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The electrochemical polymerization of methylene green and its electrocatalysis for the oxidation of NADH

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Abstract

A poly(methylene green) thin film modified electrode was successfully prepared by means of electrochemical polymerization, which was carried out with cyclic voltammetric sweeps between −0.4 and +1.2 V (vs. SCE) at a glassy carbon base electrode in methylene green aqueous solution. The pH value of the solution notably affected the process of polymerization and the characteristics of the film. Compared with modified electrodes obtained by adsorption of methylene green in aqueous solution, this polymer modified electrode exhibited a higher stability and better catalytic activity for NADH. The peak potential shifted towards a positive direction for about 180 mV in neutral medium. An obvious catalytic current could be observed for the oxidation of NADH, moreover, the polymer could reduce the oxidation overpotential of NADH by about 400 mV. The electrocatalytic current was proportional to the concentration of NADH, and a good linear relation from 0.5 to 10.0 mM with a correlation coefficient of 0.996 was obtained.

Keywords: Catalytic methods; Poly(methylene green); Electropolymerization; Electrocatalysis; NADH

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1. Introduction

Recently, the electrochemical oxidation of NADH in an aqueous solution is of increasing interest in developing amperometric biosensors for NAD$^+$-dependent dehydrogenases. The direct oxidation of NADH, whose normal formal potential is at −0.560 V vs.SCE at pH 7.0, has been extensively studied at various kinds of electrodes modified with different redox mediators such as catechols, quinones, redox dyes, metal complexes and organic conducting salts [1–6]. These mediators exhibit a significant electrocatalytic activity for the oxidation of NADH and reduce the overpotential of the electrode reaction.

In most cases, these mediators are employed by means of adsorption on the electrode surface forming a monolayer or by covalently bonding them to the functions of pretreated electrodes [7–9].

Polymer modified electrodes of the coated-wire type with a three-dimensional distribution of mediators should be preferable for the electrocatalytic oxidation of NADH because there are much more electrocatalytic reaction centers, i.e. volume effects [10], in the film compared with a monolayer membrane. Some polymers such as poly(3-

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methylthionphene), poly(thionine), poly(indole-5-carboxylic acid) and poly(methylene blue) have shown obvious electrocatalytic activity for the oxidation of NADH [11–14].

One of the most promising methods to modify an electrode is electropolymerization which can produce an adherent and conducting polymer film, and also some compounds with electrocatalytic activity can simultaneously be entrapped in conducting polymers during electrodeposition [15]. However, this method often suffers from the lack of long-term stability owing to ion exchange with the external electrolyte. A preferable but yet little examined technique would be to use a conducting polymer which is inherently electrocatalytic. Here, we prepared a new polymer modified electrode and its good catalytic activity for the oxidation of NADH was described.

2. Experimental

2.1. Reagents and materials

Methylene green(chemical grade, the XinZhong Chemical Factory, China) was used after being crystallized three times in double-distilled water. NADH was obtained from Sigma without further purification. The standard buffers were prepared with 0.05 M KH_2PO_4 and 0.05 M K_2HPO_4 containing 0.1 M potassium chloride and the pH value was adjusted by 0.02 M potassium bicarbonate. All solutions were prepared using double-distilled water.

2.2. Apparatus

Cyclic voltammetric experiments were performed using a traditional three-electrode cell. The reference electrode and counter electrode were a saturated calomel electrode (SCE) and a platinum wire electrode, respectively. A BAS 100B electrochemical Analyzer was used for all electrochemical experiments at 25°C.

2.3. Electrode modification

The glassy carbon electrode with a diameter of 5 mm was used as the working electrode. Before use, it was polished with sandpaper and 0.05 μm aluminium slurry followed by sonication in double-distilled water for 5 min. Then, the electrode was pretreated by continuous cyclic sweeps from −0.5 to +1.0 V(vs SCE) at 50 mV/s in phosphate buffer(pH 5.7) until a constant background was obtained.

Poly(methylene green) modified electrodes were prepared by cyclic sweeping at a glassy carbon electrode from −0.5 to +1.2 V at 50 mV/s in phosphate buffer containing 5.0×10^{-4} M methylene green. The film thickness depended on the number of sweeps. The modified electrodes were rinsed with phosphate buffer and stored at 4°C in the same solution for further use.

