Combination of the Microfluidic System and NiO Uric Acid Biosensor Modified by Ag Nanomaterials

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ABSTRACT There are relatively few studies on uric acid biosensors that are modified with silver nanomaterials and have high selectivity for analytes and are potentiometric uric acid biosensor. But many studies had shown that using nanomaterials thus results in a greatly enhanced sensitivity, and it could keep the activity of an enzyme. We proposed to use the silver nanowires (AgNWs) to modify the NiO uric acid biosensor. Besides, we modified the calibration circuit for the drift effect and hysteresis effect, the results showed that using the calibration circuit had improved the drift rate and the hysteresis voltage of the NiO flexible arrayed uric acid biosensors, and at least improved the drift rate by about 70%, the hysteresis voltage by about 20%, respectively.

INDEX TERMS Uric acid biosensor, nickel oxide (NiO), nanomaterials, drift effect, hysteresis effect, calibration circuit.

I. INTRODUCTION
Uric acid is a metabolite of cell nucleic acid in the body. The cause of excessive accumulation of the uric acid in the human body can be classified into the following common causes. When the human kidneys have problems, they cannot function normally to metabolize the uric acid produced in the body, or consume too much food with high purine content such as seafood and sugar-sweetened beverages, some of which are caused by stress or obesity. Those situations may cause many complications [1]. Therefore, we must pay attention to this issue. There are many types of methods for detecting uric acid, such as electrochemical method [2], UV method [3], isotope dilution mass spectrometry (IDMS) [4]. Although some of them are accurate, they are more expensive or the measurement process are more complicated. In contrast, the electrochemical method is more suitable for verifying the anti-interference characteristics of the sensor, so it is a measurement method used by a large number of research group. In this research, we developed a non-invasive sensor for detecting uric acid.

For biosensors, there are two common instability effects that affect the stability of the sensor. Potentiometric biosensors have the defects of non-ideal effects such as drift effect [5] and hysteresis effect [6]. These two effects are important factors affecting the stability of biosensors. In the long-term stability, the change of the response voltage of the biosensor with time is defined as the time drift effect. The drift effect has nothing to do with the interface of the device and the substance of the solution, but is related to the hydration process of the membrane [7]. When the sensing film is immersed in the solution, a hydration layer will be formed on the surface of the sensing film. The hydration layer will constitute an electric double layer capacitance, and the thickness will affect the interface potential [8]. Therefore, we used a calibration circuit to reduce the impact on the sensor and increase the stability of the overall measurement system and the sensor. In view of the above two kinds of non-ideal effects, our research group individually designed a calibration circuit dedicated to this non-ideal effect, effectively reduce the response voltage value measured by the effect. Therefore, this research focuses on the influence of the calibration circuit on the sensor modified with nanomaterials.

Nanomaterials could immobilize the biocatalytic by adsorption with functional groups so that they could enhance the surface area and the strength of the electrodes [9-11]. Nanowires had the unconventional catalytic activities and electrical conductivity which were used to increase the
electron transfer feature and reduced the overpotential. Silver nanoparticles (AgNPs) and silver nanowires (AgNWs) were applied to modify the arrayed electrodes based on arrayed NiO film. AgNPs can also improve the electron transfer characteristics, stabilize the enzyme [12, 13], and increase the specific surface area, which can make the film rougher. Combining with different enzymes could make various selectivity of the biosensors.

So far, nickel oxide (NiO) has shown outstanding potential in the field of biosensors. Due to the high isoelectric point (IEP = 10.7) [14], low toxicity, excellent chemical stability, and biocompatibility, NiO is an excellent material for biosensor sensing film. Therefore, in this study, we used a radio frequency (R.F.) sputtering system to deposit NiO film on a flexible substrate to prepare the sensing film of the biosensor.

