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Analysis of EGFET Based on Static Measurements and Microfluidic System for the RuO_2 Lactic Acid Biosensor

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ABSTRACT

In this study, a potential lactic acid biosensor based on ruthenium dioxide (RuO_2) film was proposed. This sensor was measured by a static voltage-time measurement system and dynamic microfluidics system. The sensitivity characteristics of the RuO_2 lactic acid (LA) biosensor under different flow rates were studied by dynamic measurement. The sensitivity characteristics were analyzed under the slow flow rates and the fast flow rates. It was found that RuO_2 LA biosensor had the better sensitivity (49.80 mV/mM) and linearity (0.996) at the flow rate of 30 μL/min. In this work, the sensitivity and linearity of RuO_2 LA biosensor at the optimal flow rate were analyzed. The addition of γ-3-aminopropyltriethoxysilane (APTES) and glutaraldehyde can not only increase the adsorption and functionalization of the enzyme, but also maintain the activity of enzyme. The sensing characteristics were improved in static and dynamic measurements. This sensor has the better sensitivity, linearity, and response time.

Keywords: Ruthenium dioxide (RuO_2), lactic acid (LA), biosensor, microfluidic, sensing characteristics, response time.

1. INTRODUCTION

In past decades, the microfluidic systems have been widely combined with sensors and applied to a variety of different scientific fields such as chemistry, biochemistry, physics, biotechnology, and nanotechnology. The biosensors based on microfluidic systems provide enhanced analytical performance, real-time detection, and rapid reaction rates (Luka et al., 2015; Reverté et al., 2016; J. Wang et al., 2009; Xing et al., 2022). The microfluidic system is developed to reduce the dilution effect and improve detection sensitivity (Leroy et al., 2015)microwave dielectric spectroscopy offers unique capabilities to investigate intracellular properties of biological cells, which are informative on many biological processes. This paper presents two prototypes of high frequency biosensors able to operate in the microwave frequency range. Both are based on capacitive detection either in a broadband or resonant mode. Thick gold metal electrodes have been investigated to perform high sensitive analysis, while a microfluidic system was implemented over the RF circuit so that flowing liquid minimally impacts global losses of the system and has hence a limited effect on sensor sensitivity. Particles to be tested are manipulated to be accurately located in the detection area using dielectrophoresis techniques. To demonstrate the proposed approach and assess the detection capabilities of such sensors, HF measurements (up to 8GHz. At present, the microfluidic system has the advantages of fast reaction speed, high sensitivity measure, low power consumption, and low cost. In this study, a microfluidic system was integrated with lactic acid (LA) biosensors to analyze the sensitivity characteristics at different flow rates. The RuO_2 is a rare precious metal material. It is an attractive electrode material for a variety of applications because of its high metal conductivity and thermodynamic stability even under anodic conditions. It also has electrochemical reversibility, good adhesion of enzymes and compounds, and is very stable in acidic solvents(Kim et al., 2014; Liao & Chou, 2008; Over, 2012). It has the properties of good thermal stability, low resistivity, good chemical resistance, and good diffusion performance (Mousli et al., 2019), and are

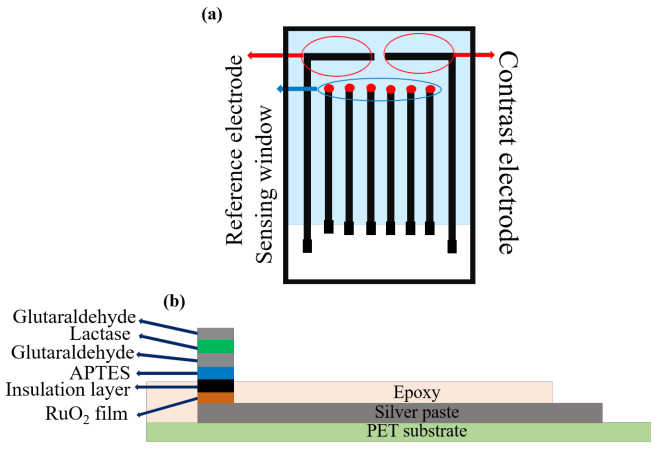

Fig. 1 The fabrication process for the arrayed RuO_2 LA biosensor with the schematic diagram and structure diagram.

