

The Micro-Electro-Mechanical-Systems, MEMS, has become more and more popular for fabricating sensor chip. Due to the recent development in biotechnology, bio-MEMS is widely incorporated into the microfluidic devices in biosensors. The sensing chips integrate the steps of sampling, reaction, separation and detection on a chip [1]. Additionally, they miniature the size and have the properties of fast response, less sample and low cost [2]. This chip is also called Lab-on-a-chip. For example, antibody-based chips for determining protein isoform [3], liquid-chromatography-based chips for detecting peptide mixture [4], and electrophoresis-based chips for sensing catechol and dopamine [5].

The materials made of the channel have many choices, such as poly(dimethylsiloxane) (PDMS) [6], poly(methyl-methacrylate) (PMMA) [7] and polycarbonate (PC) [8] … etc. PDMS offers many advantages, including high plasticity, pervious to light, bio-compatible and good mechanical stability. PDMS can be used not only for the channel mold but also for the gas-pump [9] and micro-valve [10]. By filling and releasing the gas in PDMS layer, the PDMS film can move the fluid in the micro-channel.

Glucose is a common and important biological species in human blood. The normal value of glucose is 3.5~5 mM. For getting good specific property, enzymes are widely applied as recognized molecules. However, there are many species which do affect the amperometric sensing signals. The cationic exchange membrane, Nafion®, has been proposed and put outside of the electrode to prevent the interfering species from reaching the surface of the electrode in previous work. In this study, the three-electrode pattern is formed on the glass substrate. Then it is covered by the PDMS as the channel to form sensor chip. Finally, the conducting polymer, PEDOT, was used as the matrix to entrap the glucose enzyme on the electrode [11]. Test samples can be injected into the flow system for determining the glucose concentration in series with less interference by operating with an additional working electrode.

2 EXPERIMENTAL

2.1 Fabrication of on-chip electrode

First, the design electrode shown in Fig. 1 was transferred to the film mask with a resolution of 10,000 dpi. All of the following steps were done by silicon planar technology in a lean-room environment. The glass wafer with a diameter of 4 in and a thickness of 1 mm was cleaned by acetone and coated with hexamethyldisilazane (HMDS) by vapor priming. Then the glass wafer was

covered with chemical photoresist FH-6400 by spin-coating at 1,500 rpm for 30 s and hardened at 90 ℃ for 90 s on a hot plate. After soft baking, the wafer was selectively exposed through a UV mask aligner (EVG620) to UV light (12 s, 10 mJ/cm^2) with the first mask comprised the section of the reference electrode, and the developing takes place in the FHD-5 solution for 12 s. Afterward, the wafer was cleaned by water and dried by nitrogen. The metal layers of reference are Cr, Au and Ag by sputtering in order, and the thickness is about 30, 90 and 360 nm, respectively. The unnecessary metal layers were lifted off in the acetone solution by supersonic. In the same process, the working electrode with 30 nm Cr as the adhesive layer and 100 nm Pt was prepared by the second mask.

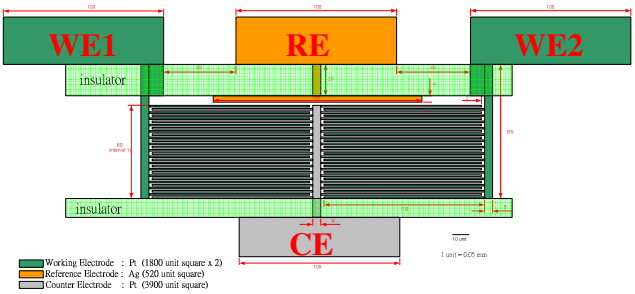


Figure 1: The schematic of the microelectrode.

2.2 Fabrication of channel and device

The fabrication process of the channel used Si molds which were the mother molds of PDMS stamp. First, silicon substrates were washed with acetone and sulfuric acid to remove any organic contaminants. All the substrates were then dried under an N_2 stream and used immediately after cleaning. Silicon wafers were coated with a 2 μm thick positive photoresist (FH-6400) by using a spin coater. They were pre-baked on a hot plate with a temperature of 90 ℃ for 90 s. The light exposure was followed for 12 s and developed for 12 s. Finally, silicon wafer was dry-etched by ICP for 100 μm deep and the patterns were transferred to the silicon substrate. A mixed viscous pre-polymer solution, PDMS (Sylgard 184, Dow Corning) and curing agent in the ratio of 10:1, was poured over the silicon mold for a period of time to remove bubbles, and then thermally cured at 60 ℃ in an oven for 20 min. After curing the pre-polymer, the PDMS mold could be peeled off from the silicon master.

The PDMS and the glass wafer were bombarded by oxygen plasma to modify their surfaces to become hydrophilic temporarily. Then PDMS was covered and glued on the glass to form the sensor chip.

