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Construction of a photovoltaic glucose sensor applying a metal-insulator-silicon structure in combination with ultra-thin polypyrrole-glucose oxidase film

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Abstract:
An enzyme-metal-insulator-silicon (EMIS) structured chemical sensor based on photovoltage technology is constructed for potentiometric biosensing of glucose in a flow-injection analysis (FIA) system. To enhance the sensibility of the sensor to glucose, an ultra-thin (60 nm) film of polypyrrole (PPy)-glucose oxidase (GOD) is formed as the glucose-sensitive membrane through a simple electropolymerization process in the absence of supporting electrolyte. The introduction of photovoltage technology and the electroimmobilization of GOD are advantageous in improving the sensitivity, selectivity, response time, linear range, stability and lifetime of potentiometric sensor for glucose measurement. Thus the photoelectric hybrid glucose sensor has extensive potential both in rapid determination of plasma glucose and long-time continuous monitoring of glucose concentration in extracellular microenvironment.

Key words: photovoltage technology, potentiometric biosensing, glucose sensor, flow-injection analysis, electropolymerization

Introduction

The detection of glucose has attracted most interest since the first publication on a glucose sensor [1]. There has been a constant increase in the number of studies devoted to glucose biosensing, among which amperometric technique is most widely used [2]. Although the amperometric glucose sensor has relatively high sensitivity and low detection limit, it seems to exhibit some disadvantages such as selectivity due to the interference of other reducible species in plasma. Besides, the electrocatalytic oxidation is not beneficial to the continuous monitoring of changes in glucose concentration. To overcome these drawbacks, an EMIS sensor based on photovoltage technology is constructed for rapid, long-time and continuous detection of glucose concentration.

Using the photovoltage technology employed in the light addressable potentiometric sensor [2], many biosensing devices are developed for enzyme based organic substances test [3,4]. The basic method lies in the pH variation induced by the enzymatic reactions as these biosensors are sensitive to pH change. However, the pH deviating from the optimum pH value would affect the enzyme activity to a certain extent, which in turn will impact the sample detection. In view of this, an electrolyte-metal-insulator-silicon structured sensor [5], which is sensitive to the redox potential determined by the ratio of oxidant and reductant in the analyte, has potential to overcome this problem.

Electropolymerized electroactive polymers have been widely used for the immobilization of enzymes in the preparation of biosensors because of the simplified technology of micro-biosensor production. In this paper the potentiometric glucose sensor is developed by modifying the metal layer of the sensor electrochemically with an ultra-thin PPy-GOD film [6]. With the application of the photovoltage technology, high sensitivity to surface potential change and continuous electrostatic measurement can be realized.

Experimental

All chemicals were of analytical grade. Glucose oxidase from *Aspergillus niger* was obtained from Sigma. Pyrrole (from Aldrich) was distilled before use, stored in a refrigerator and

protected from light. β-D-Glucose was prepared with 0.05 M phosphate buffer (pH 7). All solutions were prepared with deionized water.

The potentiometric transducer is fabricated with the layer sequence of Al/n-Si/SiO_2/Cr/Au, as shown in Fig. 1(a). 50 nm SiO_2 is grown on a sample n-type silicon with a thickness of 100 μm. Then 30 nm Cr and 150 nm Au are deposited on the SiO_2 surface, followed by a lithography process to expose the working electrode and the lead wire. Finally 300 nm Al is evaporated onto the rear side of the chip to form an ohmic contact.

The sensor surface is thoroughly cleaned before immobilization of GOD. Electropolymerization is carried out galvanostatically in stagnant solutions in a three-electrode cell with Pt auxiliary and Ag/AgCl reference electrodes. The electrical charge is set as 25 mC/cm^2 with a current density of 0.05 mA/cm^2. The ultra-thin film is formed using 0.1 M pyrrole (Py) and 20 U/ml GOD. After electropolymerization, the sensor is rinsed thoroughly with deionized water to remove any loosely bound enzyme.

The measurement circuit and flow-injection analysis system is shown in Fig. 1(c). By using photovoltage technology, the surface potential of the sensor is transformed to a photocurrent that can be detected with an external circuit. The characteristic current-voltage (I-V) curve is S shaped, as shown in Fig. 1(b).

