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ScienceDirect
Sensors and Actuators B 132 (2008) 166–171
SENSORS AND ACTUATORS B CHEMICAL
www.elsevier.com/locate/snb

Bi-functional amperometric biosensor for low concentration hydrogen peroxide measurements using polypyrrole immobilizing matrix

E.M.I. Mala Ekanayake *, D.M.G. Preethichandra, K. Kaneto

Department of Life Science and Systems Engineering, Graduate School of Life Science, Kyushu Institute of Technology, 2-4 Hibikino, Wakamatsu-ku, Kitakyushu-Shi 808-0196, Japan

Received 15 October 2007; accepted 15 January 2008
Available online 30 January 2008

Abstract

Usage of nanoporous polypyrrole on H_2O_2 bi-functional sensing after immobilizing horseradish peroxidase (HRP) is described in this paper. Nanoporous alumina membranes were used to produce nanopores on polypyrrole surface. Nanopores increased the effective surface area available for enzyme immobilizing. Further, this increased area enhances the electron flow caused by the catalytic reactions. When the sensor was deployed to sense H_2O_2 at 0.2 V a high sensitivity of 3.9 A M^{-1} cm^{-2} was observed at a fast response time of 5 s. When the working potential was reversed to −0.1 V, a wider linear range of 10 nM to 25 μM was achieved. These novel findings will lead the way to a new path of biosensor design for low substrate concentrations with enhanced characteristics.

Keywords: Polypyrrole; Nanoporous; Hydrogen peroxide; Bi-functional; Amperometric; Sensitivity

1. Introduction

Hydrogen peroxide (H_2O_2) is an ubiquitous molecule in the environment as a result of both natural and industrial processes over a long period. It is well established as a deodorizing and a bleaching agent. Other usages include organic and inorganic chemical processing, textile and pulp bleaching, metal treating, cosmetic applications, catalysis of polymerization reactions, odor control, industrial waste treatment, and control of sludge bulking in waste waters [1–3]. These usages are continuously expanding, making it a necessity not only to understand the mode of H_2O_2 application but also to sense the amount of this chemical accurately [4].

Several conventional methods such as spectrophotometry, chemiluminescent flow sensors, and oxidimetry have been used to detect H_2O_2 during the past [5,6]. There is a disadvantage of using most of these techniques for accurate measurements in biological samples as these sensors reveal only micromolar level concentrations, where the existence may be in nanomolar levels. Therefore the need for highly sensitive H_2O_2 sensors is vital in this field [7].

H_2O_2 sensors can be used to measure H_2O_2 either present in the environment or that produced as a result of another reaction. In the latter case mostly an enzyme is used as a catalytic reactor to produce H_2O_2 and these sensors are being employed to measure a different substrate concentration indirectly. In oxidase-based amperometric biosensors, H_2O_2 is produced as a by-product of the catalytic reaction and the electron flow produced by the oxidation of this H_2O_2 is measured to analyze the substrate concentration present in the analyte [8,9]. Specific amperometric sensors for detecting H_2O_2 can be developed by using the same technique.

There are many biosensors reported in literature for H_2O_2 measurements with and without using an enzyme [10,11]. Plant peroxidases, mainly, horseradish peroxidase (HRP) is the most widely used enzyme for H_2O_2 sensing [12]. H_2O_2 is getting reduced as a result of the catalytic reaction of HRP in the presence of mediators or in mediatorless manner [13]. The detection limit of these biosensors are usually more than 10 μM. Since the H_2O_2 concentration is in the range of 0.1–1 μM in biological samples (e.g. in exhaled breath condensate), the need of low concentration detectable H_2O_2 sensors is on demand [6].

* Corresponding author.
E-mail address: mala-ekanayake@edu.life.kyutech.ac.jp (E.M.I.M. Ekanayake).

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doi:10.1016/j.snb.2008.01.042

In the recent past, conducting polymers have been used as potential surfaces for enzyme immobilization and for the oxidation and reduction reactions to take place in biosensors [14,15]. Biocompatibility, low cost, ease of fabrication and the good electrical conductivity of these polymers helped to boost its usage remarkably. Within this environment, polypyrrole (PPy) has gained a distinguished popularity among other polymers because of the stability at ambient conditions together with the capability of controlling the film thickness during polymerization [16–18].

In this research a novel H_2O_2 biosensor with a high sensitivity and a fast response time using conducting polymer is reported. The novelty of this sensor is the use of nanoporous PPy for fast detection of amperometric current both in anodic and cathodic potentials. At anodic potentials H_2O_2 is directly oxidized at PPy/Pt electrode while it is getting reduced in the presence of HRP at cathodic potentials. This bi-functional sensor gives advantages of fast response, high sensitivity and low detection limits due to the special electrode structure.

