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Analytica Chimica Acta 327 (1996) 125–132

ANALYTICA CHIMICA ACTA

Properties of poly-β-aminoanthraquinone modified carbon fiber electrode as a basis for hemoglobin biosensors

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Received 4 September 1995; revised 9 January 1996; accepted 21 January 1996

Abstract

An amperometric microsensor for hemoglobin (HB) has been constructed using a carbon fiber electrode modified with poly-β-aminoanthraquinone (pAAQ). Scanning electron microscopic studies showed that the potential range and time of electrochemical polymerization had strong effects on the morphology of the polymer film at the microelectrode. The stability and the electrochemical properties of the modified electrode were studied using cyclic voltammetry. The pAAQ modified microelectrode showed an excellent electrocatalytic activity for the reduction of HB in a weakly acidic solution. The catalytic current in cyclic voltammetry increased linearly with the HB concentration between 1.0–400 μM. The amperometric response to HB had a linear range of 0.5 μM to 340 μM with a correlation coefficient of 0.990(n=18). The biosensor was stable for at least one month when it was stored in a phosphate buffer (pH 7.4) below 20°C. The obtained HB concentration in the whole blood agreed well with the one obtained with the reference instrument.

Keywords: Catalytic methods; Biosensors; Voltammetry; Poly-β-aminoanthraquinone; Hemoglobin; Blood

1. Introduction

In the last few years, the application of microelectrodes in bioelectroanalysis and biosensors has attracted a great interest due to the advantages of microelectrodes [1] and the need of miniaturization of detectors and detection devices [2]. Carbon fiber microelectrodes (CFMEs) are considered to be one of the most useful basis for microbiosensors in microflow systems and for *in vivo* measurements because of a number of unique features such as small dimension, lower double-layer capacitance and ohmic loss [1,3], high strength [4] and cheapness and readily availability in a wide variety with different properties [5], and especially rather good biocompatibility [6]. The modification of CFMEs has greatly extended its application range [2–5,7–15]. The preparation of modified CFMEs has usually been completed by using electrochemical polymerization [8], coating by polymers [4,9–11] and bovine serum albumin [5,12], noble metal deposition [13,14] or covalent modification by acylation [2,15,16]. However, little has been reported on CFMEs modified with electrochemical polymerization except for the study of the electrochemical behaviour of polyaniline film at a CFME [8]. In the present study a poly-β-aminoanthraquinone (pAAQ) modified carbon fiber

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PII S0003-2670(96)00067-0

microcylinder electrode was prepared by electrochemical polymerization.

β-aminoanthraquinone (AAQ) is an electroactive derivative of quinone molecules which are well known as mediators of electron transfer between biomolecules and electrodes [17]. Mariana *et al.* [18] studied the electrochemical reduction of AAQ by cyclic voltammetry and made a proposal for the mechanism. Hossain [19] examined the effect of AAQ chemically attached at a highly oriented pyrolytic graphite electrode on oxygen reduction to peroxide in aqueous alkaline solution and suggested that the reaction was an overall 2-electron process. A series of quinone molecular films derived from aminoquinones were obtained by electrochemical oxidation of monomers in acetonitrile [20], but never has the polymer film of AAQ been obtained in aqueous solution, and its electrocatalytic properties in redox process of biomolecules have not been reported. In this work, we describe the preparation of pAAQ film in water and present the electrochemical characterization of pAAQ. The pAAQ showed a significant electrocatalytic activity for reduction of hemoglobin (HB) in a weakly acidic solution.

Hemoglobin (HB) is an important respiratory protein in red cells. The direct electrochemical determinations of HB, particularly in a small system, is very important for clinical medicine [10]. Some studies on the direct electrochemical determination of HB have been reported [10,21,22]. However, no work on the direct electrochemical determination of HB has been studied at a microelectrode except [10], and the amperometric detection of HB with a CFME has not been reported yet. This paper reports a method of amperometric determining HB using a microelectrode applicable over a wide linear range. The result is an interesting step forward in the preparation of modified CFMEs and will be significant to develop amperometric microbiosensors.

