

H₂O₂ sensing through electrochemically deposited thionine coated ITO thin film

P. Singh¹, S. Srivastava^{1*}, S. K. Singh^{2*}¹ Department of Biotechnology, Motilal Nehru National Institute of Technology, Allahabad-211004 Uttar Pradesh, India² Centre for Animal Sciences, School of Basic and Applied Sciences, Central University of Punjab, Bathinda-151001 Punjab, IndiaCorrespondence to: singh.sunil06@gmail.com, sameers@mnnit.ac.in

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Abstract: Progression and initiation of different diseases including pulmonary diseases, alzheimer's and tumors are linked with the oxidative stress, an important cause of cell damage. Different antioxidant enzymes are involved in detoxifying reactive oxygen species including hydrogen peroxide (H₂O₂) that is generated in response to various stimuli and has important role in cell activation & bio-signaling processes. Herein, we developed hydrogen peroxide electrochemical sensor based on horseradish peroxidase (HRP) entrapped polymerized thionine (PTH) film. Electrochemical deposition of thionine (dye) on indium tin oxide (ITO) surface was carried out through chronoamperometry followed by cyclic voltammetry. Deposited thionine thin film obtained was checked for its stability at different scan rates. The PTH-modified electrodes showed linear dependence of peak current with scan rate within the range of 20 to 100 mV s⁻¹. Thionine used as electron transfer mediator between heme site of HRP and electrode. Cyclic voltammetry showed increase in the reduction peak current due to electrocatalytic reduction of H₂O₂. The sensor detection limit range from 10⁻¹ - 10² μM and limit of detection was 0.1 μM. The proposed sensor has good storage response, cost effective, high sensitivity and wide linear range that could be used for the fabrication of other enzyme based biosensors.

Key words: Thionine; Preanodization; Electrocatalytic; Cyclic voltammetry; Electrochemical.

Introduction

In the past decades, determination of hydrogen peroxide (H₂O₂) has received great attention because it's not only a byproduct of several highly selective oxidases, but also possess a fascinating antiseptic & bleaching properties and an essential mediator in food, medicine and environmental analysis (1-4). H₂O₂ is associated with generation of reactive oxygen species (ROS) which is considered as important mediators of brain damage in a number of diseases including traumatic brain injury, neurodegenerative diseases and strokes (5). Therefore determination of elevated level of H₂O₂ is an important concern. Different techniques have been employed for the analysis of H₂O₂ such as titrimetry (6), spectrometry(7), chemiluminescence (8) and electrochemical method (9). First three techniques require expensive reagents and are highly time consuming and show interferences with other reagents. Electrochemical method is free from these drawbacks and is highly promising because of its real-time measurements, simplicity, selectivity, high sensitivity and accurate determination of H₂O₂ (10). In this context, recent studies including hemoglobin (Hb) and redox enzymes (horseradish peroxidase (HRP), cytochrome c) based electrochemical sensor has been developed for the detection of H₂O₂ (11-13). Electron transfer mediators such as (Ru(NH₃)₃py)₂(14), ferrocene derivatives (15) and thionine (16) are generally been used in redox enzyme based sensor for enhancing the detection

limits as low as 10⁻⁷-10⁻⁶M.

Thionine is a phenothiazine redox dye which dissolves easily in water and ethanol (17). Both the thionine monomer and electro generated polythionine (PTH) have excellent electrocatalytic activity toward the redox of electroactive compounds. The electron transfer property of thionine has been employed in different sensors like NADH biosensor (18), carcinoembryonic antigen immunoassay (19) and H₂O₂ biosensor (20). Thionine was electrochemically deposited on glassy carbon electrode (GCE) for the detection of H₂O₂ (21). Herein, we report two step electrochemical deposition methods for thionine coating on ITO glass for H₂O₂ sensing. The electropolymerisation of thionine thin film is dependent on the preanodization voltage. During preanodization process, a positive charge accumulates on the ITO surface that is used for creating thionine cation radical to polymerize thionine. Cyclic voltammetric analysis was carried out for monitoring change in the reduction peak current due to electrocatalytic reduction of H₂O₂.