II. Experimental

A. MATERIALS

The substrate used the polyethylene terephthalate (PET) flexible substrate, which was acquired from Summit-Tech Resource Corporation Limited (Taiwan). The target of nickel oxide (NiO) of 99.95% purity was acquired from Summit-Tech Resource Corporation Limited (Taiwan), which was used as the sensing film. The uricase was acquired from Sigma-Aldrich Corporation Limited (USA). The uric acid was acquired from Sigma-Aldrich Corporation Limited (USA). The silver nanoparticles (AgNPs) were acquired from I-Mei Materials Corporation Limited (Taiwan). The silver paste was acquired from Hong-Li Corporation Limited (Taiwan), and was used as the conductive wire and reference electrodes. The epoxy thermosetting polymer (product no. JA643) was acquired from Yuan-Hong Instrument Corporation Limited (Taiwan). The deionized water (D. I.) was used for preparation of aqueous solutions (resistivity = 18.4 MΩ cm⁻¹). The measurement system consisted of the LT1167 instrumentation amplifier (Type: LT1167CN8#PBF), which was acquired from Linear Technology/Analog Devices Corporation (USA). The data acquisition (DAQ) device (Type: USB-6210) was acquired from National Instruments Corporation (USA). The program system software (Type: LabVIEW) was acquired from National Instruments Corporation (USA). The microfluidic device (Model: OB1 MK3³) was acquired from EIVESYS microfluidic innovation center (France), which was used to control the flow rate of the microfluidic system.

B. PREPARATION AND MODIFICATION OF THE NiO ARRAYED ELECTRODES

In this study, we used a radio frequency (R. F.) sputtering system to deposit NiO on polyethylene terephthalate (PET) with conductive silver wires. The deposition process parameters of the NiO film are shown in Table I. This research uses screen printing technology to use epoxy resin layer to protect the sensor, and finally successfully completed the fabrication of the NiO film arrayed electrodes. The schematic diagram of the NiO uric acid biosensor is shown in Fig. 1.

![Figure 1](image)

**FIGURE 1.** The schematic photo of the flexible arrayed NiO biosensor.

| Material                  | NiO |
|---------------------------|-----|
| Deposition pressure (torr) | 3 × 10⁻³ |
| Duration (min)            | 50  |
| Substrate temperature (°C) | Room temperature |
| Gas flow (sccm)           | Ar : O₂ = 10:3 |
| Power (W)                 | 50  |
| Thickness (nm)            | 133±1.7 |

C. PREPARATION OF THE AGNWs AND COMPOSITION OF THE URIC ACID BIOSENSOR

In this study, we used the polylol method to fabricated AgNWs [15]. First, silver nitrate (AgNO₃) and ethylene glycol (EG) were mixed to obtain a 0.128M solution, then polyvinylpyrrolidone (PVP) and sodium chloride (NaCl) crystals were mixed to obtain a powder, and then 18.4 ml of EG solution was added and stirred thoroughly. Afterwards, the AgNO₃-EG solution was titrated into the PVP-EG solution, and heated to 180°C continue stirring until it was completely dissolved and turned gray, the total time was about 20 minutes. Finally, the solution was washed 3 times with deionized (D. I.) water and centrifuged for 10 minutes each time. Finally, the precipitate obtained by centrifugation was collected and dispersed in deionized (D. I.) water for storage. Figure 2 showed the morphology of the AgNWs that was analyzed by the field emission scanning electron microscope (FE-SEM).

In this study, the enzymatic sensor uses the cross-linking method to immobilize urate oxidase (uricase) on the film. In the process of immobilization, inert proteins such as bovine serum albumin (BSA) are added to maintain enzyme activity [16, 17]. After these steps are completed, the biosensor is successfully prepared.
D. ORIGINAl MEASUREMENT CIRCUIT

Because of the presence of the uricase in the sensing area of the sensor, it will react with uric acid and accelerate the production of Allantoin, H2O2, CO2. The H2O2 will be further dissociated into hydrogen ions [18], which will increase the pH value of the solution. The pH value gradually produces a small change, resulting in a change in the surface potential. At room temperature of 25°C, the pH value can be substituted into the Nernst equation that shown in formula 2.1 to predict the change in potential to obtain the average sensitivity.

\[ E = E^0 + \frac{RT}{nF} \ln \left[ a_{H^+} \right] = E^0 - 2.303 \frac{RT}{nF} \text{pH} = E^0 - 0.05916 \text{pH} \quad (2.1) \]

where \( E \) is the membrane potential, \( E_0 \) is the standard potential for \( a_{H^+} = 1 \text{ M} \), \( R \) is the general gas constant, \( T \) is the absolute temperature by Kelvin degrees, \( n \) is the total number of charges on the ion, \( F \) is the Faraday constant and \( a_{H^+} \) is the activity of the analyte. The Nernst slope is 59.16 mV/pH at 25 °C [19].

