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INVESTIGATION ON CARBON FIBER MICROELECTRODES

XIX. HETEROGENEOUS CATALYTIC REACTION AT METHYLENE BLUE / NAFION MODIFIED MICROCYLINDER CARBON FIBER ELECTRODE AND THE DETERMINATION OF HEMOGLOBIN

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ABSTRACT: A stable methylene blue / Nafion modified carbon fiber microcylinder electrode is developed. The mediator (methylene blue) makes the oxidation of hemoglobin(HB) at this electrode be accelerated in the medium of weak acid, and shows significantly electrocatalytic activity. The catalytic current and the concentration of HB has a good linearity in the range of $5x10^{-6}$~$5x10^{-5}$mol / L. The relative variation coefficient of peak current is 3.5% for 6 determinations with $6.0x10^{-6}$mol / L HB.

In recent years, heterogeneous catalytic reactions at a modified electrode attract great interest for analytical applications [1] . However, the application of the modified electrodes has been restricted greatly due to the unstability and the passivity of modified electrodes as a result of the adsorption of biomolecules. At microelectrodes, because of its high mass transport rate, the product of electrode reaction diffuses rapidly from the electrode surface, the catalytic efficiency declines greatly [2] , and the adsorptive stability of mediator on microelectrode surface is worse. Thus, it is more difficult to study both homogeneous [3] and heterogeneous catalytic reaction with a low rate constant at microelectrodes than at normal electrodes. The study on the determination of biomolecules at a single fiber microcylinder electrode by aid of electron mediator has not made progress. In this paper, a reliable modification way is found, and a stable MB / Nafion modified carbon fiber microcylinder electrode is fabricated. The mediator MB is fixed on the surface of carbon fiber microcylinder electrode with polymer (Nafion), it reduces the mass transport rate of mediator and its reaction product and raises greatly the heterogeneous catalytic efficiency. At the modified electrode, the

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oxidation of hemoglobin can be accelerated in weak acid, it makes the oxidation peak current of MB increase. The increment of peak current is proportional to the concentration of HB. Thus, hemoglobin can be determined directly with this modified electrode. These results make the direct electrochemical determination of hemoglobin possible at a microelectrode by means of EC' electrode process.

EXPERIMENTAL

CHEMICALS

Methylene Blue (the Third Reagent Factory Shanghai) was of analytical reagent grade. Hemoglobin (ox) was produced by Shanghai Biochemistry Research Institute. The reagents making up both pH7.2 phosphate buffer solution and pH5.5 acetate buffer solution were A.R. Twice–quartz–distilled water by sub–boiling distiller was used for all solutions. Nafion (5%(w / w) ethanol solution) was obtained from Aldrich company (America).

INSTRUMENT AND PROCEDURE

Voltammetric measurements were carried out with Model–366 Bi–potentiostat, Model RE0150 Recorder (EG&G Company, America), and BAS–100B Electrochemical Analyzer (BAS Company, America). FPG–310 Color Plotter (Fujitsu Company, Japan). The 501 type thermostat (Shanghai Expermental Instrument Factory) was also used.

The microcylinder carbon fiber electrode with a radius of 5μm and a length of 2mm was used as work electrode, SCE as reference electrode and Pt wire as counter electrode. After aerating N_2 for 10min, the electrochemical measurements were carried out under an inert nitrogen atmosphere at 20°C.

RESULTS AND DISCUSSION

1. Preparation of modified electrode

The fabrication of carbon fiber microcylinder electrode was similar to that previously described [4]. After being treated electrochemically in 1.0mol / L H_2SO_4 solution, the electrode was dipped in 5% Nafion solution for several seconds, taken out and dried under the infrared lamp. The lacquering steps were repeated, and then the electrode was dipped in pH7.2 buffer solution including 1.0×10^{-3}mol / L MB, taken out after 20 min, washed with distilled water, and kept in pH7.2 buffer solution.

2. Stability of the modified electrode

When MB / Nafion modified electrode was scanned with cyclic voltammetry from −0.6V to 0.1V(v = 100mV / s) in pH5.5 acetate buffer solution, the peak currents declined continuously(Fig.1A), and the reduction currents declined following the first-order kinetic law(shown in Fig.1B), the half life was 69 hours. And the half life was 52 hours in pH 7.2 phosphate buffer solution determined with the same experiment. If the electrode was dipped in pH5.5 buffer solution for ten days (in pH7.2 buffer solution for a week), the peak current did not change before and after dipping. It is clear that the stability of the modified electrode is very good in either pH5.5 or pH7.2 solution, and the stability at pH5.5 is better than at pH 7.2.

