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Polypyrrole nanotube array sensor for enhanced adsorption of glucose oxidase in glucose biosensors

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Abstract

A novel amperometric biosensor based on polypyrrole (PPy) nanotube array deposited on a Pt plated nano-porous alumina substrate and its performances are described. Glucose oxidase (GOx) enzyme was selected as the model enzyme in this study. Commercially available nano-porous alumina discs were used to fabricate electrodes in order to study the feasibility of enzyme entrapment by physical adsorption. A PPy/PF_6^- film comprising of nanotube array was synthesized using a solution containing 0.05 M Pyrrole and 0.1 M $NaPF_6$ at a current density of 0.3 mA/cm^2 for 90 s. The immobilization was done by physical adsorption of 5 μL of GOx (from a stock solution of 2 mg/mL of 210 U/mg) on each electrode. A sensitivity of 7.4 mA cm^{-2} M^{-1}was observed with PPy nanotube array where the maximum tube diameter was 100 nm. A linear range of 500 μM–13 mM and a response time of about 3 s were observed with a nanotube array where the maximum tube diameter was 200 nm. The synthesized nanotube arrays were characterized by galvanostatic electrochemical technique. Calculated value of apparent Michaelis–Menten constant (K_m) was 7.01 mM. The use of nano-porous template electrodes leads to an efficient enzyme loading and provides an increased surface area for sensing the reaction. These factors contribute to increase the characteristic performances of the novel biosensor.

Keywords: Polypyrrole; Nanotube; Alumina; Glucose oxidase; Enzyme entrapment

1. Introduction

Enzymes have a number of distinct advantages over conventional chemical catalysts. Foremost among them are the specificity and selectivity, not only for particular reaction but also in their discrimination between similar parts of molecular or optical isomers. They catalyze only the reaction of very narrow ranges of reactants (substrates), which may consist of a small number of closely related classes of compounds or a single compound (Sara et al., 2004). There are some disadvantages in the use of enzymes which cannot be ignored and they are currently being addressed. In particular, the cost of some enzymes is highly concerned. It can be minimized if economical methods of using enzymes in biomedical applications can be found.

Since Leland C. Clark demonstrated how an enzyme could be integrated into an electrode to construct a biosensor, dramatic changes can be seen in the way to immobilize enzymes on biosensor electrodes with improved stability, selectivity and sensitivity (Brahim et al., 2002; Fiorito and de Torresei, 2001; Trojanowicz et al., 1995). Ever since, searching for promising methods of economized enzyme usage together with other factors are on demand.

In order to overcome these problems, several immobilizing techniques have been developed. Experimenting on conducting polymers as possible immobilizing matrices has drawn attention of many researchers (Ahuja et al., 2007; Yabuki and Mizutani, 2005; Gerard and Malhotra, 2005) during last few decades. Electrical conductivity can be achieved in polymer films by doping or rather inserting anionic or cationic species during the process of polymerization. The charged species formed upon doping are able to move along the carbon chain due to the double bond alteration in the conjugated polymer backbone, resulting electron movements, thus giving electronically conductive material (Sadki et al., 2000). These conducting polymers can be synthe-

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sized by various methods mainly by chemical or electrochemical oxidation.

While functioning as a three-dimensional matrix for the immobilization of active catalyst, conducting polymer's organized molecular structure on metal substrates preserve the activity for long duration. Ability of controlling thickness and morphology of conducting polymer membranes provide opportunities to deploy them in biosensors (Malhotra et al., 2006).

Currently, polypyrrole (PPy) is considered to be the most promising conducting polymer to be used as an immobilizing matrix for enzymes due to its relative stability, good conductivity and ease of preparation (Geetha et al., 2006). PPy based biosensors have found potential applications in health care, food processing, environmental monitoring, veterinary medicine, etc. (Rajesh et al., 2004; Ramanavicius et al., 2006). Among these, glucose biosensors are of utmost importance for the early detection, screening and treatment of diabetes.