3. Results and discussion

3.1. Electrochemical polymerization of methylene green

In an aqueous solution containing methylene green, the cyclic voltammograms showed two couples of redox peaks at −0.105 V and −0.256 V for cathodic process and −0.052 V and −0.214 V for anodic process at a glassy carbon electrode, respectively. These peaks were formed by a two step single electron electrode reaction of methylene green. In previous works, we found that methylene green could be adsorbed on the surface of pyrolytic graphite electrodes and act as an electron transfer mediator of NADH at −0.13 V(vs. SCE) [16]. Dong et al. also reported that the same electrode process occurred at the platinum electrode surface [17]. Fig. 1 shows that the adsorption process of methylene green at the glassy carbon electrode on cyclical sweeping is between −0.5 and +0.3 V. With an increase in the number of cycles, more and more methylene green molecules were adsorbed on the surface of the electrode, which led to an increase in the peak current. After a few cycles the adsorption gradually reached an equilibrium. Furthermore, with the increase of pH, both the cathodic (E_{pc}) and the anodic (E_{pa}) peak potential shifted in a negative direction. The slopes of $E^{0\prime}(=(E_{pa}+E_{pc})/2)$ vs. pH plots for the two redox couples were about 51 mV/pH which were less than that of Nernstian. Thus, it suggested that the electrochemical process should proceed with the participation of one hydrogen ion in each step.

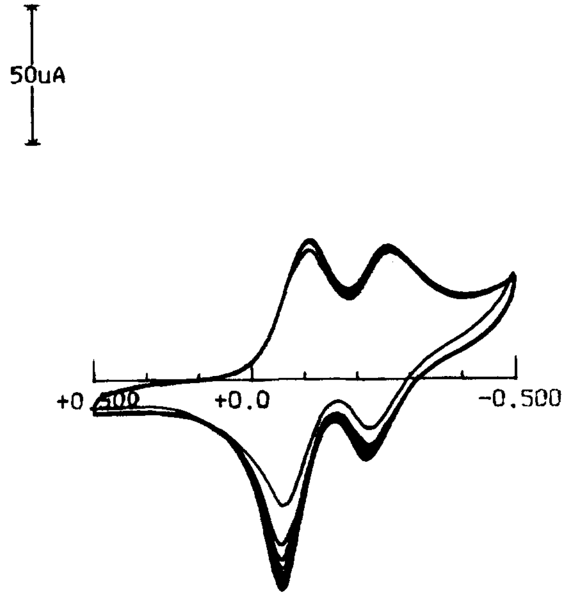

Fig. 1. Cyclic voltammograms of 5.0×10^{-4} mM methylene green in phosphate buffer (pH 7.0) between −0.5 and +0.3 V (vs. SCE); Scan rate, 50 mV/s; Scan cycle, 10.

When the anodic sweep limit was extended to +1.2 V, a shoulder appeared in the anodic process at about +0.83 V, no corresponding cathodic peak was observed, indicating that the second oxidation product is followed by a chemical reaction. Fig. 2a shows the cyclic voltammograms of methylene green between −0.50 and +1.20 V. During this process, the cathodic peaks moved towards a more positive potential and indicated a wide-range redox response, which had been observed during the polymerization process before [18]. The wide-range redox response might reflect any molecular interaction between the redox films at the electrode surface. The polymerization of some molecules without any amine groups such as thiophene, pyrrole showed a monomer's conjunction by the radical reaction within the process of electrochemical polymerization [19,20]. The same result could be obtained for the polymerization mechanism of methylene green. However, when the switching potential limit was less than +0.8 V, no change of peak currents and peak potentials could be observed, so, this process should be an adsorptive one.

In order to study the effect of experimental conditions on the formation of the polymer, we