Materials and Methods

All chemicals were analytical grade (99.99% pure), thionine (263.743 g/mol), HRP were purchased from Sigma-Aldrich; sodium dihydrogen phosphate (NaH₂PO₄), disodium hydrogen phosphate (Na₂HPO₄) from Merck. ITO glass was a product of Technistro.

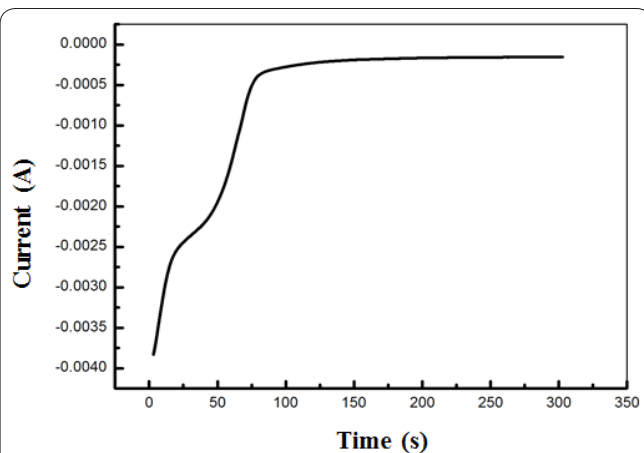


Figure 1. Deposition of poly-thionine layer through chronoamperometry.

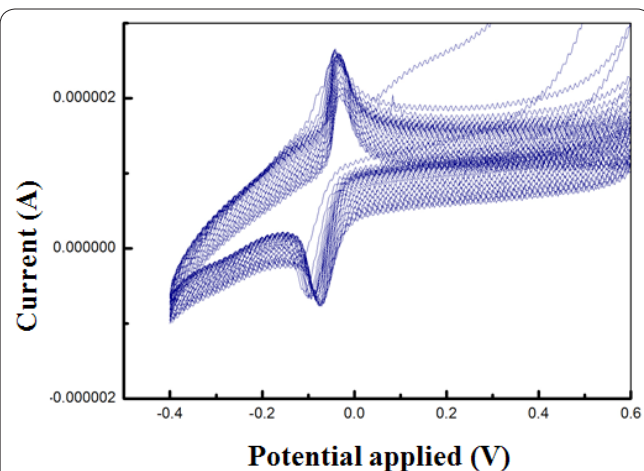


Figure 2. Deposition of poly-thionine layer through cyclic voltammetry at scan rate of (100 mV/sec).

Deposition of poly-thionine thin film on ITO plate

Electropolymerization of thionine was done through Electrochemical analyzer (Potentiostat, Autolab PGS-TAT 101) in two steps: (a) **Chronoamperometry**: Hold the ITO plate under a constant potential of -1.5 V for 5 mins in phosphate buffer solution containing thionine (0.02 M) (Figure 1) (b) **Cyclic voltammetry**: Potential cycling at scan rate of 100 mV/sec for 25 cycles between -0.4 to 0.6 V in pH 6.5 phosphate buffer solution (0.1 M) (Figure 2a) (22). Chemical reaction for the process is shown in Figure 2b.

Characterization of thin film

Surface morphology of the thin film was characterized through scanning electron microscopy (SEM) (Zeiss EVO Series) and Ellipsometer (J. A. Woollam Co.) was used to measure the thickness of the film.

Modification of thin film for hydrogen peroxide sensing

The thin film was modified by immobilizing an aliquot of HRP (1000 U cm^{-3}) solution on fabricated thionine modified electrode and incubated it for 2 hrs. After that 1% nafion solution was dropped over immobilized HRP layer and allowed to dry. Finally the electrodes were washed thoroughly and dipped in electrolytic solution to check its electrochemical behavior for H_2O_2 sensing.

