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A Hydrogen Peroxide Biosensor Combined HRP Doped Polypyrrole with Ferrocene Modified Sol-gel Derived Composite Carbon Electrode

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Abstract: A novel amperometric biosensor for the detection of hydrogen peroxide is described. The biosensor was constructed by electrodepositing HRP/PPy membrane on the surface of ferrocenecarboxylic acid mediated sol-gel derived composite carbon electrode. The biosensor gives response to hydrogen peroxide in a few seconds with detection limit of 5×10^{-7} mol • L^{-1} (based on signal : noise=3). Linear range is up to 0.2 mmol • L^{-1}.

Keywords: Hydrogen peroxide, polypyrrole, biosensor, composite carbon electrode.

Conducting polymers, because of bearing many attractive features, for example, shuttling electrons between the electrode surface and the entrapped reagents (enzymes, mediators, *etc.*) while preventing interference and electrode fouling, have been proposed as enzyme supports for electrochemical biosensors[1-4]. Polypyrrole, owing to its popularity to water solubility and the mild conditions used for electropolymerization, has been the most widely used monomer to entrap enzymes[5-8]. The sol-gel progress provides an attractive and convenient method for the incorporation of different reagents. Sol-gel derived carbon composite electrodes (CCEs)[9,10] have become increasingly useful for the design of amperometric biosensors and indicator electrodes in that the entrapped species leach out from the electrode very slowly or do not leach out while preserve their nature. Electron transfer mediators, enzymes can be readily doped into the matrixes of CCE for the development of surface renewable amperometric biosensors.

But up to date, the amperometric biosensors based on enzyme entrapped within polymer combining with mediated CCE have not been reported. In this paper, we report for the first time the amperometric hydrogen peroxide biosensor with horseradish peroxidase (HRP) doped polypyrrole based on ferrocenecarboxylic acid modified sol-gel derived CCE.

An EG&G PARC Model 273 potentiostat driven by an IBM PC with 270 software was used for electropolymerization and voltammetric measurements. A three-electrode cell with a saturated calomel reference electrode (SCE) and a platinum foil counter electrode was used. The solutions were degassed thoroughly for at least 15 min with pure N_2 and kept under a positive pressure of this gas during all experiments. All

potentials were measured and reported *versus* the SCE.

The ferrocenecarboxylic acid modified CCE was prepared as follows: silica sol was prepared by mixing 1.0 mL methyltrimethoxysilane, 0.5 mL water and 0.1 mL 0.01 mol • L^{-1} HCl. The silica sol was ultrasonicated for 10 min and stored over night. The mixture of 50 mg ferrocenecarboxylic acid and 950 mg graphite powder was added to the above stock sol. The mixture was packed into one end of a 3 mm i.d. glass tube to a length of 5 mm.

Electropolymerization was carried out in a galvanostatic mode using a current density of 0.06 mA·cm^{-2} in pH 6.8 phosphate buffer solution containing 0.25 mol • L^{-1} freshly distilled pyrrole and 3 mg • ml^{-1} HRP. The enzyme electrodes were thoroughly washed after fabrication and stored in phosphate buffer solution at 4°C when not in use.

The ferrocene group of mediators has been successfully applied to the quantitation of many compounds. Under optimum conditions the electrode had smallest background current and wide potential operating range when the ratio was 2:3 (carbon powder: sol-gel solution)[11]. The cyclic voltammograms of ferrocenecarboxylic acid modified CCEs fabricated by optimum ratio are shown in **Figure 1**. There are well-defined anodic and cathodic waves in the potential range of –0.1 to +0.6 V with the mean peak potential $E_{1/2}=(E_{pa}+E_{pc})/2$ of 275 mV.

Usually, the amounts of enzyme entrapped in polymer membranes are crucial to the response of biosensor. In order to obtain higher sensitivity thicker HRP/PPy membranes are expected. However, the background or noise current of the biosensor would be higher because of a larger effective electrode area and a higher amount of redox species in the membrane. Thus, an appropriate HRP/PPy membrane thickness was sought for higher sensitivity and lower background current by controlling the electropolymerization charge. **Figure 2** shows the calibration curve of cathodic current of the biosensor in phosphate buffer solution containing 10^{-4} mol • L^{-1} H_2O_2 to electrodeposition charge. The optimum polymer membranes were obtained at 6.79 mC • cm^{-2} under the present conditions.

Figure 1 The typical cyclic voltammograms of ferrocenecarboxylic acid modified CCEs fabricated by optimum ratio in phosphate buffer solution (pH 6.8). Scan rate, 50 mV • s^{-1}

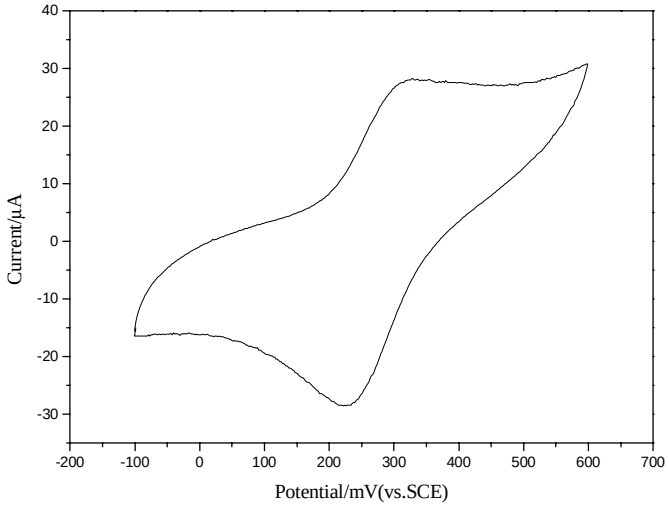


Figure 2 The calibration curve of cathodic current of the biosensor in phosphate buffer solution containing 10^{-4} mol • L^{-1} H_2O_2 to electrodeposition charge. Operating potential, 225 mV *vs.* SCE.

