

Differential Reference Electrode Applied to Arrayed pH Sensor and Arrayed Glucose Biosensor Based on Microfluidic Framework

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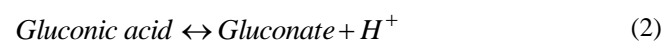
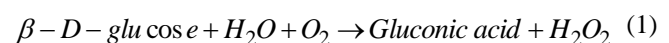
Abstract—In this study, the radio frequency (R. F.) sputtering system and screen printing technology were used to fabricate the arrayed pH sensor. And the glucose oxidase (GOx) enzyme was immobilized on the arrayed pH sensor as the arrayed glucose biosensor. Furthermore, the computer numerical control (CNC) technique was used to fabricate the microfluidic mold. The differential reference electrode can provide a stable potential in different pH solutions, which was applied in arrayed pH sensor and arrayed glucose biosensor. The arrayed pH sensor and arrayed glucose biosensor were integrated to the poly-dimethylsiloxane (PDMS) microchannel as the microfluidic device for observing the characteristics of the arrayed pH sensor and arrayed glucose biosensor at dynamical conditions. At dynamical conditions with flow rate controlled at 20 ml/min, the average sensitivity and linearity of the arrayed pH sensor were 57.27 mV/pH and 0.986, respectively. And at the flow rate controlled at 5 ml/min, the average sensitivity and linearity of

the arrayed glucose biosensor were $9.67 \text{ mV}(100 \text{ mg/dL})^{-1}$ and 0.943, respectively.

Index Terms—Arrayed pH sensor, glucose oxidase enzyme, immobilized, arrayed glucose biosensor, microfluidic mold, differential reference electrode

I. INTRODUCTION

In recent years, excess diet habits and deficient exercise lead to prevalence of diabetes in 21st century. Therefore, the biomedical industry and medical equipment become more important [1]. Hence, the high stability, reproducibility and sensitivity of the arrayed glucose biosensor can be developed to accurately control blood sugar in human blood, which is beneficial for diabetics [2-4]. The glucose is assayed by glucose oxidase (GOx) enzyme oxidation to gluconic acid and hydrogen peroxide, the reaction formula is as the following [5, 6]:



In 1970, the ion selective field effect transistor (ISFET) was proposed by P. Bergveld [7], which was used to detect pH, Na^+ , K^+ , Cl^- or other ions [8]. But the ISFET had some disadvantages, such as device instability, low sensitivity and high cost. In 1983, the extended gate field effect transistor (EGFET) was proposed by J. Van Der Spiegel et al. [9]. The sensitive layer of the EGFET was fabricated on the end of the signal line extended from the FET gate electrode. The disadvantages of ISFET can be overcome by using the EGFET. The EGFET had some advantages, such as simple package, long-term stability, low cost, temperature and light insensitivity [10, 11]. Therefore, there were many researches for several kinds of ion sensing membranes of EGFET, such as, zinc oxide (ZnO) [12], tin dioxide SnO_2 [13], ruthenium dioxide (RuO_2) [14] and vanadium pentoxide (V_2O_5) xerogel [11].

In the past decade, the microfluidic has been researched and discussed extensively. In 1990, A. Manz et al. [15, 16] presented the conception of micro total analysis system ($\mu\text{-TAS}$) to detect biological cell, that has some advantages, such as short response time, low reagent consumption, low sample volume, and portable [17, 18]. Therefore, the microfluidic device was applied to various fields, such as molecular biology [19], biochemistry [20] and genomics [21].

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In the microfluidic field, the glucose analysis is commonly based on the dynamic flow by syringe pumps [22].

II. EXPERIMENTAL

A. Chemicals and Materials

The poly-dimethylsiloxane (PDMS) silicone elastomer (product no. Sylgard® 184 A) was used as microfluidic material and purchased from Dow Corning in USA. The epoxy thermosetting polymer (product no. JA643.) as encapsulated was purchased from Everwide Chemical Co. in Taiwan, R.O.C. The silver paste (product no. 1070) used as the conductive wire was purchased from Advanced Electronic Materials Inc. (ITK) in Taiwan, R.O.C.

B. Fabrication of Microfluidic

According to previous study in our research group [23], the stainless steel master mold of microfluidic was designed by AutoCAD 2009 software. The stainless steel master mold was molded by the computer numerical control (CNC) technique. Then, the PDMS colloid was prepared by mixing main agent of PDMS and curing agent at 1:10 weight ratio. The PDMS colloid was injected in the master mold and all air bladders were removed by vacuum ball. The master mold placed in oven to solidify the PDMS, and the PDMS microfluidic was lift-off from the master mold. Finally, the inlet and outlet of microfluidic were drilled with 3 mm diameter holes. The microfluidic structure is shown in Fig. 1.

