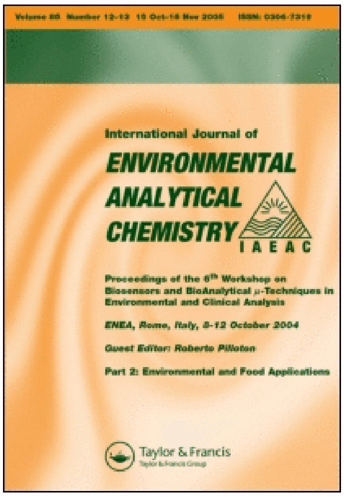

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Enhanced electron transfer by bovine serum albumin covalently attached to glassy carbon electrode and its application to determination of hydroquinone

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Enhanced electron transfer by bovine serum albumin covalently attached to glassy carbon electrode and its application to determination of hydroquinone

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Bovine serum albumin (BSA) was covalently attached to glassy carbon electrode (GCE) surface by the electrochemical method. An enhancement for the redox of hydroquinone (HQ) on BSA/GCE was confirmed by cyclic voltammetry and electrochemical impedance spectroscopy measurement. The electron transfer rate constant (k_s) on the BSA/GCE electrode is almost three orders of magnitude higher than that on bare GCE. The enhancing effect can be attributed to the electrostatic force between the positively charged HQ and negatively charged BSA. It is found that the enhanced redox process of HQ can be used to determine HQ sensitively. The oxidation current can reach 95% of its steady-state value within 30 s. The linear range for HQ determination is from 2.5×10^{-8} M to 1.325×10^{-6} M with a detection limit of 8.6×10^{-9} M at a signal-to-noise ratio of 3. The study may provide a simple, rapid and sensitive method for determination of HQ which is present in the natural environment and in chemical industry effluent.

Keywords: bovine serum albumin; hydroquinone; covalent attachment; enhanced electron transfer; sensitive determination

1. Introduction

As a kind of hydroxy derivative of benzene, hydroquinone (HQ) is a high-volume chemical product, which is used as a reducing agent, antioxidant, polymerization inhibitor, black-white film developer, anthraquinone dye, azo dyestuff, depigment agent, and as other chemical intermediates [1–3]. HQ removes naturally in wheat products, coffee, tea, fruits such as cranberry, various vegetables, red wine and some beers [4–6]. Furthermore, if handled improperly, this organic pollutant poses short- and long-term damage and danger to the health of human beings and the natural environment. It could enter into the human body via the respiratory and digestive organs or by direct contact to affect skin, mucous membranes, digestive, hepatic and renal systems. High concentration of HQ may incur headache, fatigue, tachycardia, decompensation, kidney damage and even death. Long-term respiration in the atmosphere containing low concentration of HQ may cause coughing, dizziness, anorexia, nausea, vomiting and the pigmentation of the eye [7].

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In this respect, there is an urgent need for rapid, low cost and possibly direct methods to degrade and quantitate these pollutants. Several analytical techniques including titration, spectrophotometry and high-performance liquid chromatography (HPLC) have been used for determination of HQ [8–11]. However, these procedures are usually time-costing with complicated sample pretreatment and high costs. In comparison with the other traditional methods, the electrochemical method is relatively simple, rapid and effective, and has attracted intense and widespread attention from many scientists. Electrocatalytic oxidation of phenolic compounds was realised on a carbon paste modified with Ni(II) porphyrin adsorbed on SiO_2/Nb_2O_5-phosphate and it was applied to determine HQ and 4-aminophenol with good sensitivities [12]. An organic-phase biosensor based on paraffin/graphite modified with sweet potato tissue as the source of peroxidase was developed and used for determining HQ in cosmetic creams [13]. A simple and highly selective electrochemical method has been developed for simultaneous determination of HQ and catechol at glassy carbon electrode modified with multiwall carbon nanotubes [14].

