Glucose Biosensor Based on Poly(N-methylaniline)/Chitosan Composite

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Abstract

Amperomertic glucose sensors were prepared by the electrochemical synthesis of new poly(N-methylaniline)/Chitosan(PNMANI)/Ch composites in 0.10 M H_2SO_4 acid medium. Glucose oxidase (GOD) was immobilized on to the surface of these composites by physical adsorption method. The composite was characterized by FTIR and UV-visible spectrophotometric methods and detailed response studies were performed with respect to glucose concentration and pH. The PNMANI/Ch/GOD electrode was utilized for glucose sensing for concentrations in the range of 0.06- 1.831 mM by the amperometric method. The electrode was found to have a response time of 70 s and the Michaelis-Menten constant was calculated as 1.71 mM.

Introduction

The biopolymer chitosan is a type of matrix for enzyme immobilization with such attractive properties as excellent film-forming ability, high permeability for water, good adhesion, nontoxicity, and biocompatibility (1, 2). However, its poor electrical conductivity results in a poor response time and a high operational voltage limits its applicability in devices. Composites have been prepared by incorporating a rigid conducting polymer (such as PANI) into a flexible matrix (such as chitosan) combining the good processability of the matrix and the electrical conductivity of the conducting polymer (3). Additionally, conducting polymers have the ability to efficiently transfer the electric charges produced by biochemical reactions to electronic circuits(4).

Biosensors prepared by using a conducting polymer as a support material have a fast response time and a high storage and operational stability (5). Polyaniline is extensively researched for its electrical, optical, chemical and electrochemical properties due to its simple synthesis method, stability in air, and potential range of applications (6). It is the best known semi-flexible rod-like conducting polymer system with chemical and structural flexibility surrounding its amine nitrogen linkages for binding with biological materials (7). However, the major disadvantages of PANI are its insolubility in common organic solvents and its infusibility. There are some possible methods for preparing soluble PANIs. One of these methods is to substitute one or more hydrogens by an alkyl, an alkoxy, an aryl hydroxyl, or an amino group, or halogen in an aniline nucleus (8,9). As known from the literature both chitosan and polyanilines are sensitive to different enzymes such as lactate (10), horseradish (11), glucose oxidase (12).

The main goal of this work is to electrochemically synthesize poly(N-methylaniline)/Chitosan (PNMANI/Ch) composites in 0.10 M H_2SO_4 acid medium and to investigate their biosensing application to glucose sensing. The objective is to improve the response time and sensitivity of chitosan *via* the PNMANI/Ch composite. Glucose oxidase was selected as a model enzyme to construct an amperometric glucose biosensor, to explore for the first time, applicability of this composite polymer as a matrix for enzyme immobilization. For biosensor application, glucose oxidase (GOD) was immobilized on the surface of the composite by the physical adsorption method. A biosensor for glucose was developed based on the electrochemical oxidation of H_2O_2. Influence of pH on the activity of the PNMANI/Ch-GOD electrode was also studied.

Experimental Procedure

All electrochemical experiments were carried out with the conventional three-electrode cell, using the modified Pt electrode as a working electrode, a saturated calomel electrode (SCE) as a reference electrode, and a platinum wire as an auxiliary electrode. The working electrode was a platinum electrode with 1 mm^2 of surface area. 4μL of 0.01g chitosan/ml solution in 2 wt% acetic acid was dropped onto the Pt and dryed at room temperature. Then, 0.2 ml NMANI was added to 10 ml of 0.10 M H_2SO_4 acid. The potential was cycled between 0 and 1.05 V versus saturated calomel reference electrode at a scan rate of 50 mV/s for 30 cycles. The amount of

PNMANI/Ch composite was increased for characterization using a platinum electrode with bigger surface area. The composite was washed with 2 wt% acetic acid and deionized water until it became colorless. The final composite was dried at 50 ^{o}C for 24 hours. Electrochemical measurements were carried out with a Gamry PCI4/750 potentiostat

Result and Discussion

Figure 1 shows the cyclic voltammogram recorded between 0.00 and 1.05 V, with a scan rate 50 mVs^{-1} during the synthesis of PNMANI using a modified Pt electrode with chitosan, as a working electrode. The obtained electrode was ready for use in sensing after the final wash with water and denoted as PNMANI/Ch/GOD. The cyclic voltammetric growth of PNMANI shows only one well-defined anodic peak at 0.38 V (13).