also used as electrode materials for supercapacitors (Li et al., 2018). The measurement of LA in the human body has been discussed recently because LA levels are related to tissue oxygenation (de Keijzer et al., 1999). The lactate concentration is im-

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portant for continuous monitoring in surgery (Bakker et al., 1996) septic shock frequently evolves into multiple system organ failure (MSOF, sports medicine (Parra et al., 2006), and first aid (J. Wang, 1999). The normal LA concentration range is 0.5 - 1.5 mM (Chou, Yan, Liao, Lai, Chen, et al., 2018). Lactic acidosis occurs when the concentration of LA in the blood rises to 5 mM (Phypers & Pierce, 2006). Thus, the detection of LA is important for clinical diagnosis, medicine, and food analysis (Arakawa et al., 2022; Arivazhagan et al., 2023; Ferraraccio & Bertoncello, 2023; Mazzei et al., 1996; Yamada et al., 2022; Zhang et al., 2022)2023; Mazzei et al., 1996; Yamada et al., 2022; Zhang et al., 2022.

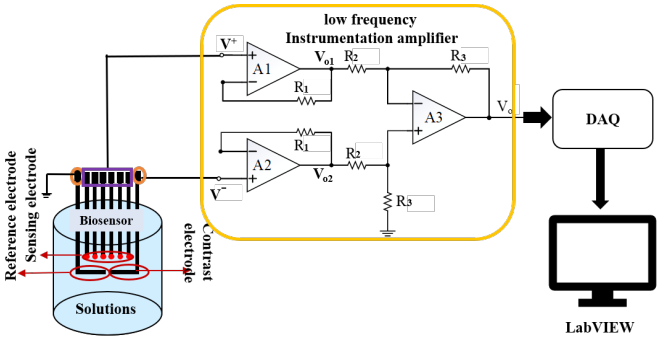


Fig. 2 Schematic diagram of biosensor measurement system.

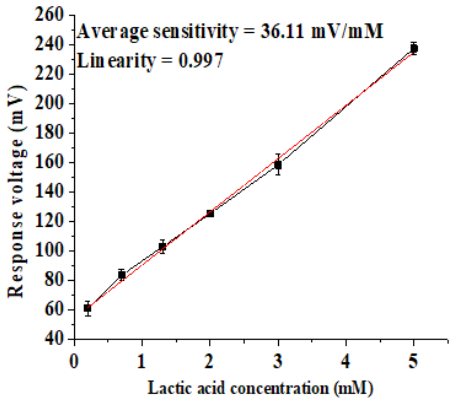


Fig. 3 The average sensitivity and linearity of the RuO_2 LA biosensor at the static measurements.

2. EXPERIMENTAL

2.1 Preparation Process of Flexible Arrayed Biosensor based on RuO_2 LA

In this paper, the lactic acid biosensor was fabricated according to previous research of our research groups (Kuo et al., 2022)a low-frequency instrumentation amplifier (LFIA. The silver adhesive is printed on PET substrate through screen printing technology. The RuO_2 sensing films are deposited by a radio frequency (R.F.) sputtering system. After encapsulation using epoxy resin as an insulating layer, leaving only six sensing windows and reference electrodes. Then, it was baked in the oven for an hour. LDH was used and it is found in whole tissues, such as blood cells and heart muscle. Moreover, with the help of coenzyme nicotinamide adenine dinucleotide (NAD^+), it has a high catalytic activity for the transformation of LA. The fabrication process of the RuO_2 LA biosensor was shown in Fig. 1.