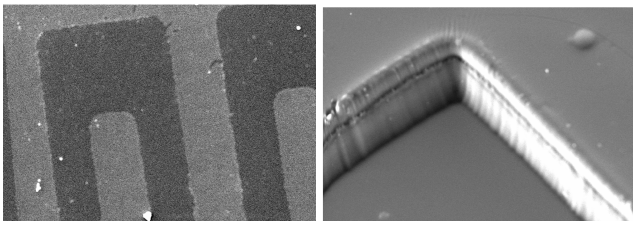
2.3 Preparation of enzyme-modified film

The polymerization condition is similar to the one mentioned in our previous work [11]. The conducting polymer, PEDOT, was used as the matrix to entrap the glucose oxidase for immobilization. The PEDOT was electropolymerized on "WE1" in Fig. 1 in 0.02 mM phosphate buffer solution (PBS, pH=7.4) containing 2,000 U/ml glucose oxidase and 0.3 M KCl at a flow rate of 5 ml/hr by cyclic voltammetry with the potential scanned between 0.2 and 1.2 V (*vs.* Ag) for 15 cycles.

3 RESULTS AND DISCUSSIONS

3.1 Surface of electrode and channel

In each 4 in glass wafer, it can be cut into two piece chips (70 mm × 35 mm) of microelectrode arrays by a diamond cutter. The microelectrode arrays can be divided into four sections (shown in Fig. 1), including the two working electrodes, WE1 and WE2, with the same area of 4.5 mm^2, the reference electrode with an area of 1.3 mm^2 and the counter electrode with an area of 9.75 mm^2. The surface metal layer of the working, reference and counter electrodes are platinum, silver and platinum, respectively. The microfluidic channel height of 100 μm on the chip was formed by means of combining the cover of PDMS, which was built-up by a stamp of silicon wafer. The SEM pictures of the electrode array and the PDMS channel were shown in Fig. 2. In Fig. 2a, the electrode and the gap are almost the same width as that of the designed mask. In Fig. 2b, the channel with a smooth surface has a pit, which is the same as the silicon stamp.



(a) (b)

Figure 2: (a) The pattern of the electrode (500X)
(b) The PDMS mold (500X in 40^0)

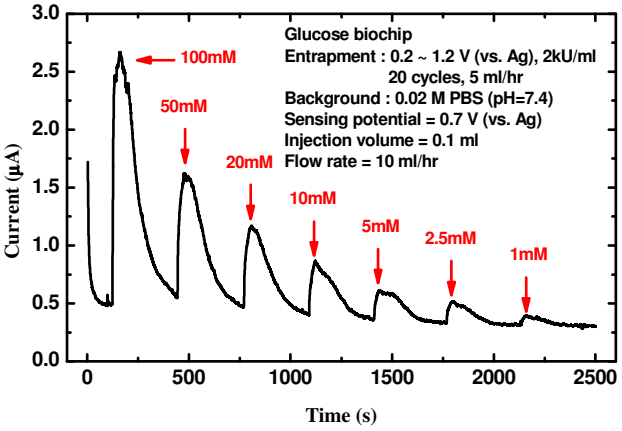
3.2 Performance of the sensor chip

The sensing signals are obtained indirectly by detecting H_2O_2 at a 0.7 V (vs. Ag). The glucose oxidase entrapped in the polymer catalyzes the glucose to produce H_2O_2. In a flow system, adjustment on parameters could either make the performance better or poorer. For example, the higher the flow rate, the faster the response time, but it also gives a lower sensing signal. In order to get a balanced performance, several flow rates (1.2, 3, 6, 12 ml/hr) were tested (not shown here), and a flow rate at 10 ml/hr was chosen for the following tests.

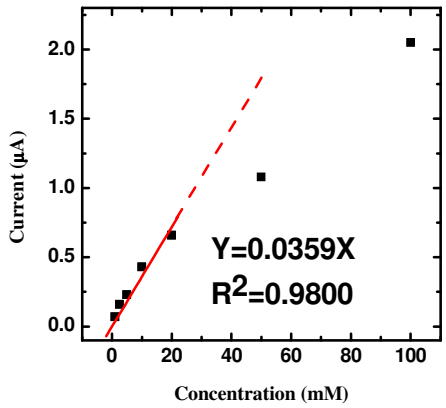
At a flow rate of 10 ml/hr, the PEDOT modified enzyme electrode as the working electrode was applied 0.7 V vs. Ag reference electrode in the background solution

NSTI-Nanotech 2007, www.nsti.org, ISBN 1420061836 Vol. 2, 2007

(PBS) by injecting different concentrations of glucose samples, each with 100 µl. The pulse signals of the sensing current are shown in Fig. 3(a). Fig. 3(b) shows the relationship between the net current response and the glucose concentration with the vibration noise of sensing current lower than 10 nA. The linear regression from 0.1 to 20 mM, which includes the range of normal human blood, with a sensitivity of 8 nA mm^{-2} mM^{-1}, is shown in Fig. 3(b). The sampling time, the time takes from each injection of the sample to the pulse current returns approximately to the background level, was about 5 min. Besides, in Fig. 3(a), the response time and the recovery time, defined as the times take for current reaching 95% of the steady-state, are about 30 s and 230 s, respectively.



