



ELSEVIER

Journal of Electroanalytical Chemistry 380 (1995) 283–285

JOURNAL OF
ELECTROANALYTICAL
CHEMISTRY

Short communication

Heterogeneous catalytic reaction at a methylene blue/Nafion[®] modified carbon fiber microcylinder electrode

Huangxian Ju, Yigang Xun, Hongyuan Chen

Department of Chemistry, Nanjing University, Nanjing 210008, China

Received 14 April 1994; in revised form 9 June 1994

Keywords: Nafion[®]; Heterogeneous catalytic reactions; Microcylinder electrode

1. Introduction

Microelectrodes have attracted great interest for analytical applications [1] and the study of homogeneous reaction kinetics [2]. Recently, the study of heterogeneous catalytic reactions at microelectrodes modified with many kinds of mediators or promoters has been reported because of the need to determine the nature of biomolecules in special small environments [3–6]. However, because of the high mass transport rate of microelectrodes [1,7], the product of the electrode reaction diffuses rapidly from the electrode surface and the catalytic efficiency declines greatly [8]. Therefore it is generally not easy to study the catalytic reactions at a microelectrode unless exceptionally high scan rates are employed or a coating polymer is used to fix the mediators or promoters efficiently.

Hemoglobin (HB) is an important respiratory protein in red cells. The determination of HB is very significant in clinical medicine. In general, it is carried out by spectrophotometry, but this method often requires very toxic KCN (in the HiCN method) and considerable time. Thus it is desirable to determine HB by a direct electrochemical method. Although studies on the electrocatalytic reduction or oxidation of HB at modified electrodes [9–11] and the properties of methylene blue (MB) at a bare or Nafion[®] modified normal size electrode [11–15] have been reported, to our knowledge the distribution of MB between a Nafion[®] film and solution and the direct determination of HB at a modified microelectrode have not been described. In this work, we used MB as an electron mediator, which was immobilized by Nafion[®] on the surface of a carbon fiber microcylinder; the basic properties of this chemically modified electrode were studied. The MB/Nafion[®] modified microelectrode has

been applied to determine HB conveniently in clinical human blood without special treatment.

2. Experimental

The carbon fiber (type PAN) with a diameter of 6–8 μm (obtained from Shanghai Synthetic Fiber Research Institute, China) was chosen as matrix material to fabricate the carbon fiber microcylinder electrode. Nafion[®] modified microelectrodes were prepared by twice dipping the carbon fiber microcylinder electrodes (length, 5.0 mm) in an alcoholic solution of 5 wt.% Nafion[®] 117 (equivalent weight, 1100; Aldrich Chemical Company, USA) for several seconds; after removal, the samples were dried under an IR lamp. Before dip-coating with Nafion[®], the electrodes were pretreated electrochemically in 1.0 mol l⁻¹ H₂SO₄ solution with a triangular-wave sweep from -1.0 V to +2.0 V ($\nu = 20\,000\text{ mV s}^{-1}$) for 5 min. The Nafion[®] film was well distributed on the electrode surface, observed with a scanning electron microscope. MB/Nafion[®] modified electrodes were fabricated by dipping Nafion[®] modified electrodes in 0.2 mol l⁻¹ phosphate buffer solution (PBS) at pH 7.2 containing $1.0 \times 10^{-3}\text{ mol l}^{-1}$ MB for 20 min; after removal, the unadsorbed species were washed off with doubly distilled water. MB was adsorbed evenly on the electrode surface as observed from its micrograph. The electrodes were kept in pH 7.2 PBS.

Pt wire and a saturated calomel electrode (SCE) were used as counter- and reference electrodes respectively. The electrolyte solution was deaerated with pure N₂ for 10 min before each electrochemical experiment which was carried out with an M366 bipotentiostat

(EG&G, USA) and a BAS-100B electrochemical analyzer (BAS Co., USA) at 20 ± 0.1 °C.