Serum albumins, one of the most available and extensively studied proteins, are the most abundant proteins in humans and other mammals. BSA is a large globular protein with a good essential amino acid profile. It consists of 583 amino acids in a single polypeptide chain and it is an active molecule composed of a polypeptide with abundant disulfide bonds and active amino acids. It has been reported that BSA can be adsorbed on metal and other electrode surfaces and the interaction of protein with organic molecules or metal substrates has been investigated [15–18].

As is known to us, a chemically stable covalent linkage between the nitrogen atom of the amine group in BSA and the edge plane sites of the carbon electrode surface can be formed. However, to the best of our knowledge, the covalent attachment of BSA onto the surface of solid electrode is rarely reported. In this study, BSA is covalently linked to GCE surface through C–N bond by the electrochemical method to form a well-arranged molecular layer. The enhanced electron transfer process of HQ at BSA/GCE is investigated, so that a method for determination of HQ with high sensitivity based on this enhancement is developed.

2. Experimental

2.1 *Reagents*

BSA purchased from Bovogen Biological Pty Ltd (Australia) was used as received. Hydroquinone and the other chemicals were of analytical grade and were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The supporting electrolyte was 0.2 M acetate buffer solution (ABS) (pH 5.0). All solutions were prepared with doubly distilled water.

2.2 *Immobilisation of BSA onto GCE and its electrochemical characterisation*

A GCE was polished with 0.3 μm and 0.05 μm alumina slurry with microcloth, and sonicated thoroughly in ethanol and doubly distilled water, respectively. Then GCE was activated in 0.5 M H_2SO_4 solution by cycling between 0 V and +1.6 V at a sweep rate of $100\,mV\,s^{-1}$ until stable cylic voltammograms (CV) were observed. The pretreated GCE was immersed in 10 mM BSA solution and cycled from −0.6 V to +1.5 V at a sweep rate of 50 mV s^{-1}, rinsed with buffer solution and distilled water, then dried in nitrogen atmosphere. We denoted this electrode as BSA/GCE, which was characterised by cyclic voltammetry and electrochemical impedance spectroscopy.

2.3 *Electrochemical measurements*

The electrochemical redox behaviour of HQ on BSA/GCE was investigated by cyclic voltammetry. Differential pulse voltammetry was used to the selective determination of HQ from catechol and amperometry was used for the high sensitive determination of HQ. All the electrochemical experiments were carried out with a CHI660A electrochemical workstation (CH Instrument, USA) in a three-electrode system at room temperature (about 25°C). A platinum wire was employed as the counter electrode, and a saturated calomel electrode (SCE) as the reference electrode. All potentials are referred vs. SCE unless otherwise specified. Prior to each experiment, pure nitrogen was bubbled through the solution for 15 min and afterwards an inert atmosphere was maintained inside the electrochemical cell by flushing with nitrogen gas.

3. Results and discussion

3.1 *Modification of GCE with BSA and its electrochemical characterisation*

The modification of BSA on GCE was carried out by several cycles of potential cycling between −0.6 and 1.5 V at 50 mV s^{-1}. The multi-cycle cyclic voltammograms (CVs) are shown in Figure 1. No redox peak is observed on the first scan. Interestingly, an oxidation peak at about +1.1 V can be seen in the next cycle, but no reduction peak appears in the reverse cathodic sweeping, indicating that BSA was immobilised on GCE surface. The oxidation peak near +1.1 V increases from cycle to cycle. The CV almost reaches a

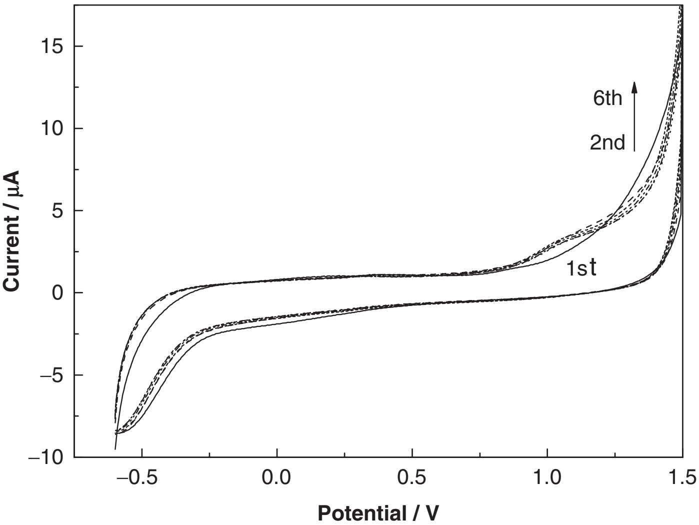

Figure 1. The multi-cycle CV of GCE in ABS containing 10 mM BSA. Sweep rate: 50 mV s^{-1}. Note: (A–F) The first to sixth cycle, respectively.