C. Fabrication of Arrayed pH Sensor and Arrayed Glucose Biosensor

In this study, the polyethylene terephthalate (PET) was used as substrate. First, the PET substrate was cut to dimension 35 mm × 20 mm, which was cleaned by ethanol and distilled (D. I.) water in ultrasonic vibrator for 10 minutes. Secondly, the ruthenium dioxide (RuO₂) thin films were deposited on PET substrate by radio frequency (R. F.) sputtering. Silver paste and epoxy paste were coated on PET substrate as conductive wires and isolation layer by using screen-printer and baked at 120 °C for 30 and 90 minutes, respectively. Then, the arrayed pH sensor was completed.

The glucose sensing membranes were prepared by the mixing of Nafion and glucose oxidase (GOx) enzyme, and dropping on arrayed pH sensor to prepare arrayed glucose biosensor. After the GOx enzyme was immobilized, the arrayed glucose biosensor was stored at 4 °C in a refrigerator. The schematic diagram of the completed fabrication process is shown in Fig. 2.

D. Differential Reference Electrode

According to previous study in our research group [24], the polypyrrole of conductive polymer was deposited on silver paste by electrodeposited method, and the processes were described as follows: (1) The KH₂PO₄ and K₂HPO₄ were mixed as phosphate buffer solution. (2) Allocating the electrolytes of potassium chloride (KCl) and acetonitrile were 0.1M and 1M, respectively. (3) Allocating the concentration of the pyrrole was 0.3M. (4) The above described solutions were mixed by magnetic stirrer for 5 minutes as polymerization solution. (5) The polypyrrole of conductive

polymer was deposited on silver paste by electrodeposited method for 30 minutes. And then the sensor was taken out from the electrodepositing solution and immersed in the deionized water (D.I.) for 30 minutes removing the imperfect and remaining pyrrole. (6) The sensor was immersed in 6M NaOH for 30 seconds removing the OH⁻ group in PPy film. The schematic diagram of differential reference electrode is shown in Fig. 3.

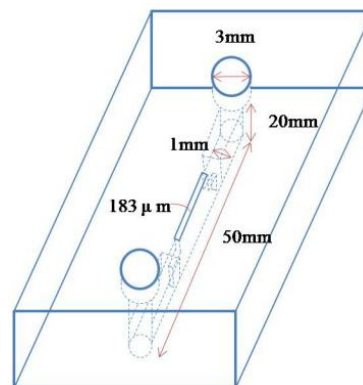


Figure 1. Schematic diagram of the microfluidic.

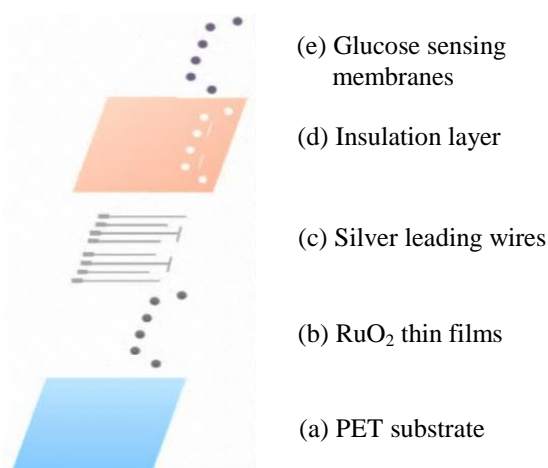


Figure 2. Schematic diagram of the arrayed glucose biosensor.

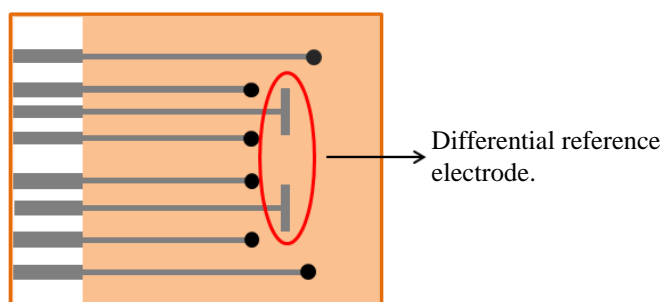


Figure 3. Schematic diagram of the differential reference electrode.