In this research, we used the voltage-time (V-T) measurement system to measure the change in response voltage of the sensor under different concentrations of uric acid solution to calculate the average sensitivity of the sensor. Figure 3 is the structure diagram of the V-T measurement system. There are 6 instrumentation amplifiers LT1167 on this original measurement circuit. The reason for choosing the instrumentation amplifiers LT1167 is that it has high input impedance and low output impedance, good stability, and can be used to filter out noise. The signal from the sensor, after passing through these 6 instrumentation amplifiers LT1167, will be transmitted to DAQ Card. The DAQ Card can convert the analog signal into a digital signal. Finally, we used LabVIEW system to present the data on the computer screen in the form of graphs.

E. CALIBRATION CIRCUIT

We used a calibration circuit to reduce non-ideal effects, such as drift effect and hysteresis effect. The calibration circuit can improve the stability of the biosensor, so that the biosensor has a better performance improvement in the long-term measurement. The calibration standard of the calibration circuit varies slightly with the response voltage of the biosensor. The calibration circuits for the drift effect and hysteresis effect are shown in Fig. 4 and Fig. 5 [20, 21]. \( A_1 \) and \( A_2 \) represent error amplifiers. \( R_1, R_2, R_3 \) and \( R_4 \) represent resistance. The non-inverting amplifier of this circuit is composed of \( A_1, R_1, R_2 \). \( M_p \) represents the P-MOSFET pass transistor. The negative feedback network is composed of \( A_2, R_3, R_4 \). \( C_{out} \) represents the output voltage capacitance. The operating principle of this circuit is to use a non-inverting amplifier to amplify the tiny signal of the biosensor. In this calibration circuit, if \( V_c \) determined that it is at a high level at this time, the current flowing into the transistor will be reduced. If the circuit determines that the \( V_c \) at the negative level at this time, \( M_p \) and \( M_n \) provide a stable current to charge \( C_{out} \) and provide a stable response voltage. The signal that was amplified at the beginning, and finally through the use of \( R_7 \) and \( R_8 \), to reduce the multiplier of the non-inverting amplifier. The operating principles of the two correction circuits are the same. The difference between the calibration circuit used for the drift effect and the calibration circuit used for the hysteresis effect is the input of \( V_{ref2} \). \( V_{ref2} \) is the applied voltage, which can affect the stability of the sensor. In the drift effect, because the measured solution concentration does not change, \( V_{ref2} \) is determined by the user based on the response voltage measured by the sensor at that concentration. In the hysteresis effect, because different solutions concentrations are used for measurement, the calibration circuit will automatically adjust the value of \( V_{ref2} \) according to the response voltage of the sensor at different concentrations.
B. DYNAMIC MEASUREMENTS OF THE AGNPS-URICASE/AGNWs/NIO FILMS URIC ACID BIOSENSOR

In this work, the dynamic measurement was used to track the enzyme loading and interface resistance since they affect the average sensitivity and linearity of the biosensor [24]. The dynamic measurement was conducted with an injection device to analyze the response voltage of the biosensor. In dynamic measurement, the enzyme loading and interface resistance affect the average sensitivity and linearity of the biosensor [24]. When the sensor is in a low flow rate environment, the enzyme can react effectively with the analyte, so you will find that the sensor has better average sensitivity and linearity at this time. In addition, the average sensitivity and linearity of the uric acid biosensor based on AgNPs-uricase/AgNWs/NiO films under microfluid flow is better than the result of static measurement. On the other hand, at fast flow rates, although the average sensitivity of the sensor decreases, it is still better than static measurement. The reason may be that there is not enough time for the catalytic reaction between the analyte and uricase, so the average sensitivity will decrease. Table II showed the average sensitivities and linearities of the uric acid biosensors based on AgNPs-uricase/AgNWs/NiO films under the slow flow rates from 1.4 μL/min to 7.0 μL/min, and the average sensitivities and linearities of the sensor under the fast flow rates from 10 μL/min to 50 μL/min. After experimentation, we finally chose to measure under the condition of 10 μL/min because it has the highest average sensitivity.