steady pattern after 40th scan, so that the optimal cycle number is estimated as 40. According to Porter and Lin, the carbon-nitrogen links can be formed and BSA was covalently attached on GCE surface [19,20].

One of the most useful electron transfer indicators, $Fe(CN)_6^{3-}$, was employed to electrochemically characterise the resulting BSA/GCE. The CVs of 5 mM $Fe(CN)_6^{3-}$ obtained at (1) bare GCE and (2) BSA/GCE are depicted in Figure 2(a). We can see that the peak current of the $Fe(CN)_6^{3-}$ decreases obviously and the peak–peak separation increases when the BSA is attached to the electrode surface. The decrease of current may be ascribed to the electrostatic repulsion between the negatively charged BSA and $Fe(CN)_6^{3-}$, which results in the block to the electron transfer of $Fe(CN)_6^{3-}$ at BSA/GCE surface.

Figure 2(b) shows the electrochemical impedance spectra (EIS) of $Fe(CN)_6^{3-}$ at (1) bare GCE and (2) BSA/GCE. The typical shape of Nyquist impedance spectra includes a

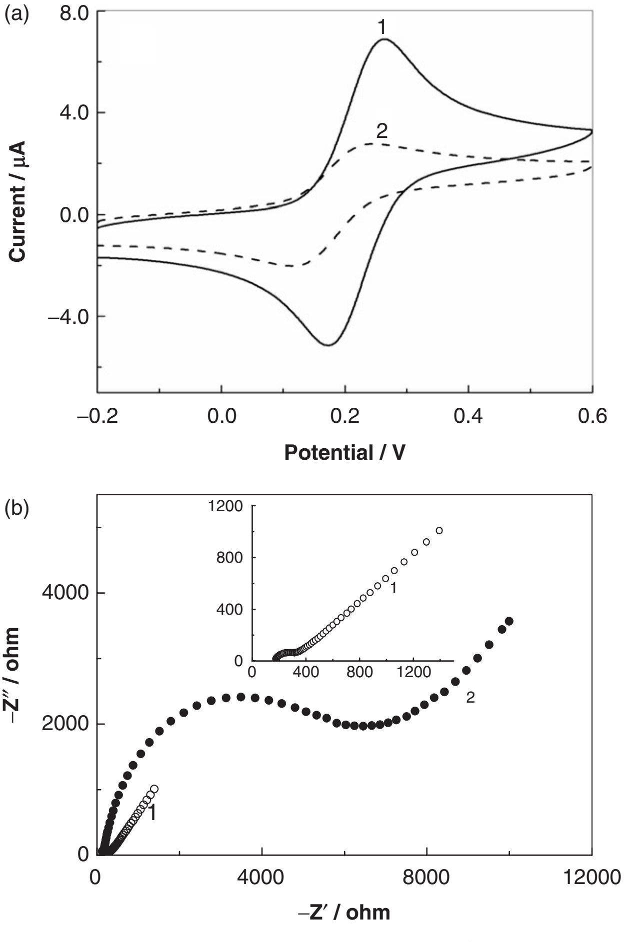

Figure 2. Cyclic voltammograms. (a) (Sweep rate: 100 mV s^{-1}) and Nyquist plots (b) (Initial potential: 0.2 V; Frequency range: $1 \times 10^{-2} \sim 1 \times 10^{5}$ Hz; Amplitude: 0.005 V) obtained at bare GCE and BSA/GCE in 5 mM ferricyanide solution.
Notes: 1 – Bare GCE; 2 – BSA/GCE.