III. RESULTS AND DISCUSSION

A. Reliability of Differential Reference Electrode

In this study, the output voltage of differential system was expressed as equation (3). And the differential measurement system is shown in Fig 4 [25].

$$V_{out} = V_{out1} - V_{out2} = (V_{s1} - V_{ref}) - (V_{s2} - V_{ref}) = V_{s1} - V_{s2} \quad (3)$$

where the V_{ref} is the potential of the reference electrode, V_{s1} is

the potential of the working electrode, V_{s2} is the potential of the contrast electrode, V_{out1} is the potential difference between the working electrode and the reference electrode, and V_{out2} is the potential difference between the contrast electrode and the reference electrode. According to above description, the reference electrode does not affect the sensitivity of this system and provides the basic potential for this device. In order to confirm characteristics of polypyrrole film, the differential reference electrode was measured in different pH solutions. The measurement results show that the sensitivity of differential reference electrode was 0.705 mV/pH, and are shown in Fig. 5. Suppose the pH sensor has ideal Nernst effect (pH sensitivity = 59.2 mV/pH), the ratio of voltage change for the differential reference electrode could be obtained. It was 1.19%, which was smaller 5 %, so it was confirmed that had stability characteristic. And the stability of differential reference electrode was better than other literatures [26-28]. The quasi-reference electrodes with dip coating technology that the pH sensitivity was 2.5 mV/pH [26] and the ratio of voltage change was 4.22 %, and the drop casting technology that the pH sensitivity was 0.55 mV/pH [27] and the ratio of voltage change was 0.92 %. The best sensitivity of the literatures was 0.01 mV/pH by thin film technology [28], which the ratio of voltage change was 0.01 %, but it was disposable structure, as shown in Table I. The experimental results show that the differential reference electrode has good characteristics and that can provide stable potential in different pH solutions.

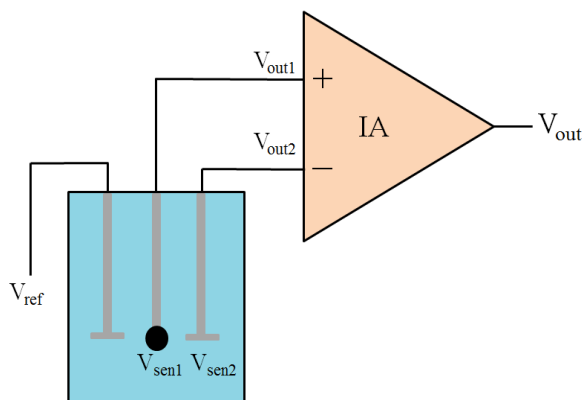


Figure 4. Sensing diagram of differential measurement system.

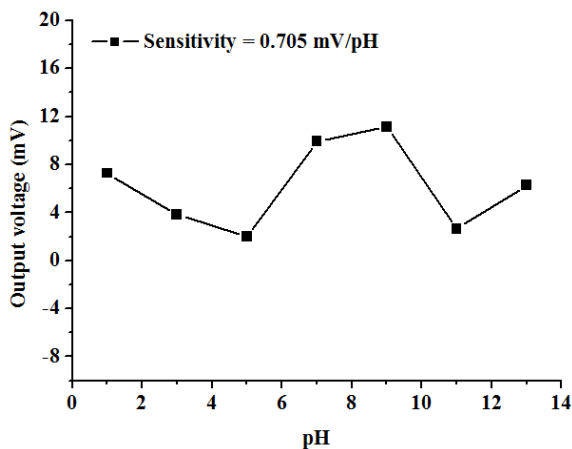


Figure 5. Characteristics of the differential reference electrode in different pH solutions.

B. Arrayed pH Sensor Based on Different Reference Electrodes

In order to obtain reliability of differential reference electrode, the arrayed pH sensor based on differential reference electrode and commercial Ag/AgCl reference electrode were measured in pH1 to pH13. The experimental results are shown in Fig. 6, the average sensitivity and linearity of arrayed pH sensor based on differential reference electrode were 56.77 mV/pH and 0.977, respectively. The average sensitivity and linearity of arrayed pH sensor based on commercial Ag/AgCl reference electrode were 55.88 mV/pH and 0.999, respectively. The linearity of arrayed pH sensor based on differential reference electrode is lower than the commercial Ag/AgCl reference electrode but the average sensitivity of arrayed pH sensor based on the differential reference electrode is higher than the commercial Ag/AgCl reference electrode. Therefore, the differential reference electrode seems is more suitable in differential framework.

