



## Electrocatalytic reduction of dioxygen on hemin based carbon paste electrode

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### Abstract

A hemin-modified carbon paste electrode was constructed by a simple, rapid and effective method. The electrochemical behaviour of the modified electrode was characterized by cyclic voltammetry. The modified electrode obtained was very stable and exhibited electrocatalytic response for the reduction of oxygen. The possible mechanism for the catalytic reduction of dioxygen is discussed. The dioxygen is reduced via a one-step reduction accompanying four electrons and four protons transfer at pH 7–11.

### 1. Introduction

Determination of dioxygen is an important analytic target in the field of biochemistry [1], neuroscience [2], and physiology [3]. It can be measured using electrochemical methods due to the advantage of high sensitivity and selectivity. The metalloporphyrins have superior chemical reactivities when coupled with specific protein environments as found in nature [4]. For example, cytochrome oxidase, which contains four metalloporphyrins in an enzyme unit, plays an important role in oxygen reduction in such an environment. Metalloporphyrins and substituted phthalocyanine have been extensively examined as catalysts for electrochemical reduction of dioxygen because of their similarity to cytochrome oxidase. Many kinds of strategies have been employed to produce chemically modified electrodes (CMEs) to study the electrochemical reduction of dioxygen [5–11]. In these works, covalent bonding, adsorption and electropolymerization of porphyrins have been employed to modify the carbon based or metal electrode surfaces. Arifuku reported an electrochemical study concerning the catalytic dioxygen reduction on glassy carbon electrodes modified by covalent bonding of hemin [12]. Many systems, using hemin or hemoglobin as mediator for electrocatalysing reduction of hydrogen peroxide or dioxygen, were reported [13–17]. Unfortunately, the construction of such CMEs is complicated and time-consuming; furthermore, most of these systems are not stable because of the fragility of the chemical bond linking the porphyrin and the electrode surface or porphyrin leaching from the electrode surface or the denaturing of the enzymes. These are barriers to the application and study of the electrochemistry of

porphyrin. The preparation of bulk modified electrodes that physically trap the compound of interest in a matrix that also binds the conductive particles has achieved some success in preparation of enzyme modified electrodes. A variety of trapping compounds including silicon [18] epoxy [19] oil [20] and wax [21] have been employed. But up to now, the study of the electrocatalytic reduction of dioxygen on carbon paste electrodes modified by inclusion of iron protoporphyrin IX (hemin) has not been reported. In this work, an amperometric electrode for dioxygen was developed via a simple and effective method.

### 2. Experimental details

#### 2.1. Materials

Iron protoporphyrin IX (hemin) was purchased from the Shanghai Lizhu Reagent Company, solid wax was from Beijing Chemical Works, carbon powder was spectral purity, 0.03 mm in diameter; other reagents were of analytical grade and were used as-received. The buffer solutions, which also served as supporting electrolytes, were prepared from phosphate, borate and citrate. The pH adjustment was achieved by using HCl or NaOH. All solutions were prepared with double deionized water.

#### 2.2. Apparatus

Voltammetric measurements were performed on a CHI812 Electrochemical Analyser (Shanghai, China). All experiments were made using a conventional

three-electrode cell with a modified or unmodified carbon paste working electrode, a standard reference electrode (RE3V; BSAJ) and a platinum wire auxiliary electrode. Experiments were conducted at room temperature ( $\sim 18^\circ\text{C}$ ).

### 2.3. Fabrication of electrode

Carbon powder was immersed in  $8\text{ mol dm}^{-3}$  hydrochloric acid solution for 8 h, washed by deionized water until no  $\text{Cl}^-$  could be detected, and then kept in alcohol. Treated carbon powder was filtered and dried in the air. The hemin containing electrode (HCPE) was prepared as follows: 22% (by weight) of hemin and 54% of carbon powder and 24% of solid wax were thoroughly mixed in a mortar with a pestle, then heated on a hot plate at  $55^\circ\text{C}$  to make a paste. While hot, a portion of the paste was packed into a Teflon tube, 1.6 mm in diameter, until it contacted a copper wire placed in the end of the tube. Before the measurements, the surface of the electrode was renewed and polished on a smooth paper sheet. The carbon paste electrode (CPE), used for comparison, was prepared in exactly the same manner as that of the HCPE, except for the absence of hemin, and thus the percentage of carbon powder was 76%.