semicircle followed by a straight line. In general, two frequency regions can be distinguished in the presence of electroactive species. The semicircle portion observed at higher frequencies corresponds to electron-transfer-limited process, while the straight line represents the diffusion-limited, electron transfer process. It is found that EIS (shown in the insert) observed at bare GCE is approximately a straight line with the slope of 1, and only a very small semicircle is obtained at high frequency section in Nyquist curve. However, a much larger semicircle owing to heterogeneous electron transfer resistance can be found at high frequency for that of BSA/GCE, indicating that charge transfer resistance is greatly increased. It is evidenced that BSA is immobilised on GCE surface by electrochemical method and forms a stable thin-layer at the interface between the electrode and solution, and the electron exchange ability on the electrode surface has become weaker, therefore, the heterogeneous electron-transfer velocity between $Fe(CN)_6^{3-}$ and GCE is greatly decreased.

An inconsistency between the current scales of voltammograms for ferricyanide with charge transfer resistance may be observed. It is known that electrochemical resistance is dependent on the feature of analyte and electrode surface (including thickness of membrane growth on electrode surface, compactness of the membrane, etc.). However, the redox current depends on the redox ability of the analyte and the redox catalysis of electrode to the analyte. Therefore, decrease in redox current does not match the increase of charge transfer resistance for ferricyanide on bare GCE and BSA/GCE.

3.2 *The enhanced redox process of HQ by BSA*

CVs of HQ at bare GCE and BSA/GCE are shown in Figure 3. Compared with that obtained at bare GCE (Figure 3a), we can see that a pair of enhanced redox peaks appear at BSA/GCE (Figure 3c). This pair of redox peaks is attributed to the electrochemical oxidation and reduction of HQ, because no peak is obtained at BSA/GCE in the buffer solution without HQ (Figure 3d). The peak-to-peak separation (ΔE_p) of HQ obtained at

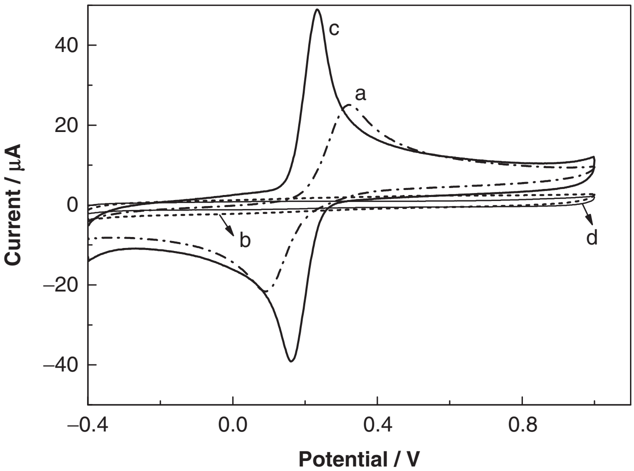

Figure 3. The impact of BSA on the Cyclic voltammograms of 1 mM HQ.
Notes: (a) Hydroquinone on bare GCE; (b) GCE in acetate buffer solution; (c) Hydroquinone on BSA/GCE; and (d) BSA/GCE in acetate buffer solution.

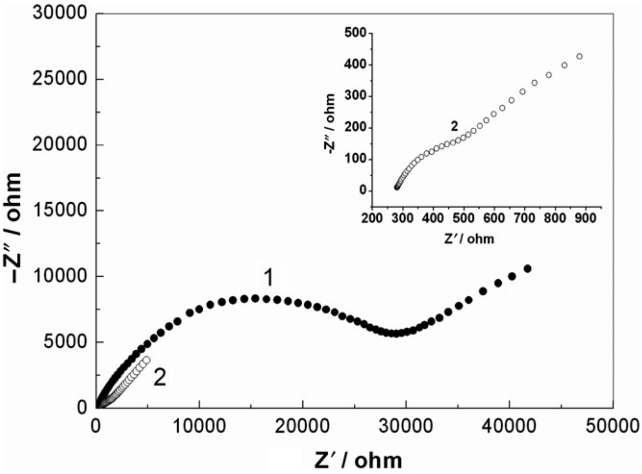

Figure 4. Nyquist plots obtained at GCE and BSA/GCE in 1 mM HQ.
Notes: Initial potential: 0.2 V; Frequency range: $1 \times 10^{-2} \sim 1 \times 10^{5}$ Hz; Amplitude: 0.005 V; 1 – Bare GCE; 2 – BSA/GCE.