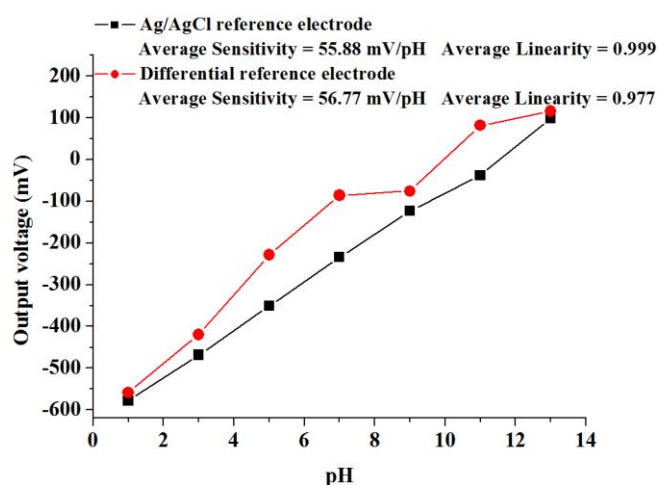


Figure 6. Characteristics of the arrayed pH sensor based on differential reference electrode and Ag/AgCl reference electrode.

C. Dynamical Measurement of Arrayed pH Sensor Based on Differential Reference Electrodes

In order to observe the stability of differential reference electrode at dynamical conditions, the arrayed pH sensor was integrated in PDMS microfluidic to measure different flow rates of pH solutions. Table II shows that the average sensitivity and linearity of the pH sensor array at flow rates from 5 ml/min to 30 ml/min. The results demonstrate that the average sensitivity of pH sensor array were 55.16 mV/pH to 57.27 mV/pH, the average linearity of pH sensor array were 0.981 to 0.986, respectively. And the best flow rate was 20 ml/min, in this flow rate, the average sensitivity and linearity of the pH sensor array were 57.27 mV/pH and 0.983, respectively. The measurement result is shown in Fig. 7. The results demonstrated that the average sensitivity and linearity of the pH sensor array at dynamical conditions were better than at static conditions.

TABLE I
COMPARISONS OF THE DIFFERENT REFERENCE ELECTRODES BASED ON DIFFERENT TECHNOLOGIES.

Category	Fabrication method	Sensitivity (mV/pH)	Preservation	References
Differential reference electrode	Electrodeposited method	0.705	Dry store	In this study
Lithium lanthanum titanium oxide	Dip coating technology	2.500	Dry store	[26]
Cadmium	Drop casting technology	0.550	Dry store	[27]
Ag/AgCl	Thin film technology	0.010	Disposable	[28]

TABLE II
COMPARISON OF THE AVERAGE SENSITIVITY AND LINEARITY FOR ARRAYED pH SENSOR WITH DIFFERENT FLOW RATES

Flow rate (ml/min)	Average sensitivity (mV/pH)	Average linearity
5	56.48	0.981
10	56.69	0.983
15	56.52	0.981
20	57.27	0.986
25	56.00	0.983
30	55.16	0.983

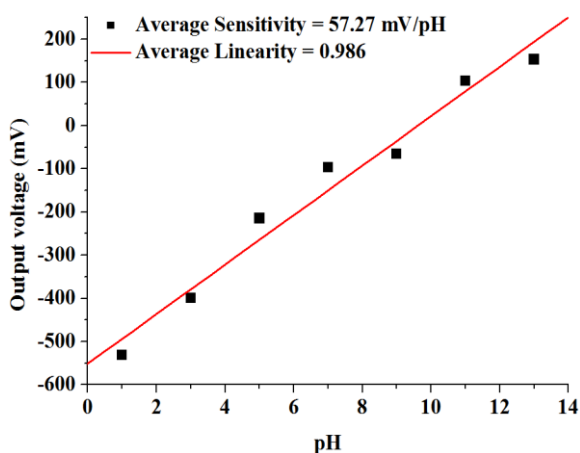


Figure 7. Characteristics of the arrayed pH sensor correspond to pH solutions at flow rate of 20 ml/min.

D. Static Measurement of Arrayed Glucose Biosensor

The GOx enzyme was immobilized on the arrayed pH sensor as the arrayed glucose biosensor. And the arrayed glucose biosensor was measured in different glucose solutions. The experimental results are shown in Fig. 8. According to the experimental results, the average sensitivity and linearity of the arrayed glucose biosensor were $4.47 \text{ mV}(100 \text{ mg/dL})^{-1}$ and 0.973, respectively.