Preliminary experiments demonstrated that CPE, which displayed low background, low noise and high reactivity to ascorbic acid and reversible oxidation–reduction to potassium ferrocyanide, were produced when the carbon content was from 50% to 76%. The best unmodified CPE were produced with 76%.

### 2.4. Procedure for preparation of solutions with known $\text{O}_2$ concentration

The supporting electrolyte solution was deaerated for 30 min with high purity nitrogen gas or oxygen gas. Fixed-potential amperometry experiments were carried out in five different concentrations of oxygen buffer solutions: oxygen-free ( $0\ \mu\text{mol dm}^{-3}$ ), air-saturated ( $200\ \mu\text{mol dm}^{-3}$ ) or oxygen-saturated solution ( $1260\ \mu\text{mol dm}^{-3}$ ), additional oxygen standard solution containing  $100\ \mu\text{mol dm}^{-3}$  and  $630\ \mu\text{mol dm}^{-3}$  oxygen were prepared by mixing  $\text{N}_2$ -saturated with air-saturated and nitrogen-saturated with oxygen-saturated solutions, respectively [22].

## 3. Results and discussion

### 3.1. Electrochemistry of the modified electrode

Figure 1(a) and (b) shows cyclic voltammograms of the HCPE and the CPE in nitrogen-saturated  $0.1\text{ mol dm}^{-3}$   $\text{Na}_2\text{HPO}_4\text{--NaH}_2\text{PO}_4$  buffer solution (pH 7.42). It can be seen that in the potential range  $+0.3$  to  $-1.0$  V, no redox peak at the CPE is observed, while at the HCPE, one redox peak appears. The mean peak potential  $E_{1/2} = (E_{pa} + E_{pc})/2$  is  $-0.39$  V with peak potential

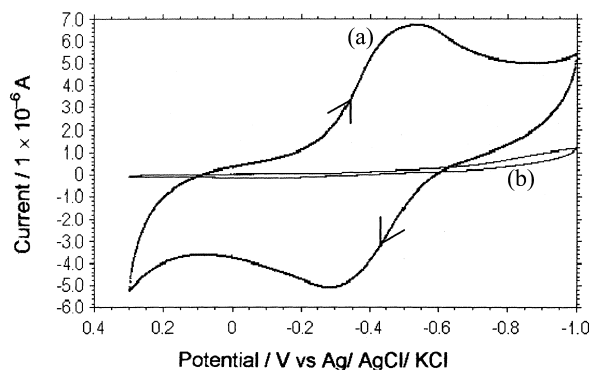


Fig. 1. Cyclic voltammograms of HCPE (a) and CPE (b) in nitrogen-saturated  $0.1\text{ mol dm}^{-3}$   $\text{Na}_2\text{HPO}_4\text{--NaH}_2\text{PO}_4$  buffer solution (pH 7.42). Scan rate  $100\text{ mV s}^{-1}$ .

separation of  $0.236$  V. According to the literature [10] this redox peak may represent a single electron process of iron at the core of the hemin. Figure 2(a) shows the cyclic voltammograms of the HCPE at different scan rates in the potential range  $+0.3$  to  $-1.0$  V in nitrogen-saturated  $0.1\text{ mol dm}^{-3}$   $\text{Na}_2\text{HPO}_4\text{--NaH}_2\text{PO}_4$  buffer solution (pH 7.42). From Figure 2(b) it can be seen that the cathodic peak current increases linearly with the

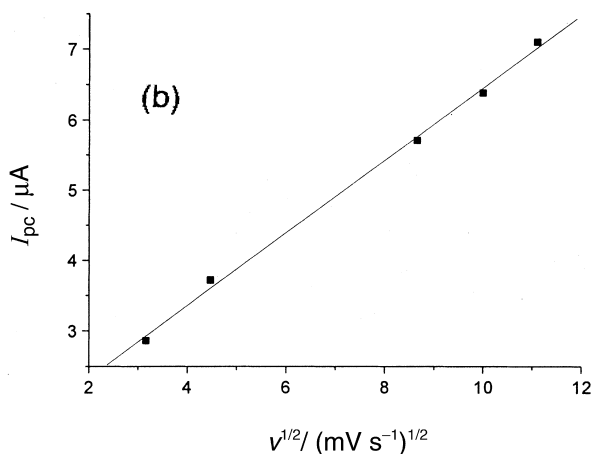
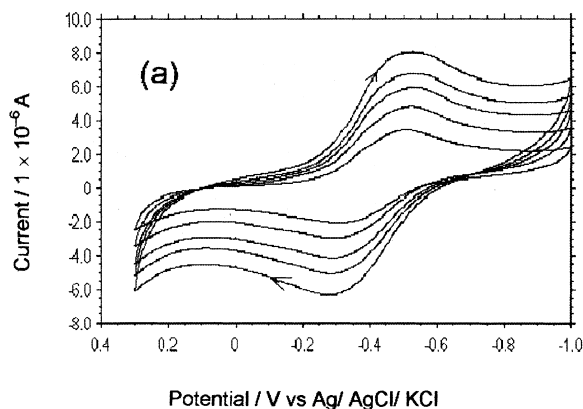


