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Reaction of NiO film on flexible substrates with buffer solutions and application to flexible arrayed lactate biosensor

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ABSTRACT

In this study, we proposed a flexible arrayed lactate biosensor based on pH-sensitive nickel oxide (NiO) films, and also investigated the adhesion between NiO film and different flexible substrates under reactions with different solutions. L-lactate dehydrogenase (LDH) and nicotinamide adenine dinucleotide (NAD^+) were co-immobilized on pH-sensitive NiO film by using a crosslinking reagent, 3-glycidoxypropyltrimethoxysilane (GPTS). It was found that GPTS concentration would affect the sensing characteristics for the lactate biosensor. The lactate biosensor with 1:2 volume ratio of GPTS-toluene mixture had the best average sensitivity and linearity. In the range of lactate concentration from 0.2 mM to 3 mM, arrayed flexible lactate biosensor based on the NiO/PET structure had 38.218 mV/mM of average sensitivity. In addition, the average sensitivity of the sensor based on the NiO/Pt/PET structure was 32.483 mV/mM.

1. Introduction

Nickel oxide (NiO) is one of p-type semiconducting materials, and which has been used to develop gas sensor, biosensor, dye-sensitized solar cell and even electrochromic device [1–7]. For sensing material of biosensor, NiO has a big potential due to its several advantages, such as high chemical stability, biocompatibility, high electron transfer feature, oxygen ion conductivity, nontoxicity and high electrocatalytic properties [4].

In this study, we proposed a flexible arrayed lactate biosensor based on NiO film, where sensing film array and reference electrode were miniaturized on flexible substrate. The degree of NiO film peeling off was also investigated during the film reacted with buffer solution for a long time.

Lactate is a key metabolite during anaerobic metabolism in human body [8,9]. When the energy in tissues is insufficient from aerobic respiration, an increase in lactate concentration will occur from the anaerobic metabolism [10]. The normal range of lactate concentration in human blood is from 0.5 mM to 1.5 mM [8]. The detection for lactate is important for clinical diagnostic, medicine, and food analysis [8,11].

Normally, most of enzymatic lactate biosensors were based on L-lactate dehydrogenase (LDH) and L-lactate oxidase (LOD) due to their simple enzymatic reaction and simple design fabrication. The enzymatic lactate biosensors based on LOD reaction had some drawbacks, such as a high oxidation potential required, an interference caused by electro-oxidizable species, a fluctuation of oxygen concentration in the solution, an instable detection limit, and a difficult task for miniaturization in practical application [8]. LDH presents throughout the tissues, such as blood cells and heart muscle, and has a high catalytic activity for conversion of lactate with an aid of a coenzyme (nicotinamide adenine dinucleotide, NAD^+). The reactions of LDH involved in biosensors were shown in Eqs. (1) and (2) [8].

$$\text{Lactate} + NAD^+ \xrightarrow{\text{LDH}} \text{Pyruvate} + \text{NADH} + H^+ \quad (1)$$

$$\text{NADH} \rightarrow NAD^+ + H^+ + 2e^- \quad (2)$$

Several methods of enzyme immobilization have been investigated, such as entrapment, crosslinking and adsorption [8]. In this study, LDH and NAD^+ were used to achieve catalytic reaction of lactate, and 3-glycidoxypropyltrimethoxysilane (GPTS) was used as crosslinking reagent to bind LDH and NAD^+ onto NiO film. Moreover, the GPTS concentration was also investigated and compared to obtain the optimal sensing characteristics.

2. Experiment

2.1. Materials and reagents

The polyimide (PI), polyethylene naphthalate (PEN), polyethylene terephthalate (PET) and indium tin oxide (ITO)-coated PET substrates

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were purchased from Zencatec Corporation (Taiwan). The nickel oxide (NiO) and platinum (Pt) target with 99.95% purity was purchased from Ultimate Materials Technology Co., Ltd. (Taiwan), used as a material of sensing film. The standard pH butter solutions were purchased from Sigma Aldrich Chemical Company (USA). Phosphate monobasic (KH_2PO_4) powders and potassium phosphate dibasic (K_2HPO_4) powders were purchased form Darmstadt, Germany and Katayama Chemical Co., Ltd. (Japan), used to prepare 100 mM, 60 mM, 20 mM and 4 mM phosphate buffer solutions (PBS) with pH 7.

The silver paste was purchased from Advanced Electronic Material Inc. (Taiwan), which was used as conductive wire and reference electrode. The epoxy thermosetting polymer (product no.JA643) was purchased from Sil-More Industrial, Ltd. (Taiwan), which was used as insulation layer. Lactate was purchased from Sigma-Aldrich Co. (USA), used to prepare the test solutions which lactate concentrations were 0.2, 0.7, 1.3, 2, 3, 5 and 10 mM. L-Lactate dehydrogenase (LDH) and β-nicotinamide adenine dinucleotide hydrate (NAD^+) were purchased from Sigma-Aldrich Co. (USA), which were respectively used as enzyme and coenzyme to catalyze the reaction of lactate. 3-Glycidoxypropyl-trimethoxysilane (GPTS) and toluene were purchased from Sigma-Aldrich Co. (USA), which were used for the enzymatic immobilization.

