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A disposable amperometric ethanol biosensor based on screen-printed carbon electrodes mediated with ferricyanide-magnetic nanoparticle mixture

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Abstract

Magnetic Fe_3O_4 nanoparticles were prepared by co-precipitation method and used to develop a reagentless disposable amperometric ethanol (EtOH) biosensor. The electrochemical characteristics of modified processes were analyzed by cyclic voltammetry (CV) and chronoamperometry (CA). Results showed that the presence of Fe_3O_4 nanoparticles could enhance the peak currents of redox species. Moreover, the alcohol biosensor exhibited an excellent sensitivity and fast response time for EtOH with a wide linear response range from 1.0 to 9.0 mM.

Keywords: Fe_3O_4 nanoparticles; EtOH biosensor; Screen-printed carbon electrodes (SPCEs)

1. Introduction

Sensitive and selective amperometric biosensors are required for EtOH determination in fermentation and distillation processes, as well as in the clinical diagnostic analyses [1,2]. The amperometric EtOH biosensing response is based on the oxidation of NADH, which was generated in the enzymatic reaction of ethanol with NAD^+/YADH system, by a mediator (e.g. ferricyanide, Ferri) [2–5]. The overall electrochemically biosensing scheme is as shown in Fig. 1. However, the YADH-based EtOH biosensors suffer rapid activity loss [4–6] with the dependence on a soluble cofactor (NAD^+/NADH) [7], and also a high overpotential for the direct oxidation of NADH at a bare electrode surface [1,3,5].

Since nano-sized magnetic bioconjugated materials could potentially result in many unique properties such as large surface area, higher bioactivity, excellent conformation stability, and better contact between biocatalyst and its substrate [8,9], there are growing interests in their applications for construction of biosensor devices.

In this study, magnetite (Fe_3O_4) nanoparticles were prepared by co-precipitation method and a reagentless disposable amperometric biosensor, constructed by simple surface coatings of yeast YADH, NAD^+ cofactor and mixture of Ferri-Fe_3O_4 nanoparticles onto SPCEs, was developed for ethanol monitoring. The modified processes of the SPCEs and the related electrochemical characteristics were analyzed by CV. In addition, the optimal YADH loading was also evaluated by CA measurements.

2. Experimental

YADH from baker's yeast (EC 1.1.1.1, 450 U mg^{-1} solid) and β-NAD^+ were purchased from Sigma. Ferricyanide (Ferri) was obtained from Merck. All other chemicals were the analytic grade reagents and used without further purification.

CV and CA measurements were performed with Model 440 Electrochemical Workstation (CH Instruments, USA).

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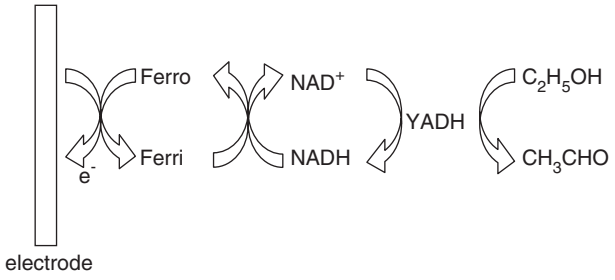

Fig. 1. NAD^+-YADH/Ferri-based ethanol-biosensing mechanism.

All measurements were performed at room temperature. The size and morphology of the Fe_3O_4 nanoparticles were observed by TEM (JEOL model JEM-2010 at 200 kV). XRD measurement was performed on an X-ray diffractometer (MAC Sience/M 21 X, Japan) using CuK_α radiation ($\lambda = 0.1542$ nm). Disposable SPCEs were supplied by Apex Bio-technol. Corp.

Magnetic Fe_3O_4 nanoparticles were prepared by co-precipitation method according to the procedure described by Liao and Chen [6]. In total, 60 mg of Fe_3O_4 nanoparticles were dispersed in 5 ml phosphate buffer solution (PBS, 0.1 M, pH 8.8) containing 0.1 M Ferri, and then the dispersed solution was sonicated for 1 h. Ten microliters of Ferri-Fe_3O_4 solution was dropped onto SPCEs and dried at 50 °C for 15 min. Eight mg of YADH and 8 mg of NAD^+ were dissolved in 4 ml of PBS ($[YADH] = 2\,mg\,ml^{-1}$, $[NAD^+] = 3$ mM). Ten microliters of YADH/NAD^+ solution was dropped onto Ferri-Fe_3O_4 SPCEs and dried at 30 °C. The disposable NAD^+-YADH/Ferri-Fe_3O_4 deposited SPCEs were ready to use.

3. Results and discussion

The Fe_3O_4 nanoparticles synthesized in this study were essentially very fine and monodisperse with a mean diameter of ca. 9.8 nm from TEM image (not shown). In addition, six characteristic peaks for Fe_3O_4 ($2\theta = 30.72$, 35.94°, 43.74°, 54.12°, 57.54° and 63.20°) were observed in the XRD patterns for the Fe_3O_4 nanoparticles (data not shown). From these diffraction data, it can be inferred that a spinel structure Fe_3O_4 has been synthesized [6].

CV is a simple and easy mean to show the changes of electrode behaviour in the modification process, because the electron transfer between the solution species and the electrode must occur by tunneling through either the barrier or the defects in the barrier. Fig. 2 shows the cyclic voltammograms of the modified process of Ferri (curve a), NAD^+-YADH/Ferri (curve b), Ferri-Fe_3O_4 (curve c) and NAD^+-YADH/Ferri-Fe_3O_4 (curve d) deposited SPCEs, respectively. It could be found that the peak currents of the curves (b) and (d) were decreased comparing with curves (a) and (c), respectively. This may originate from the insulating enzyme protein layer on the electrode that retards the electron transfer between redox probe and electrode surface. After Fe_3O_4 nanoparticles being deposited on the SPCEs, the peak currents were greatly increased. It might be suggested that the Fe_3O_4 nanoparticles could provide a favorable micro-environment for the enzyme to directly transfer electrons with electrodes [10].