3. Results and discussion

When an MB/Nafion[®] modified microcylinder electrode was scanned continuously by cyclic voltammetry from 0.0 to -0.6 V with a scan rate ν of 100 mV s^{-1} in pH 7.2 PBS, a pair of peaks was found at -0.41 V and -0.34 V; the peak currents declined continuously following a first-order kinetic expression, and the half-life was 52 h. No change in peak currents appeared after the electrode was dipped in pH 7.2 PBS for a week, indicating the absence of dissolution of MB molecules from the polymer film into the aqueous solution. This suggests that the mediator, MB, is strongly immobilized on the electrode surface by means of the coated Nafion[®] film.

The peak current of the cyclic voltammogram at the above electrode is proportional to ν in 0.2 mol l^{-1} PBS in the pH range 2.2–10.7 in the absence of MB. This indicates that the electrode process is not controlled by diffusion, and obeys the relationship in a thin film, which is similar to that in a thin-layer cell [16].

The distribution of MB in the Nafion[®] film and in solution was studied using a glassy carbon electrode (radius, 0.25 cm), because it was difficult to calculate quantitatively the thickness of the Nafion[®] film at the carbon fiber microcylinder electrode. The glassy carbon electrode was modified with $3 \mu\text{l}$ of Nafion[®] solution. The thickness of the Nafion[®] film is $0.5 \mu\text{m}$, obtained from its density (about 2.0 g cm^{-3} [17]) and the quantity used. The concentration c_p of MB incorporated into the Nafion[®] film can be determined by chronocoulometry with the formula $c_p = Q/nFA\ell$, where A is the electrode area, Q is the charge consumed in complete oxidation of the film species and $n = 2$ [12]. c_p is relevant to the concentration c^* of MB in solution, and tends to a constant $c_{p,\text{max}}$ with increasing immersion time in a fixed concentration of MB. The ratio K of $c_{p,\text{max}}$ to c^* , defined as the extraction coefficient [17], is a constant of 110 within the solution concentration range of 8×10^{-6} to $1.5 \times 10^{-3} \text{ mol l}^{-1}$. Beyond $1.5 \times 10^{-3} \text{ mol l}^{-1}$, the ratio decreases with increasing c^* , suggesting that the saturation of MB in the Nafion[®] film occurs. In Ref. [13], it was found to be inadequate, if the concentration of MB in the Nafion[®] film was replaced with c^* , to calculate the apparent diffusion coefficient D_{app} of MB in the Nafion[®] film by the Cottrell equation. Since the value of K is relatively large, and the rate of equilibrium of MB between the Nafion[®] film and solution is relatively slow, this suggests that the loss of MB by diffusion from the film to the solution in a short time is very small [17]. Thus the MB/Nafion[®] modified carbon

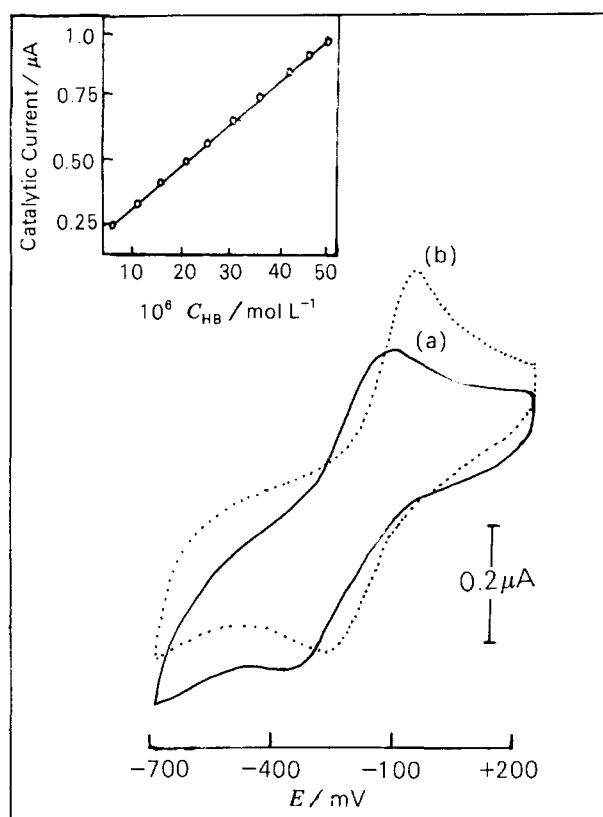


Fig. 1. Cyclic voltammograms of MB/Nafion[®] modified microcylinder electrode in pH 5.5 acetate buffer solution (a) and buffer plus $1.0 \times 10^{-5} \text{ mol l}^{-1}$ HB (b); inset: relation between catalytic peak current and concentration of HB, $\nu = 100 \text{ mV s}^{-1}$.

fiber microcylinder electrode will be considerably stable.

