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Ceramic Carbon/Polypyrrole Materials for the Construction of Bienzymatic Amperometric Biosensor for Glucose

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Abstract: A novel amperometric glucose biosensor was constructed by electrochemical formation of a polypyrrole (PPy) membrane in the presence of glucose oxidase (GOD) on the surface of a horseradish peroxidase (HRP) modified ferrocenecarboxylic acid (FCA) mediated sol-gel derived ceramic carbon electrode. The amperometric detection of glucose was carried out at +0.16 V (*vs*. SCE) in 0.1 mol/L phosphate buffer solution (pH 6.9) with a linear response range between 8.0×10^{-5} and 1.3×10^{-3} mol/L of glucose. The biosensor showed a good suppression of interference and a negligible deviation in the amperometric detection.

Keywords: Biosensor for glucose, polypyrrole, ceramic carbon electrode, sol-gel.

Amperometric enzymatic biosensors have high selectivity and simplicity in use. It has advantages over other analytical methods in biochemistry, pharmacology, so it evokes strong interests[1,2]. Generally, the detection mode involved in oxidase based biosensors is often based on the electrochemical detection of hydrogen peroxide directly[3,4]. However the direct oxidation of hydrogen peroxide requires a relative high working potential (exceeding *ca*. 0.6 V *vs*. SCE), at which many biological substances can also be electrochemically oxidized. General methods used to suppress the interferences involved: (1) utilization of selective electrocatalyst as electron mediator[5] to reduce the overpotential; (2) construction of an additional membrane[6,7] on the tip of the electrode to prevent the diffusion of interferences toward the surface of biosensor. An attractive alternative approach to suppress the interferences has been developed by the construction of bienzymatic peroxidase/hydrogen peroxide producing oxidase amperometric biosensors in the past few years[8,9]. In such bienzymatic biosensors, hydrogen peroxide (which enzymatically produced in the presence of oxidase substrate and dissolved oxygen) is selectively reduced by peroxidase.

Since the pioneering works of Lev's group, sol-gel derived composite carbon electrodes (CCEs)[10-12] have been widely used to develop all kinds of amperometric biosensors. In this paper, we take advantages of sol-gel process to construct a bienzymatic amperometric biosensor for glucose by coating a GOD/PPy membrane on the surface of ferrocenecarboxylic acid mediated and HRP modified CCE.

An EG&G PARC Model 273 potentiostat driven by an IBM PC with 270 software was used for all electrochemistry experiments. A three-electrode cell, equipped with a

saturated calomel reference electrode (SCE) and a platinum foil counter electrode was used. The solutions were deaerated thoroughly for at least 15 min with pure N_2. All potentials were measured and reported *versus* the SCE.

The HRP modified ferrocenecarboxylic acid mediated CCEs were prepared as follows: A mixture of 1.0 mL methyltrimethoxysilane, 0.5 mL water and 0.1 mL HCl (0.01 mol/L) was sonicated for 10 min. Then the homogeneous silica solution was stored over night at 4°C in a refrigerator. 160 mg graphite powder, 20 mg HRP, 20 mg FCA and 0.3 mL silica sol-gel solution were thoroughly mixed. The mixture was packed into one end of a 3 mm i.d. glass tube to a length of 5 mm, subsequently allowed to dry and gel for at least 5 days at 4°C. After being polished on weighing paper the surface of resulting HRP modified FCA mediated CCEs was smooth and shiny. Copper wire, inserted from the other end of glass tube, provided the electrical contact.

The formation of GOD/PPy membrane was carried out in a galvanostatic mode with a current density of 0.06 mA/cm^2 in 0.1 mol/L phosphate buffer solution (pH 7.0) containing 0.25 mol/L pyrrole and 3.5 mg/mL GOD. The solution was deaerated with N_2 for 15 min and left unstirred prior to electropolymerization. The bienzymatic biosensors were thoroughly washed after preparation and stored in phosphate buffer at 4°C when it is not in use.

Figure 1 shows the cyclic voltammograms of HRP modified FCA mediated CCE at different scan rates. There are well-defined anodic and cathodic waves with mean peak potential $E_{1/2}$ =(Epa+Epc)/2 of 278 mV. Inset shows the plot of peak current *vs*. the square root of scan rate.

