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Multianalyte Biosensors for the Simultaneous Determination of Glucose and Galactose Based on Thin Film Electrodes

Neng Qin JIA[1]*, Zong Rang ZHANG[1], Jiang Zhong ZHU[2], Guo Xiong ZHANG[2]

[1] Department of Chemistry, Shanghai Normal University , Shanghai 200234
[2] State Key Lab of Transducer Technology, Shanghai Institute of Metallurgy , Chinese Academy of Sciences, Shanghai 200050

Abstract: A multianalyte biosensor for the simultaneous determination of glucose and galactose was developed by immobilizing glucose oxidase (GOD) and galactose oxidase (GAO) on Nafion-modified thin film platinum disk electrodes. The dual Pt working electrodes with disk shape and the surrounding ring shaped counter electrode were fabricated by thin film technology, which were integrated onto the same microchip. The response of the designed biosensor for glucose and galactose were linear up to 6.0 mmol/L and 3.5 mmol/L with sensitivities of 0.3 μA/mmol/L and 0.12 μA/mmol/L, respectively. No cross-talking effect was observed.

Keywords: Multianalyte biosensor, glucoe, galactose, Nafion, thin film technology.

There is an increased demand of multianalyte sensing devices having potential applications in biomedical, industrial and environmental fields[1-2]. The multianalyte biosensors become one of the top research subjects in the past few years. It attracts great interests in fabricating multianalyte biosensors by various methods[3-8]. Recently, thin film technology, adopted from the microelectronics industry, has been turned to be an attractive technology for the fabrication of chemical sensors and biosensors due to ease, quality, reproducibility and low cost of manufacturing[9-10].

In this paper, glucose and galactose, which are important parameters in medical applications, were chosen as model analytes. An amperometric multianalyte biosensor for the simultaneous determination of glucose and galactose had been developed by thin film technology and a compatible immobilization process of enzyme with some additional treatments. Thin film technology was used to fabricate sensor chips, where two Pt disk working electrodes and Pt ring counter electrode were integrated on one chip. Nafion polymer, as anti-interference membrane, was modified on the electrode surface. GOD and GAO were immobilized on the Nafion-modified dual disk Pt electrodes.

Electrochemical measurements were carried out on a home-made bipotentiostat and EG&G Princeton Applied Research Corporation(PARC)model 173 Potentiostat/ Galvanostat with a LM 20A X-Y recorder (Shanghai, China). Ag/AgCl and plantium ring on the chip were used as reference and counter electrodes, respectively.

Galactose oxidase (GAO) and glucose oxidase (GOD) were obtained from Sigma.

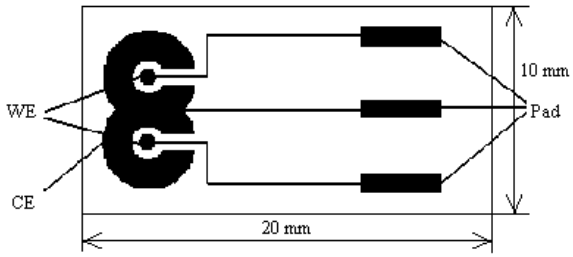
* E-mail: nqjia@shtu.edu.cn

5% Nafion-117 solution was from Fluka. All other reagents were analytical grade. All solutions were prepared with doubly distilled water.

A typical sensor chip layout employed in constructing glucose/galactose sensors was shown in **Figure 1**. The microfabrication process was as follows: an alumina wafer (1mm thick, optically polished on single side) was used as substrate. 50 nm Ti layer and 300nm Pt layer were evaporated by an electron gun at a substrate temperature of 25^{o}C. Titanum served as adhension promoters to the wafer. The electrode geometries were patterned using the lift-off technique. Areas to be insulated were covered by a silicon nitride film. The one-chip layout consists of dual disk shaped Pt (1.5 mm diameter, 3.0 mm electrode gap) working electrodes and a Pt ringcounter electrode(18.8 mm^2) with leads of 0.4 mm and 1.8×5 mm contact pads.

The Nafion films on the electrodes were prepared by casting 4 μL of 0.1% Nafion solution onto the central Pt disk electrodes and then dried. 50 μL of 0.1 mol/L phosphate buffer (pH 7.2) solution containing 4 mg GAO or GOD and 4 mg BSA were mixed carefully with 10 μL of 5% glutaraldehyde solution. 4 μL of GOD or GAO final solution were pipetted carefully onto the Nafion modified electrodes avoiding any mixing of the two solutions. After air-drying at room temperature, the multianalyte biosensors were washed and stored at 4°C.