Fig. 2. (a) Cyclic voltammograms of HCPE in nitrogen-saturated  $\text{Na}_2\text{HPO}_4\text{--NaH}_2\text{PO}_4$  buffer solution (pH 7.42) at different scan rates. (From inner curve to out curve: 10, 20, 75, 100 and  $125\text{ mV s}^{-1}$ ). (b) Linearity of the currents with the square root of scan rate.

square root of scan rate between 10 and 200  $\text{mV s}^{-1}$ , suggesting a diffusion-controlled process. Consistent with the above, when the scan rate was increased, the cathodic and anodic peak potentials moved in the negative and positive directions, respectively.

It is reported that most electrocatalytic systems of porphyrin and phthalocyanine are not very stable [7]. This limits their usefulness. Compared to the chemically modified electrodes obtained by other methods, the carbon paste electrode using solid wax as binder has certain advantages. In contrast to the electrode prepared by absorbing hemin onto the surface, the stability of carbon paste electrodes is high. For example, in the potential range from +0.3 to  $-1.0$  V, in nitrogen-saturated  $0.1 \text{ mol dm}^{-3}$   $\text{Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$  buffer solution (pH 7.42) with a scan rate of  $100 \text{ mV s}^{-1}$ , the potential and the peak current remained almost unchanged after successive scanning for about 12 h. When the modified electrode was stored in the atmosphere for two months, the current response remained unchanged. We postulate that the high stability of HCPE is related to both the insolubility of hemin under acidic and neutral conditions, coupled with its physical incorporation into the solid wax matrix that prevents the hemin from leaching out of the electrode surface. Wang et al. [23] also reported the thermal stabilization of enzymes immobilized within carbon paste electrodes. For testing reproducibility, six HCPE were prepared using the same method and immersed in buffer solution. The RSD of the cathodic peak current in  $\text{N}_2$ -bubbled buffer solution (pH 7.42), obtained at a scan rate of  $100 \text{ mV s}^{-1}$ , is 7.8%. These results indicate that the HCPE have good stability and reproducibility.

### 3.2. Electrocatalytic behaviour of the HCPE

We found that the HCPE exhibited similar electrocatalytic behaviour to that previously observed for the reduction of dioxygen at glassy carbon [12]. Figure 3(a) shows cyclic voltammograms for the electrocatalysis of dioxygen reduction at HCPE in oxygen-saturated  $0.1 \text{ mol dm}^{-3}$   $\text{Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$  buffer solution at pH 7.42. Compared with the CPE (b), the peak poten-

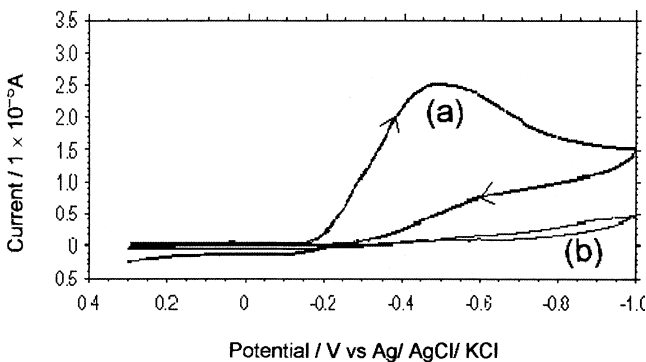


Fig. 3. Cyclic voltammograms of HCPE (a) and CPE (b) in oxygen-saturated  $0.1 \text{ mol dm}^{-3}$   $\text{Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$  buffer solution (pH 7.42). Scan rate  $100 \text{ mV s}^{-1}$ .