Figure 1 Cyclic voltammograms of HRP modified ferrocenecarboxylic acid mediated CCE in phosphate buffer solution (pH 7.0) at different scan rates

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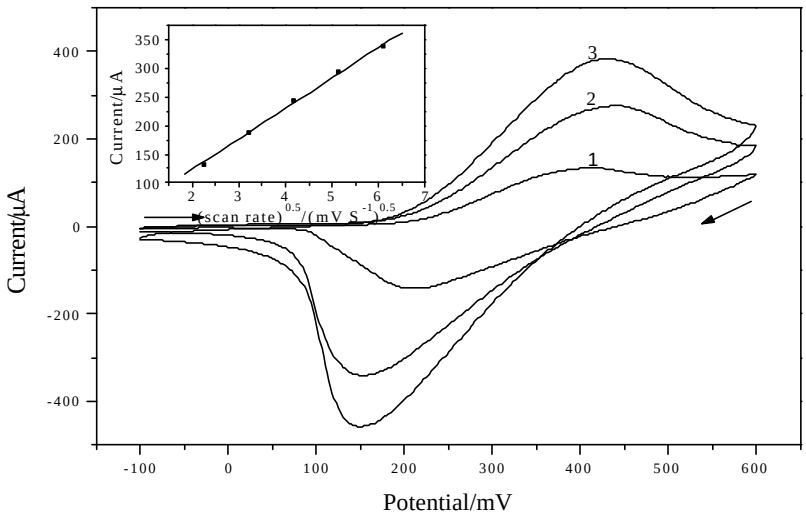


(1) 5 mV/s; (2) 20 mV/s; (3) 50 mV/s.
The inset shows the plot of anodic peak current *vs*. the square root of scan rate.

Figure 2 shows the effect of electropolymerization charge to the response of bienzymatic biosensor toward 10^{-4} mol/L glucose. It can be seen that a maximum

response was achieved when the charge for electropolymerization was 10.18 mC/cm^2.

Figure 2 Effect of passing charge during electropolymerization on the response of bienzymatic glucose biosensor towards 1.0×10^{-4} mol/L glucose

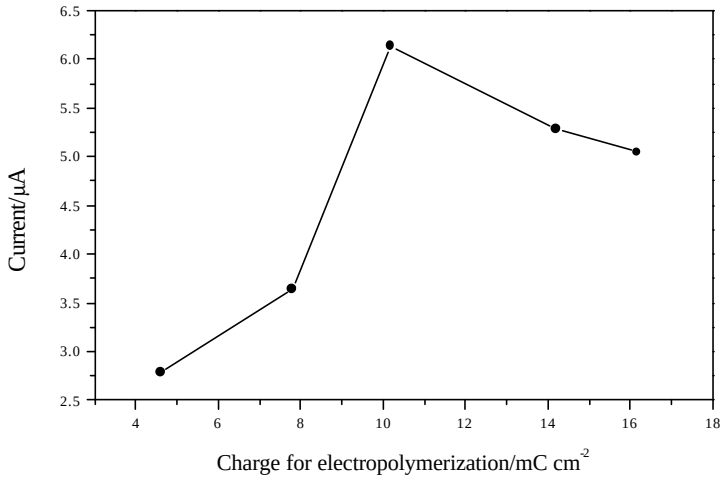


Figure 3 Response mechanism of bienzymatic biosensor for glucose detection

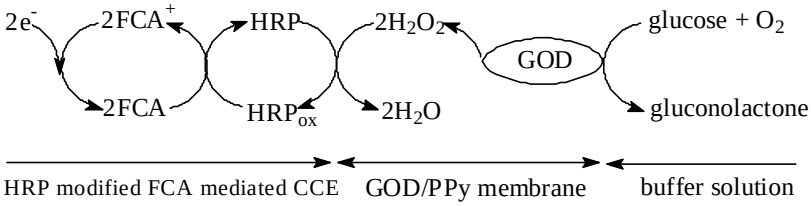
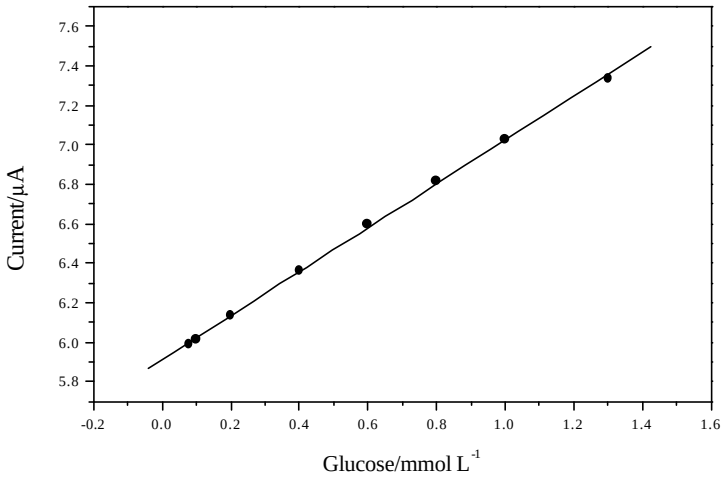