2. Experimental

2.1. Reagents and materials

β-aminoanthraquinone (AAQ) (Dr. Theodor Schuchardt Gmbh, München, Germany) with a purity of 96.2% was obtained from the Shanghai Chemical Factory in China without further purification. HB (from ox blood) was from the Shanghai Biochemistry Research Institute. The reagents preparing 0.2 M buffer solutions, pH 3–12 (phosphate) and pH 5.5 (acetate) were of analytical reagent grade. Water used in experiments was twice-quartz-distilled. Human blood samples were obtained from Jiangsu Tumor Hospital without any pretreatment. Carbon fiber (PAN type) with 6–7μm diameter came from the Shanghai Synthetic Fiber Research Institute. Epon 812 epoxy resin (New York, U.S.A) was used to seal the electrodes.

2.2. Apparatus

Electrochemical measurements were carried out with a BAS-100B Electrochemical Analyzer equipped with a PA-1 Preamplifier (BAS, USA) which was used to amplify the current and to filter out noise, and an FPG-310 Color Plotter (Fujitsu, Japan) which was used to record the voltammograms. Scanning electron microscopic photographs (amplification 10000×) were obtained with an X-560 scanning electron microscope (Hitachi, Japan) by cutting the carbon fibers in various stages of the modifying process on the sample stand, then treating them by spraying gold. The experimental temperature was controlled at 20±0.1°C.

2.3. Preparation of modified microelectrode

The base electrodes were single carbon fiber microcylinder electrodes with a length of 6–10 mm which were usually similar to that given in a previous paper [10]. After the carbon fiber microcylinder electrode was washed thoroughly with acetone and water, it was electrochemically pretreated in fresh 1.0 M H_2SO_4 solution with triangular-wave potential sweep from −1.0 V to +2.0 V at a scan rate of 200 mV s^{-1} for10 min. The pretreated electrode was washed with water again, and then immersed in 0.5 M AAQ solution prepared in ethyl alcohol and pH 7.0 phosphate buffer with 1:1 (v/v) for electrochemical polymerization. The electrochemical polymerization was completed with cyclic voltammetry at 500 mV s^{-1} in various potential ranges for different numbers of sweep cycles.

2.4. Electrochemical procedures

A three-electrode system with saturated calomel electrode (SCE) as reference electrode, Pt wire as counter electrode and the above modified electrode as working electrode was employed. Behaviour and optimization was studied in 5 ml buffer solutions after deaerating with pure N_2 for 10 min. Amperometric determinations of HB were carried out after a small volume(μl) HB was added in 2.0 ml deaerated pH 5.5 acetate buffer while stirring at constant rate and applying a fixed voltage of −0.7 V *vs.* SCE. The current–time responses following single additions of HB were recorded. All electrochemical measurements were performed under a nitrogen atmosphere inside a faraday cage.

3. Results and discussion

3.1. Surface morphology and effect of potential sweep range

At an electrochemically pretreated carbon fiber microcylinder electrode, the cyclic voltammograms of AAQ in pH 7.0 PBS showed various phenomena at different potential sweep ranges (Fig. 1). When the range was from +1.0 to −0.5 V, the successive voltammograms overlapped and no peak occurred. Its microscopic photograph (Fig. 2A) showed a lot of explicit stripes on the carbon fiber surface. However, when changing the negative limiting of sweep range into −1.0 V, all voltammograms showed a couple of peaks at −0.65 V and −0.54 V (Fig. 1). With increasing number of sweep cycles, both cathodic and anodic peak currents increased, furthermore, the increasing rate decreased and the peak currents tended to become constant after continuously sweeping for ten cycles, indicating the formation of a polymer film of AAQ. The positive limitation of the sweep range had a very slight effect on the voltammogram and the effect decreased with increasing number of sweep cycles. Comparing Fig. 2A with 2B and 2C, the stripes on the carbon fiber disappear after the negative potential limiting changed to −1.0 V. In Fig. 2B and C these stripes have been covered by polymer film formed in cyclic sweeps. Fig. 2B and C also show that the effect of positive potential limiting

