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Study on the enhancement of catalytic activity for hemoglobin by quinhydrone in poly(*o*-aminophenol) film

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Abstract

Hemoglobin (Hb) and quinhydrone (QHQ) were incorporated in poly(*o*-aminophenol) [*o*-AP, POAP] film by electropolymerization of *o*-aminophenol in a weak acid solution containing Hb and QHQ. The nonconducting polymer film was found to be nearly rigid by piezoelectric quartz crystal (PQC) impedance. Therefore, the thickness of the Hb–QHQ–POAP film was estimated as about 104±10 nm by quartz crystal microbalance (QCM). The QHQ mediation effects on the biomacromolecule Hb entrapped in the POAP film were investigated by using cyclic voltammetry, amperometric technique and kinetic study. Cyclic voltammograms showed that the redox peaks in the Hb–QHQ–POAP film are much more reversible than those in the Hb–POAP film. The response current of the Hb–QHQ–POAP film to H_2O_2 was almost twice than that of the Hb–POAP film. The Michaelis–Menten constant and the activation energy of Hb in the Hb–QHQ–POAP film are 7.47 mM and 13.91 kJ/mol, respectively, both are smaller than that in the Hb–POAP film. These results showed that the immobilized Hb in POAP film exhibited higher catalytic activity to H_2O_2 due to the mediation of QHQ.

Keywords: Hemoglobin; Poly(*o*-aminophenol); Quinhydrone; Hydrogen peroxide; Piezoelectric quartz crystal impedance

1. Introduction

Hemoglobin (Hb), containing four-polypeptide chain, each of which has one electroactive iron heme group, can store and transport molecular oxygen in mammalian blood. Although Hb does not play a role as an electron carrier in biological systems, it has been reported to possess "pseudo-peroxidase" activity [1,2] due to its close similarity with peroxidases. The conventional methods to immobilize Hb include that of electrostatic absorption [3], entrapment in composite films [4] and polymer film [5].

Although Hb possesses functional groups that can be easily oxidized or reduced by redox reagents, it is generally difficult to obtain direct electron transfer between Hb and an electrode. An appropriate electron donor can mediate the electron transfer between the Hb and an electrode, and hence, such a mediator is expected to improve the performance of the pseudo-peroxidase-based hydrogen peroxide sensor. Ferrocene [6] and quinhydrone (QHQ) [7] have been reported as mediators for horseradish peroxidase to construct H_2O_2 sensor. Methylene blue [8] has been used as a mediator for the reduction of Hb in nafion film on microcylinder carbon fiber electrode.

Since the oscillation of piezoelectric quartz crystal (PQC) in liquid medium was successfully realized in 1980s, the quartz crystal microbalance (QCM) technique has been used in many fields, such as ion-selective electrode and molecular imprinting polymer technology, etc. However, the frequency shift was not only affected by the mass change on the electrode but also by the physicochemical properties of the liquid around the electrode surface, such as conductance and viscosity–density. Therefore, piezoelectric quartz crystal impedance PQCI was introduced. In our lab, PQCI has been successfully used to monitor the growth of bacteria [9] and study the viscoelasticity of molecular

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imprinting polymer film [10]. However, the biomacromolecules entrapped in poly(*o*-aminophenol) [*o*-AP, POAP] films are still not investigated by PQCI.

Therefore, in this paper, Hb was immobilized simultaneously with QHQ, which is used as an electron-transfer mediator, in POAP film. POAP film was selected because of its relative cheapness, easy preparation, and successful use in the development of uric acid, lactate, and hydrogen peroxide biosensors [6,11,12]. The viscoelasticity and the thickness of Hb–QHQ–POAP film were estimated by PQCI and QCM, respectively. Meanwhile, the effects of mediator QHQ on Hb entrapped in POAP film were investigated by studying its catalytic activity to H_2O_2. The effects of experimental parameters including the scanning number of electropolymerization, pH of the solution, and the applied potential were investigated in detail.

2. Experimental

2.1. Reagents

Hemoglobin (bovine red cells) was purchased from Worthington Biochemical (USA). *o*-Aminophenol (*o*-AP) [99%] and quinhydrone were purchased from Shanghai Chemical Reagent (China) and used as received. All other chemicals were of analytical grade. Hydrogen peroxide solutions were prepared daily and standardized by potassium permanganate titration. Double distilled water was used throughout.

2.2. Apparatus

AT-cut 9-MHz piezoelectric quartz crystals (diameter: 12.5 mm) with gold electrodes (diameter: 6.0 mm) on both sides were used. The quartz crystal was fixed to a glass tube using silicone rubber, and only one side of the crystal was coated with film and allowed to contact the solution. An HP 4395A network/spectrum/impedance analyzer was employed to obtain the conductance spectrum.