tial for dioxygen reduction at modified electrodes is much more positive, and the catalytic reaction starts at the cathodic peak of hemin. It can be clearly seen that the cathodic current rises while the anodic current decreases and disappears. This is due to the presence of an electrochemical (EC) catalytic regeneration process on the catalytic reduction of dioxygen [24]. Figure 4(a) shows the cyclic voltammograms of the HCPE at different scan rates in the potential range from  $-0.3$  to  $-1.0$  V in oxygen-saturated buffer solutions (pH 7.42). From Figure 4(b) it can be seen that catalytic reduction current increases linearly with the square root of scan rate between 75 and  $200 \text{ mV s}^{-1}$ , in agreement with a diffusion-controlled process. Moreover, with increasing scan rate, the reduction peak potentials moved in the negative direction. In the scan rate range from 35 to  $500 \text{ mV s}^{-1}$ , the peak reduction potential is linear with the log of scan rate:

$$E_{pc} = -0.035 \ln v - 0.5597 \quad (1)$$

with a correlation coefficient of 0.9973. According to the literature [25–29],

$$E_{pc} = E^{o'} - \frac{RT}{\alpha n_x F} \left[ 0.780 + \ln \frac{D_R^{1/2}}{K_s} + \ln \left( \frac{\alpha n_x F v}{RT} \right)^{1/2} \right] \quad (2)$$

From Equations 1 and 2 we obtain  $\alpha n_x = 0.36$ , where theory determines that a value of  $\alpha$  such that

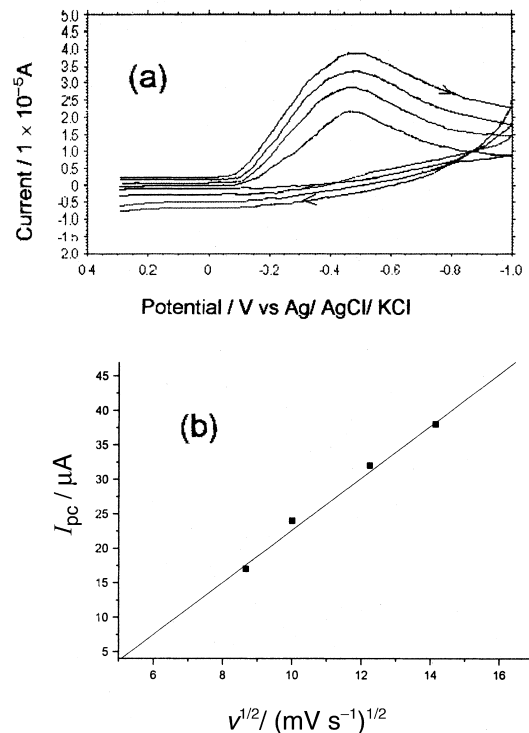


Fig. 4. (a) Cyclic voltammograms of HCPE in oxygen-saturated  $\text{Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$  buffer solution (pH 7.42) at different scan rates. (From inner curve to out curve: 75, 100, 150 and  $200 \text{ mV s}^{-1}$ ). (b) The linearity of the currents with the square root of scan rate.

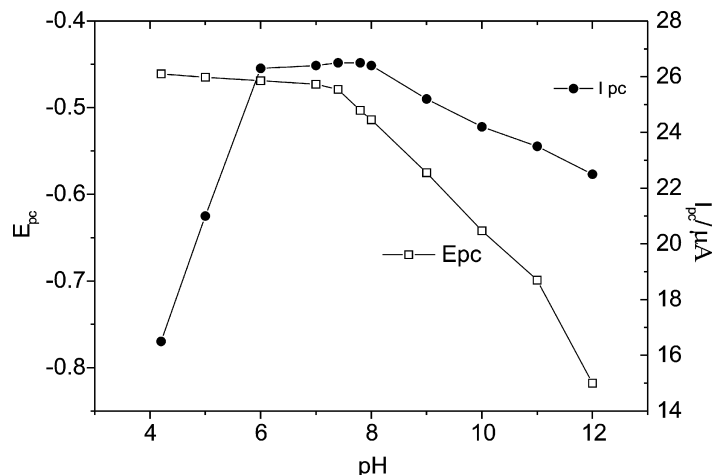
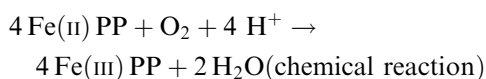
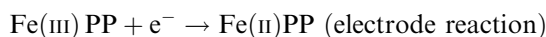


Fig. 5. pH dependence of the peak potential and peak current for dioxygen reduction in oxygen-saturated buffer solutions ( $\nu = 100 \text{ mV s}^{-1}$ ).