Results and discussion

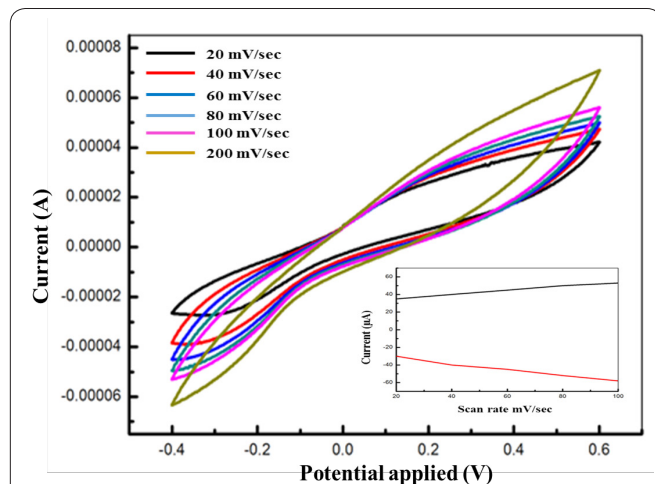


Figure 3. Cyclic voltammograms of a poly-thionine modified ITO plate in 0.1 M phosphate buffer (pH 6.5) at scan rates of 20, 40, 60, 80, 100, 200 mV/sec. Inset showing [variation in current at different scan rates].

Redox behaviour of the thin film

Redox behaviour of the deposited film was checked by applying potential cycling in 0.1 M phosphate buffer solution (pH 6.5) at different scan rates of 20, 40, 60, 80, 100, 200 mV/sec. The anodic and cathodic peak current was plotted against scan rate and linear relationship behavior between them confirms its redox behavior representing stability of the deposited thin film (Figure 3).

Characterization of the deposited film

SEM micrograph revealed clear morphological distinction between the bare ITO electrode and thionine deposited electrode. The thionine film is composed of randomly oriented closely packed spherical particles, about 30-40 nm in diameter as shown in Figure 4. Thickness of the deposited film was ~ 48 nm measured through Ellipsometer.

H_2O_2 sensing

Deposited thionine film electrode was used for the electrochemical analysis of H_2O_2 . In absence of H_2O_2 , the enzyme immobilized electrode does not contribute in catalytic reduction of H_2O_2 in phosphate buffer. Enzyme immobilized electrode showed decrease in electrochemical redox response as compared to characteristic thionine redox peak observed in figure 3 at 100 mV/sec confirms the successful immobilisation of HRP on poly-thionine film. Observed decrease in conductance (redox response) was due to the insulating nature of HRP biomolecule. But in the presence of H_2O_2 modified electrode showed increase in the reduction peak

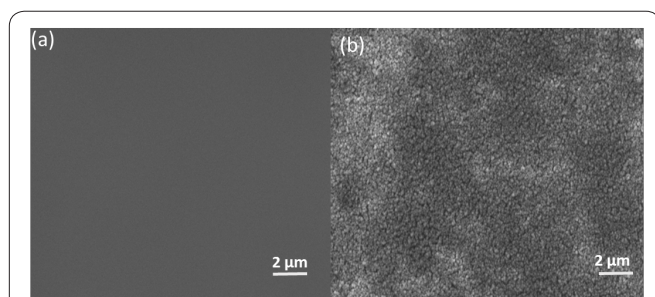


Figure 4. SEM micrograph of (a) bare ITO plate (b) thionine coated ITO plate.