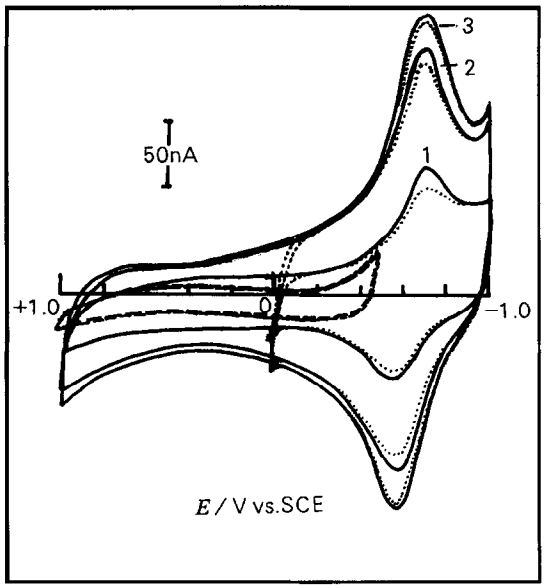

Fig. 1. Cyclic voltammograms of 0.5 M AAQ in ethyl alcohol and pH 7.0 phosphate buffer with 1:1 (v/v) at 500 mV s^{-1}. (1) first cycle, (2) fifth cycle and (3) tenth cycle.

on the formation of polymer film is very slight. These results are different from films of aniline polymerization which were formed by the amine oxidation at positive potential [23]. At the same time, the results are also different from those of [20] in which the aminoquinone polymer was formed by the amine oxidation at an oxidation potential more positive than +1.2 V. Here the polymerization of AAQ is formed by the reduction of quinone in aqueous solution instead of the oxidation of amine in acetonitrile solution. Although the peak currents did not change after ten successive cyclic sweeps, the polymer film thickened (*e.g.* Fig. 2D for fifty cycles) and some holes did appear causing unstable electrochemical behaviour. Therefore, the preparation of pAAQ modified microelectrode was completed by cyclic sweeps between +1.0 V to −1.0 V at 500 mV s^{-1} for ten cycles.

3.2. Stability of pAAQ modified electrode

The successive cyclic voltammetric experiments indicated that at the beginning of the cyclic voltammetric sweeps, the currents of cathodic and anodic peaks at the potentials of −0.485 V and −0.594 V