2.2 Instrumentation Amplifier Measurement System

The biomedical signal of the sensing windows was sensed by the instrumentation amplifier (LT1167), and the sensed voltage was input to the data acquisition equipment (DAQ) as shown in Fig. 2. Thus, the sensed voltage was converted into digital data. Finally, the measurement data were analyzed by the LabVIEW software (Kuo et al., 2021). By applying this measurement system, the sensing characteristics of the LA biosensor can be obtained. As shown in Fig. 3, the average sensitivity and linearity of the LA biosensor was 36.11 mV/mM, and the linearity was 0.997.

2.3 Microfluidic Measurement System

According to previous research (Chou et al., 2019), the LA biosensor was placed in a microfluidic channel made by polydimethylsiloxane (PDMS). The structure of the microfluidic device is shown in Fig. 4. In this figure, the 2D and 3D drawings of the microfluidic channel were demonstrated. The complete microfluidic measurement system is shown in

Fig. 5. A flow controller was used to provide different flow rates, and the flow rates were adjusted by the software. Then, the nitrogen was used to accurately drive the various t flow rates. The microfluidic flow controller controlling its flow rates will measure the solution injected into the microfluidic channel. The response voltage of the LA biosensor was read out by the instrumentation amplifier, and the data was collected by DAQ equipment and transmitted to LabVIEW.

3. RESULTS AND DISCUSSION

3-1 The Basic Principle of LA Biosensor

In general, most lactate biosensors are based on LDH and L-lactate oxidase (LOD) due to the simplicity of enzymatic reactions and the simplicity of design and manufacture. Enzymatic LA biosensor based on LOD reaction has some disadvantages such as high oxidation potential, large fluctuation of oxygen concentration in solution, and unstable detection limit. The LDH is an H^+ transfer oxidoreductase. The enzyme catalyzes the reversible conversion of LA to pyruvate. It reduced NAD^+ to NADH, which can then produce H^+. Through the accumulation of hydrogen ions on the surface of the sensor film, different response voltages were obtained due to different LA concentrations. The reactions of LDH involved in biosensors were shown in formula (7) and (8) (Chou, Yan, Liao, Lai, Wu, et al., 2018).

$$Lactate + NAD^+ \rightarrow Pyruvate + NADH + H^+ \quad (7)$$

$$NADH \rightarrow NAD^+ + H^+ + 2e^- \quad (8)$$

3-2 The Static and Dynamic Measurement Analysis

The normal LA concentration range in human blood is 0.5 - 1.5 mM. Thus, the prepared concentration range were 0.2 mM, 0.7 mM, 1.3 mM, 2 mM, 3 mM, and 5mM. First, a LA biosensor was immersed in a PBS solution containing LA. After standing for 60 seconds, the average sensitivity of static measurement was 36.11 mV/mM and the linearity was 0.997 as shown in Fig. 3. The sensitivity is defined as the change between response signals of biosensors at different concentrations of analytes(Forootan et al., 2017) better known as qPCR, is the most sensitive and specific technique we have for the detection of nucleic acids. Even though it has been around for more than 30 years and is preferred in research applications, it has yet to win broad acceptance in routine practice. This requires a means to unambiguously assess the performance of specific qPCR analyses. Here we present methods to determine the limit of detection (LoD. The sensitivity of biosensors is very important in the analysis of analyte concentration. The effect of analyte per unit concentration on the sensor is defined as average sensitivity. The average sensitivity can be calculated as follows (11) (Indrayanto, 2018):

$$S_0 = \frac{\Delta V}{R} \tag{11}$$

where S_o is the average sensitivity, R is the linear range, and ΔV is the potential difference between the highest and lowest LA concentrations. ΔV is 17.82 mV and R is the linear range between 0.2 mM to 5 mM. Linearity is an important criterion, which can be used to judge the accuracy of sensors. The higher the linearity, the higher the reliability of the measurement results as shown in Fig. 3.