$0.3 < \alpha < 0.7$  is consistent with a single electron transferred in the control process of the electrode reaction (i.e.  $n_\alpha = 1$ ).

To interpret the origin of the reduction process, we studied the effect of pH on peak potential. Figure 5 shows the relationship between  $E_{pc}$  and pH. In the pH range 4 to 7, the peak potential is virtually independent of pH. In the pH range from 7–11, the peak potential shifts negatively with increase in pH. When the pH increases by one unit,  $E_{pc}$  shifts negatively about 55 mV indicating that a proton is involved in the electrocatalytic process over this pH range. From the discussion above and reports on the study of hemin to the electrocatalytic reduction of dioxygen [9, 12, 30], the electrocatalytic reduction of  $O_2$  by HCPE in the pH range 7–11 can be described by the following mechanism:



The  $O_2$  is reduced by the one-electron reduced species [Fe(II) PP] in the presence of protons to yield water, and

the Fe(III) PP is regenerated. Thus, in the situation of high oxygen concentration, the anodic current disappears completely.

Figure 5 also shows the relationship between the reduction current and pH. Clearly, the current is nearly constant in the range pH 6–8, very suitable for the measurement of  $O_2$  under physiological conditions.

### 3.3. Application

On the basis of the voltammetric results described, it appears likely that amperometric detection of  $O_2$  by HCPE is possible. We use fixed-potential amperometry to measure dissolved  $O_2$  concentrations. The electrode potential was fixed at  $-0.55 \text{ V}$ , five concentrations of oxygen at room temperature were tested. Figure 6 shows the amperogram for the determination of dioxygen, and the electrode response was linear for dissolved  $O_2$  [0 to  $1260 \mu\text{mol dm}^{-3}$   $O_2$ ; mean five point standard curve, current  $i$  ( $\mu\text{A}$ ) =  $1.35 + 0.0026 \times O_2$  concentration ( $\mu\text{mol dm}^{-3}$ ),  $r = 0.9972$ ].

Interference effects were investigated by testing the cyclic voltammetric responses of HCPE to glucose, ascorbic acid and histidine, which usually exist in

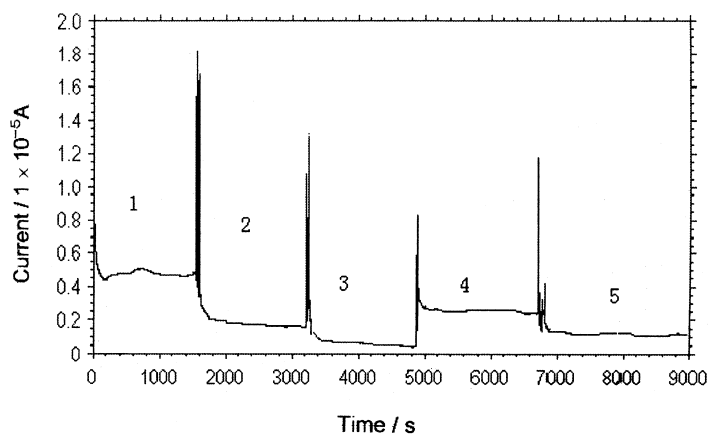


Fig. 6. Amperometric response of oxygen reduction in five concentration oxygen buffer solutions (pH 7.42), fixed potential at  $-0.55 \text{ V}$ . (1) 1260, (2) 200, (3) 0, (4) 630 and (5)  $100 \mu\text{mol dm}^{-3}$ .

biosamples. In experiments, we found that  $10^{-2}$  mol  $\text{dm}^{-3}$  of glucose; histidine and  $10^{-3}$  mol  $\text{dm}^{-3}$  of ascorbic acid did not interfere with the detection of dissolved oxygen. These favourable results indicate that the hemin modified carbon paste electrode may be used for the determination of dissolved oxygen in biosamples.

#### 4. Conclusion

Hemin modified carbon paste electrodes were constructed and their electrochemical behaviour was characterized by cyclic voltammetry. It has been demonstrated that the electrode has many advantages, such as simple and rapid preparation, good chemical and mechanical stability and good reproducibility. The modified electrode possesses high catalytic activity for oxygen reduction over a wide range of pH buffer solutions. This research will be helpful for the manufacturing of amperometric sensors for the determination of dioxygen in biosamples.

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