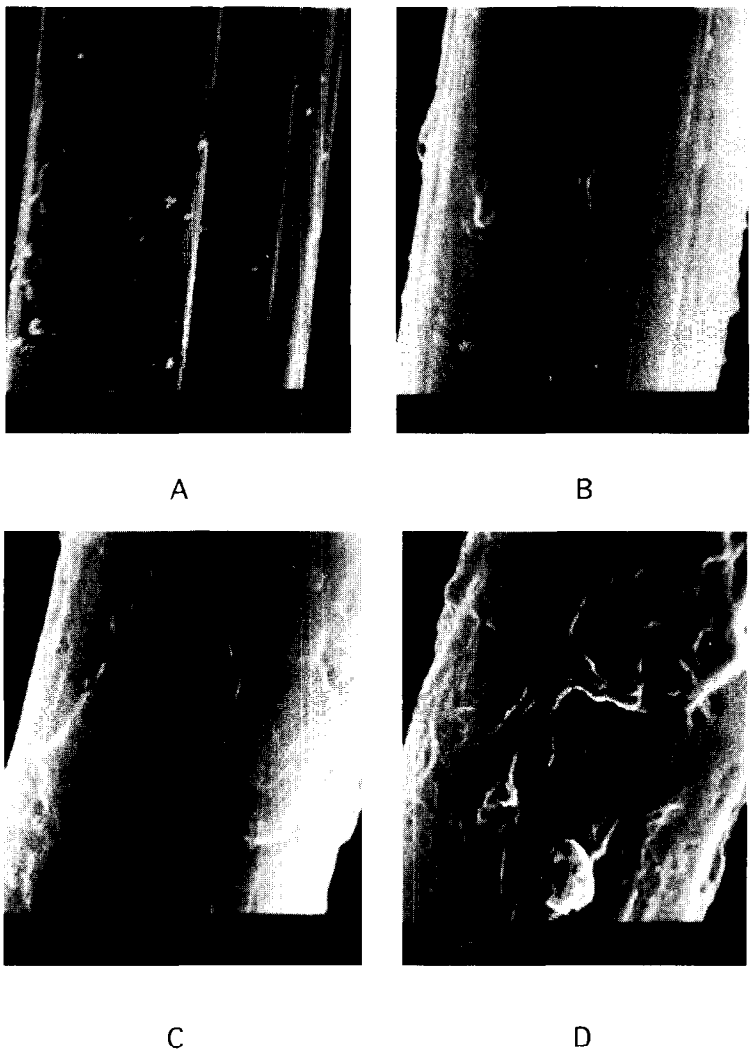

Fig. 2. Scanning electron microscopic photographs of carbon fibers successively sweeped in 0.5 M AAQ solution at 500 mV s^{-1} in the potential ranges of (A) +1.0 to −0.5 V, (B) 0 to −1.0 V and (C) +1.0 to −1.0 V for ten cycles as well as (D) +1.0 to −1.0 V for fifty cycles.

slightly decreased, then gradually dropped down to a constant value and kept that value for a very long time (*e.g.* Fig. 3 for continuous cyclic sweeps of 6 h). After the modified microelectrode was dipped in pH 7.0 buffer solution for more than one month, no change of peak currents appeared, indicating that the stability of pAAQ modified CFME was very good. The little decrease of peak currents at the beginning was due to the desorption of few AAQ monomers adsorbed on the polymer film.

3.3. Kinetic behaviour of pAAQ modified electrode

The cyclic voltammograms at various scan rates showed that, while increasing the scan rate, not only the peak currents linearly increased, but also the peak potentials slightly shifted (Fig. 4). Thus, the electrode reaction was a surface-controlled process with slower electron transfer rate. On increasing the logarithmic value of the scan rate, the anodic peak potential shifted linearly in positive direction, and the cathodic

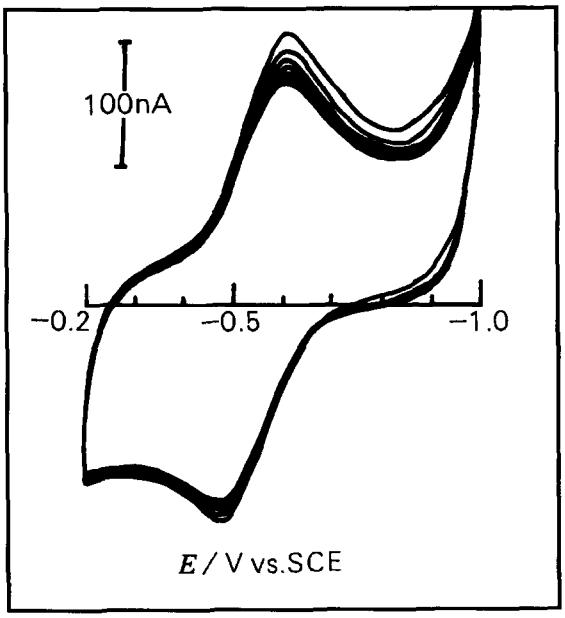

Fig. 3. Cyclic voltammogram of pAAQ modified electrode at pH 5.5 and 100 mV s^{-1}.