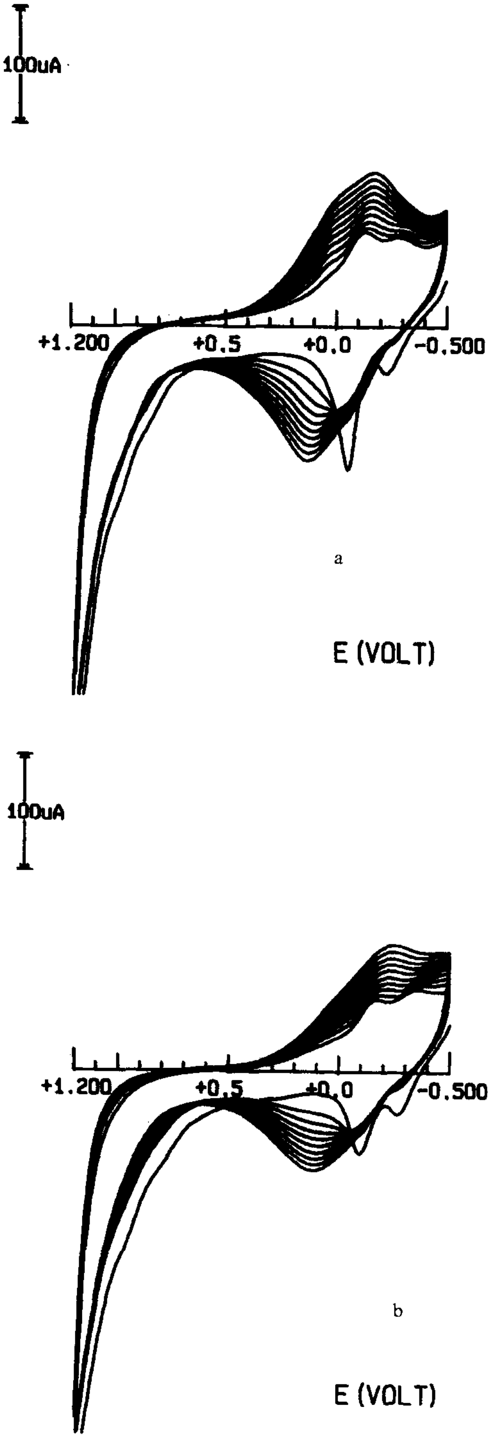

Fig. 2. Cyclic voltammograms of 5.0×10^{-4} mM methylene green in phosphate buffer between −0.5 and +1.2 V (vs. SCE); Scan rate, 50 mV/s; Scan cycle, 10; (a) pH=7.0, (b) pH=8.76.

polymerized methylene green at a fresh electrode and an adsorbed one by cycling in methylene green solution between +0.5 and −0.5 V, respectively. At a fresh electrode the cyclic voltammogram of the first cycle showed two clear couples of redox peaks, which are produced by the redox reaction of the monomer of methylene green (Fig. 2a), but in the following cyclic voltammograms the two couples of peaks disappeared, and at the same time, wide range peaks in more positive direction was obtained due to the polymerization. Furthermore, the peak currents quickly increased with an increase in the number of cycles. However, the cyclic voltammograms at an adsorbed electrode showed distinct redox peaks of monomers even if the electrode had been swept in methylene green solution in the range of +1.20 to −0.50 V for a few cycles. After continuously sweeping for longer time, the redox peaks gradually decreased and finally a wide-range polymerizing peak occurred, indicating that the amount of monomer of methylene green on the electrode surface gradually decreased (Fig. 3). Finally only monomers of methylene green was oxidized on the polymer surface. In contrast, there is no direct oxidation of monomer at a fresh electrode after the second cycle, the polymer film increased quickly.

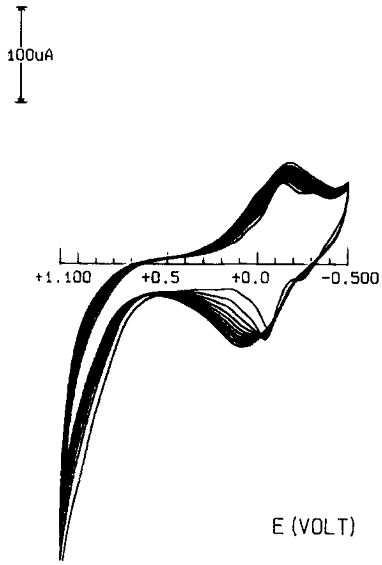

Fig. 3. Cyclic voltammograms of 5.0×10^{-4} mM methylene green in pH 7.0 phosphate buffer between −0.5 and +1.2 V (vs. SCE) at an electrode modified by adsorption. Scan rate, 50 mV/s.