Electrochemical (EC) measurements were performed at a CHI 660 Electrochemical Workstation (Shanghai Chenhua Instrument, China) using a conventional three-electrode cell. The gold microelectrodes were constructed by sealing gold wire (diameter: 0.2 mm, >99.99%) in a glass tube with epoxy resin and used as a working electrode. A platinum wire was applied as the counterelectrode, and a saturated calomel electrode (SCE) was used as a reference electrode. All the potentials in this paper were in respect to SCE. All measurements were carried out at room temperature (18±2 °C).

2.3. Preparation of the Hb–POAP and the Hb–QHQ–POAP modified electrode

The Hb–QHQ–POAP modified electrode was prepared by continuous cyclic scanning from −0.2 to 0.8 V (15 cycles) at a scan rate of 50 mV/s in 0.2 M fresh prepared acetate buffer solution (pH 5.0) containing 1×10^{-2} M *o*-AP, 4×10^{-5} M Hb, and 1×10^{-3} M QHQ. The Hb–POAP modified electrode was fabricated in a similar way. The solution was deaerated with pure nitrogen prior to electropolymerization. After the polymerization procedure, the modified electrode was rinsed with pH 5.0 acetate buffer solution to remove the physically adsorbed species and kept in the phosphate buffer (pH 7.2) at 4 °C before use.

2.4. Procedures

Amperometric measurements were performed under stirred conditions. Aliquots of standard solution of H_2O_2 were successively added to the buffer solution. Prior to the experiments, the buffer and sample solutions were purged with pure nitrogen for at least 15 min to remove oxygen.

3. Results and discussion

3.1. Characterization and performance of POAP, Hb–POAP, and Hb–QHQ–POAP films

The Hb–QHQ–POAP film was electropolymerized in a 0.2 M acetate buffer solution (pH 5.0) containing 1×10^{-2} M *o*-AP, 4×10^{-5} M Hb, and 1×10^{-3} M QHQ. There was only one anodic peak at ca. 0.55 V (vs. SCE) on the first cycle, which was suppressed during the second cycle. This shows that the polymer film blocks further access of the monomer to the electrode surface and the film becomes electro-inactive. Hence, a very thin electropolymerized membrane is resulted on the electrode surface. The Hb–POAP film and the POAP film were prepared in a similar way.

3.1.1. Viscoelasticity effect of the films

Most redox polymer films behave neither as a rigid layer nor an ideally viscous (Newtonian) membrane and therefore have to be treated as viscoelastic polymer films [13]. The viscoelasticity of the film is evaluated by measuring the conductance using the PQCI technique [14]. Therefore, we investigated in detail if there was any difference in the half bandwidth of the conductance spectrum between the bare and the modified electrodes. The conductance (G) at half peak height and half bandwidth of the conductance spectrum ($\Delta f_{G_{1/2}}$) can be calculated as [15]:

$$G = G_{\max}/2 = 1/(2R_1) \quad (1)$$

$$\Delta f_{G_{1/2}} = f_{HG_{1/2}} - f_{LG_{1/2}} = R_1/2\pi L_1 \quad (2)$$

where $f_{HG_{1/2}}$ and $f_{LG_{1/2}}$ are the higher and lower frequencies at half peak height (G=$G_{\max}$/2) in the conductance

spectrum, respectively; R_1 and L_1 are the motional resistance and the motional inductance, respectively.

Fig. 1 shows the conductance spectrum for the bare and the modified electrodes. The calculated G_{max} and $\Delta f_{G1/2}$ values are given in Table 1. As can be seen from Fig. 1A, curves *a* and *b* show the conductance spectrum corresponding to the bare PQC and the POAP modified PQC in the air, respectively. No appreciable change in both the height of the peak and the half bandwidth of the conductance spectrum can be found. It can be concluded that the POAP film is nearly rigid and viscoelastic effects can be negligible, which agrees with the report of Ortega [16]. Curves *c* and *d* show the conductance spectrum corresponding to the bare PQC and the POAP modified PQC in 0.2 M acetate buffer (pH 5.0), respectively. The fact that no significant change in the half bandwidth may further prove that the POAP is nearly rigid under the experimental conditions used in this work. Similar phenomena were observed not only between the Hb–POAP-modified PQC and the bare PQC but also between the Hb–QHQ–POAP-modified PQC and the bare PQC (see Fig. 1B,C). Hence, it is possible to conclude that Hb–POAP film and Hb–QHQ–POAP film are nearly rigid and viscoelastic effects can be negligible. It is worth to note that the frequency shift changes with respect to their bare electrode increase in the order of film POAP, Hb–POAP, and Hb–QHQ–POAP. These changes may have been resulted from the introduction of the QHQ and Hb in the polymer when copolymerized.