When c^* was $1.0 \times 10^{-3} \text{ mol l}^{-1}$, we obtained a concentration of MB in the Nafion[®] film of 0.11 mol l^{-1} . Thus the D^{app} value of MB in the film was $5.3 \times 10^{-9} \text{ cm}^2 \text{ s}^{-1}$, determined by chronoamperometry using a microcylinder electrode, and $D^{\text{app}} = 5.0 \times 10^{-9} \text{ cm}^2 \text{ s}^{-1}$ was obtained using a glassy carbon electrode. These values are in good agreement and about 1000 times less than that in solution ($7.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ [12]). The results are similar to those obtained for other species in polymer or solution [17]. The very much lower diffusion coefficient indicates a much smaller mobility within the Nafion[®] film, facilitating the fast immobilization of MB in the film. Thus the Nafion[®] film plays an important role in preventing the loss of the mediator MB.

The MB/Nafion[®] modified carbon fiber microcylinder electrode can electrocatalyze the oxidation of HB (HbFe(II)) in pH 5.5 acetate buffer solution (0.20 mol l^{-1} acetic acid + 0.20 mol l^{-1} sodium acetate) through the electron transfer reaction at the heterogeneous boundary layer between MB and HB. It regenerates the electroactive reactant MB_{red} , enhances the anodic peak currents and decreases the cathodic peak

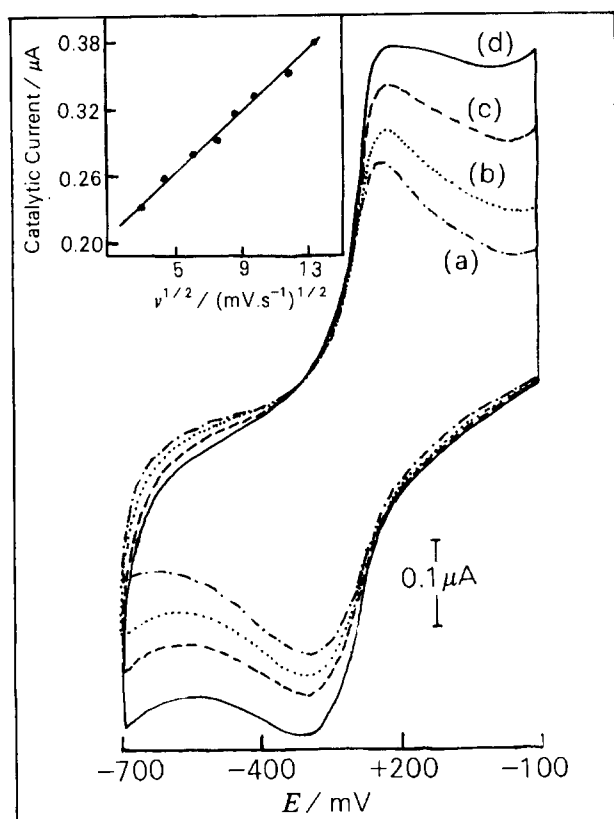


Fig. 2. Cyclic voltammograms of the modified electrode in 1.0×10^{-5} mol l^{-1} HB solution (pH 5.5) at $\nu = 10$ (a), 40 (b), 100 (c) and 200 $mV s^{-1}$ (d); inset: plots of the peak current vs. $\nu^{1/2}$.

currents in the cyclic voltammogram (Fig. 1). When HB_{red} sample solution is added to the pH 5.5 buffer solution, the peak potentials of the redox couple MB_{ox}/MB_{red} in both anodic and cathodic processes shift in the positive direction by about 80 mV because of the catalytic reaction between HB and MB, which possibly changes the rate of electron self-exchange between MB_{red} and MB_{ox} in the film. The experimental results indicate that HB does not permeate into the Nafion[®] film or adsorb onto the film surface, and c_p of MB in the film does not decrease. The catalytic anodic peak currents are proportional to $\nu^{1/2}$, and the peak potential is independent of ν (Fig. 2), indicating that the electrode process is controlled by the diffusion of HB in solution [18], the interfacial chemical reaction rate between HB and MB_{ox} is large, and the electrode process is similar to the direct electrochemical oxidation of HB at the electrode.