2. Experimental

2.1. Materials and apparatus

All the chemicals were of analytical grade. Horseradish peroxidase (EC 1.11.1.7, 225 U/mg), pyrrole monomer, sodium hexafluorophospate and H_2O_2 (30%) were purchased from Wako, Japan and pyrrole monomer was distilled before use. Phosphate buffer solution (PBS) was freshly prepared by using KH_2PO_4 and Na_2HPO_4 and the pH was adjusted to 6.50 by using a Cyberscan pH 100/RS232 portable pH meter. Alumina Anodisc^{TM}s of 200 nm maximum pore diameter (25–50% pore density) were purchased from Whatman Japan Ltd. Metal electrodes (Pt and Ag) used in the three electrode cell were from Nilaco Co. Pt was sputtered on alumina electrodes by using a JEOL quick coater (JFC 1500) plasma sputtering equipment. The electrochemical polymerization, cyclic voltammetry and the amperometric measurements were carried out using a Hokuto Denko HSV-100 automatic polarization system. Scanning electron microscope (SEM) images were taken with a Shimadzu-superscan (model SS-550). All the procedures were carried out at $25 \pm 2\,^{\circ}C$ unless otherwise stated.

2.2. Electrode preparation

Alumina discs were sputtered with Pt (50 nm) through a mask to make them conducting and this thin layer of Pt served as the working electrode for polymerization. PPy polymerization was completed according to a procedure described elsewhere [19]. Fig. 1(a) and (b) shows the images of alumina Anodisc^{TM} and alumina/Pt electrode after polymerisation, respectively. Electrodes were rinsed with PBS after polymerization and allowed to be dried at 4 °C. To analyze the depth of PPy nanopores through SEM, alumina disc was dissolved by immersing the sensor in 1 M NaOH (Fig. 2).

2.3. Enzyme immobilization

HRP immobilization on the prepared electrode was carried out by drop coating an aliquot of enzyme (2 μl from a stock solution of 1 mg ml^{-1}) on the prepared electrode, allowing it to be physically adsorbed for 30 min at 4 °C and subsequently washing it with excess phosphate buffer. The prepared Pt/PPy/HRP sensor was stored at 4 °C in an incubator under dry condition when not in use.

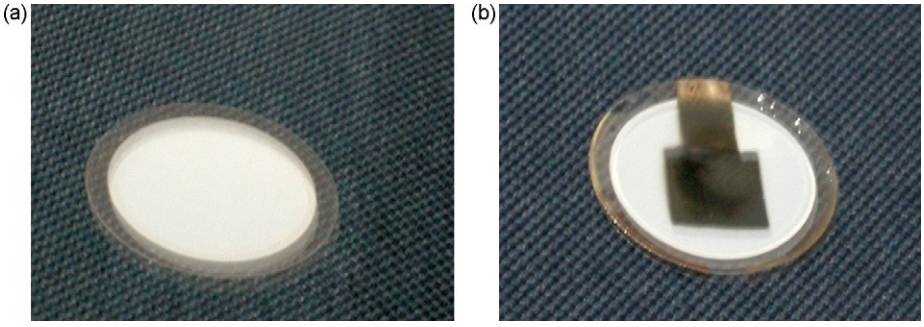

Fig. 1. (a) Anodisc^{TM} before Pt sputtering; (b) alumina/Pt/PPy sensor after polymerization. The sensor area is 1 cm^2.

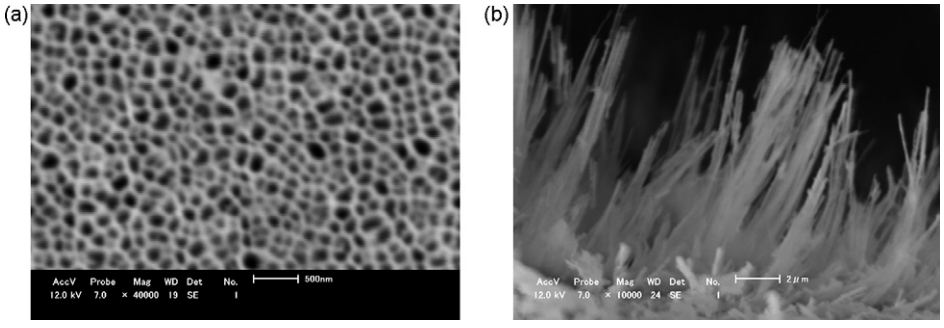

Fig. 2. (a) SEM image of alumina/Pt/PPy sensor and (b) PPy nanoporous film after dissolving alumina disc.