

A NOVEL MICRODIALYSIS GLUCOSE SENSOR SYSTEM BASED ON CO-IMMOBILIZING ON AU MICRO-ELECTRODE BY SOL-GEL TECHNIQUE

Ping Yu, Weiting Liu, Yuquan Chen, Guang Li

Biosensor National Special Lab. Dept. of Biomedical Engineering,
Zhejiang University, Hangzhou, Zhejiang, 310027, P.R.China

Abstract-A microdialysis sensor system for continuous glucose measurements has been developed. The sensor is based on co-immobilizing glucose oxidase (GOD) with the catalase by sol-gel technique on the surface of the silicon bases with two pattern of Au microelectrodes. A graduated (“sandwich”) immobilizing method is performed to improve the stability and the life time of the co-enzyme system. The response of the system was in good order. Above all, we developed a new geometry of bispiral microelectrode and numeric analysis its electrochemical character.

Keywords-microdialysis,co-enzyme,sol-gel, microelectrode

I. INTRODUCTION

Continuous glucose monitoring has been the focus of much research, especially in the clinical management of diabetes, since the first “enzyme electrode” of glucose was reported by Updike and Hicks in 1967. The studies to improve the character of the biosensors and the measuring system have been reported recently. A feasible method to monitor the glucose concentration *in vivo* is to implant a biosensor in the s.c. tissue, however, the function of glucose electrode such as a hydrogen peroxide detecting amperometric electrode is impaired evidently in human s.c. tissue and plasma, the insulating protein shell surrounding the sensor causes the fouling of the electrode or the gradual loss of enzyme activity, which can lead the long-term drift of the sensor and affect its sensitivity. Furthermore, the method to retain the activity and the stability of the enzyme immobilized on the electrode is also very significant.

It is known that the microdialysis system is opting for coping with the problem that occurs in subcutaneous adipose tissue, prevent electrode from being insulated by protein in the s.c. tissue for longtime monitoring. We presented here a microdialysis system (Fig1) using an interval pump which perfuses at the rate of 10 ul/min for 15min and stops for about 15 min. It is used to let the glucose concentration in the subcutaneous tissue recover absolutely, so that it can be possible to use the correction *in vitro* with the impulse microdialysis method in the glucose detection.

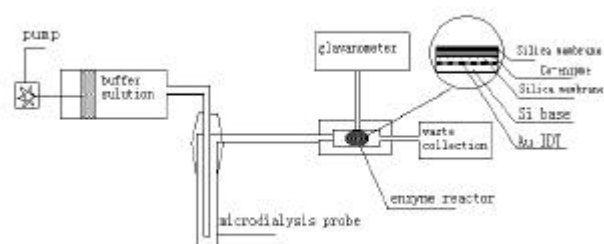


fig1.the configure of the micor sensor system

In our earlier work it was show that the glucose sensor co-immobilized with the glucose oxidase and the catalase based on the “sandwich” configuration of sol-gel-derived method has a very good linearity and responsibility over the whole measuring range. This work presents a novel glucose sensor system to examine the amperometric value for H_2O_2 detection based on co-immobilized on an interdigital microelectrode or a dispiral microelectrode. The microelectrode can be defined as the electrodes having dimensions of a micrometer or less (nm). Due to the advantages of a microelectrode (or its array), such as high current densities and high diffusion rates, the importance of the use of microelectrodes has been widely accepted in the field of electroanalytical chemistry. In the recent topics, a lot of researches were on the focus of the interdigital microelectrode (IDA) and disk microarray electrode. In recent years the former was in widely used due to its convenient fabrication and good electrochemical character, such as promise of further enhancements of signal/noise ration and selectivity. The steady-state current response for the IDA can be calculated by solution of equation

$$\frac{1}{D} \frac{\partial C}{\partial t} = \frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial z^2} \quad (1)$$