The catalytic peak currents depend greatly on the pH of the solution, because the interaction between the Nafion[®] film and HB is obviously affected by the pH of the solution. A suitable pH value of 5.5 was chosen for the determination of HB. In pH 5.5 acetate buffer solution, the catalytic peak current vs. the con-

centration of HB is linear from 5×10^{-6} to 5×10^{-5} mol l^{-1} (inset in Fig. 1) with a correlation coefficient of 0.998 and a relative variation coefficient of 3.5% for six determinations at 6×10^{-6} mol l^{-1} HB.

A clinical human blood sample, diluted 100-fold with pH 5.5 acetate buffer solution, was determined directly using the MB/Nafion[®] modified electrode without interference. The average value of three determinations was 2.4×10^{-3} mol l^{-1} . The result is close to the value of 2.5×10^{-3} mol l^{-1} obtained by spectrophotometry (HiCN method) at a wavelength of 540 nm. Experiments showed that the catalytic current of HB_{red} only fell to 96% after the electrode had been used for 2 h. This proves that the performance of the mediator (MB) modified microelectrode can be greatly improved by the use of Nafion[®] and can be applied well in practice.

Acknowledgement

The National Natural Science Foundation of China is gratefully acknowledged for financial support.

References

- [1] R.M. Wightman and D.O. Wipf, in A.J. Bard (ed.), *Electroanalytical Chemistry*, Vol. 15, Marcel Dekker, New York, 1989, p. 267.
- [2] A.J. Bard, J.A. Crayston, G.P. Kittleson, T.V. Shea and M.S. Wrighton, *Anal. Chem.*, 59 (1987) 2101.
- [3] M.K. Dayton, A.G. Ewing and R.M. Wightman, *Anal. Chem.*, 52 (1980) 2392.
- [4] E.N. Navera, M. Suzuki, E. Tamiya, T. Takeuchi and I. Karube, *Electroanalysis*, 5 (1993) 17.
- [5] K. Yokoyama, K. Nakajima, S. Uchiyama, S. Suzuki, M. Suzuki, T. Takeuchi, E. Tamiya and I. Karube, *Electroanalysis*, 4 (1992) 859.
- [6] M.G. Garguilo, N. Huynh, A. Proctor and A.C. Michael, *Anal. Chem.*, 65 (1993) 523.
- [7] H.X. Ju, H.Y. Chen and H. Gao, *J. Electroanal. Chem.*, 361 (1993) 251.
- [8] M.K. Dayton, A.G. Ewing and R.M. Wightman, *Anal. Chem.*, 52 (1980) 2392.
- [9] J. Zhou and E. Wang, *Bull. Sci. (Chin.)*, 38 (1991) 240.
- [10] J. Ye and R. Baldwin, *Anal. Chem.*, 60 (1988) 2263.
- [11] S. Song and S. Dong, *Bioelectrochem. Bioenerg.*, 19 (1988) 337.
- [12] H.W. Robert and I. Shain, *Anal. Chem.*, 39 (1967) 1527.
- [13] Z. Lu and S. Dong, *J. Chem. Soc., Faraday Trans. 1*, 84 (9) (1988) 2979.
- [14] V. Kuban, P.K. Dasgupta and J.N. Marx, *Anal. Chem.*, 64 (1992) 36.
- [15] A.R. Guadalupe, K.E. Liu and H.D. Abruna, *Electrochim. Acta*, 36 (1991) 881.
- [16] A.J. Bard and L.R. Faulkner, *Electrochemical Methods Fundamentals and Applications*, Wiley, New York, 1980, pp. 409–413.
- [17] H.S. White and A.J. Bard, *J. Am. Chem. Soc.*, 104 (1982) 4811.
- [18] K. Aoki, K. Tokuda and H. Matsuda, *J. Electroanal. Chem.*, 199 (1986) 69.