BSA/GCE is 82 mV, while that observed at bare GCE is 239 mV, as demonstrated in Figure 3. The redox reversibility is enhanced and the peak current is greatly increased, indicating that the electron transfer between HQ and electrode is facilitated by the attached BSA. It suggests that BSA may act as a kind of functional bridge molecule between HQ and GCE, although it blocks the electron transfer between $Fe(CN)_6^{3-}$ and GCE, as mentioned above. As we know, the isoelectric point of BSA is 4.7, in acetate buffer solution of pH = 5.0, the protoned HQ may be positively charged and could adsorb onto negatively charged BSA immobilised on GCE surface by electrostatic interaction, resulting in a decrease in distance between HQ and electrode surface, and subsequently, an enhancement in the electron transfer between HQ and electrode is obtained.

This explanation is also confirmed by EIS, as shown in Figure 4. The charge-transfer resistance (R_{ct}) of HQ obtained at bare GCE is relatively large, (i.e., $>10^4\,\Omega$). However, the R_{ct} is greatly decreased (about $10^2\,\Omega$) with the adsorption of BSA onto GCE surface, which is again indicative of that BSA can promote electron transfer between HQ and GCE.

3.3 *Kinetics of electrode reaction of HQ*

CVs of HQ obtained on bare GCE and BSA/GCE at different potential sweep rates are depicted in Figure 5. Both the peak currents and the peak potential separation (ΔE_p) between the cathodic and anodic peaks increase as the sweep rate increases. The linear relationship between the redox peak current and sweep rate in the range of 10–100 mV s^{-1} (Figure 6) indicates that the electron transfer between HQ and bare GCE and BSA/GCE is a surface-controlled quasi-reversible process. However, it can be seen a non-zero intercept exists in plots of redox peak current versus sweep rate. Maybe this is caused by the quasireversibility of electrochemical reaction. This phenomenon can also be observed in researches reported by Vaze [21] and Chen and colleagues [22].

