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Specific Determination of Hydrogen Peroxide With A Catalase Biosensor Based on Mercury Thin Film Electrodes

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A biosensor for specific determination of hydrogen peroxide was developed using catalase (EC 1.11.1.6) in combination with a mercury film electrode. Catalase was immobilized with gelatine by means of glutaraldehyde on the electrode surface. The biosensor response was monitored by following the reduction peak of dissolved oxygen at around -0.24 V. The peak current changes upon addition of hydrogen peroxide and gives a linear response in a concentration range of 1-50 x 10^{-6} M within a response time of 3 minutes.

Introduction

Peroxides exhibit bleaching, oxidizing and catalytic abilities, hence their widespread use as industrial reagents. The environmental and health problems associated with the scale of use of this material are enormous. H_2O_2 is the main bleaching agent in detergents, pulp and paper, cellulose, domestic bleach, textiles and wine cork manufacturing and also in the food industry. It is used in small quantities as a mild disinfectant and antiseptic in pharmaceutical and cosmetic products. Some of these products, such as toothpaste, teeth whitener and mouthwash are for oral application, and the amount of H_2O_2 content is critical in the formulations[1,2]. Most of the analytical techniques for peroxide determination, such as chromatographic, polarographic, chemical reduction, colorimetric and photometric methods are generally time-consuming and not very suitable for routine or on-line analysis[3,4]. Amperometric methods have been used for its determination but the major drawback of these methods is that the electrode surface can be subjected to other redox reactions. Therefore, more selective methods based on amperometric biosensors have been developed. Many oxidase enzymes based amperometric biosensors were investigated for the determination of hydrogen peroxide since it is an end product of the biologically important substrates. A biosensor prepared by immobilizing the catalase on a dissolved oxygen probe was reported to give a linear response in a concentration range of 10^{-5} - 10^{-3} M[5]. Another catalase based biosensor was prepared by immobilizing within gelatine by means of glutaraldehyde and fixing on a pre-treated teflon membrane that served as an enzyme electrode[6].

According to the reaction given below, hydrogen peroxide is converted into oxygen by catalase dispersed in the gelatine layer and the amount of oxygen produced can then be detected amperometrically.

$$H_2O_2 \overset{Catalase}{\rightarrow} 1/2\ O_2 + H_2O$$

Maximum electrode response was obtained in pH 7.0 phosphate buffer (0.05 M) at 35 °C with a response time of 30 s. Thus, H_2O_2 can be determined selectively in a concentration range of $1\times\ 10^{-5}$- $3\times\ 10^{-3}$ M.

The aim of the present study was to develop an alternative procedure for monitoring the dissolved oxygen for the determination of hydrogen peroxide concentration. It is well known that oxygen dissolved in aqueous solutions gives two reduction waves on the polarogram obtained with a dropping mercury electrode. The first wave corresponds to the reduction of dissolved oxygen into H_2O_2 and occurs at 0.05 V vs SCE. Mercury thin film electrodes (MTFE) behave like a complete mercury electrode and provide a flat surface suitable for immobilization. Since it is sensitive to mechanical deterioration, a rather delicate procedure is required for immobilization. In this study a novel MTFE-based biosensor was developed by adding the gelatine and the enzyme mixture over the electrode surface. It was shown that the electrode can be used for the determination of oxygen evolved by the above reaction.

Experimental

Chemicals

The chemicals used were of analytical reagent grade. Catalase (EC 1.11.1.6) (from bovine liver), 225 bloom calf skin gelatine and glutaraldehyde were obtained from Sigma.

Apparatus

Voltammetric analyses were carried out with a Metrohm 693 VA Processor and 694 Stand. A three electrode system was used. Potential was measured against a Ag/AgCl reference electrode and a platinum wire was used as the auxiliary electrode. The working electrode was a glassy carbon electrode (GCE) supplied from Metrohm with a 3 mm diameter.

Electrode preparation

The GCE was polished on a piece of cloth by using alumina slurry and rinsed with distilled water and then ultrasonically cleaned. Mercury deposition was performed ex situ. The plating solution was prepared by simply mixing 8 ml of distilled water with 1 ml of HCl solution (% 30) and 1 ml of Hg^{2+} solution (1 g/L). Nitrogen gas was bubbled through the solution for 5 minutes and then the surface of the electrode cleaned electrochemically by scanning the potential between (-1200)-(-100) mV several times. The mercury was deposited by applying the potential of -0.8 V for 5 minutes while stirring the electrode at a rate of 1800 rpm. Any metal contamination was removed from the surface by scanning the potential in deposition intervals.