(a)



(b)

Figure 3: (a) The sensing signals of the biosensor at difference concentrations by flow injection analysis.
(b) The relationship between current and concentration.

3.3 Interference test

Although the linear range covers the glucose level of normal human, the oxidation-favored interferences in blood, such as ascorbic acid (AA), still raise the sensing currents at a high voltage of 0.7 V. As a result, the pre-reaction section, "WE2" in Fig. 1, is designed to lower interference in this microsystem. In a flow injection analysis, WE2 was also applied at 0.7 V (*vs.* Ag) to electrocatalyze the oxidation-favored materials of the injecting samples [12]. In Fig. 4, for a single-potential test on WE1, both 0.08 mM AA solution and a blend of 0.08 mM AA plus 10 mM Glu sample reached 31.3 % and 145.5 % of the sensing current obtained for 10 mM Glu, respectively. AA contributes a significant current to the total current on the PEDOT enzyme-modified film. In contrast, the sensing current of the blend sample reached 99.6 % that of 10 mM Glu response in a bi-potential test. The concentration of the oxidation-favored species in the injecting sample was reduced in the first electrode. It is concluded that the bi-potential operation can reduce the interferences in a flow injection system. This microsystem offers a good selectivity by providing the pre-reaction section and the specificity of an enzyme.

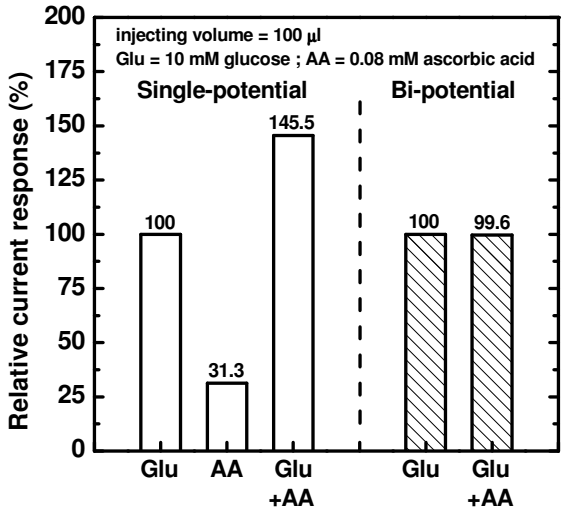


Figure 4: The relative current response of the interference for on-chip biosensor with single-potential (blank bar) and bi-potential (slash bar) operations.

4 CONCLUSIONS

In a single potential operation, AA is almost oxidized on the surface of the electrode to get 45% extra current. By adding the second working electrode, the effect of the interference is reduced significantly. This is because AA near the surface of the first electrode is pre-reacted electrochemically, so as to achieve an interference-free sensor at the second electrode.

5 ACKNOWLEDGEMENTS

This work was supported by the National Research Council of Republic of China, under grant nos. NSC 94-2224-E-002-021 and NSC 95-2221-E-002-307.

REFERENCES

[1] T. Richter, L. Loranelle, O. D. Richtard, U. Bilitewski and D. J. Harrison, Sens. and Actua. B, 81, 369, 2002.

[2] P. A. Auroux, D. Iossifidis and D. R. Reyes, Anal. Chem., 74, 2637, 2002.

[3] M. Lonnberg and J. Carlsson, J. Chromatogr. A, 1127, 175, 2006.

[4] J. Xie, Y. Miao, J. Shih,Y.-C. Tai and T. D. Lee, Anal. Chem., 77, 6947, 2005.

[5] M. J. Schoning, M. Jacobs, A. Muck, D.-T. Knobbe, J. Wang, M. chatrathi and S. Spillmann, Sens. Actua. B, 108, 688, 2005.

[6] J. C. McDonald, D. C. Duffy, J. R. Anderson, D. T. Chiu, H. Wu, O. J. A. Schueller and G. M. Whitesides, Electrophoresis, 21, 27, 2000.

[7] S. M. Ford, B. Kar, S. Mcwhorter, J. Davies, S. A. Soper, M. Klopf, G. Calderon and V. J. Saile, Microcolumn, 10, 413, 1998.

[8] Y. Liu, d. Ganser, A. Schneider, R. Liu, P. Grodzinski and N. Kroutchinina, Anal. Chem., 73, 4196, 2001.

[9] M. A. Unger, H. P. Chou, T. Thorsen, A. Scherer and S. R. Quake, Science, 288, 113, 2000

[10]K. Hosokawa and R. Madea, J. Micromech. Microeng., 10, 415, 2000

[11] P.-C. Nien, T.-S. Tung and K.-C. Ho, Electroanal., 18, 1408, 2006.

[12] M. Hashimoto, S. Upadhyay and H. Suzuki, Biosen. Bioelectron., 21, 2224, 2006.