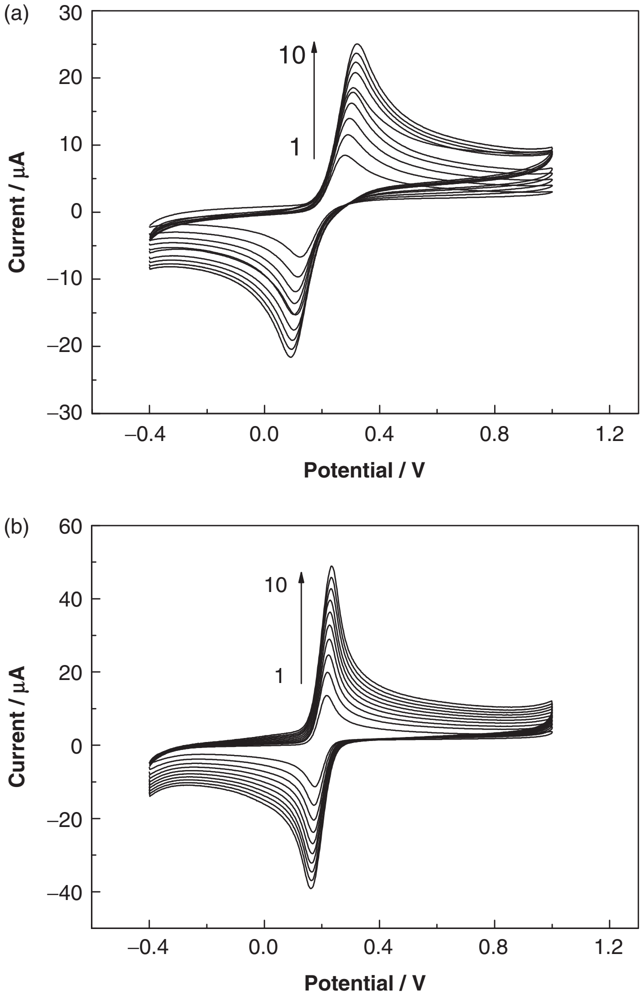

Figure 5. Cyclic voltammograms of 1 mM HQ on bare GCE (a) and BSA/GCE (b) with different potential sweep rates.
Notes: 1 ~ 10 sweep rates 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 mV s^{-1}.

From the data demonstrated in Figure 5(a), according to the Laviron equation [23], when the peak-to-peak separation is larger than 200 mV n^{-1}, the relationship between the peak potential E_p and the sweep rate can be expressed by Equation (1):

$$E_p = f(\lg \upsilon) \tag{1}$$

For the cathodic peak, the slope value is $-2.3RT/\alpha nF$, and for the anodic peak, $2.3\,RT/(1-\alpha)nF$. From the results shown in Figure 7, α can be obtained. And k_s, the standard rate constant of reaction, is expressed as

$$\lg k_s = \alpha \lg(1-\alpha) + (1-\alpha)\lg\alpha - \lg\frac{RT}{nF\upsilon} - \alpha(1-\alpha)\frac{nF\Delta E_p}{2.3RT} \tag{2}$$

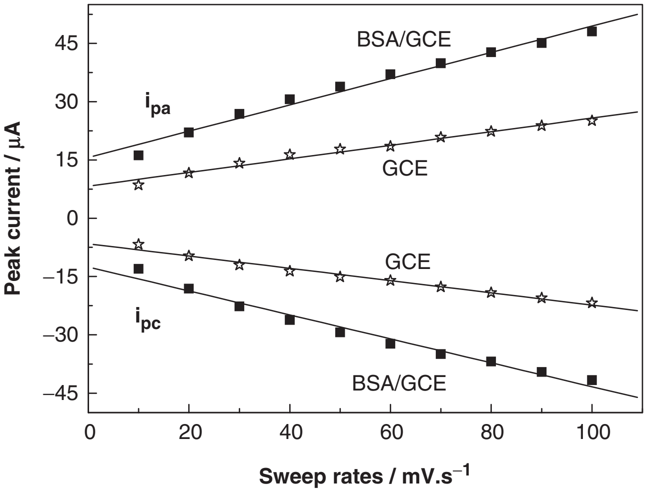

Figure 6. The plot of redox peak current against sweep rates for 1 mM HQ on bare GCE and BSA/GCE.

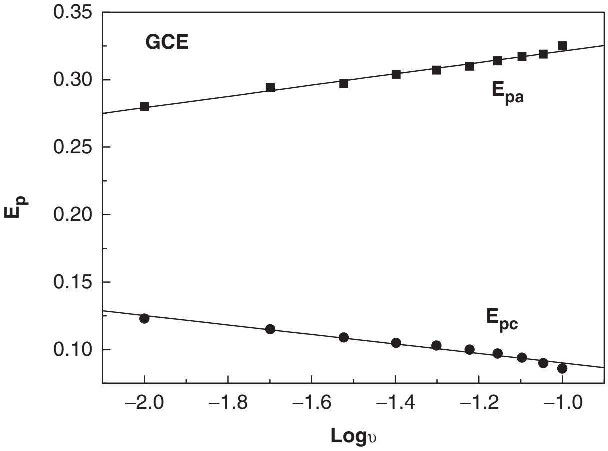

Figure 7. The plot of peak potential verse the logarithm of sweep rates for 1 mM HQ on bare GCE.

where α is the transfer coefficient, n is the number of electrons involved in the reaction, and ΔE_p is the peak-to-peak separation. The calculated value of the electron transfer rate constant (k_s) between HQ and bare GCE is 0.041 s^{-1}.