Catalase (28000 IU/ml) and 225 bloom gelatine (11 mg/ml) were mixed at 38 °C in 0.05 M phosphate buffer (pH 7.0) and 100 μl of this solution was added to cover the electrode surface and allowed to dry at 4 °C for 1 hour. Finally the electrode was immersed in 2.5 % glutaraldehyde solution in 0.05 M phosphate buffer (pH 7.0) for 5 minutes.

Procedure

20 ml of 0.05 M phosphate buffer (pH 7.0) was placed in the voltammetric cell. The electrode was immersed in the solution and the stirrer was switched on for a predetermined time. After the solution became

quiescent, the potential was scanned in the cathodic direction by differential pulse (dp) mode with a 50 mV pulse amplitude. Unless otherwise stated, the experiments were performed in non-deaerated solutions and the peak currents were corrected for the blank.

As stated above, enzymatic activity does not change significantly in ambient conditions and shows a decrease at temperatures higher than 35 °C. Therefore, the experiments were carried out in the range between 28 and 31 °C .

Results and Discussion

Mercury thin film electrodes (MTFE) behave as a true mercury electrode. Figure 1 shows the dp voltammogram of MTFE obtained with 0.05 M phosphate buffer solution.

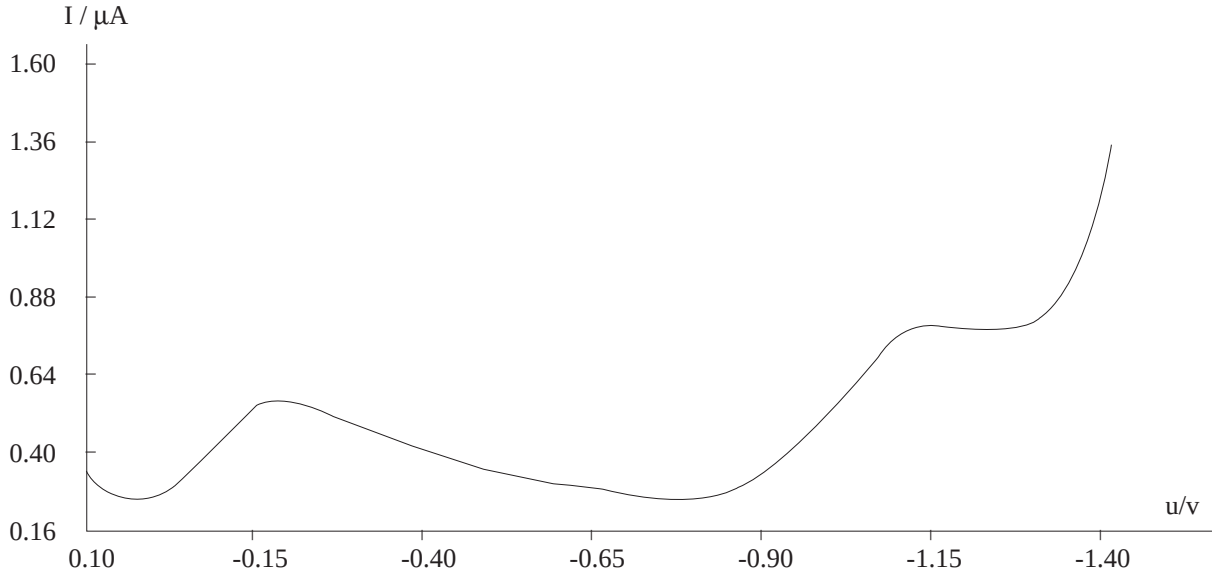

Figure 1. The dp voltammogram obtained with the MTFE in the buffer solution.

Two distinct reduction peaks appeared on the potential range studied. Upon deaerating the solution with nitrogen, the peaks were diminished indicating that these peaks correspond to the reduction of dissolved oxygen in two steps at around -0.15 V and -1.1 V. Following the modification with gelatine, the electrode was immersed in the buffer system and then the potential was scanned between 0.05-0.60 V. The first peak was observed at -0.24 V. This shift in the peak potential can be attributed to the changed surface characteristics of the electrode. Upon addition of hydrogen peroxide to the buffer solution, this peak was increased due to the increased amount of oxygen between the MTFE surface and the bioactive layer. Since the electrode response will depend on this equilibrium concentration of dissolved oxygen, the time required for obtaining a steady response was studied. Figure 2 shows the change in peak current at -0.24 V obtained for the buffer solution spiked with 1×10^{-6} M H_2O_2.