III. RESULTS AND DISCUSSION

A. STATIC MEASUREMENT OF THE AGNPS-URICASE/AGNWs/NIO FILMS URIC ACID BIOSENSOR

In this work, the flexible arrayed uric acid biosensor based on AgNPs-uricase/AgNWs/NiO films was immersed in PBS solution (pH 7.4) with the uric acid concentrations from 2 mg/dL to 10 mg/dL (the range in the human blood), and the data were recorded by using the voltage time (V-T) measurement system. Figure 6 showed the average sensitivity and linearity of the uric acid biosensor based on AgNPs-uricase/AgNWs/NiO films were 49.32 mV/(mg/dL) and 0.980, respectively.

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Drift effect with the calibration circuit of the flexible arrayed uric acid biosensor based on AgNPs-uricase/AgNWs/NiO films

The biosensor during the long time measurement process was usually accompanied by a non-ideal effect which was called drift rate. The drift rate was an important indication for the stability of the biosensors, but most research might ignore this issue. The output response voltage of the biosensor at the 5th and 12th hours is divided by the elapsed time (7 hours), and the value obtained is the drift rate [25]. In this experiment, we immersed the AgNPs-Uricase/AgNWs/NiO films uric acid biosensor in a 10mg/dL uric acid buffer solution for 12 hours, and use the V-T measurement system and calibration circuit to investigate the change in its response voltage. According to Fig. 9, the drift rate of the AgNPs-uricase/AgNWs/NiO sensor is 1.02 mV/hr, and the drift rate of the calibration circuit is 0.31 mV/hr. Table III presents the detailed values of the response voltage changes after using the calibration circuit. The experimental results confirmed that this NiO uric acid biosensor successfully reduced the drift rate by using the calibration circuit, and the improvement rate was as high as 70%. The response voltage is maintained at a value close to the horizontal line.

C. DRIFT EFFECT WITH THE CALIBRATION CIRCUIT OF THE FLEXIBLE ARRAYED URIC ACID BIOSENSOR BASED ON AGNPS-URICASE/AGNW/NI O FILMS

The biosensor during the long time measurement process was usually accompanied by a non-ideal effect which was called drift rate. The drift rate was an important indication for the stability of the biosensors, but most research might ignore this issue. The output response voltage of the biosensor at the 5th and 12th hours is divided by the elapsed time (7 hours), and the value obtained is the drift rate [25]. In this experiment, we immersed the AgNPs-Uricase/AgNWs/NiO films uric acid biosensor in a 10mg/dL uric acid buffer solution for 12 hours, and use the V-T measurement system and calibration circuit to investigate the change in its response voltage. According to Fig. 9, the drift rate of the AgNPs-uricase/AgNWs/NiO sensor is 1.02 mV/hr, and the drift rate of the calibration circuit is 0.31 mV/hr. Table III presents the detailed values of the response voltage changes after using the calibration circuit. The experimental results confirmed that this NiO uric acid biosensor successfully reduced the drift rate by using the calibration circuit, and the improvement rate was as high as 70%. The response voltage is maintained at a value close to the horizontal line.

D. HYSTERESIS EFFECT WITH THE CALIBRATION CIRCUIT OF THE FLEXIBLE ARRAYED URIC ACID BIOSENSOR BASED ON AGNPS-URICASE/AGNW/NI O FILMS

The hysteresis effect can be regarded as the stable recognition analysis of the sensor, also known as reversibility. The method of operation is to immerse the working electrode of the sensor in the object to be measured, and then the sensor responds to changes in voltage under different concentration cycles. Finally, the final response voltage of the sensor and the initial response voltage will shift, which is called hysteresis Voltage (Vh).

In order to verify the stability of the sensor, we set up the experiments of the forward loop and the reverse loop respectively for verification. The sensor is placed in the forward loop of 6 mg/dL → 2 mg/dL → 6 mg/dL → 10 mg/dL → 6 mg/dL, and in the reverse loop of 6 mg/dL → 10 mg/dL → 6 mg/dL → 2 mg/dL → 6 mg/dL for measurement [26]. In this study, we use the V-T measurement system to analyze the flexible arrayed uric acid biosensor based on AgNPs-uricase/AgNWs/NiO films. As shown in Fig. 10, the hysteresis voltages of the forward loop and the reverse loop of the flexible arrayed uric acid biosensor based on AgNPs-uricase/AgNWs/NiO films are 2.37 mV and 1.13 mV, respectively. The result of the experiment showed that the hysteresis voltage in the reverse loop is relatively high. This is because the enzyme on the sensor will be degraded after multiple measurements, making the sensor unstable [27]. After using the calibration circuit, it can be found that the hysteresis voltage of the sensor dropped significantly.
Figure 11 and Fig. 12 showed the hysteresis voltage of the calibrated circuit for the flexible arrayed uric acid biosensor based on AgNPs-uricase/AgNWs/NiO films, which are 1.93 mV and 0.82 mV in the forward loop and in the reverse loop, respectively. Experimental results confirmed that by using the calibration circuit, the hysteresis voltages were reduced. The hysteresis voltages based on AgNPs-uricase/AgNWs/NiO films were reduced by 18.57% and 27.44%, respectively. The detailed values are shown in Table IV.