The process of polymerization of methylene green was also related to the pH value of buffer solution. At various pH values, polymer films of different structures could be obtained. As for the electrochemical polymerization of methylene blue, no polymerization for methylene blue could be obtained at a platinum electrode in acidic solution [18] because a reverse peak appeared corresponding to the reduction of the second cation. But, in alkaline solution the polymerization of methylene blue could be obtained at a glassy carbon electrode due to the presence of hydroxide ions [21]. In our experiments, both neutral and alkaline conditions could produce a good polymerization for methylene green at a glassy carbon electrode (e.g. Fig. 2b for alkaline solution). Compared with methylene blue, methylene green possessed an additional $-NO_2$ group, which reduces the attraction of hydroxide ions for methylene green. In general, the influence of hydroxide ions could be understood in terms of both nucleophilic catalysis and a shielding effect for methylene blue. Consequently, it can be concluded that the N-heteroatom participated in the electrochemical polymerization of Methylene green.

Under neutral conditions, an interesting polymerization process was observed for methylene green. A shoulder appeared at +0.1 V and all peak currents increased on subsequent sweeps. We can control the film thickness according to the number of cycles. The monomer peak has hardly been observed. The polymerization process is rapid and the surface of electrode is covered by a thin polymeric film.

3.2. Characteristics of poly(methylene green) modified electrode

Different cyclic voltammetric curves were shown in Fig. 4 when the poly(methylene green) thin film modified electrode were swept in pH 7 phosphate buffer, which are obtained under different conditions. It can be seen that there is a high base current at the modified electrode prepared in acid solution, moreover, the peak shape of the cathodic peak is distorted (Fig. 4a). However, the modified electrode prepared in neutral solution exhibits two couples of cyclic voltammetric peaks (Fig. 4b), and the peaks overlapped each other, so only a couple of peaks can be observed. When the electrode was prepared in

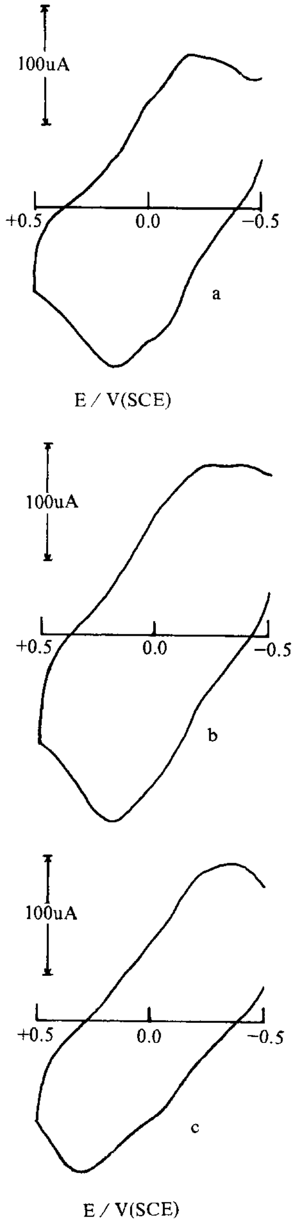

Fig. 4. Cyclic voltammograms of poly(methylene green) modified electrodes in pH 7.0 phosphate buffer. The electrodes were prepared at (a) pH 5.8, (b) pH 7.0 and (c) pH 8.76 between −0.5 and +0.5 V(vs SCE); Scan rate, 50 mV/s.

alkaline solution, not only the base current was the same as that in acid solution, but also its difference of peak potential was large (Fig. 4c). All these results indicated that the structure of the polymer film in the process of polymerization was closely related to the pH values of the solution. The participation of hydrogen ions was more easy for the film structure which was obtained in neutral buffer solution. The experiments indicated that the modified electrode prepared in neutral medium was the best. So, this modified electrode was used in the following experiments.

Fig. 5 shows the cyclic voltammograms for the polymer film at pH 7.0 with different scan rates. There were two couples of peaks appearing and the peak potentials were +0.10 V and −0.09 V for E_{pa}, +0.035 V and −0.154 V for E_{pc}, respectively. The peak current was proportional to the scan rates which indicated a surface process. According to the equation, $I_p = n^2F^2\Gamma Av/4RT$ and $Q_\Gamma = nFA\Gamma$, it can be obtained that the electron transfer number was 1 for each redox couple and the surface coverage was 4.1×10^{-10} mol/cm^2. The thickness of film is related to the surface concentration. The number of layers of electroactive redox species can be obtained from the ratio of Γ/Γ_{mono}. Thus, the thickness of the film can be estimated by the molecular size of MG and its layer numbers. It has been assumed that the size of MG is identical with that of methylene blue (MB) due to the similar structures. The size of MB and Γ_{mono} is