The boundary condition is

Report Documentation Page

Report Date 25 Oct 2001	Report Type N/A	Dates Covered (from... to) -
Title and Subtitle A Novel Microdialysis Glucose Sensor System Based on Coimmobilizing on AU Micro-Electrode by SOL-GEL Technique	Contract Number	
	Grant Number	
	Program Element Number	
Author(s)	Project Number	
	Task Number	
	Work Unit Number	
Performing Organization Name(s) and Address(es) Biosensor National Special Lab Department of Biomedical Engineering Zhejiang University Hangzhou Zhejiang, 310027, P.R. China	Performing Organization Report Number	
	Sponsor/Monitor's Acronym(s)	
Sponsoring/Monitoring Agency Name(s) and Address(es) US Army Research, Development & Standardization Group (UK) PSC 802 Box 15 FPO AE 09499-1500	Sponsor/Monitor's Report Number(s)	
	Distribution/Availability Statement Approved for public release, distribution unlimited	
Supplementary Notes Papers from 23rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society, October 25-28, 2001, held in Istanbul, Turkey. See also ADM001351 for entire conference on cd-rom.		
Abstract		
Subject Terms		
Report Classification unclassified	Classification of this page unclassified	
Classification of Abstract unclassified	Limitation of Abstract UU	
Number of Pages 3		

$$\left. \begin{aligned}
z = 0, 0 \leq x \leq x_1, \text{Cathode} \quad , C_o / C_R = \exp \frac{nF}{RT} (E_c - E^0) \\
z = 0, x_2 \leq x \leq W, \text{Anode} \quad , C_o / C_R = \exp \frac{nF}{RT} (E_z - E^0) \\
0 \leq x \leq x_1, x_2 \leq x \leq W, D \left(\frac{\partial C_R}{\partial z} + \frac{\partial C_o}{\partial z} \right) = 0 \\
x_1 \leq x \leq x_2, z = 0, \frac{\partial C}{\partial z} = 0 \\
x = 0, x = W, \frac{\partial C}{\partial x} = 0
\end{aligned} \right\}$$

where D is the diffusion coefficient of the electroactive species, C is the concentration, z is the direction of diffusion perpendicular to flow, x is the direction of flow, W is the width of the unit element, $2x_1$, $2(W-x_2)$, and x_2-x_1 is the length of the cathode, the anode and the insulation, respectively. We optimized the width, the length and the space of the electrode cell to get more effective diffusive field. But we found the influence of the discontinuity of the diffusive field on the extreme points of the bind microarray electrode represented obviously by the diminution of the size of the IDA, which cause the stability of the measurement decrease.

Some researches were reported that the ring microelectrode has good responsibility because of its non-linearity diffusion. In our new geometry of bispiral microelectrode (Fig 2), which remains the character of the geometry of the ring and has the current additive property as the microelectrode array. Its current response can be described as the equation

$$\frac{\partial C}{\partial t} = D \left(\frac{1}{r} \frac{\partial C}{\partial r} + \frac{\partial^2 C}{\partial r^2} + \frac{1}{r^2} \frac{\partial^2 C}{\partial \theta^2} + \frac{\partial^2 C}{\partial z^2} \right) \quad (2)$$

with the boundary condition

$$\left. \begin{aligned}
t = 0, z \geq 0, C_R = C^*, C_o = 0 \\
t > 0, z = 0, r = aq, D \left(\frac{\partial C_R}{\partial z} + \frac{\partial C_o}{\partial z} \right) = 0 \\
t > 0, z = 0, r < aq, \text{ or } r > aq, \frac{\partial C}{\partial z} = 0 \\
t > 0, z \rightarrow \infty, C = C^* \\
t > 0, z \geq 0, r \rightarrow \infty, C = C^*
\end{aligned} \right\}$$

whose solution can be calculated by FAM method. Since it has not been reported anywhere before, we designed the same total length of the bispire compared with the length of the interdigital electrode present above. It indicated that the response current was higher than the contrastive IDA. That is, to obtain the same response current, the area of the bispiral microelectrode much smaller than the IDA. Therefore, it has great advantage of the development of miniaturization, especially in the use of nanometer application. Experiment also showed its good stability.