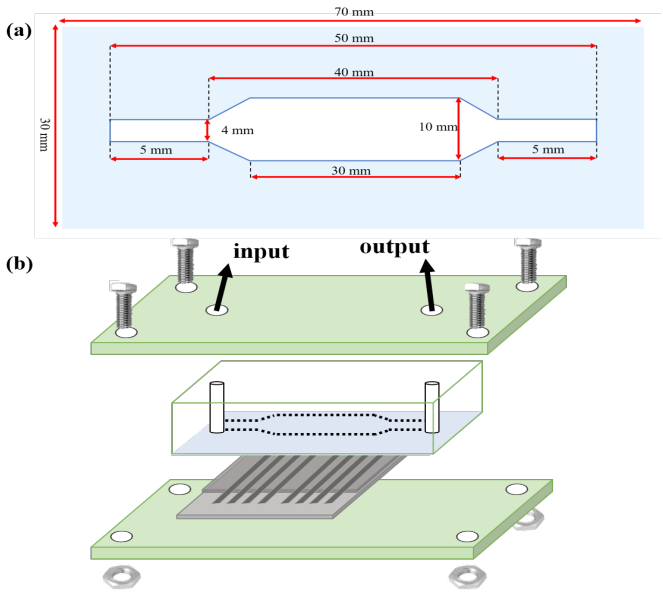


Fig. 4 The structure of the microfluidic device. (a) The 2D and (b) 3D drawing of the microfluidic channel.

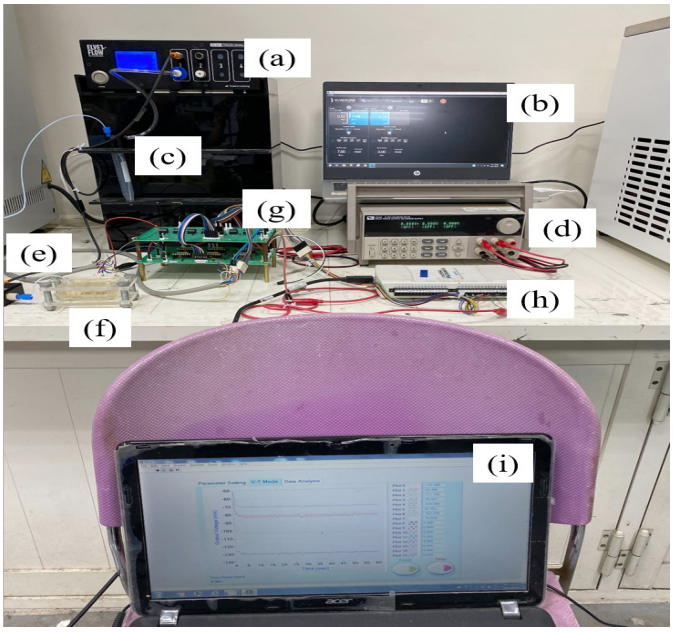


Fig. 5 The complete microfluidic measurement system. (a) microfluidic control system, (b) flow control software, (c) solution test tube, (d) power supply, (e) flow controller, (f) microfluidic channel, (g) readout circuit, (h) DAQ, (i) LabVIEW.

The limit of detection (LOD) is an important sensing characteristic of the biosensor. It means the minimum concentration of the analyte which the biosensor can detect. According to previous research, the LOD of the biosensor can be calculated by the 3-sigma method as follows (12)(Indrayanto, 2018; Rajaković et al., 2012)DDHs are chemically complex mixtures, and second, the chemical contents of raw plant materials are affected by the site of cultivation, age of plants, methods of harvesting, and processing. QC is used by manufacturers to ensure the consistency, safety, and efficacy of the DDHs. QC of DDHs can be performed by two approaches, namely, marker-oriented and chemical pattern-oriented (metabolite profiling:

$$C_{LOD} = \frac{3\sigma}{S_0} \tag{12}$$

where the C_{LOD} is the LOD of the concentration, σ is the standard deviation of response voltage while the pure PBS solution was measured, and S_0 is the average sensitivity. The average sensitivity of the RuO_2 LA biosensor was shown in Fig. 3. The response voltage of the flexible arrayed RuO_2 LA biosensor in pure PBS solution was 62.29 mV. The standard deviation of the measured response voltage of the flexible arrayed RuO_2 LA biosensor in pure PBS solution was 0.05 mV.