Figure 1 Schematic view of the sensor chips.



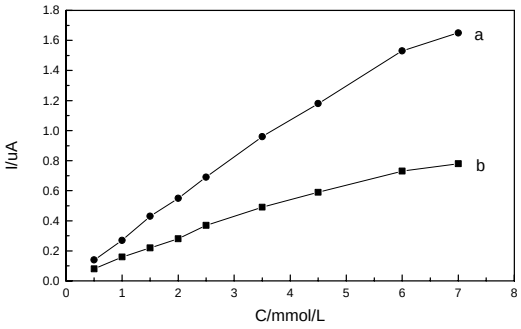
WE: working electrode CE: counter electrode

Before enzyme immobilization, cyclic voltammetry was used to study the electrochemical behaviors of the sensor microchips and to evaluate the reproducibility between sensor chips. Experiments showed that microfabricated thin film electrodes had reversible electrochemical behaviors. In addition, they had good reproducibility among sensor mirochips. Therefore , the microfabricated thin film electrodes could be used as the substrate electrodes of the biosensors.

The effect of pH on the multianalyte biosensor reponse was investigated. The response current increased with increasing pH values from 5.5 to 8.75 and reached a maximum at pH 7.2, then decreased gradually with further increasing of the pH values. So, pH 7.2 was selected in the subsequent experiments. The response from multianalyte biosensor was also measured in the presence of possible interfering substance (0.2mmol/L ascorbic acid) during the determination of 1 mmol/L glucose and galactose solution. Ascorbic acid caused hardly any interference on the response of biosensor. This was attributed to the selectivity of Nafion film.

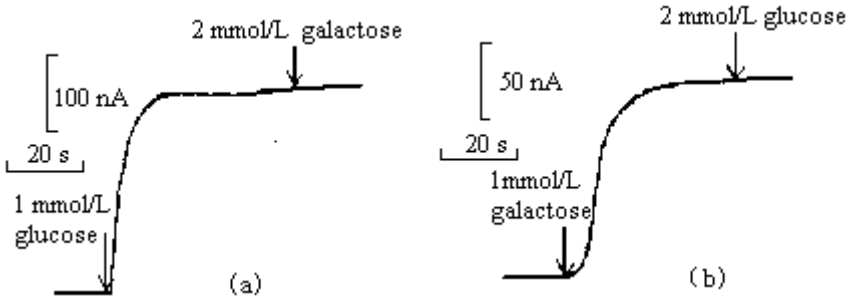
The calibration curves of the designed biosensor were obtained (**Figure 2**) by measuring simultaneously glucose and galactose in the 0.1 mol/L phosphate buffer solution (pH=7.2). It shows that the biosensor exhibits a linearity of 6.0 mmol/L for glucose and 3.5 mmol/L for galactose. The sensitivities and the response times were 0.3 μA/mmol/L, 30 s for glucose and 0.12 μA/mmol/L, 40 s for galactose.

Figure 2 **C**alibration curves of the multianalyte biosensor in phosphate buffer solution (pH 7.2)



(a).glucose and (b) galactose; Operating potential, 700 mV *vs.* Ag/AgCl.

Figure 3 Response of multianalyte biosensor on alternately added amounts of glucose and galactose.



(a) Response of the glucose sensor ; (b) Response of the galactose sensor

The enzymatic oxidation of the analytes glucose and galactose produced hydrogen peroxide within the enzyme membranes of the sensors. Due to the diffusion of biocatalytically generated H_2O_2, there are potential cross-talk problems on amperometric multianalyte biosensors[6-7]. Experiments were conducted to determine if cross-talk occurred on the biosensors. As can be seen from **Figure 3**, glucose and galactose were added alternately in order to check the response of the multianalyte biosensor, no response was observed either at the glucose sensor when galactose was injected, or at the galactose sensor when glucose was injected. Therefore, no cross-talk could be observed during the determination, probably due to the geometrical arrangement of the sensors[5,8].

The results show that thin film technology holds great promise on the fabrication of biosensors for simultaneous monitoring of multianalytes. Further studies are in

progress on the other characteristics and the applications of the multianalyte biosensors basing microfabrication technology.

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