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Analytical Methods

An amperometric sensor based on Prussian blue and poly(*o*-phenylenediamine) modified glassy carbon electrode for the determination of hydrogen peroxide in beverages

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ABSTRACT

An amperometric sensor based on Prussian blue (PB) and poly(*o*-phenylenediamine) (POPD) modified glassy carbon electrode (GCE) was developed for the determination of hydrogen peroxide (H_2O_2). The PB film was electrodeposited onto the GCE surface by amperometry, while the POPD film was formed on the top of PB layer by cyclic voltammetry. It was found that the POPD film remarkably improved the stability and selectivity of PB-based sensor. Under the optimised conditions, the developed electrode demonstrated a wide linear range from 0.1 μM to 0.12 mM with the detection limit of 0.05 μM. Furthermore, the developed electrode was applied for the amperometric determination of H_2O_2 in 10 different commercial beverages. The pretreatment of the beverage samples was the adjustment of pH value. Experimental results showed that the proposed electrode could be a useful tool to detect H_2O_2 in aseptically packaged beverages.

1. Introduction

Hydrogen peroxide (H_2O_2) is the main product of oxidase catalysed reactions, and is an important parameter for monitoring the bio-processes (Kiyoshi, Tadayuki, & Yuko, 1995; Ping, Ru, Fan, Wu, & Ying, 2010). Meanwhile, H_2O_2 is also released into the environment in either large or small quantities as it is widely used in many industrial processes, for example, as the oxidising, bleaching or sterilising agent in packaged materials (Alpat, Alpat, Dursun, & Telefoncu, 2009; Mehmet, Ayşegül, & Bekir, 2004; Susana, María, & José, 2005). However, high concentration of H_2O_2 would be irritative to the skin and affect human health. Therefore, the determination of H_2O_2 is of great importance in many areas including biochemistry, food and environment (Ülfet & Güler, 1999; Zhang, Zhai, Gao, Wen, & Dong, 2008).

Several methods have been developed for the determination of H_2O_2 such as fluorescence (Luo, Yin, Gao, & Wang, 2009), chemiluminescence (Lu, Jun, & Leslie, 2009) and spectrophotometry (Sunil & Narayana, 2008). These methods, however, require complex instrumentations which are not suitable for on-site applications. The electrochemical sensors have been widely used for H_2O_2 determination due to their high sensitivity, low cost, fast response and simplicity (Upadhyay, Ting, & Chen, 2009; Zhao, Xu, & Chen, 2006). The direct detection of H_2O_2 is usually accomplished by the oxidation at anodic potential (+0.6 V versus Ag/AgCl). However, electrochemically active interfering species which are usually present in biological fluids, such as ascorbic acid and uric acid, are easily oxidised at that potential and produce an interfering current (Hoshi et al., 2001; Rami & Heidari, 2009; Salimi, Noorbakhsh, Mamkhezri, & Ghavami, 2007). One effective method is using the enzyme-based biosensors, but the enzymes are relatively expensive and unstable (Di, Zhang, Yao, & Bi, 2006; Wang, Yang, Feng, Jiao, & Li, 2009). Another promising alternative is to explore the electron mediator to reduce the reduction potential of H_2O_2 (de Mattos, Gorton, Laurell, Malinauskas, & Karyakin, 2000; Wang, 2005). One such mediator is Prussian blue (PB), which is known as "artificial peroxidase" and exhibits high electrocatalytic activity for H_2O_2 reduction at the low potential (Karyakin, 2001; Karyakin, Gitelmacher, & Karyakina, 1995; Karyakin, Karyakina, & Gorton, 2000). However, the PB film has a serious limitation due to its rapid desorption from electrode surface resulted in losing its catalytic efficiency (de Mattos, Gorton, Ruzgas, & Karyakin, 2000; Karyakin, Karyakina, & Gorton, 1999; Ricci & Palleschi, 2005; Vidal, Espuelas, Esperanza, & Castillo, 2004). In order to overcome this problem, polymer (Garjonyte & Malinauskas, 1999; Gerard, Chaubey, & Malhotra, 2002), ionic conductor (de Mattos & da Cunha Areias, 2005), additive (de Mattos, Gorton, & Ruzgas, 2003) and self-assembled monolayer (Vidal et al., 2004) have been employed to enhance the stability of PB film.

Recently, Hsu et al. reported an amperometric sensor based on a palladium electrode to detect H_2O_2 in beverages (Hsu, Chang, & Kuo, 2008). However, the beverage samples had to been filtered before the measurement in order to prevent the electrode fouling.

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In this work, a glassy carbon electrode (GCE) modified with PB and poly(*o*-phenylenediamine) (POPD) films was developed to determine H_2O_2 in commercially aseptic beverages. This film modified electrode was prepared by electrodepositing the PB and *o*-phenylenediamine onto the GCE surface, respectively. Results showed that the POPD layer on the top of PB film could remarkably improve the operational stability of PB film. Furthermore, the POPD layer could effectively prevented the macromolecular in the beverage samples from adsorbing to the PB film. Therefore, no filtration operation was required, and the only pretreatment for the beverage samples was the adjustment of pH value which was done by adding the 0.1 M HCl or KOH solution. According to our knowledge, it was the first time that using the PB and POPD films modified GCE (GCE/PB/POPD) to determine H_2O_2 in the aseptically packaged beverages.