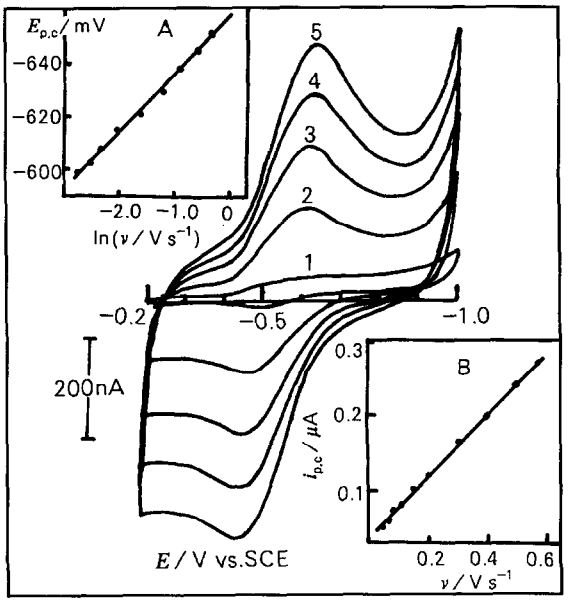

Fig. 4. Cyclic voltammograms of pAAQ electrode in pH 5.5 solution at (1) 10, (2) 100, (3) 200, (4) 300 and (5) 400 mV s^{-1}. Inset A: $E_{p,c}$ *vs.* ln ν; Inset B: plots of $i_{p,c}$ *vs.* ν.

peak potential shifted linearly in negative direction (*e.g.* Fig. 4A for $E_{p,c}$). From the slope value of −23 mV and the equation of $E_{p,c}$= constant − (0.5RT/α nF)ln ν, the charge transfer coefficient α=0.57 can be obtained.

With increasing pH, the peak potentials of both anodic and cathodic peaks shifted in the negative direction. The formal potential $E^{o'}$, which was calculated according to $E^{o'}=E_{p,a}-\alpha(E_{p,a}-E_{p,c})$ [24] and using α of 0.57, shifted in the negative direction with a slope of 56 mV pH^{-1}, as shown in Fig. 5. The reaction was an overall 2-electron process [19], and thus, the number of H^+ participating in this electrode process is 2 at pH 3–12.

The increase of pH led to an increment of the peak potential difference of the cyclic voltammograms, furthermore, the voltammogram was distorted at higher pH, indicating that the rates of electron transfer reaction between pAAQ and electrode surface, and electron self-exchange reaction between the reduction-state and the oxidation-state pAAQ in the polymer film decreased due to reduction of the H^+ concentration. The apparent surface electron transfer rate constant k can be approximately obtained from the equation of Laviron [25] and the value of α (0.57). The results were shown in Table 1.

3.4. *Electrocatalytic reduction of hemoglobin at pAAQ modified electrode*

Fig. 6 shows the electrocatalysis of pAAQ modified electrode to the reduction of HB in pH 5.5 acetate buffer solution. After 8.0 μl 1.0 mM HB was added in 2.0 ml buffer solution, the anodic peak current decreased, the cathodic peak current largely increased. The cathodic peak potential shifted about 50 mV in the negative direction because the catalytic reaction between pAAQ and HB in solution, which possibly changes the rate of electron self-exchange reaction between the reduction-state and the oxidation-state pAAQ in the polymer film. The catalytic mechanism can be expressed as follows:

$$pAAQ_{Ox} + 2e \rightleftharpoons pAAQ_{Red}$$

$$HbFe(III) + pAAQ_{Red} \rightarrow pAAQ_{Ox} + HbFe(II)$$

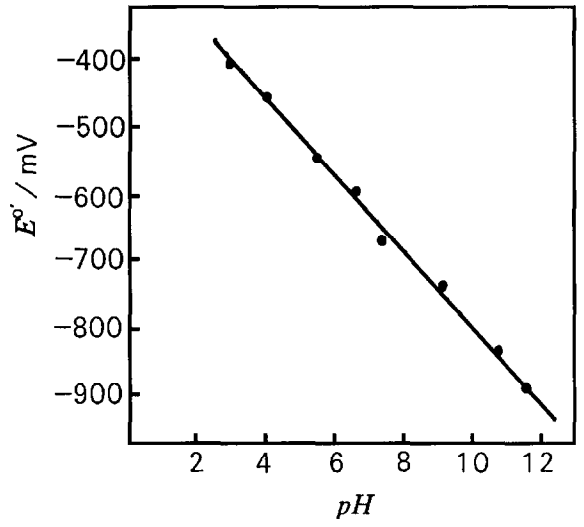

Fig. 5. Dependence of $E^{o'}$ of pAAQ at carbon fiber electrode on pH.