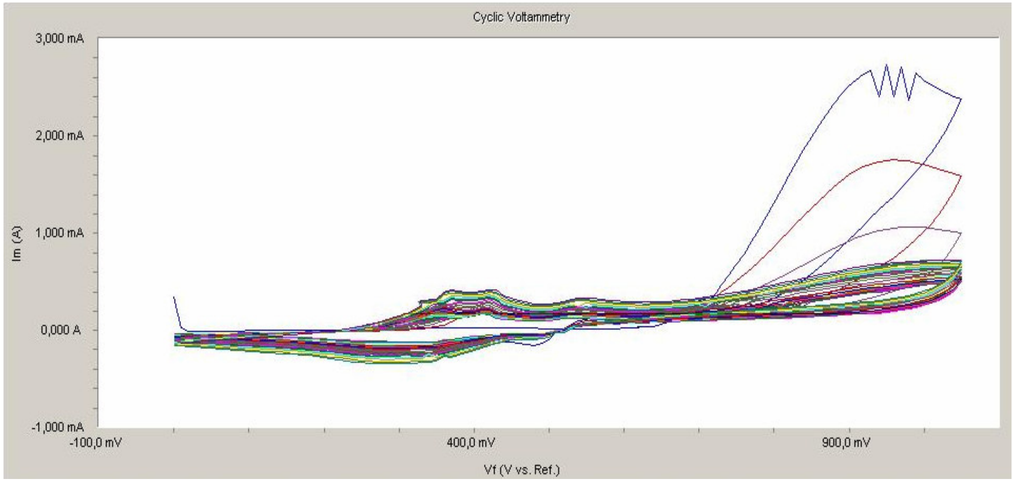

Figure 1 Electrochemical polymerization cycles of PNMANI/Ch

Electrochemically synthesized films of PNMANI/Ch composite were prepared in 0.10 M H_2SO_4 for immobilizing glucose oxidase (GOD). The enzyme electrode was prepared by immersing the PNMANI/Ch composite film into 5mg/ml glucose oxidase in 0.10 M phosphate buffer solution for 20 minutes. Then, the enzyme electrode was washed with 0.10 M phosphate buffer after which, it was ready to use. Free enzyme electrodes and chitosan enzyme electrodes (without any conducting polymer) were also prepared for comparison of glucose sensing capabilities.

The effect of pH on the behavior of the enzyme electrode was studied with 0.10 M phosphate buffer solution and 5 mg/ml GOD at the steady state potential at 0.6 V as a function of the pH. The electrochemical response is quite good at pH ranging from 7.0-8.0 and the maximum potential was observed at pH 7.4 in phosphate buffer.

The effect of the glucose concentration was investigated on the response of chitosan, PNMANI/Ch and free enzyme electrodes. The response current increases somewhat linearly with glucose concentration from 0.06 mM to 0.60 mM for the chitosan/GOD, from 0.06 mM to 1.54 mM for free enzyme and from 0.06 mM to 1.83 mM in the case of PNMANI/Ch/GOD. The PNMANI/Ch/GOD composite biosensor has a bigger linear range and higher sensitivity (larger slope) than free enzyme and chitosan/GOD biosensors (Figure 2).

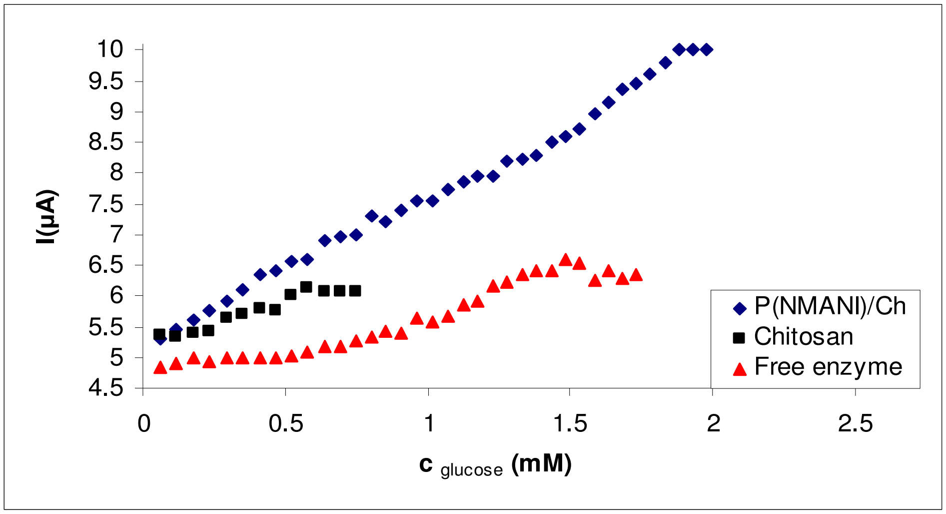

Figure 2. Changes in the response of free enzyme, chitosan/GOD, PNMANI/Ch/GOD enzyme electrode with glucose concentration (at 0.6 V in 0.10 M phosphate buffer solution (pH 7.4), 5 mg/ml GOD, 25 ^{o}C)

According to the lineweaver-Burk form of the Michaelis– Menten equation, the relation between the reciprocal of the response current and the reciprocal of glucose concentration is linear. The apparent Michaelis–Menten constant Km_{app} (slope of this line) of the biosensor based on the PNMANI/Ch/GOD electrode is fitted to be 1.707 mM glucose.

Conclusions

Upon immobilizing glucose oxidase phy physical adsorption methods, an electrode for glucose sensing, the PNMANI/Ch/GOD electrode, was prepared and tested for its performance in amperometric sensing of glucose. The PNMANI/Ch/GOD composite electrode shows wider linear range, higher sensitivity, and smaller response time, when compared to the Ch/GOD or free GOD electrodes due to the increased conductivity of the composite material. This glucose sensor material is expected to have superior biocompatibility, which can be revealed by further studies.

Acknowledgements

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