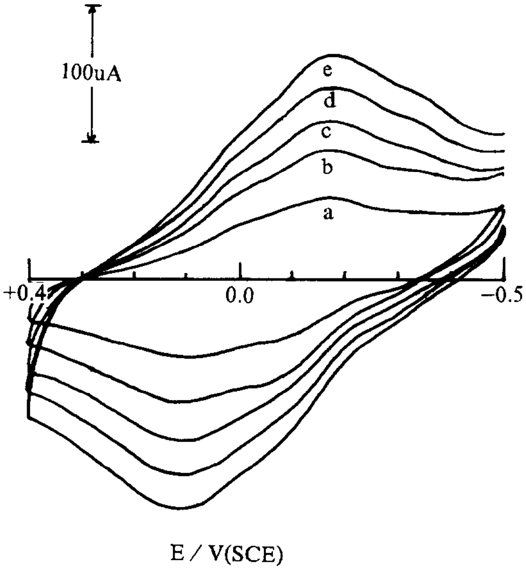

Fig. 5. Cyclic voltammograms of poly(methylene green) modified electrode prepared at pH 7.0 (a) 10 mV/s, (b) 30 mV/s, (c) 50 mV/s, (d) 70 mV/s and (e) 90 mV/s.

0.8 nm^2 and 2.0×10^{-10} mol/cm^2 respectively which has been obtained [22]. Therefore the thickness of this film was about 1.8 nm.

3.3. Electrochemical oxidation of NADH at poly(methylene green) modified electrode

The cyclic voltammograms of the poly(methylene green) modified glassy carbon electrode in the absence and presence of NADH solutions were shown in Fig. 6. The oxidation of NADH was accompanied by an increase of the anodic current at +0.1 V (vs. SCE) corresponding to the oxidation of the poly(methylene green) film. The observed increase of anodic current was due to the increase of reduced polymer which was regenerated by NADH during the scan, and there was an increase in the anodic region. Simultaneously, the cathodic current decreased. As for the more negative couple of peaks, no change was found. It indicated that this couple of peaks had no influence on the oxidation of NADH.

The effect of pH on the anodic current (with 0.5 mM NADH) was investigated from pH 4.5 to 9.0 (Fig. 7). The optimum pH was found to be 6.6~7.5.

The amperometric response of the poly(methylene green) modified electrode for NADH in aqueous solutions has been intensively examined. The calibration curve was obtained by measuring the steady-state current at a fixed potential of ca. 0.1 V/SCE upon the increase of NADH concentration by injecting the stock solution. The catalytic currents were proportional to the concentration of NADH in the range 0.5–10.0 mM with a correlation coefficient of 0.996, (see Fig. 8). The detection limit is 1.0×10^{-5} M. The relative standard deviation of results was 5.5% for six successive determinations at 0.5 mM NADH.

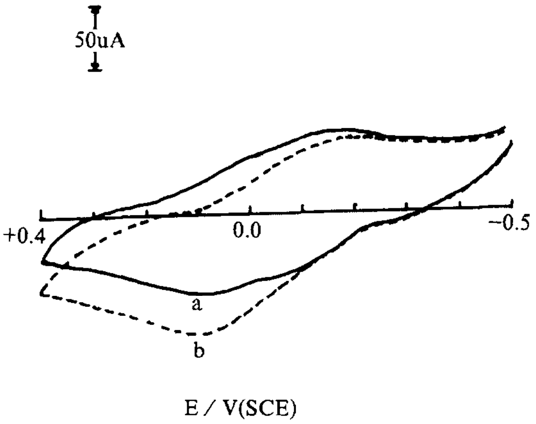

Fig. 6. Cyclic voltammograms of poly(methylene green) in (a) pH 7.0 phosphate buffer, (b) a + 2.0 mM NADH; Scan rate, 10 mV/s.

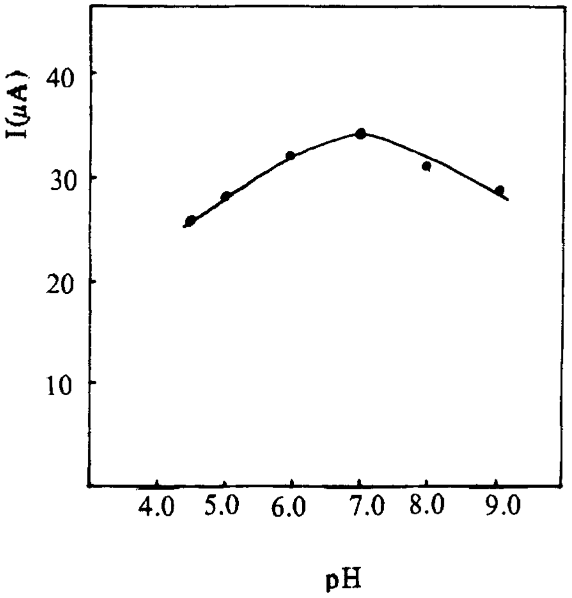

Fig. 7. The effect of pH for the catalytic oxidation of 2.0 mM NADH at a poly(methylene green) modified electrode.