As shown in Figure 5(b), the peak-to-peak separations of the cyclic voltammograms of HQ from 10 to100 mV s^{-1} are 43, 46, 54, 59, 61, 63, 67, 72, 77, and 82 mV, respectively. The charge transfer coefficient can be supposed to be between 0.3 and 0.7, so the electron transfer rate constant between HQ and BSA/GCE is estimated to be 67.5 s^{-1} according to Laviron's model with the formula:

$$k_s = \frac{mnF\upsilon}{RT} \tag{3}$$

where m is a parameter related to the peak-to-peak separation [23].

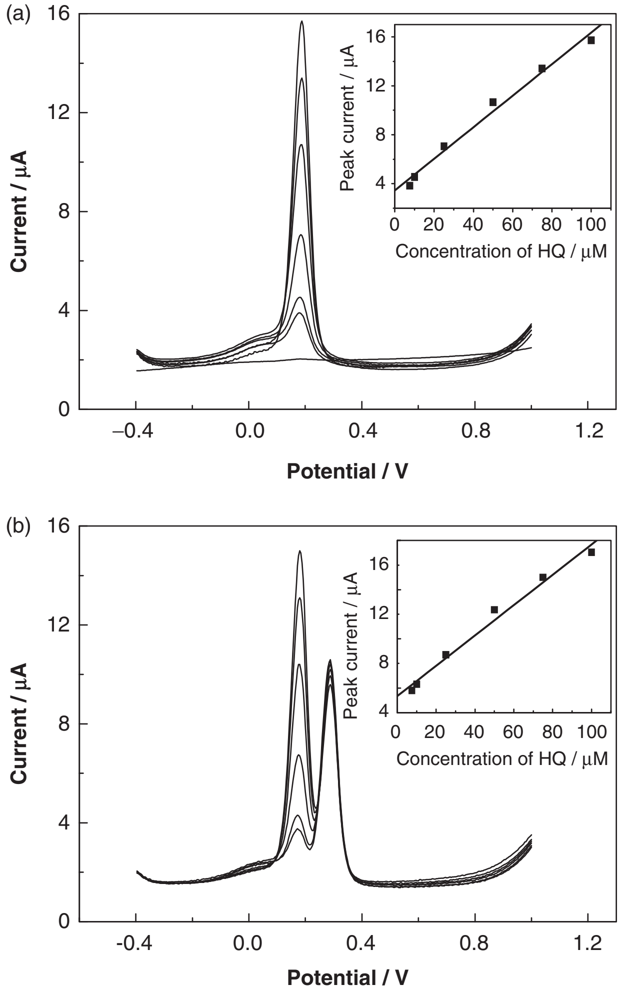

Figure 8. Differential pulse voltammograms for different concentration of HQ within the range from 7.5 μM to 100 μM (a) without and (b) with 50 μM catechol (Inset: calibration plots for HQ).

With the results, we can see that the standard rate constant k_s of HQ is increased from $0.041\,s^{-1}$ to $67.5\,s^{-1}$ through the promotion of BSA; indicating that the electron transfer of HQ at BSA/GCE surface is remarkably enhanced.

3.4 *Determination of HQ*

Based on enhanced oxidation of HQ by BSA, the application of BSA/GCE to determine HQ pollutants was further investigated. First, a usual electroanalytical method with low non-faradic current and high sensitivity and good resolution, differential pulse voltammetry, was employed. Differential pulse voltammograms (DPVs) of different concentration of HQ obtained at BSA/GCE are depicted in Figure 8(a) and the calibration plots are shown in the insert. We can see that the oxidation peak currents of HQ are linear