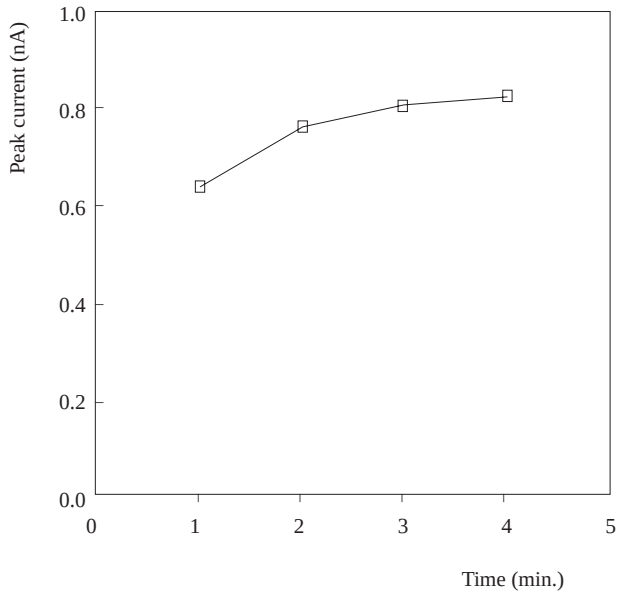

Figure 2. The effect of reaction time on the electrode response. The peak currents were obtained for the buffer solution spiked with 1×10^{-6} M H_2O_2.

It can be seen that 3 to 4 minutes are needed to reach the equilibrium state. Under these conditions (0.05 M phosphate buffer, pH 7.0, 3 minutes response time), the change in the peak current at -0.24 V with substrate addition was investigated. The peak current was plotted against the hydrogen peroxide concentration and the calibration curve was obtained in a concentration range of 1-50 $\times 10^{-6}$ M (Figure 3)

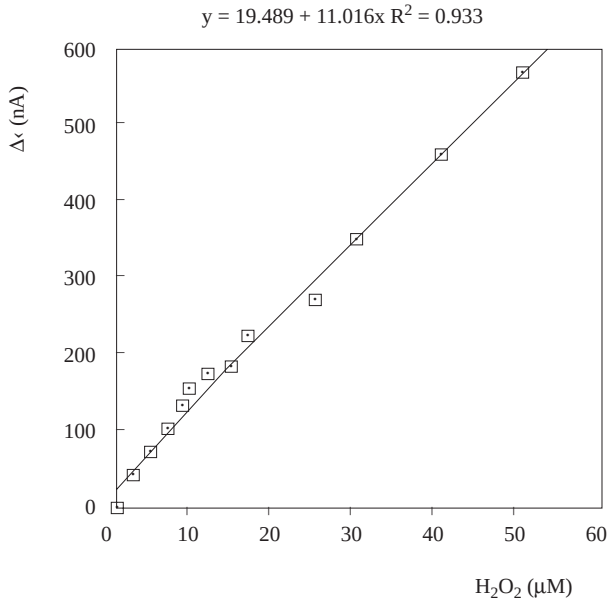

Figure 3. The calibration curve for H_2O_2 added pH 7.0 phosphate buffer.

The reproducibility of the method was tested by repeating the procedure for the buffer solution spiked with 5×10^{-5} M. The standard deviation and variation coefficient were found to be $\pm$ 0.056 and 4.68 %, respectively for 5 measurements. The stability of the electrode is mostly affected by the bioactive membrane

rather than the mercury film electrode. After being used several times, the gelatine layer over the MTFE surface was carefully wiped out with a piece of cloth and close examination showed that the electrode surface retains its homogeneous film shape. The experiment was repeated with this electrode using 5×10^{-5} M H_2O_2 solution and buffer alone. No significant difference was found in the peak currents in the absence of the bioactive membrane indicating the enhanced sensitivity of the method developed. The application of the method to the determination of H_2O_2 in milk samples is under study. The advantage of this analytical method is the use of an enzyme peroxide sensor as the indicator electrode.

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