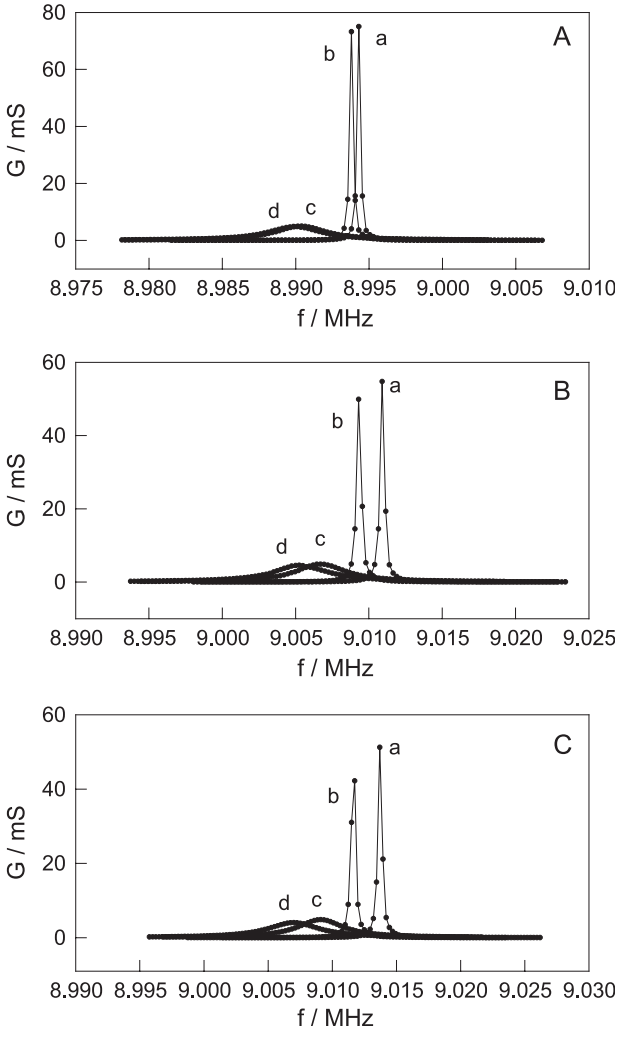


Fig. 1. Effect of the presence of the film and pH 5.0 acetate buffer solution on the conductance spectrum of the quartz crystal. (A) a, c: bare crystal in air and in buffer, respectively; b, d: POAP film-modified crystal in air and in buffer, respectively. (B) a, c: bare crystal in air and in buffer, respectively; b, d: Hb–POAP film-modified crystal in air and in buffer, respectively. (C) a, c: bare crystal in air and in buffer, respectively; b, d: Hb–QHQ–POAP film-modified crystal in air and in buffer, respectively.

Usually, the absolute value of $\Delta f_0/\Delta R_1$ is used to reflect whether the frequency shift is dominated by mass loading or by the viscosity–density of the solution near the interface. If the value is more than 10 Hz/Ω for the 9 MHz quartz crystal used in this work [17], the frequency shift is dominated by mass loading. Otherwise, the frequency shift is dominated by the viscosity–density of the solution near the interface. The absolute values of $\Delta f_0/\Delta R_1$ in this work are also shown in Table 1. From Table 1, the values are all much greater than 10 Hz/Ω. This indicates that the frequency shift of the above three films is dominated by the mass loading and that the viscosity–density effect of the solution near the interface can be negligible.

3.1.2. Estimation of the film thickness

Because the electropolymerized film is nearly rigid, the Sauerbrey equation [18] can be applied and rewritten as:

$$\Delta f = -2.26 \times 10^6 f^2 \Delta m \quad (3)$$

where Δf (Hz) is the frequency shift, f (MHz) is the fundamental frequency, and Δm (g/cm^2) is the mass loading density. The thickness of the films, d (cm), can be expressed by

$$d = \Delta m/\rho_f \quad (4)$$

where ρ_f is the density of the polymer film (g/cm^3). Combined with Eq. (3), the relationship between d and Δf would be:

$$d = -\left(5.485 \times 10^{-9}\right)\Delta f/\rho_f \quad (5)$$

In this work, the density of the polymer films was assumed to be that of the monomer (ρ=1.0 g/cm^3) [19].