CA measurements were examined with YADH loading on the SPCEs as a parameter. Amount of YADH loading was varied in the range from 0.002 to 0.050 mg solid. The response current of the modified electrode was significantly dependent on the amount of enzyme loaded on the SPCEs, as shown in Fig. 3. An increase in the response current was observed up to 0.020 mg. Therefore, the loaded enzyme in further experiments was selected with 0.020 mg YADH for each SPCEs-test strip.

The NAD^+-YADH/Ferri-Fe_3O_4-based biosensors showed an excellent sensitivity for ethanol in a wide linear response range (Fig. 4). The calibration curve was obtained from 1 to 9.0 mM of ethanol in 0.1 M PBS at pH 8.8 and

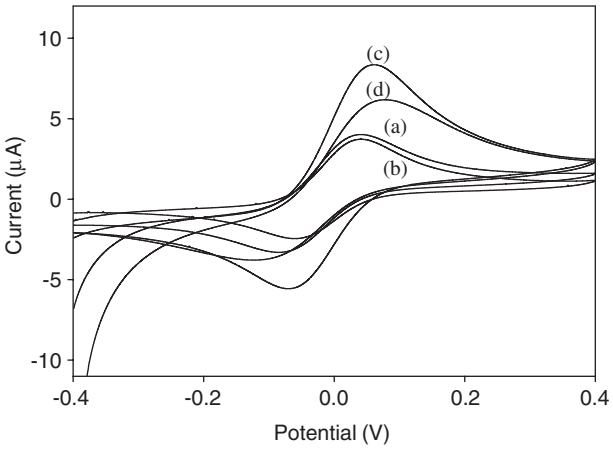

Fig. 2. Cyclic voltammograms of (a) Ferri, (b) NAD^+-YADH/Ferri, (c) Ferri-Fe_3O_4 and (d) NAD^+-YADH/Ferri-Fe_3O_4 deposited SPCEs, respectively, in PBS (0.1 M, pH 8.8). Scan rate: $10\,mV\,s^{-1}$.

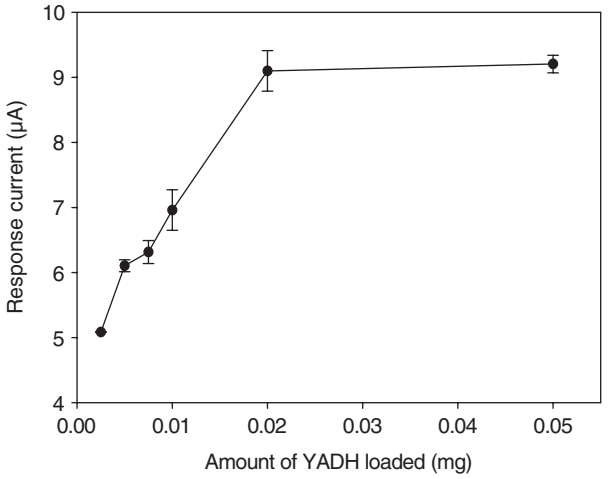

Fig. 3. Influence of the amount of YADH loaded on the response current of NAD^+-YADH/Ferri-Fe_3O_4 deposited SPCEs. Applied potential: 0.2 V.

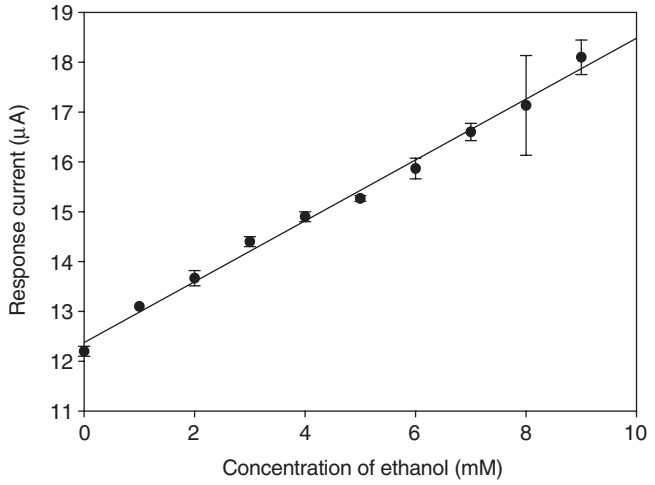

Fig. 4. Calibration curve for ethanol obtained from CVs by NAD^+-YADH/Ferri-Fe_3O_4-based biosensors.

the following linear equation is obtained: Response current (μA) = 0.61 [ethanol] + 12.38, $R^2 = 0.9929$.

4. Conclusions

Fe_3O_4 nanoparticles were successfully prepared by co-precipitation method and they were used to develop a disposable, reagentless biosensor for alcohol determination in a simple and effective way. It was found that using Fe_3O_4-deposited SPCEs, the response currents of redox species by CV measurement could be enhanced. Also, the NAD^+-YADH/Ferri-Fe_3O_4-based biosensor showed an excellent sensitivity (0.61 $\mu A\,mM^{-1}$) and fast response time (20 s) for EtOH in 0.1 M PBS (pH 8.8) with a wide linear response range from 1.0 to 9.0 mM.

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