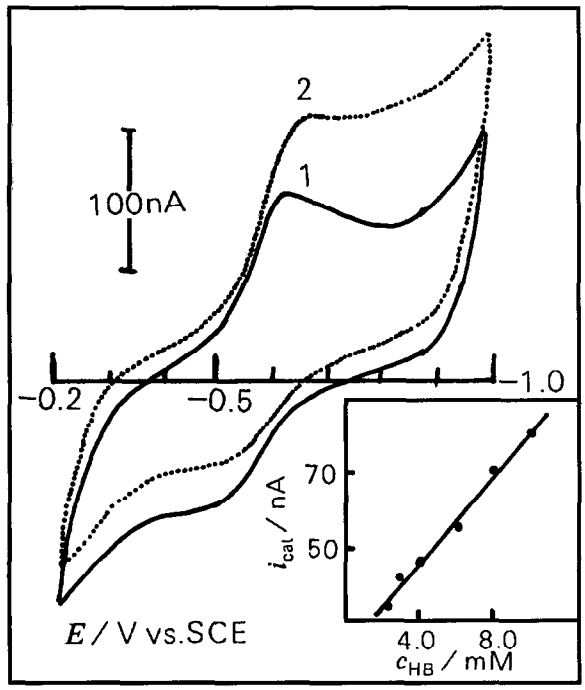

Fig. 6. Cyclic voltammograms of pAAQ modified electrode in pH 5.5 buffer solution (———) and pH 5.5 buffer including 4.0μM HB (···) at v=100 mV s^{-1}. Inset: plots of i_{cat} *vs.* concentration of HB.

Table 1
Effects of pH on ΔE_p and electron transfer rate constant

pH	ΔE_p mV^{-1}	$k^{o'}$ s^{-1}
3.0	49	1.48
4.0	68	1.03
5.5	109	0.46
6.5	125	0.34
7.4	138	0.26
7.8	153	0.20
9.1	194	0.088
10.8	213	0.061
11.6	238	0.038

With an increase of the HB concentration, the increment of the cathodic peak (*i.e.* catalytic peak current i_{cat} =$(i_{p,c})_2$ −$(i_{p,c})_1$) linearly increased (inset in Fig. 6). The linear range was from 1.0 μM to 400 μM with a correlation coefficient of 0.984.

3.5. Amperometric response of the pAAQ modified electrode to hemoglobin

In amperometric measurements, the potential dependence of HB response (dynamic potential voltammogram) indicated that the modified electrode had an amperometric response to HB at potentials more negative than −0.53 V in weakly acidic solution. With a negative shift of the applied potential, the response rose sharply up to −0.70 V, and then reached a constant value (Fig. 7). The amperometric response to HB was seriously affected by the pH value of the solution. With increasing pH, the response enhanced in the range of pH 3.0 to 4.5, and remained constant at pH 4.5 to 6.8, thereafter the response decreased. Therefore, the amperometric determination was controlled at −0.70 V in a pH 5.5 buffer solution.