Fig. 6. The response voltages of the LA biosensor based on the lactase/RuO_2 sensing film under (a) slow flow rates (b) fast flow rates.

The average sensitivity of our sensor is 36.11 mV/mM. The response voltage is measured for 60 seconds and n is 60 points. According to the formula (12), it can be calculated that LOD is about 4.1 µM.

The dynamical measurement is performed by a microfluidic control system and the flow rate at low and high velocities were measured. The microfluidic control system (OB1 MK3^{+}) was bought from (Elvesys Microfluidic Innovation Center, France). It was employed to control the flow rate of the microfluidic system. For microfluidic quantification, cell, blood, and DNA trace detection can be performed with fast, stable, and pulse-free microfluidic flow. Moreover, this control system achieved a 9 ms response time, 0.005% pressure control accuracy, and 0.006% resolution. Then, the nitrogen was used to accurately drive the different flow rates. A flow controller was used to provide different flow rates, and the flow rates were controlled by flow control software. The slow flow rates were 1.4 µL/min, 2.8 µL/min, 4.2 µL/min, 5.6 µL/min, and 7.0 µL/min, respectively. The fast flow rates were 10 µL/min, 20 µL/min, 30 µL/min, 40 µL/min, and 50 µL/min, respectively. The response voltages of the LA biosensor under slow flow rates and fast flow rates are shown in Fig. 6. Fig. 6(a) shows the response voltage of the LA biosensor based on the lactase/RuO_2 sensing film in LA solution at slow flow rates. Fig. 6(b) shows the response voltage of the LA biosensor based on the lactase/RuO_2 sensing film in LA solution at fast flow rates.

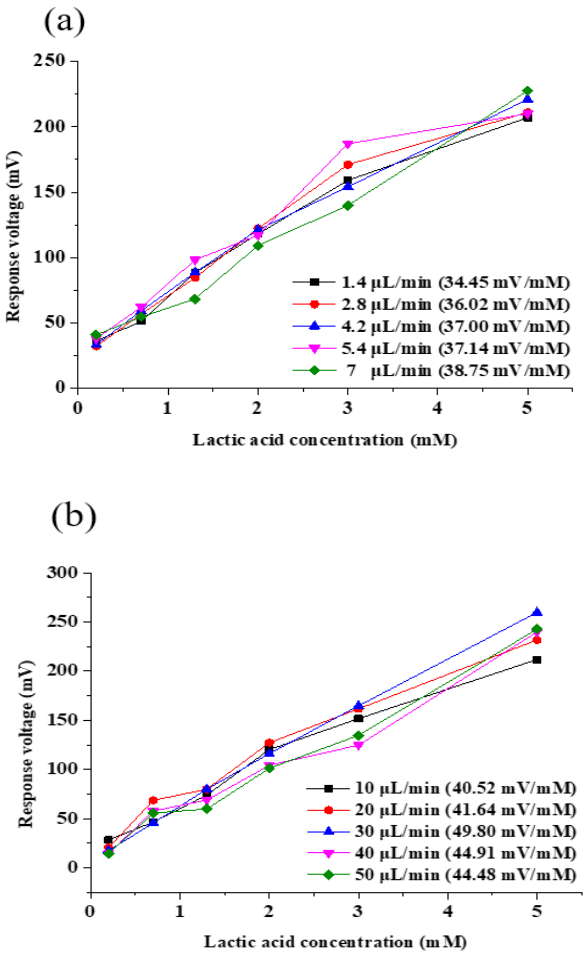


Fig. 6 The response voltages of the LA biosensor based on the lactase/RuO_2 sensing film under (a) slow flow rates (b) fast flow rates.