The electrochemical (EC) method was also used to verify the value calculated from the QCM. According to the linear relationship between total charge density (Q/A) and film thickness (d) [20, 21], the film thickness was also obtained. The results by using these two methods are shown in Table 2. As can be seen, the thickness of the polymer film by QCM is similar to that by the EC method. The thickness of the POAP film is about 44±6 and 42±4 nm by QCM and the EC method, respectively. Compared with that of POAP film, the thickness of Hb–POAP film is much thicker. The reason may be that the

Table 1
Comparison of the bare and modified PQC in air and in buffer by PQCI

Electrode no.		In air			In buffer		
		G_{max}	$\Delta f_{G_{1/2}}$	$\Delta f_{0g}/\Delta R_1$	G_{max}	$\Delta f_{G_{1/2}}$	$\Delta f_{0L}/\Delta R_1$
1	Bare	75.04±1.02	238±1		4.99±0.45	4227±2	
	POAP	73.23±0.98	245±1	1097±3	4.83±0.40	4440±2	61±4
2	Bare	54.74±2.00	310±3		4.87±0.62	4192±3	
	Hb–POAP	49.88±0.86	335±2	1040±5	4.24±0.58	4865±3	40±5
3	Bare	51.21±1.82	336±3		4.85±0.74	4309±4	
	Hb–QHQ–POAP	42.20±1.04	348±4	1237±7	4.05±0.60	4926±4	51±8

G_{max}: the conductance, mS; $\Delta f_{G_{1/2}}$: half bandwidth of the conductance spectrum, Hz.
Δf_{0g}: the frequency shift in air; Δf_{0L}: the frequency shift in buffer.
ΔR_1: change in the motional resistance.
$\Delta f_0/\Delta R_1$: the ratio of the frequency shift to change in the motional resistance, Hz/Ω.

existence of Hb has an effect on the thickness of the polymer films. It can also be seen that the Hb–QHQ–POAP film is the thickest among the three polymer films. The thickness of the Hb–QHQ–POAP film is 104±10 nm, which is still very thin.

3.1.3. Permeability of POAP films to interferents

Many organic compounds may cause interference with the H_2O_2 determination, e.g., ascorbic acid and uric acid. Fig. 2A shows cyclic voltammograms obtained in 0.2 M acetate buffer (pH 5.0) containing 8×10^{-4} M ascorbic acid at the bare electrode (curve *a*), POAP electrode (curve *b*), and Hb–QHQ–POAP electrode (curve *c*). Obviously, the interference of ascorbic acid is completely suppressed at the two polymer-modified electrodes. The oxidation current of uric acid is evidently decreased at the two polymer-modified electrodes (see Fig. 2B). The reason may be that the POAP film, owing to its low conductivity and small pore size, does not allow relatively large molecules such as ascorbic acid and uric acid to access the electrode surface, just like the case of poly(*o*-phenylenediamine). Therefore, the nonconductive POAP films can avoid the above interferents.

3.2. Comparison of the Hb–POAP film with the Hb–QHQ–POAP film

3.2.1. Effect of the mediator in the film

Fig. 3A depicts the cyclic voltammograms at the Hb–POAP (curve *a*), Hb–QHQ–POAP (curve *b*), and QHQ–POAP (curve *c*) electrodes in 0.2 M acetate buffer solution (pH 5.0). It can be seen from curve *a* that a quasi-reversible peak was observed. Compared with curve *a* in Fig. 3A, curve *b* shows that a pair of well-defined and reversible redox peaks appears at the Hb–QHQ–POAP electrode. The formal potential (E_0) is −0.2 V while the separation between the anodic and cathodic peaks is 0.15 V. Additionally, a pair of redox peaks at about −0.2 V can be observed on the QHQ–POAP electrode (curve *c*). So, the pair of redox peaks located at −0.2 V is attributed to the QHQ redox process.

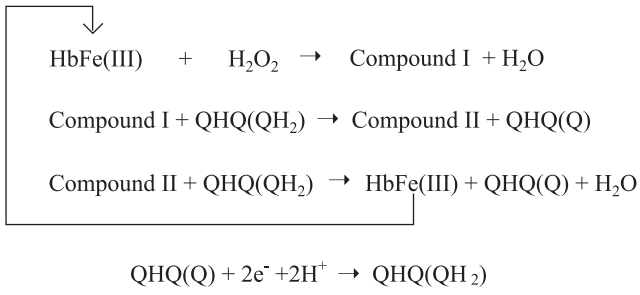
Fig. 3B shows the cyclic voltammograms of the Hb–QHQ–POAP electrode at different scan rates. The relationship between the peak current and scan rate is shown in Fig. 3C. It can be found that the peak current is linearly related to the scan rate in the range between 10 and 50 mV/s, indicating that this is a surface electron transfer process [1].

Table 2
Comparison of the QCM method with electrochemistry (EC) method for the calculation of the film thickness

Films	Average *d*/nm	
	QCM	EC
POAP	44±6	42±4
Hb–POAP	91±9	87±9
Hb–QHQ–POAP	104±10	104±10

3.2.2. Amperometric response of Hb–POAP and Hb–QHQ–POAP films

The mediated catalytic mechanism can be expressed by the following schemes [3,7]:



$$QHQ(Q) + 2e^- + 2H^+ \rightarrow QHQ(QH_2)$$

where compound I (oxidation state +5) and compound II (oxidation state +4) represent the intermediates in the reactions.