Researchers try to improve the accuracy, stability, sensitivity, linear range, reproducibility and several other factors determining the quality of glucose biosensors by different techniques of immobilization of glucose oxidase or glucose dehydrogenase enzyme on suitable electrodes with PPy membranes. Up-to-date several physical adsorption, cross linking and electro polymerization methods have been tried to fulfill this requirement (Cho et al., 1996). Glutaraldehyde is widely used as a cross-linking agent that can prevent leaching out (Gade et al., 2006). Copolymerization on top of an existing thin film of PPy is another method widely being used.

Immobilization by physical adsorption has the advantages of simplicity, general applicability, high yield and ability to reload the enzyme when the catalytic activity of the immobilized enzyme has decreased below an acceptable level. Another major advantage of this method is to prevent the denaturing of the enzyme. However, a limitation of this technique is the need to control the working conditions in order to prevent the desorption of the immobilized enzyme (Wilson and Walker, 2000). This can be done easily by using a water insoluble polymer matrix like PPy. Enzyme entrapment can be maximized while minimizing the leaching out of enzyme by selecting a porous polymer membrane.

Methods of fabricating micro-porous PPy films by ion exchanging or other means of chemical interferences have been reported (Ma et al., 2005; Dayal and Godjevargova, 2006). These need to undergo several rigorous and time consuming steps to maintain the porosity at least in micro scale. Still these porous structures are not well organized and have several drawbacks in bulk preparation.

Our novel approach is to use a nano-porous electrode to fabricate a nanotube array of PPy and thereby enhancing enzyme entrapment. Nano scale tubes provide a higher exposed surface area compared to the flat surface electrodes. This results a high capacity of enzyme adsorption and enables fast transport of the gaseous by-product. This is a unique novel fabrication technique for a PPy nanotube array and leads to a very simple and fast fabrication method with all other requirements of a quality biosensor with a very low consumption of enzyme. This will fortify the proposed biosensor a high performance device among the existing biosensors.

2. Experimental

2.1. Selection of materials

In this new method, a thin film of PPy is fabricated in and around the pores of a nano-porous electrode by electropolymerization. By controlling the thickness of the film, a PPy nano film, which comprises of an array of nanotubes grown inside the pores can be achieved. Extra care has to be taken and the polymerizing time has to be optimized in order to retain the pore structure without overgrowing the film that may cause a flat morphology on solution side.

Here we have selected glucose oxidase (GOx) as the sample enzyme to fabricate a glucose biosensor considering the facts of low cost and higher stability of this particular enzyme (Kaimori et al., 2006).

When a glucose biosensor comprises a highly porous PPy film, the enzyme immobilization by physical adsorption will be strengthened and hydrogen peroxide produced as a result of the catalytic reaction below can reach the electrode at a high rate for detection.

$$\text{Glucose} + O_2 \xrightarrow{\text{GOx}} \text{Gluconic Acid} + H_2O_2$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$$

To satisfy this requirement, we have selected nano-porous alumina discs (Anodisc^{TM} from Whatman) coated with a thin layer of Pt as our electrodes to electro-polymerize a thin layer of PPy. Anodisc^{TM}s are hydrophilic thin membrane filters with non-interconnected parallel nano-pores running from one surface to the other. According to the manufacturer, Anodisc^{TM}s have a certain level of protein binding property and it is another reason to select it as our base substrate to develop the sensor. The porosity of these discs is found to be 25–50%. Alumina and animal charcoal had been tried as substrates for enzyme entrapment in the past and found that though they have entrapment property, their depletion rate is very high (Wilson and Walker, 2000).

PF_6^- itself acts as a good dopant to produce porous films is used in this study to dope PPy. The enzyme loading and the amperometric response of our electrode is remarkably improved by the combination of selected substrate material, polymer and dopant. In this article it is reported a much improved approach of physical adsorption for the preparation of Pt/PPy/GOx electrode based on nano-porous alumina Anodisc^{TM}s.

2.2. Material, methods and apparatus

Glucose oxidase (E.C.1.1.3.4., 210 U/mg) from *Aspergillus niger*, Pyrrole monomer, ascorbic acid, uric acid and sodium tetrafluorophosphate were purchased from Wako, Japan. D-Glucose was obtained from Sigma–Aldrich. Pyrrole monomer was distilled and sodium tetrafluoro phosphate solution was