From Table I, the average sensitivity was 38.75 mV/mM and linearity were 0.997 at a flow rate of 7.0 µL/min with slow flow rate conditions. Table II shows that the average sensitivity was 49.80 mV/mM and linearity was 0.996 at a flow rate of 30 µL/min with fast flow rate conditions. It is also proved that the average sensitivity of the LA biosensor under the dynamic measurement was better than that under the static measurement. From the measurement results under different flow rates, the average sensitivities and linearities under the dynamic measurement are better than those under the static measurement. By observing the dynamic and static measurements, the average sensitivity increased from 36.11 mV/mM to 49.80 mV/mM. At a flow rate of 40 µL/min, the average sensitivity and linearity decreased to 44.91 mV/mM and 0.975, respectively. The decrease in average sensitivities at flow rates above 30 µL/min is due to the separation of the lactase from the working electrode at higher flow rates, resulting in a decrease in the sensitivities of the lactate biosensor. The average sensitivity decreased with the increase of flow rates, due to the decrease of diffusion resistance under dynamic conditions (Chou et al., 2012) array chlorine ion sensor was integrated to the poly-dimethylsiloxane (PDMS. It can be concluded that the sensing characteristics of LA biosensor were more stable at a high flow rate (Chou et al., 2013). According to previous studies, the thickness of the boundary layer will decrease and the diffusion of the boundary layer will continue to increase with the increase of microfluidic velocity. As a result, the number of enzyme molecules increases and the diffusion time decreases (Samphao et al., 2015). Hydrogen ions catalyzed by LA are not easily absorbed by the membrane due to the high flow rate. In addition, the average sensitivity decreased when the flow rate increased more than 30 µL/min. It can be speculated that this phenomenon refers to fluid dynamics with high velocity, which may cause flow-induced vibration (FIV) in microfluidics. This may cause a fluid shock in microfluidic devices.

Table 1 The Average Sensitivity and Linearity of the Fabricated LA Biosensor at Slow Flow Rates.

Film	Flow rate (µL/min)	Average sensitivity (mV/mM)	Linearity
Lactase/RuO_2	0	36.11	0.997
	1.4	34.45	0.962
	2.8	36.02	0.975
	4.2	37.00	0.979
	5.6	37.14	0.988
	7.0	38.75	0.997

Table 2 The Average Sensitivity and Linearity of the Fabricated LA Biosensor at Fast Flow Rates.

Film	Flow rate (µL/min)	Average sensitivity (mV/mM)	Linearity
Lactase/RuO_2	10	40.52	0.981
	20	41.64	0.985
	30	49.80	0.996
	40	44.91	0.975
	50	44.48	0.967

Table 3 Comparison of Sensing Characteristics for Different LA Biosensors

Sensing film	Linear range (mV/mM)	Average Sensitivity (mV/mM)	Linearity	Response time (second)	Ref.
Lactase/RuO_2 (static)	0.2 - 5	36.11	0.997	5	This Work
Lactase/RuO_2 (dynamic)	0.2 - 5	49.80	0.996	N/A	This Work
LDH/NAD^+/MBs/GPTS/ GO/NiO	0.2 - 3	45.40	0.992	21	(Chou, Yan, Liao, Lai, Wu, et al., 2018)
FePt NPs-g-C_3N_4/CZO	0.2 - 10	11.12	0.998	11	(Diallo et al., 2013)
LOD/Si_3N_4	1 - 6	20.00	N/A	N/A	(Xu et al., 2005)