Fig. 4A shows the typical steady-state response current to the H_2O_2 at the Hb–POAP (curve *a*) and Hb–QHQ–POAP (curve *b*) electrodes under optimum conditions. Standard H_2O_2 solution was added stepwise (corresponding to a concentration increment of 6.9×10^{-5} M) until a final concentration of 7.6×10^{-4} M H_2O_2 was reached. From Fig. 4A, the response current at Hb–QHQ–POAP electrode is almost twice than that at the Hb–POAP electrode. This

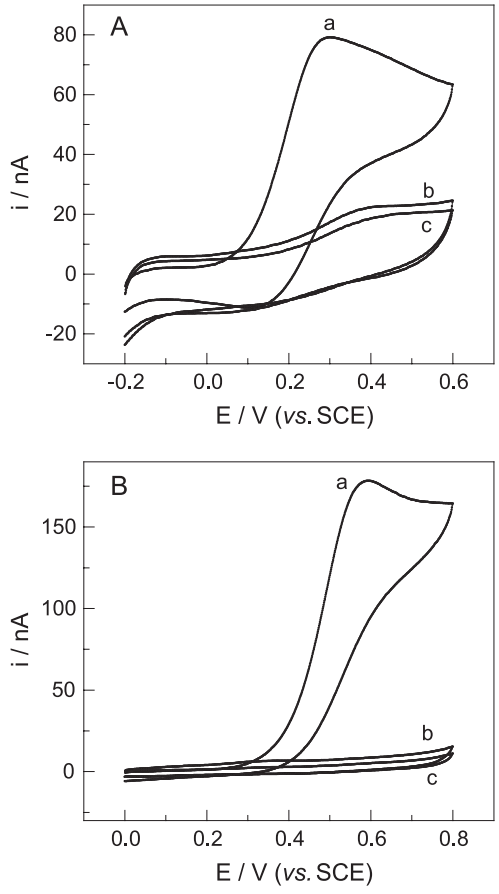


Fig. 2. (A) Cyclic voltammograms for the oxidation of 8×10^{-4} M ascorbic acid in 0.2 M acetate buffer (pH 5.0) at bare electrode (a), POAP electrode (b), and Hb–QHQ–POAP electrode (c). (B) Cyclic voltammograms for the oxidation of 5×10^{-3} M uric acid in 0.2 M acetate buffer (pH 5.0) at bare electrode (a), POAP electrode (b), and Hb–QHQ–POAP electrode (c). Scan rate: 50 mV/s.

indicates that the addition of QHQ results in the increase of the response current. In addition, the sensitivity of the Hb–QHQ–POAP electrode to H_2O_2 can also be calculated to be 0.023 A M^{-1} cm^{-2}, which is higher than that of 0.004 A M^{-1} cm^{-2} obtained at an HRP-incorporated carbon paste electrode [22].

From the calibration curve *b* shown in Fig. 4B, the steady-state reduction current is linear with the H_2O_2 concentration over the range of 1.4×10^{-5} to 5.7×10^{-3} M. The regression equation is i_{ss} (nA)=4.35+3.06 [H_2O_2] (mM), with a correlation coefficient of 0.9990. Additionally, the response time was less than 2 s.

3.2.3. Kinetic parameters of Hb–POAP and Hb–QHQ–POAP films

The maximum reaction rate V_{max} and the apparent Michaelis–Menten constant K'_m were determined using the following Lineweaver–Burk equation [23]:

$$\frac{1}{i_{ss}} = \frac{K'_m}{i_{max}}\frac{1}{C} + \frac{1}{i_{max}} \quad (6)$$