2. Experimental

2.1. Reagents

All chemicals were of at least analytical grade and used without any further purification. H_2O_2 (30%, w/w) was purchased from Sigma, and the concentration of diluted H_2O_2 prepared from this material was determined by the classic potassium permanganate titration method. Solution of catalase (EC 1.11.1.6, 4000 U mg^{-1}) and *o*-phenylenediamine (OPD) were also obtained from Sigma. Ascorbic acid and uric acid were obtained from Sinopham Chemical Reagent Co., Ltd. (China). Millipore-Q (18.2 MΩ cm) water was used for all experiments. Ten aseptically packaged beverages were obtained from a local supermarket (Hangzhou, China). The components and pH value of the beverage samples were summarised in Table 1.

2.2. Preparation of electrode

Prior to the surface modification, the GCE (3 mm diameter) was manually polished with alumina powder (Al_2O_3, 1 μM) until a mirror finish was obtained, and then left in sonication for 5 min (in pure water and ethanol, respectively) to further eliminate microparticles absorbed on the electrode surface. The PB film was deposited in a solution containing 2.5 mM $FeCl_3$, 2.5 mM $K_3Fe(CN)_6$, 0.1 M KCl and 0.1 M HCl at a working potential of 0.4 V versus Ag/AgCl applied for 40 s. Then the electrode was immersed into a solution containing 0.1 M KCl and 0.1 M HCl, and electrochemically cycled for 20 times between 0.35 and −0.05 V at a scan rate of 0.05 V/s. After washed with deionised water, it was dried for 1 h at 100 °C in an oven. The electropolymerisation of POPD layer on the top of PB film was performed by cycling the applied potential from −0.05 to 0.8 V for 15 cycles at a scan rate of 0.01 V/s in a phosphate buffer solution (PBS, pH 5.5, 0.01 M) containing 5.0 mM OPD monomer. Thereafter, the developed electrode was placed into the PBS for 20 min to achieve equilibrium of the films.

2.3. Apparatus

All the electrochemical measurements were carried out on CHI 440 electrochemical workstation (CH Instruments, USA). A conventional three-electrode system, consisting of the prepared GCE/PB/POPD as the working electrode, and a saturated Ag/AgCl electrode as a reference electrode, a platinum wire as an auxiliary electrode, was employed. The amperometric measurement of the H_2O_2 response was carried out at the potential of −0.05 V versus Ag/AgCl with stirring the solution gently. The PBS (pH 5.5, 0.05 M, K_2HPO_4/KH_2PO_4) containing 0.1 M KCl was used as the electrolyte solution. All the measurements were carried out at room temperature.

2.4. Determination of H_2O_2 in real samples

The real samples analysis was performed in 10 different beverages (see Table 1). The only sample pretreatment required in all cases was the adjustment of pH value to 5.5 which was done by adding appropriate amount of 0.1 M HCl or KOH solution. Before the determination of H_2O_2 in the beverage samples, the catalase was employed to confirm whether the samples contained the endogenous H_2O_2. For the determination of H_2O_2 concentration in the beverage samples, various standard concentrations of H_2O_2 were injected into the test solution and the mixed samples were analysed using the GCE/PB/POPD. The response current was recorded when the steady state was reached. The difference between the baseline and the steady state current was used to calculate the concentration of H_2O_2.

3. Results and discussion

3.1. Electrochemistry of the GCE/PB/POPD

Fig. 1A shows the cyclic voltammograms (CVs) of the GCE/PB/POPD in the absence and presence of 3.0 mM H_2O_2 in the PBS. In the absence of H_2O_2, the reversible electrochemical behaviour of PB was observed at the GCE/PB/POPD (Fig. 1A(a)). This redox response was stable during the repetitive scans, suggesting the presence of POPD layer could improve the stability of the PB film. After

Table 1
The components and pH value of the aseptically packaged beverages.

Sample	Beverages	Components	pH Value
1	Lemon Flavour Tea	Red tea extract, sucrose, citrate, ascorbic acid, lemon juice and water	5.24
2	Green Tea	Green tea extract, sucrose, honey, ascorbic acid, citrate and water	5.37
3	Tie Guanyin Tea	Tie Guanyin Tea extract, sucrose, essence, D-sodium isoascorbiate, ascorbic acid, sodium citrate, sodium hexametaphosphate and water	6.15
4	Jasmine Tea	Jasmine Tea extract, green tea extract, sucrose, essence, sodium citrate, ascorbic acid, sodium hexametaphosphate and water	5.96
5	Ice Red Tea	Red Tea extract, sucrose, fructose syrup, citrate, sodium citrate, essence, ascorbic acid, caramel and water	3.52
6	Apple Juice	Apple Juice concentrate, sucrose, citrate, malic acid, carboxymethylcellulose sodium, essence, caramel and water	3.20
7	Pineapple Juice	Pineapple Juice concentrate, sucrose, sodium chloride, citrate, essence, xanthan, pectin, carboxymethylcellulose sodium, carotene and water	2.98
8	Honey Peach Juice	Peach Juice concentrate, sucrose, citrate, essence, D-sodium isoascorbiate, carotene, carboxymethylcellulose sodium and water	3.12
9	Grape Flavour Soft Drink	Sucrose, citrate, sodium benzoate, sodium hexametaphosphate, citrine, neotame, essence and water	4.22
10	Watermelon Flavour Soft Drink	Watermelon juice concentrate, sucrose, citrate, citrine, sodium benzoate, sucralose, essence and water	3.98