Fig2. Schematic top view of the electrodes

II. EXPERIMENTAL DETAILS

A. Materials

The following chemicals were used: Tetramethoxysilane (TMOS) was obtained from Fluka. Glucose oxidase (GOx, EC 1.1.3.4, from *Aspergillus niger*, 250 units/mg) and catalase (EC 1.11.1.6, from *bovine liver*, 260,000 units/ml) was purchased from Roche. PEG400 was obtained from the Shanghai Pudong Gaonan reagent factory (Shanghai, China). All other chemicals were of analytical grade, and aqueous solutions were prepared in doubly distilled water.

B. Apparatus

A commercially available microdialysis probe is used with a molecule cut-off 20,000 Dalton, which is controlled by a stepping-motor-controlled syringe pump, offering the perfusion rate of 10 ul/min and the interval time of 15min. Au interdigital microelectrode and bispiral microelectrode cells with the base of silicon were fabricated under the design. The amperometric detection of glucose was performed by applying a potential of +600mv.

C. Preparation of enzyme electrode

The sol-gel solution was prepared by mixing 5ml of TMOS, 9ml of H₂O, 2ml of PEG400, and 10mM NaF in a glass vial. The mixture was sonicated for 30min and then a clear and homogeneous solution was obtained. This solution was stored at room temperature for use.

To 1.0mL the stock solution was diluted by water. A sol-gel film without enzyme was spin cast onto the substrate of the interdigital electrode. After drying under ambient conditions for 24h, 30μL of the co-enzyme sol-gel solution (1.0mL of the stock solution, 30mg of GOx and 45μL of catalase, 1.0mL of water) was dropped onto the top glassy layer and spun to yield the second co-enzyme thick layer. In order to retain the activity of the enzymes, the electrode was dried at 40°C for 24h.

III. RESULTS AND DISCUSSION

A. Response characteristics of the micro-dialysis system.

Fig 3. shows a typical response curve. The rise time (10-90%) of the total system is found to be <1.5min. the second

scheme of the bispireal showed a little shorter response time and higher current value. Since some investigations have reported the wide span of the subcutaneous glucose level

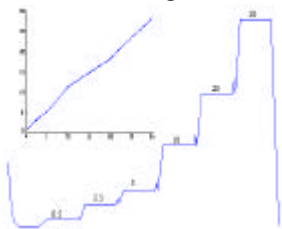


Fig 3. Response characteristics of the glucose monitoring system with microdialysis sampling.

(between 20 and 90% of the intravenous level) may be explained by the development of a glucose depletion region around the measuring site. The interval pump scheme can well deal with this problem.

B.Characteristics of the electrodes.

The calibration curve ranges from 0.5 to 50mM glucose in buffer. The “sandwich” sensor modified with the two scheme of micro-electrodes has a very good linearity over the whole measuring range. It might indicated that the linearity has little relativity with the pattern of the microelectrode.

The stability of the glucose sensor was tested by amperometric measurement over a period of two month using 5mM glucose. The co-enzyme sandwich sensor showed lower decrease in response. It is suggested that co-immobilizing catalase with Gox might effect the stability of the sensor.

IV. CONCLUSIONS

In this article we present a small and wearable microdialysis system developed by co-enzyme sol-gel thin film technology. The amended interval pump of the microdialysis system makes the baseline measurement accurate and convenient, which can improves the response current for the glucose concentration. On the other hand, the sensor immobilized by GOx with catalase exhibits good relativity of the current to the glucose concentration and high linear range from 2.5 to 30mM. The detection limits were on the order of 0.25mM. The response time of the whole systme is within 1.5min.

Forthermore, the developed geometry of the bispireal microelectrode showed significant potential for miniaturized device

V.ACKNOWLEDGEMENT

This project was supported by the National Nature Science Foundation and Zhejiang Province Foundation.

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