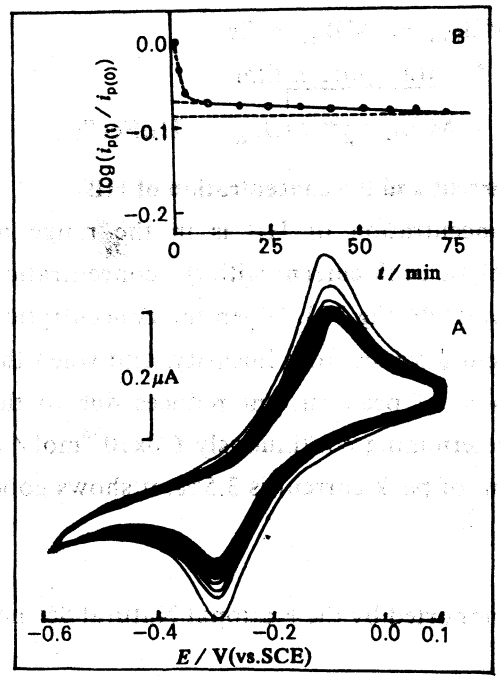

Fig.1 The cyclic voltammogram (A) as well as the relation between $\log(i_{p(t)} / i_{p,0})$ and scan time (B) in pH5.5 PBS, the $i_{p,0}$ is the peak current of the first sweep, the $i_{p(t)}$ is the peak current determined at time t, v = 100mV / s.

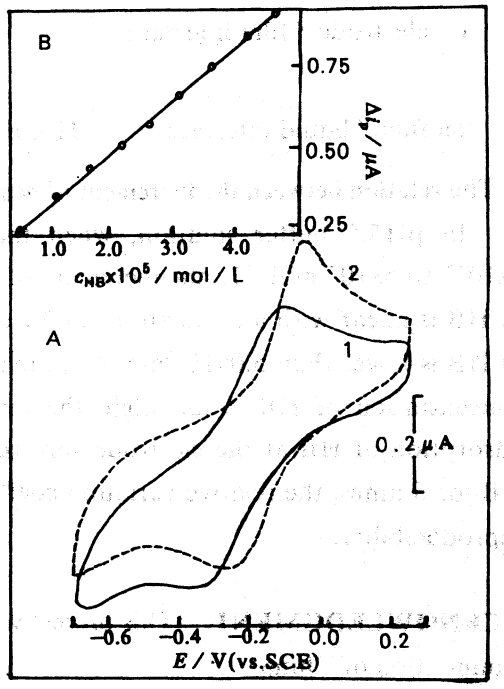

Fig.2 The electrocatalysis of modified electrode to HB (A), 1. in pH5.5 buffer solution, 2. in A+1.0x10^{-5}mol / L HB; as well as the relation between increment of peak current and concentration of HB (B).

3. Catalysis of modified electrode to the oxidation of hemoglobin

Hemoglobin(HB) is an important respiratory protein in red cell. The determination of HB is very important in clinics. However, due to its enormous structure and the

passivation of electrode by the adsorption of HB and other biomelocules on its surface, the direct electrochemical determination of HB is very difficult. The glassy carbon electrode has been modified with toluidine and MB to determine HB in the literature [5–7]. Our work shows that the MB / Nafion modified carbon fiber microcylinder electrode has electrocatalytic activity for the oxidation of HB in weak acid medium, and the electron transfer reaction occurs between HB and MB. The process regenerates the reactant MB_{red}, raises the oxidation peak current, and declines the reduction peak current due to the decrease of MB_{ox} concentration in the film(in Fig.2A). The catalytic mechanism can be shown as follows (for the sake of simplicity, the H^+ participating electrode reaction and the electricity of MB are neglected):

on electrode / film interface $\quad MB_{Red} \rightleftharpoons MB_{Ox} + 2e$

transport in flim

on film / liquid interface $\quad HbFe(II) + MB_{Ox} \xrightarrow{k} MB_{Red} + HbFe(III)$

4. The relation between the increment of peak current and the concentration of HB

In pH5.5 buffer solution, when the concentration of HB is in the range of $5x10^{-6}$ to $5x10^{-5}$mol / L, the increment of catalytic peak current with the concentration of HB is linear with a correlation coefficient of 0.998(Fig.2B). When the concentration of HB is lower than $5.0x10^{-6}$mol / L, the current deviates from linearity, and when the concentration of HB is too high, the increment of peak current reduces due to the adsorption of HB at the electrode surface. Determining continuously $6.0x10^{-6}$mol / L HB for 6 times, the relative variation coefficient of peak current is 3.5%, it shows good reproducibility.

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