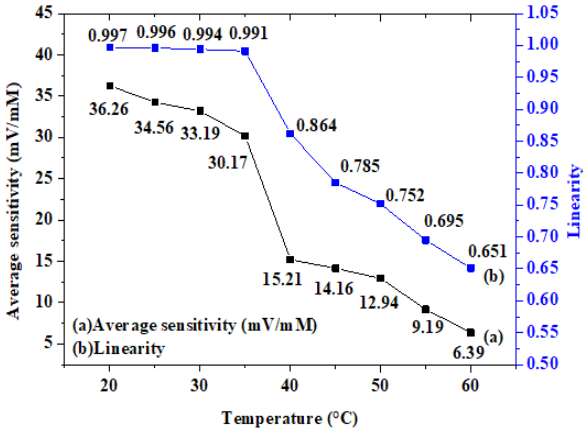
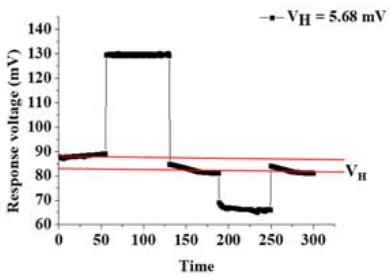


Fig. 7 The average sensitivity and linearity of the RuO_2 LA biosensor at different temperatures under static measurement.

3.3 Temperature Effect

The temperature effect of RuO_2 LA biosensor was measured by a hot plate/stirrer (PC400D, USA). The average sensitivity and linearity of the RuO_2 LA biosensor at different temperatures measured in static measurement were shown in Fig. 7. When the temperatures were above 40 ℃, the average sensitivity and linearity were decreased. In the range of 20℃- 35 ℃, it can be noted that the average sensitivity had little change with the temperature increase. At 60 ℃, the sensor had the worst performance on average sensitivity and linearity. It can be seen that this result indicates that LA has lost its activity at this temperature. The temperature was higher when the solution was heated. The structure of the LA will change, and the enzyme or protein will be easily thermal denaturation, resulting in a decrease in the detection rate (Y. T. Wang et al., 2010). In this case, the LA molecules will not readily adsorb to the sensor. Thus, the sensor was suitable to be placed between 25 ℃ and 35℃.

(a)



(b)

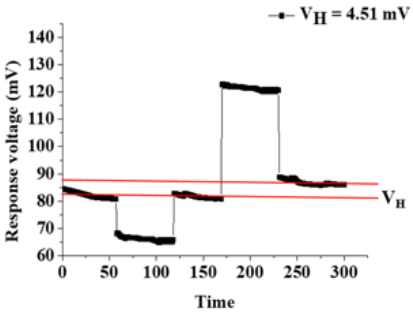


Fig. 8 The hysteresis voltages of the RuO2 LA biosensor (a) forward cycle, (b) reverse cycle.

3-4 Hysteresis Effect

The V-T measurement system was used to measure the response voltage. The measurement of the hysteresis effect confirmed the initial concentration of the LA. When the concentration of the analyte solution was changed and then restored to the original concentration, the measurement response voltage was different from the solution before and after the two times. The difference between the initial response voltage and the final response voltage of the same LA solution is called hysteresis voltage. The output voltage difference voltage at the initial and final response was the hysteresis voltages (Nien et al., 2021)and the CZO sensing film was deposited using radio frequency (R.F.. When the hysteresis was smaller, the sensing film of the sensor was less susceptible to the influence of the solution. Fig. 8 (a) showed the hysteresis curves of the RuO_2 LA biosensor in the forward cycle. After the sensor was soaked in the solution for 5 minutes, the sensor is measured for 60 seconds. At the concentration under a forward cycle of 1.3 mM → 3 mM → 1.3 mM → 0.2 mM → 1.3 mM, the hysteresis voltage V_H was 5.68 mV. Fig. 8(b) showed the hysteresis curves of the RuO_2 LA biosensor in the reverse cycle. At the concentration under a reverse cycle of 1.3 mM → 0.2 mM → 1.3 mM → 3 mM → 1.3 mM, the hysteresis voltage V_H was 4.51 mV. The sensing window with high concentration first remained more hydrogen ions, so the variation was large.