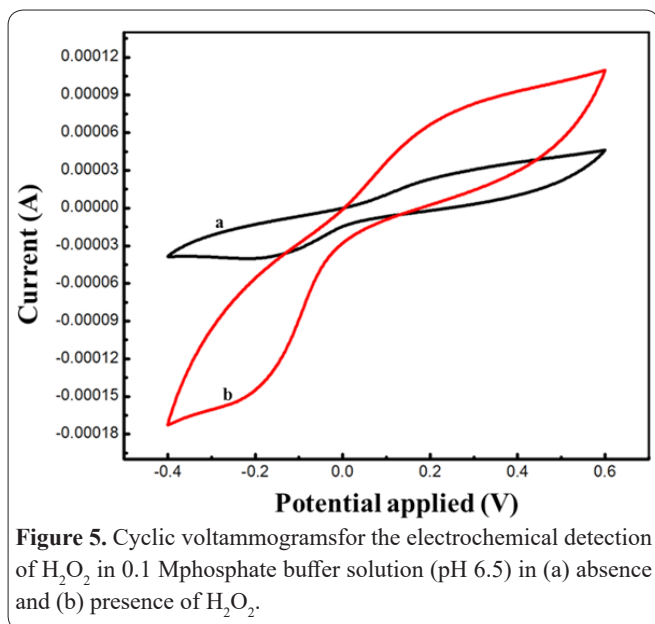


Figure 5. Cyclic voltammograms for the electrochemical detection of H₂O₂ in 0.1 M phosphate buffer solution (pH 6.5) in (a) absence and (b) presence of H₂O₂.

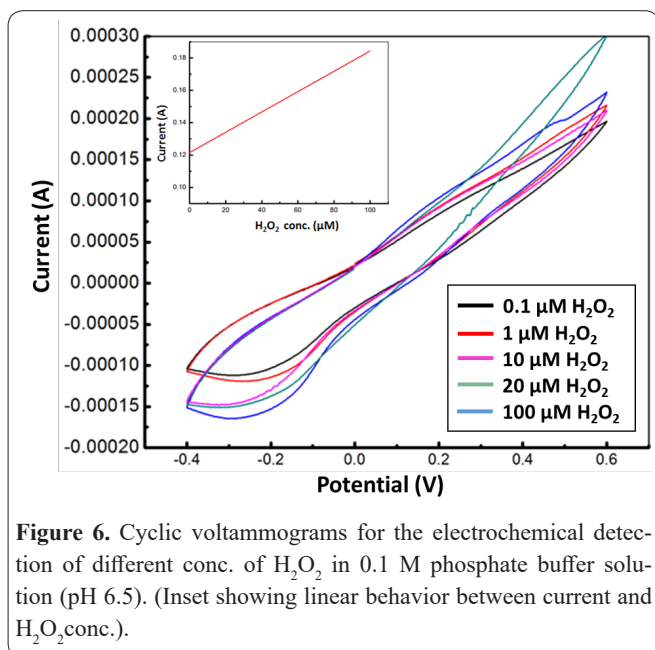


Figure 6. Cyclic voltammograms for the electrochemical detection of different conc. of H₂O₂ in 0.1 M phosphate buffer solution (pH 6.5). (Inset showing linear behavior between current and H₂O₂ conc.).

current confirming reduction of H₂O₂ (Figure 5). The sensor detection limit range from 10⁻¹–10² μM and limit of detection was 0.1 μM (Figure 6). This clearly demonstrates that PTH efficiently shuttles electrons between the redox center of HRP and the working electrode. The stability of the electrodes were monitored continuously for a month and it has given response with only 5% less activity than the initial.

Conclusion

In this study, we developed poly-thionine deposited ITO electrode based biosensor that is cost effective, portable, highly stable, fast response and provide excellent platform for adsorption of HRP enzyme in determination of hydrogen peroxide. Besides, suitability for surface characterization based studies has made poly-thionine based ITO electrode more preferred over GCE for the electrocatalytic reduction of H₂O₂. Change in reduction current observed through cyclic voltammetry reflects the presence of H₂O₂ in the electrolytic solution. This method could be very well used for other enzymes in the preparation of other amperometric electrodes.

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