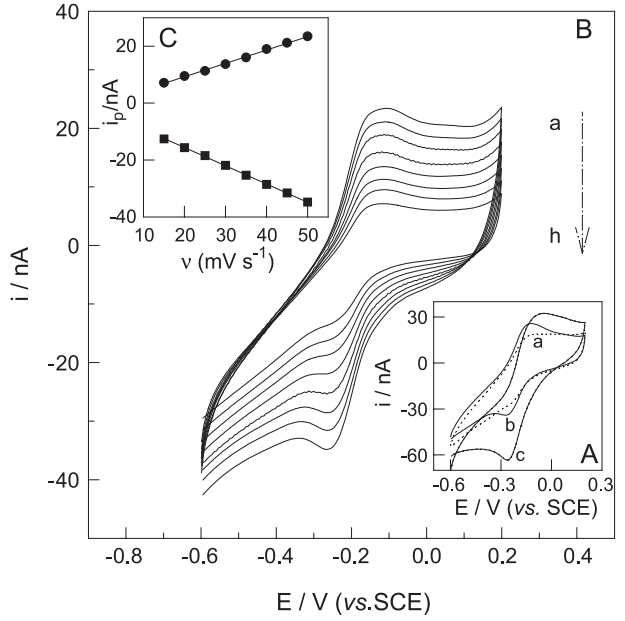


Fig. 3. (A) Cyclic voltammograms at the (a) Hb–POAP, (b) Hb–QHQ–POAP, and (c) QHQ–POAP electrodes in 0.2 M acetate buffer (pH 5.0). Scan rate: 50 mV/s. (B) Cyclic voltammograms of the Hb–QHQ–POAP electrode at scan rates of (a) 15, (b) 20, (c) 25, (d) 30, (e) 35, (f) 40, (g) 45, and (h) 50 mV/s. (C) The relationship between peak currents and scan rate for the Hb–QHQ–POAP electrode in pH 5.0 acetate buffer.

where i_{ss} is the steady-state current after the addition of substrate, c is the bulk concentration of substrate, and i_{max} is the maximum current measured under conditions of

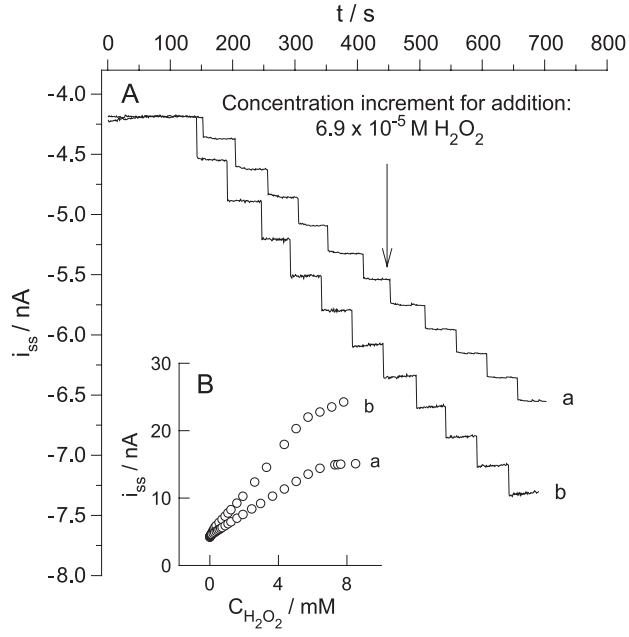


Fig. 4. (A) Amperograms of the Hb–POAP electrode (a) and the Hb–QHQ–POAP electrode (b) with successive addition of 6.9×10^{-5} M H_2O_2 in pH 5.0 acetate buffer at an operation potential of −0.25 V. (B) Calibration curve for the amperometric determination of H_2O_2 at the Hb–POAP electrode (a) and the Hb–QHQ–POAP electrode (b).

substrate saturation. The V_{max} and K'_m of the Hb–POAP electrode are 36.34 nA and 8.45 mM, respectively, while the V_{max} and K'_m of the newly developed Hb–QHQ–POAP electrode are 48.33 nA and 7.47 mM, respectively (see Fig. 5). Significant differences were obtained. The low value of K'_m indicates that the immobilized Hb retains its bioactivity and possesses a high biological affinity to H_2O_2.

The activation energy, E_a, can be calculated according to the Arrhenius equation [24]:

$$\log k = -\frac{E_a}{2.303RT}\frac{1}{T} + \log B \quad (7)$$

where k is the reaction rate constant, E_a is the activation energy, R is the gas constant, T is the absolute temperature, and B is a constant. Eq. (7) can be rewritten as the following equation:

$$E_a = \frac{2.303RT_1T_2}{T_2 - T_1}\log\frac{k_2}{k_1} \quad (8)$$

while the concentration of reactant is identical, k_2/k_1 can be substituted by V_2/V_1 because the reaction rate is in direct proportion to the reaction rate constant [24]. In this work, the temperatures were selected as 22 and 32 °C. So, the calculated values of E_a at the Hb–POAP and Hb–QHQ–POAP electrodes are 30.80 and 13.91 kJ/mol (n=3), respectively. The latter is similar to that reported by Aydemir and Kuru [25]. The lower value of E_a of the Hb–QHQ–POAP electrode indicates that QHQ indeed enhances the catalytic activity of Hb to H_2O_2 and increases the response current.