3-5 Response Time

The response time is defined as the time required for the response voltage to change from its initial state to 95% steady state after the sensor is immersed in the analytic. The RuO_2 LA biosensor was immersed in a pure PBS solution. The response voltage remains stable for 30 s, then the LA solution of 2 mM was added in PBS solution. If the response time is shorter, the response for the sensor responses to the analyte is faster. The response time the

RuO_2 LA biosensor was shown in Fig. 9.

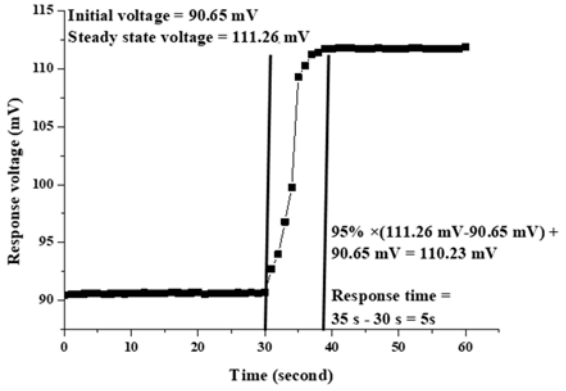


Fig. 9. The response time for the RuO_2 LA biosensor.

The initial voltage of the sensor was 90.65 mV and the response voltage of steady state was 111.26 mV. The initial state to 95% of the steady state was 19.57 mV. The measurement results were shown in Fig. 9, the response time of the RuO_2 LA biosensor was 5 seconds.

The comparison of sensing characteristics for different LA biosensors were shown in Table III. In a previous study, Chou et al. (Chou, Yan, Liao, Lai, Wu, et al., 2018) proposed a LA biosensor based on NiO film. The graphene oxide and magnetic are used to improve the characteristics of biosensors. The sensing characteristics of biosensor were analyzed by potentiometric method. The average sensitivity was 45.4 mV/mM and response time was 21 seconds. However, the response time was large. Nien et al. (Diallo et al., 2013) presented a LA biosensor based on CZO film. Three different concentrations of FePt NPs were added to modify the CZO film based LA sensor. The response time was significantly improved. The A. K. Diallo research group (Xu et al., 2005) investigated the design and manufacture process of ElecFET microdevices, which combine potential and amperometric detection/transduction principles at the micro scale. For the detection of LA, this biosensor can only achieve 20.00 mV/mM average sensitivity. In this study, the average sensitivity and linearity of RuO_2 LA biosensor were analyzed under static and dynamic measurement. The average sensitivity and linearity were 36.11 mV/mM and 0.997 under static measurement. The average sensitivity and linearity were 49.80 mV/mM and 0.996 under dynamic measurement. The biosensor achieved better sensing characteristics under dynamic measurement. Moreover, the biosensor has the small response time of 5 seconds. From the sensing measurement, stability and response time of the proposed LA biosensor have better performance compared with other counterparts.

4. CONCLUSION

In this paper, a static and dynamic measurement method was presented to analyze the sensing characteristics of LA biosensor. In the static measurement, the sensitivity characteristics of the biosensor were analyzed by V-T measurement system. The experimental results show that the average sensitivity is 36.11 mV/mM. In the dynamic measurement, a microfluidic measurement system was used to analyze the sensitivity characteristics of the biosensor under different flow rates. According to the dynamic experimental results, the average sensitivity increased from 36.11 mV/mM to 49.80 mV/mM due to the improvement of ion diffusion. The best average sensitivity was 49.80 mV/mM at the flow rate of 30 μL/min. Moreover, the lactase/RuO_2 sensing films have better average sensitivity and linearity over temperatures ranging from 20°C to 35°C. Compared with other enzyme biosensors, the proposed LA biosensor has better anti-interference, sensing characteristics, and static and dynamic stability.

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