Figure 3 describes the proposed reaction mechanism of bienzymatic amperometric biosensor for the electroenzymatic detection of glucose. Glucose, which diffuses from bulk solution into the GOD/PPy membrane, is catalyzed by GOD and dissolved dioxygen. The oxidized form of HRP (HRP_{ox}) is produced by the enzymatic reaction of hydrogen peroxide and HRP, and subsequently reduced by the ferrocenecarboxylic acid to regenerate HRP. The resulting ferricinium ion (FCA^+) is electro-reduced to regenerate ferrocenecarboxylic acid, producing the response current at the same time.

The response from the bienzymatic glucose biosensor was also measured in the presence of some possible interfering substances (urate, lactate, oxalate and ascorbic acid). The ratio of the amperometric response of mixtures of 1.0×10^{-4} mol/L each interfering substance in the presence of 1.0×10^{-4} mol/L glucose compared to that of 1.0×10^{-4} mol/L glucose alone is taken as the criterion for the selectivity of the glucose biosensor. Those substances cause hardly any interference on the response of the biosensor. This is attributed to the selectively enzymatic reduction of hydrogen peroxide by HRP and the low operating potential for amperometric detection.

The calibration curve for the bienzymatic glucose biosensor (**Figure 4**) obtained by

Figure 4 Linear range of the calibration curve of bienzymatic glucose biosensor in phosphate buffer solution (pH 6.9) at 160 mV *vs.* SCE



an amperometric response under optimized experimental conditions shows a linear range between 8.0×10^{-5} and 1.3×10^{-3} mol/L of glucose with a sensitivity of 1.11 μA/mmol·L^{-1} and a correlation coefficient of 0.998. The response time of biosensor towards glucose is less than 25 s. The detection limit, estimated as three times of the noise, is 1.0×10^{-5} mol/L for glucose.

A novel bienzymatic amperometric biosensor for glucose has been developed by electrodepositing GOD/PPy membrane on the surface of HRP modified ferrocene-carboxylic acid mediated CCE. The heterobilayer glucose biosensor showed a good suppression of interferences (such as urate, oxalate, ascorbic acid, *etc.*) with favorable sensitivity towards glucose. The method combining electropolymerization with sol-gel derived composite carbon electrode provides feasible choice for developing novel biosensors.

References

1. J. Wang, *Anal. Chem.*, **1999,** *71*, 328R.
2. G. S. Wilson, Y. Hu, *Chem. Rev.*, **2000,** *100*, 2693.
3. P. N. Bartlett, P. R. Birkin, *Anal. Chem.*, **1994**, *66*, 1552.
4. S. A. Jaffari, A. P. F. Turner, *Biosen. Bioelectron.*, **1997**, *12*, 1.
5. S. A. Wring, J. P. Hart, *Analyst*, **1992**, *117*, 1215.
6. T. Yao, K. Takashima, *Biosen. Bioelectron.*, **1998**, *13*, 67.
7. G. G. Wallace, M. Smyth, H. Zhao, *TrAC-Trend. Anal. Chem.*, **1999**, *18*, 245.
8. M. C. Shin, H. C. Yoon, H. S. Kim, *Anal. Chim. Acta*, **1996**, *329*, 223.
9. L. Mao, K. Yamamoto, *Anal. Chim. Acta*, **2000**, *415*, 143.
10. M. Tsionsky, G. Gun, V. Glezer, O. Lev, *Anal. Chem.*, 1994, *66*, 1747.
11. Lev, M. Tsionsky, L. Rabinovich, V. Glezer, S. Sampath, I. Pankratov, J. Gun, *Anal. Chem.*, **1995**, *67*, 22A.
12. D. Avnir, S. Braun, O. Lev, M. Ottolenghi, *Chem. Mater.*, **1994**, *6*, 1605.

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