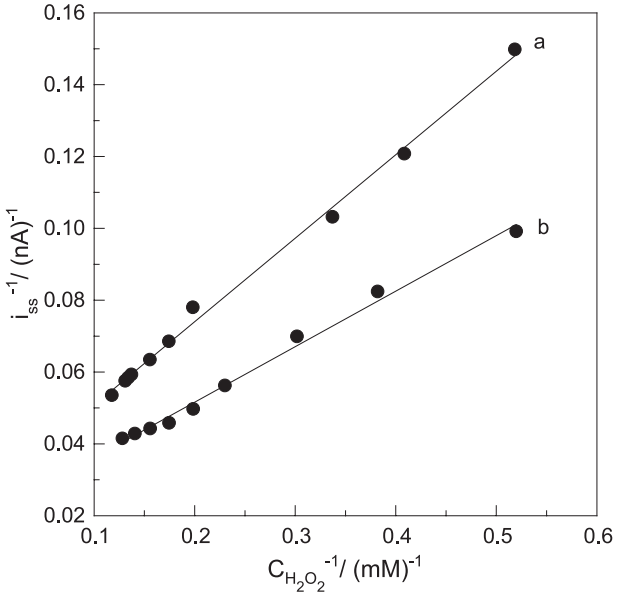


Fig. 5. Determination of K_m: Hb–POAP electrode (a) and Hb–QHQ–POAP electrode (b).

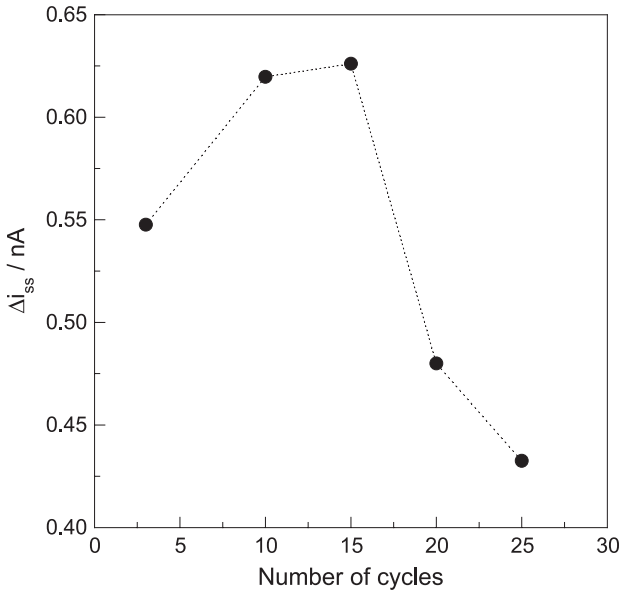


Fig. 6. Effect of number of cycles on the response current to pH 5.0 acetate buffers containing 1.7×10^{-4} M H_2O_2. Operating potential: −0.25 V.

3.3. Optimization of parameters for Hb–QHQ–POAP electrode

3.3.1. The effect of the scan number in electropolymerization

In order to explore the influence of the scan number during electropolymerization on response current, a set of modified electrodes was prepared with a number of potential scans ranging from 3 to 25 cycles. The response current to 1.7×10^{-4} M H_2O_2 in 0.2 M acetate buffer (pH 5.0) is shown in Fig. 6. It can be found that the response current increases with the increasing scan number and a maximum can be observed at 15 scans. When the scan number is less than 15, the amount of Hb entrapped in the POAP film increases with the increasing scan number. After the scan number is more than 15, a decrease in the response current takes place. It may be explained that the further increase of the polymer thickness might slow down the electron transfer rate between the mediator and the surface of the electrode. So, a scan number of 15 was selected in the following electropolymerization process.

3.3.2. The effect of pH

Fig. 7 shows the effect of pH on response current to H_2O_2 at the Hb–QHQ–POAP electrode. The response current decreases with increasing pH in the pH range from 3.6 to 8.0, then it increases slightly when pH value is above 8.0. This phenomenon is similar to the result of Han et al. [2]. Although the peak current is higher at lower pH solution, such buffer solution should not be employed due to the poor stability of the Hb–QHQ–POAP film in such condition. It has been reported that Hb keeps

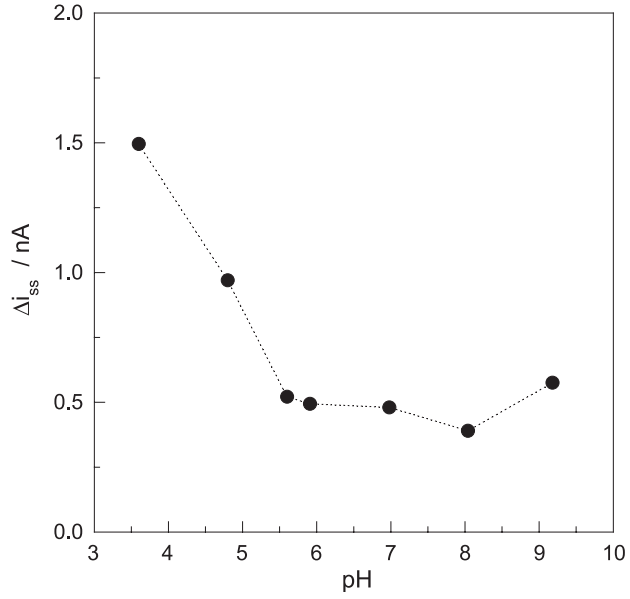


Fig. 7. Dependence of the response current on pH at the Hb–QHQ–POAP electrode in 0.2 M acetate buffer solution containing 1.7×10^{-4} M H_2O_2. Operating potential: −0.25 V.