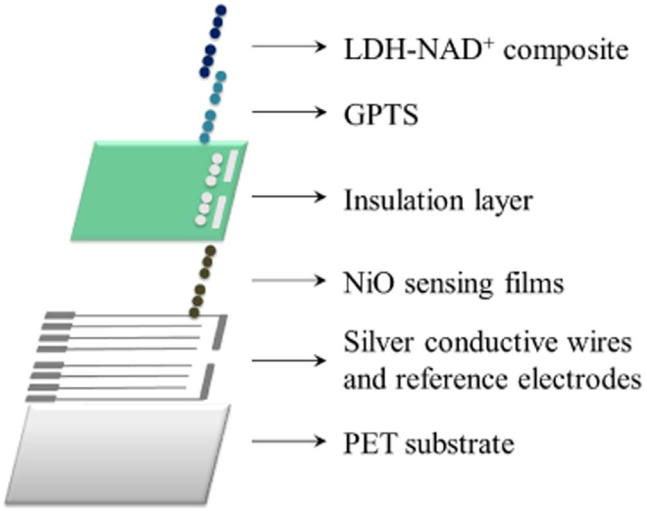

Fig. 1. Schematic structural diagram of flexible arrayed lactate biosensor [12].

2.2. NiO films deposited on different substrates

The radio frequency (R. F.) sputtering system was used to deposit NiO films on different substrates such as PET, ITO-coated PET, Pt-coated PET, PEN, PI, glass and silicon (Si) substrates. The (Ar) and oxygen (O_2) gases were introduced into sputtering chamber, and which oxygen content was at about 27%. The sputtering power and working pressure were set at 50 W and 3 mTorr, respectively. The deposition time was 50 min. The thickness of NiO film was 355 nm. In addition, deposition parameters of Pt film fabricated on PET substrate were as follows: the flow rate of Ar gas controlled at 10 sccm, 60 watt sputtering power, 30 mTorr working pressure and 5 min deposition time. The thickness of Pt film was 75 nm.

2.3. NiO films on different substrates immersed into different solutions

On the purpose of imitating human blood in this study, it was investigated that the PBS concentration affected the NiO film on different substrates. The pH value of each PBS was all set at 7, and their concentrations were 100 mM, 60 mM, 20 mM and 4 mM, respectively. Then, NiO films on different substrates were immersed into different concentration PBS.

In addition to PBS concentration, the pH value thereof was also taken into consideration. Therefore, NiO films on different substrates were immersed into standard pH buffer solutions which pH values were 1, 7 and 13, respectively.

2.4. Preparation of flexible arrayed lactate biosensor

First, the silver paste were printed on flexible PET substrate by using the screen-printing technique to prepare the silver reference electrodes and conductive wires, not only replacing traditional glass reference electrode but also achieving the miniaturization of reference electrode and conductive wires. Second, pH-sensitive NiO films were deposited on the end of conductive wires by using R. F. sputtering system. Subsequently, the sensor was packaged by using the epoxy and the screen-printing technique. The epoxy layer was used to define the areas of sensing films, and it was also as an insulation layer to prevent silver conductive wires from corroding of test solution.

To immobilize enzyme on pH-sensitive NiO film, GPTS was adopted as crosslinking reagent for binding enzyme onto NiO film. First, each 2 μl GPTS-toluene mixture was dropped onto NiO film and then left in oven at 120 °C for 1 h. The volume ratios of GPTS-toluene mixture were set at 1:1, 1:2 and 1:4 to investigate that GPTS content affect the sensing characteristics of lactate biosensor. Second, each 2 μl LDH-NAD^+ composite was dropped onto NiO film and then stored in refrigerator at 4 °C for one day. Finally, LDH-NAD^+ composite was successfully co-immobilized on NiO film by cross-linking method. The schematic structural diagram of the lactate biosensor was shown in Fig. 1 [12]. The photo of the lactate biosensor was shown in Fig. 2, and which size was about 3.5 cm * 3.0 cm.

2.5. Potentiometric measurement system

The response voltages of flexible arrayed sensor immersed in test solution were measured by using potentiometric measurement system. First, the response voltages were through six instrument amplifiers (LT1167) with benefit of high input impedance to lower input noise. After that, the signals of response voltages were sent to computer through data acquisition (DAQ) card. Finally, the data of response voltages were accessed via LabVIEW software. The potentiometric measurement system was shown in Fig. 3 [12]. Moreover, the stability of silver reference electrode was investigated for pH and lactate concentrations. From Fig. 4 (a) and (b), no significant variation occurred on response

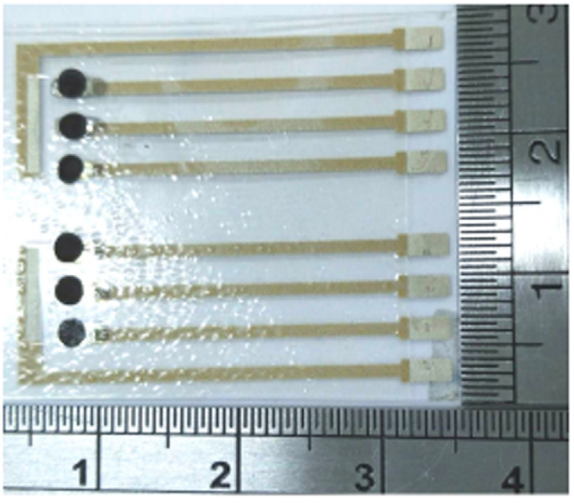

Fig. 2. Photo of flexible arrayed lactate biosensor.

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