In pH 5.5 solution, with the injecting of HB, the current of amperometric response increased. The curve of steady state response is shown in Fig. 8. It can be seen from Fig. 8 that the response time is very short and that the response has a linear increment with increasing concentration of HB in the range of 0.5 μM to 340 μM with a correlation coefficient of 0.990 (inset in Fig. 8, n=18). The average current response was 23.2 nA (SD=2.3%) at a HB concentra-

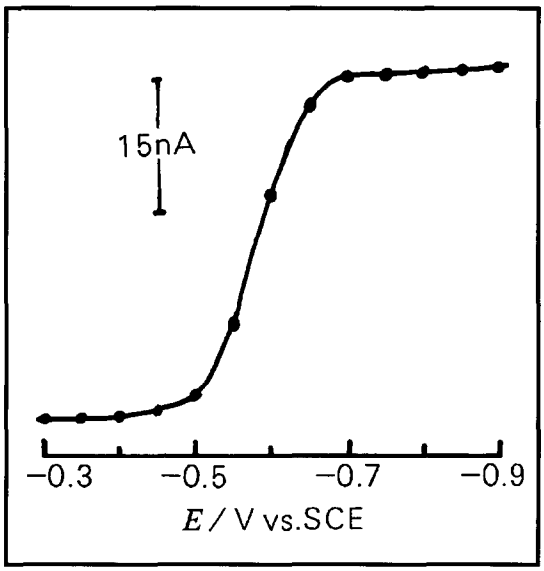

Fig. 7. Dynamic potential voltammogram in 10 μM HB solution at pH 5.5.

tion of 8.0 μM for five successive determinations. When the HB concentration was larger than 340 μM, the current deviated from linearity. In solutions containing HB concentrations lower than 0.3 mM, the electrode can be successively used for more than two weeks. After the sensor had been stored in pH 7.4 buffer solution below 20°C for one month, the amperometric response to HB had only decreased by 5.7%. This is very advantageous to clinic analysis of HB.

3.6. *Amperometric determination of hemoglobin in clinic blood*

After a 4.0 μl clinical blood sample was 500-fold diluted with pH 5.5 buffer solution, the amperometric response of pAAQ modified electrode can be used to detect the concentration of HB at −0.70 V. The average current value of three determinations in an interval 2 h was 13.2 nA (SD=3.6%). The HB concentration in the diluted solution was 4.73 μM, and thus the concentration of HB in whole blood was 2.36 mM (153 $g L^{-1}$). The result was close to the value of 158 $g L^{-1}$ obtained with a blood corpuscle autometer (Coulter, USA).

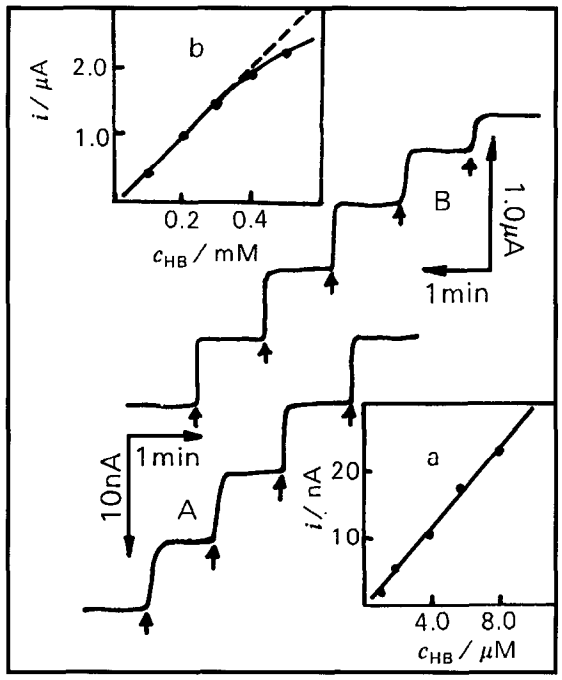

Fig. 8. Amperometric response of pAAQ modified microelectrode to successive increments of 4.0 μM (A) and 0.10 mM (B) HB with an interval of 1 min at −0.70 V and pH 5.5. Inset: plots of current *vs.* HB concentration, (a) for lower and (b) for higher concentration.

Acknowledgements

The project was supported by National Natural Science Young Foundation of China. Gui-Feng Yan at Jiangsu Tumor Hospital is gratefully acknowledged for her kind assistance in supplying human blood samples and verifying the experimental data.

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