its highest bioactivity in the acetate buffer of pH 5.5 [26]. Therefore, the pH value of 5.0 was adopted in the experiment.

3.3.3. The effect of applied potential

In order to optimize the applied potential for the H_2O_2 determination, the effect of applied potential on the response current has been studied. The relationship between steady-state current and applied potential in pH 5.0 acetate buffer is shown in Fig. 8. The response current gradually increases with the applied potential from 0 to −0.45 V and changes slowly below −0.45 V. This indicates that a negative applied potential facilitates the reduction of the oxidized state of QHQ. This means that the electron transfer rate between compound I and compound II is accelerated (see the scheme in Section 3.2.2). Considering the possible interference from oxygen at a more negative potential and the operation stability of the electrode, the potential of −0.25 V was selected for the study.

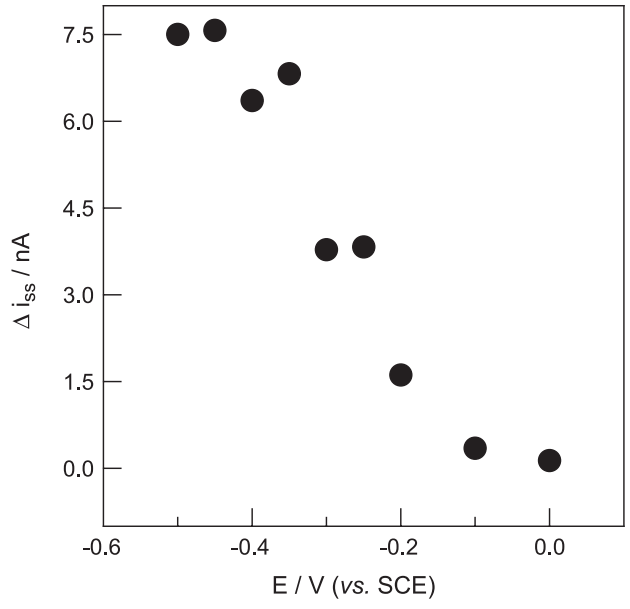


Fig. 8. Response current of the Hb–QHQ–POAP electrode related to the applied potential in pH 5.0 acetate buffer containing 6.9×10^{-4} M H_2O_2.

Table 3
The reproducibility of the Hb–QHQ–POAP electrode

Electrode no.	Δi_{ss}/nA	Means	R.S.D.
1	0.4777±0.0064		
2	0.4863±0.0065		
3	0.4687±0.0071	0.4781±0.0097	±2.0%
4	0.4893±0.0085		
5	0.4683±0.0097		

Δi_{ss}: the change of the steady-state current after the injection of 1.7×10^{-4} M H_2O_2.
R.S.D: relative standard deviation (n=5).

3.4. Stability and reproducibility of the Hb–QHQ–POAP electrode

The stability of the proposed Hb–QHQ–POAP electrode was studied by repeatedly recording the response current to H_2O_2. Measurements were performed once a day during the first week and subsequently every 2 days. The modified electrodes were stored in phosphate buffer solution (pH 7.2) under refrigeration at 4 °C. The modified electrode retained more than 85% of its initial sensitivity to H_2O_2 after 2 weeks.

To investigate the reproducibility of the Hb–QHQ–POAP electrode, the response current to H_2O_2 at five electrodes prepared in the same way was recorded. The result is shown in Table 3. The relative standard deviation (R.S.D.) was 2.0%.

4. Conclusion

In this paper, PQCI showed that the different POAP films were nearly rigid and that the effect of viscoelasticity could be ignored. Thus, the thickness of these polymers was estimated by QCM. The introduction of QHQ enhanced the catalytic activity of Hb to H_2O_2, which was confirmed by cyclic voltammetry, amperometric technique, and kinetic study. The Hb–QHQ–POAP electrode offered fast response, good permselectivity, stability, and reproducibility for hydrogen peroxide detection. The Hb–QHQ–POAP electrode may be used for the fabrication of other biosensors, e.g., glucose and xanthine sensors, to avoid interference from other commonly oxidizable species, such as L-ascorbic acid and uric acid.

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