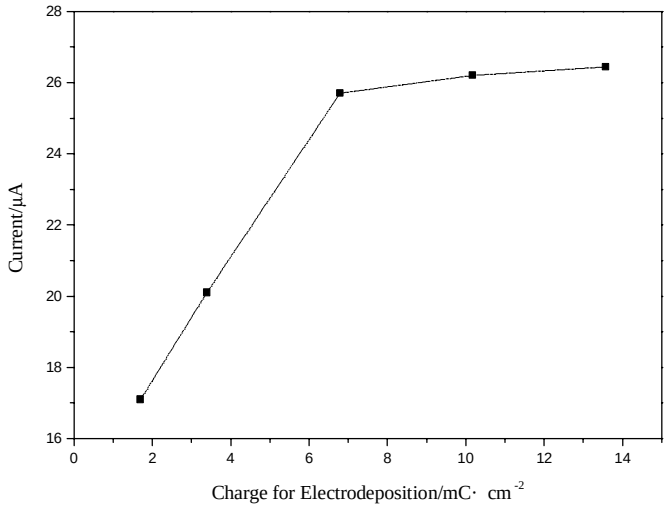
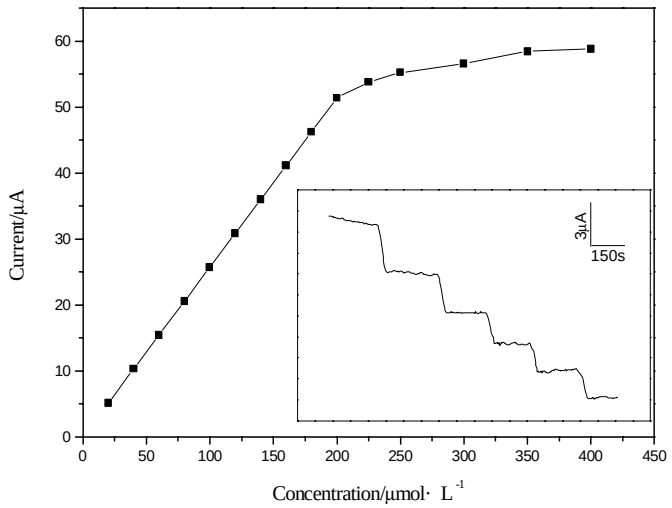


Figure 3 The calibration curve of hydrogen peroxide biosensor response by amperometric detection.[a]



[a] Inset shows the steady-state current–time response curve with addition in steps of 10^{-4} mol • L^{-1} H_2O_2 in phosphate buffer (pH 6.8) at 225mV *vs.* SCE.

The calibration curve of hydrogen peroxide biosensor response by amperometric detection is shown in **Figure 3**. The inset shows the steady-state current–time response of hydrogen peroxide biosensor with increasing H_2O_2 concentration in 10^{-4} mol •L^{-1} steps. Linear range is up to 0.2 mmol • L^{-1} with a correlation coefficient of 0.996 and a sensitivity of 0.24 μA •μmol •L^{-1}. Response time is less than 20 s. The detection limit of 5×10^{-7} mol • L^{-1} was obtained based on signal: noise=3.

The enzymatic reaction in HRP/PPy layer and the electrode reaction may be expressed as follows[12]:

$2H_2O_2 + HRP \rightarrow$ compound I + $2H_2O$
compound I + 2Fc $\rightarrow$ HRP + $2Fc^+$
$2Fc^+ + 2e \rightarrow 2Fc$

Where Fc and Fc^+ are the reduced and oxidized forms of ferrocenecarboxylic acid as mediator.

The construction of hydrogen peroxide biosensor with HRP/PPy coating on ferrocenecarboxylic acid mediated sol-gel derived CCE surface is described. The biosensor responses to hydrogen peroxide in a few seconds with satisfactory detection limit and sensitivity. The method by combining surface coating with sol-gel derived composite carbon electrode may be efficient for the development of biosensor.

References

1. M. Umana, J. Waller, *Anal. Chem.*, **1986**, *58*, 2979.
2. S. B. Adeloju, J. N. Barisci, G. G. Wallace, *Anal. Chim. Acta*, **1996**, *332*, 145.
3. O. A. Sadik, G. G. Wallace, *Anal. Chim. Acta*, **1993**, *279*, 209.
4. D. Compagnone, G. Federici, J. Bannister, *Electroanalysis*, **1995**, *7*, 1151.
5. D. Belanger, J. Nadreau, G. Fortier, *J. Elecrtoanal. Chem.*, **1989**, *274*, 143.
6. J. M. Cooper, D. Bloor, *Electroanalysis*, **1993**, *5*, 883.
7. A. Seillou, N. Jaffrezic, C. Martlet, S. Cosnier, *Anal. Chim. Acta*, **1999**, *401*, 117.
8. M. Yasuzawa, T. Matsuki, H. Mitsui, A. Kunugi, T. Nakaya, *Sens. Actuators B*, **2000**, *66*, 25.
9. M. Tsionsky, G. Gun, V. Glezer, O. Lev, *Anal. Chem.*, **1994**, *66*, 1747.
10. S. Sampath, O. Lev, *Anal. Chem.*, **1996**, *68*, 2015.
11. J. Li, L. S. Chia, N. K. Goh, S. N. Tan, *J. Electroanal. Chem.*, **1999**, *460*, 234.
12. J. E. Few, M. A. Harmer, H. A. O. Hill, S. I. Libor, *J. Electroanal. Chem.*, **1986**, *201*, 1.

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