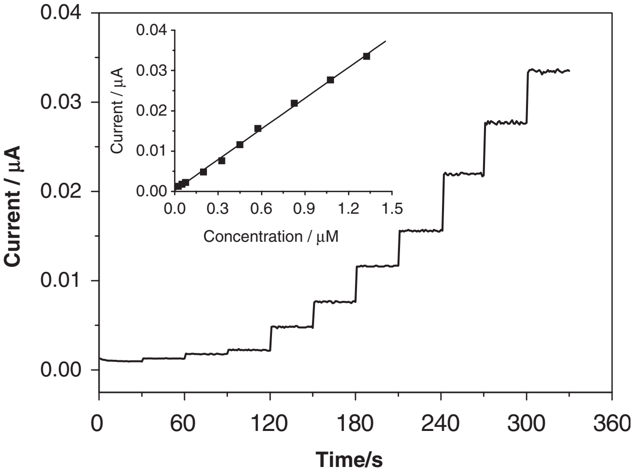

Figure 9. Current–time response curve of the modified glassy-carbon electrode on increasing the hydroquinone concentration in ABS.

with the concentrations of HQ within the range from 100 µM to 7.5 µM, and the regression equation is $i_p\,(\mu A) = 0.988 + 0.131\,C\,(\mu M)$ with the correlation coefficient of 0.996. Ten continuous measurements of 50 µM HQ showed good reproducibility with RSD of 0.091%.

The influence of a kind of interference, catechol, which is an isomer of HQ and often coexists in environment samples with HQ, was tested by analysing a series of binary mixtures of different concentration of HQ from 100 µM to 7.5 µM and 50 µM catechol. From the results shown in Figure 8(b), it is found that the oxidation peak current of HQ is still linear with its concentrations, indicating that the determination of HQ is not interfered by the presence of catechol. We could selectively determine HQ with coexisting catechol. The regression equation obtained is $i_p\,(\mu A) = 3.347 + 0.124\,C\,(\mu M)$ with the correlation coefficient of 0.992.

A more sensitive technique was used to evaluate HQ. Figure 9 shows a typical current-time plot of the BSA/GCE electrode after the successive addition of aliquots of HQ to the stirring ABS solution. With the increase of the concentration of substrate, the modified electrode responds rapidly to the substrates and achieves 95% of the steady-state current within 30 s. A linear relationship between the current response and the HQ concentration ranging from 2.5×10^{-8} M to 1.325×10^{-6} M is obtained. The calibration plots of response current and the concentration of HQ are shown in the Figure 9 insert. The corresponding linear equation is $i\,(\mu A) = 0.0003 + 0.0254C\,(\mu M)$ and the correlation coefficient is 0.9989. The detection limit (three times the signal blank/slope) is 8.6×10^{-9} M. The results are better than the range of $1.0 \times 10^{-7} \sim 1.375 \times 10^{-4}$ M and detection limit of 1.5×10^{-8} M observed on a hydroquinone biosensor based on the immobilisation of laccase on the surface of magnetic core-shell nanoparticles modified carbon paste electrode [24] and upper and lower ranges of 2×10^{-3} M and 2×10^{-6} M respectively with detection limits of 2×10^{-7} M HQ as reported by using microdialysis sampling coupled with high-performance liquid [25], as the comparative results shown in Table 1. The results show that BSA/GCE may be used to determine HQ with simplicity and high sensitivity.

Table 1. Comparison of BSA method and other methods.

	Responding time	Linear concentration range of hydroquinone	Detection limit
BSA/GCE	30 s	$2.5 \times 10^{-8} \sim 1.325 \times 10^{-6}$ M	8.6×10^{-9} M
Laccase/Fe_3O_4–SiO_2/CPE [24]	60 s	$1.7 \times 10^{-7} \sim 1.375 \times 10^{-4}$ M	1.5×10^{-8} M
Microdialysis/HPLC	160 s	$2 \times 10^{-3} \sim 2 \times 10^{-6}$ M	2×10^{-7} M

4. Conclusion

BSA covalently attached to GCE enhances the electron transfer between HQ and GCE by about 1,600 times, indicating that BSA immobilised onto GCE functions as a favourable molecular bridge between HQ and GCE. The sensing oxidation peak currents of HQ are linear with the HQ concentrations within the range of 2.5×10^{-8} M to 1.325×10^{-6} M, and the detection limit achieves 8.6×10^{-9} M (3N/S). The selective determination of HQ and catechol is also realised at this BSA/GCE. This study provides a simple and rapid method for selective and sensitive determination of environmental pollutants.

Acknowledgements

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