3.4. The stability of the modified electrode

Fig. 9 shows the cyclic voltammograms of the poly(methylene green) modified glassy carbon electrode when it was cyclically swept in pH 7.0 phosphate buffer between −0.5 and +0.4 V (vs. SCE) for 100 cycles. It can be concluded that the peak currents did not decline when the electrode was used for a long time. There was about 10% decrease when it was continuously oxidized at +0.1 V in pH 7.0 phosphate buffer containing 2 mM NADH for 4 h partially due to the exhausted electrolysis.

An electrode was also tested for prolonged use. For this purpose, it was placed in 4°C refrigerator for 15 days. The response current for NADH did not exhibit an obvious change before and after storage. So, the modified electrode had a good stability and the

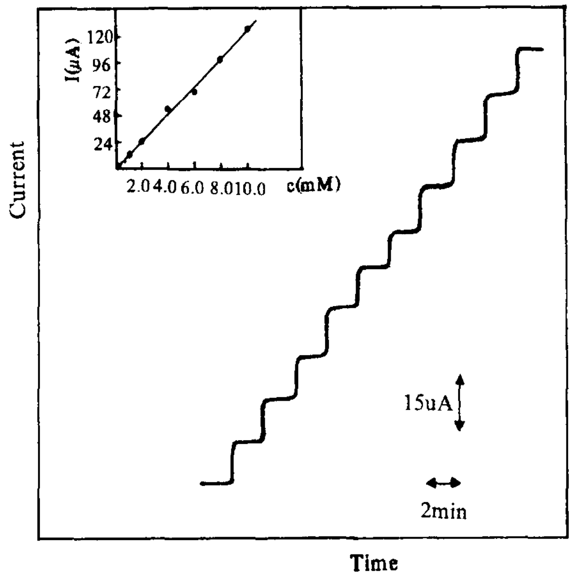

Fig. 8. Amperometric response of the poly(methylene green) modified electrode for successive additions of 0.5 mM NADH. Applied potential, +100 mV; Stirring rate, 300 rpm. The resulting calibration plot is shown(inset).

polymer strongly adheres to the surface of the electrode.

4. Conclusions

Poly(methylene green) modified electrodes could be obtained by means of electrochemical polymerization in neutral buffer solutions. This polymer modified electrode exhibited a significant catalytic activity for the oxidation of NADH, meanwhile, a good stability could be obtained for a long term use. Because of its linear catalytical response for NADH, it could be used for the determination of NADH. Moreover, an amperometric determination depending on various dehydrogenases could be developed at this modified electrode according to its polymeric conditions. This is under way in our laboratory.

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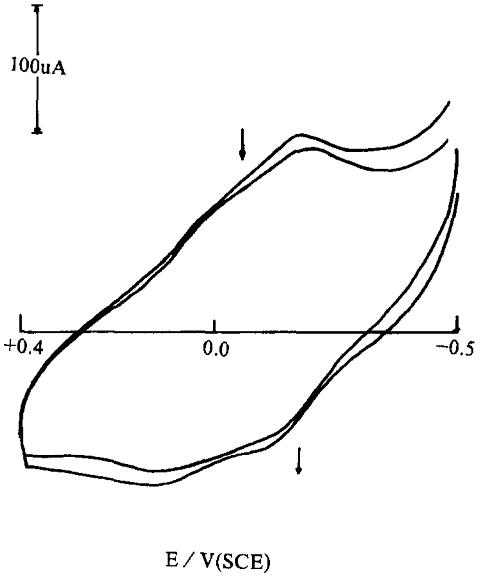

Fig. 9. The stability of poly(methylene green) modified electrode cyclic sweeping in pH 7.0 phosphate buffer (a) the first cycle; (b) the 100th cycle. Scan rate, 50 mV/s.

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