When the sensor surface contacts a glucose solution, an equilibrium surface potential is formed because of the enzyme-catalyzed reaction

$$\text{glulcose} + O_2 \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2 \quad (1)$$

And the oxidation-reduction (redox) pairs of O_2 and H_2O_2 will generate an equilibrium redox potential on the Au surface. Thus a stable I-V curve can be obtained. Different glucose solutions will produce different I-V curves, and the shift of I-V curves along the bias voltage (ΔV) reflects the glucose concentration change. In continuous monitoring, the bias voltage is usually set at a fixed value, and the photocurrent variation (ΔI) also reflects the glucose concentration change.

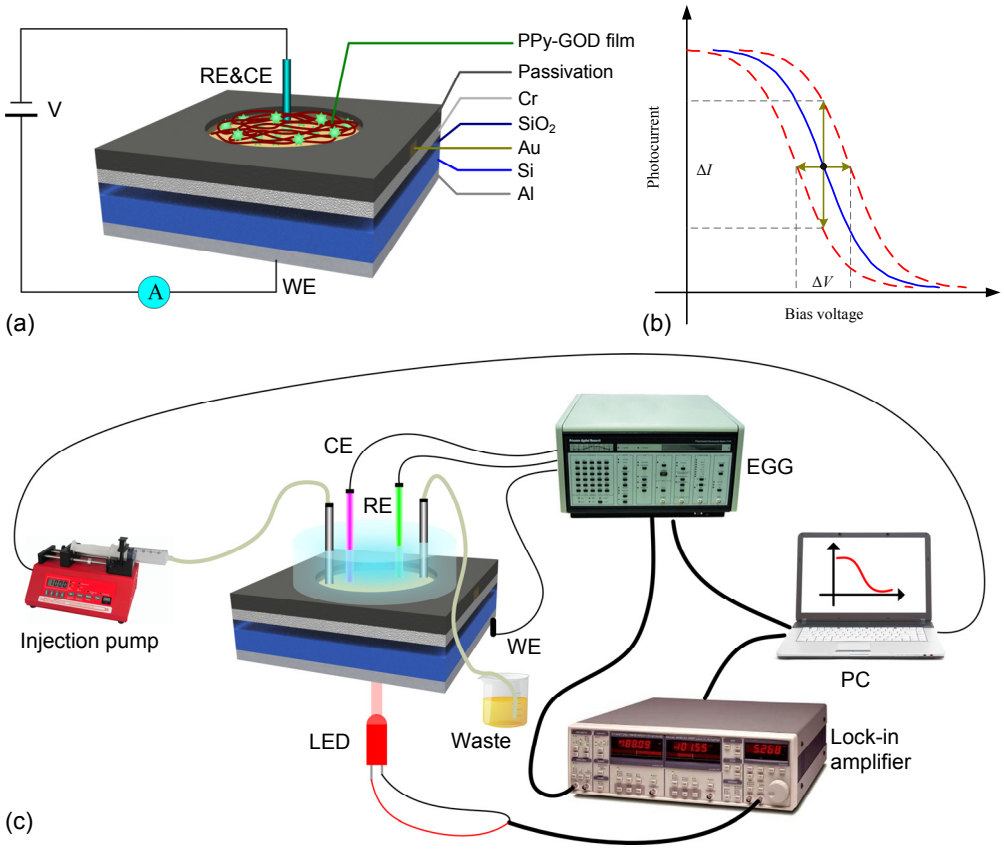
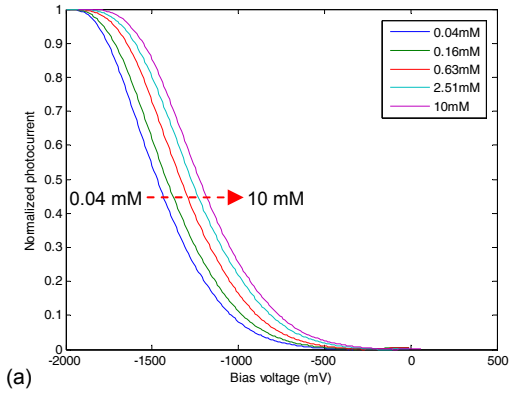