| Cycle          | Hysteresis voltage (mV) | Improvement (%) |
|----------------|-------------------------|-----------------|
| Forward loop   | 2.37                    | 18.57           |
| Reverse loop   | 1.13                    | 27.44           |

E. LIFETIME OF THE FLEXIBLE ARRAYED URIC ACID BIOSENSOR BASED ON AGNPS-URICASE/AGNWs/NiO FILMS

Figure 13 was the average sensitivities of the uric acid biosensor based on AgNPs-Uricase/AgNWs/NiO films at different storge time. We conducted this experiment to make a more durable biosensor. Lifetime was defined as the time elapsed when the average sensitivity was less than 50% [28-30]. We immersed the biosensor in a uric acid solution of 2 mg/dL to 10 mg/dL, and used the V-T measurement system to measure the average sensitivity of the sensor every three days. Each measurement was performed simultaneously in the same environment to ensure that the biosensor was not affected by the environment. After each measurement, we placed the biosensor in a 4°C environment to maintain the enzyme activity. The experimental results in Fig. 13 confirmed that the average sensitivity of the biosensor on the 24th day was less than 50%, so the lifetime of the AgNPs-uricase/AgNWs/NiO uric acid biosensor was 24 days.

F. REPRODUCIBILITY OF THE FLEXIBLE ARRAYED URIC ACID BIOSENSOR BASED ON AGNPS-URICASE/AGNWs/NiO FILMS

For enzymatic biosensors, the stability and reproducibility were very important characteristics. When repeating the measurement, the enzyme stability was poor...
and the activity may decrease [31]. In Fig. 14, we explored whether the average sensitivity of the AgNPs-uricase/AgNWs/NiO uric acid biosensor was affected after 10 repeated measurements. The results in Fig. 14 show that the average sensitivity of the biosensor under these 10 measurements was similar, with a relative standard deviation (RSD) of 1.6%. The main reason is that silver nanomaterials can prevent the loss of enzymes [32, 33]. Finally, according to the above experimental results, the quality of the flexible array uric acid biosensor based on AgNPs-Uricase/AgNWs/NiO films was quite excellent and has high accuracy. The good reproducibility of the sensor can be classified into the following two reasons. The first modifier on the sensing area is the AgNWs, which are made by using the same batch of processes, and the AgNPs that we used are commercially available with a size of about 10nm. Therefore, on the surface of nanomaterials, we ensured that the source of the materials was very stable and had high quality. On the other hand, when the R. F. sputtering system deposited the film of the sensor, we used a mask to control the deposition range, so that the deposition range is limited to the tiny area of the working electrode of the sensor, so that the quality of the sensor should be as consistent as possible.

FIGURE 14. The reducibility of the flexible arrayed uric acid biosensor based on AgNPs-uricase/AgNWs/NiO films.

IV. CONCLUSION

In conclusion, we successfully applied the calibration circuit to measure the NiO uric acid biosensor based on AgNPs-uricase/AgNWs/NiO films in this study. Both of the drift rate and the hysteresis voltage had a limited decrease by 69.29 % and 23.00 % ± 4.44. In terms of stability, the lifetime of the NiO uric acid biosensor based on AgNPs-uricase/AgNWs/NiO films was shown about 24 days; the repeatability of the NiO uric acid biosensor based on AgNPs-uricase/AgNWs/NiO films was shown the good accuracy, and the RSD of the sensor was 0.16. In the microfluidic experiments, the average sensitivity and the linearity of the slow flow rates were shown an outstanding trend due to the biosensor was enough time to cause catalyzed reactions under slow flow rate. On the contrary, in terms of fast flow rate might be that the uricase did not have enough time to catalyze the reaction so that lead to the decrease of the average sensitivity.

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