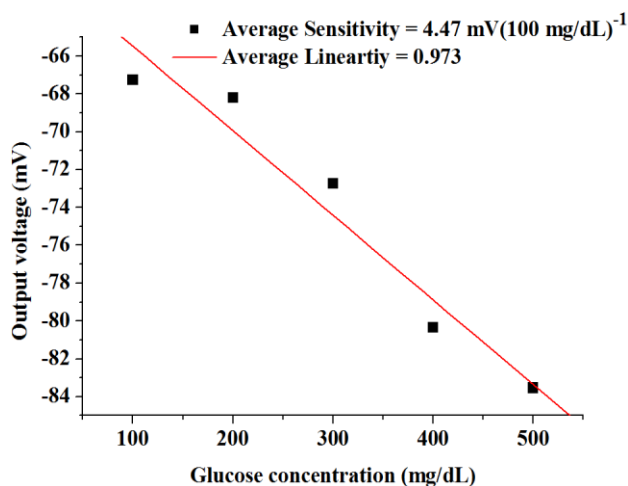


Figure 8. Characteristics of the arrayed glucose biosensor correspond to glucose solutions.

E. Dynamical Measurement of Arrayed Glucose Biosensor

The arrayed glucose biosensor was integrated in PDMS microfluidic to measure the average sensitivity and linearity of the arrayed glucose biosensor at dynamical conditions. Table III shows the average sensitivity and linearity of the arrayed glucose biosensor at flow rates from 5 ml/min to 30 ml/min. The results demonstrate that the best flow rate was 5 ml/min, which average sensitivity and linearity of the arrayed glucose biosensor were $9.67 \text{ mV}(100 \text{ mg/dL})^{-1}$ and 0.943, as shown in Fig. 9. The results demonstrate that the characteristics of the arrayed glucose biosensor at static conditions were better than at dynamical conditions. Because the diffusion resistance of sensing film exists in the outer of sensing membrane and reactant transports to the internal of sensing membrane by diffusion method. The diffusion efficiency was influenced by diffusion resistance of sensing film, and the diffusion resistance of sensing film can be decreased by increasing the flow rate. Furthermore, the high flow rate would make the membrane to fall off and causes the lower sensitivity and linearity [29-31].

TABLE III
COMPARISON OF THE AVERAGE SENSITIVITY AND LINEARITY FOR ARRAYED GLUCOSE SENSOR WITH DIFFERENT FLOW RATES

Flow rate (ml/min)	Average sensitivity (mV/(100 mg/dL) ⁻¹)	Average linearity
5	9.67	0.943
10	7.35	0.948
15	6.15	0.923
20	3.01	0.856
25	1.53	0.633
30	3.25	0.487

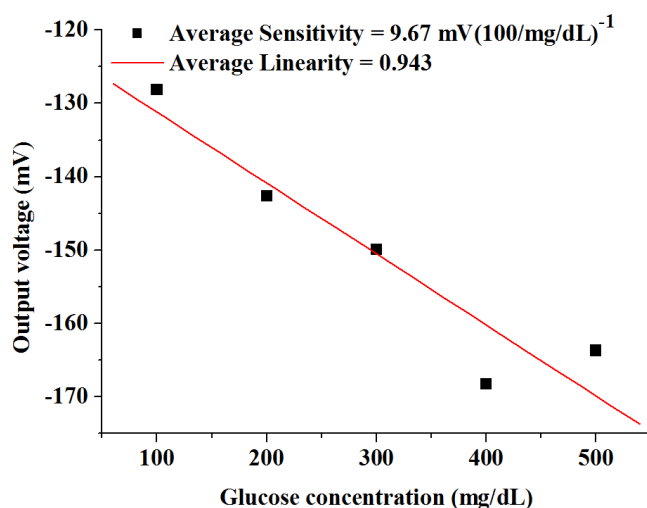


Figure 9. Characteristics of the arrayed glucose biosensor correspond to glucose solutions at flow rate of 5 ml/min.

IV. CONCLUSIONS

In this study, the sensitivity of differential reference electrode was 0.705 mV/pH. It can provide a stable potential in different pH solutions. And the differential reference electrode was applied in the arrayed pH sensor and arrayed glucose biosensor. The arrayed pH sensor and arrayed glucose biosensor were analyzed. The average sensitivities and linearities of arrayed pH sensor and arrayed glucose biosensor were 56.77 mV/pH, 4.47 mV(100 mg/dL)⁻¹ and 0.977, 0.943, respectively. At dynamical conditions, the average sensitivities and linearities of arrayed pH sensor and arrayed glucose biosensor were 57.27 mV/pH, 9.67 mV(100 mg/dL)⁻¹ and 0.986, 0.943, respectively.

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