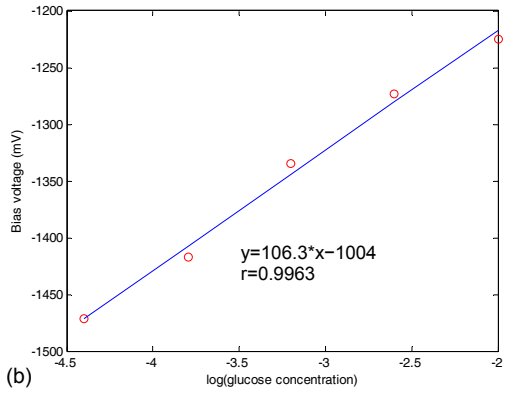
Fig. 1. Schematic diagram of experimental set up. (a) Sensor structure with sequence of Al/n-Si/SiO_2/Cr/Au/ PPy-GOD; (b) Characteristic curve of photovoltaic glucose sensor; (c) Measurement system and flow-injection analysis system.

Results and Discussion

When in contact with glucose solution with different concentrations, the enzyme-catalyzed oxidation of glucose induces the surface potential change on the Au layer, which can be reflected through the I-V curve shift along the bias voltage. A potentiometric sensor is used for glucose detection immediately after electropolymerization. And the measurements are carried out in stagnant solutions. Fig. 2(a) shows the I-V curves of 5 glucose solutions with different concentrations (0.04 mM, 0.16 mM, 0.63 mM, 2.51 mM and 10 mM). The relationship between the bias voltage at the inflection point of I-V curve and the logarithm of glucose concentration is shown in Fig. 2(b). The sensitivity is 106.3 mV/dec. Besides, the linear correlation coefficient is about 0.9963, which means the bias voltage has good linear relationship with log(glucose concentration) within the range of about 40 μM~10 mM. The linear range of the ultra-thin PPy-GOD photovoltaic glucose sensor is significantly better than the range of 1~3 mM and 1.5~10 mM obtained in other studies.



(a)



(b)

Fig. 2. Experimental result for glucose detection immediately after electropolymerization. (a) Normalized I-V curves for different glucose concentration. The legend shows the concentration; (b) Linear relationship between bias voltage and log(glucose concentration).

The result of continuous glucose monitoring is shown in Fig. 3. The bias voltage was fixed at −1300 mV in measurement. The responsive photocurrent shows a gradient curve for different glucose concentrations, which demonstrates the detection capability of the Au-PPy-GOD film in the range of 40 μM~10 mM. The response time for glucose sensing is about 5 minutes. As the samples are measured in stagnant solutions, another 5 minutes are taken to obtain stable potentials, while a longer period of time is needed for high glucose concentrations. This may be due to the limited catalytic activity of the enzyme under the activity units of 20 U/ml.

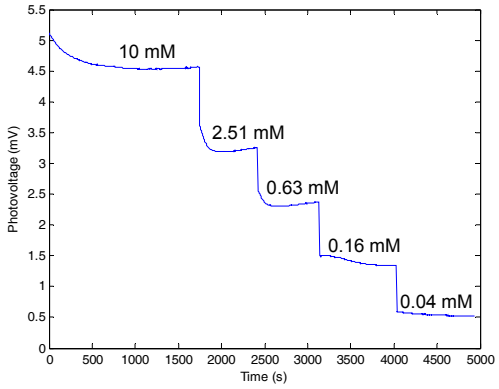


Fig. 3. Continuous monitoring of glucose concentration. The values of concentration are marked on the curve.

Conclusions

In this study a photovoltaic glucose sensor with an ultra-thin PPy-GOD film was developed. The Au layer of an EMIS structured sensor was modified with the PPy-GOD film through a simple electropolymerization process. The sensitive film Au-PPy-GOD showed good linearity and stability in the range of 40 μM~10 mM with sensitivity of 106.3 mV/dec. The photoelectric hybrid glucose sensor has extensive potential both in rapid determination of plasma glucose and long-time continuous monitoring of glucose concentration in extracellular microenvironment.

Acknowledgements

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