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Characterization of ZrO_2/PEG Composite Film as Immobilization Matrix for Glucose Oxidase

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***Abstract*—**A biosensor based on glucose oxidase (GOx) immobilized onto nanoparticles zirconium oxide with polyethylene nanocomposite for glucose monitoring has been designed. The CTAB/PEG/ZrO_2/GOx nanocomposite was deposited onto screen printed carbon paste (SPCE) electrode via spin coating technique. The properties of CTAB/PEG/ZrO_2/GOx were study using scanning electron microscopy (SEM). The SPE modified with the CTAB/PEG/ZrO_2/GOx showed electrocatalytical response to the oxidation of glucose when ferrocene carboxaldehyde was used as an artificial redox mediator, which was studied by cyclic voltammetry (CV). Several parameters such as working potential, effect of pH and effect of ZrO_2/PEG layers that governed the analytical performance of the biosensor, have been studied. The biosensor was applied to detect glucose with a linear range of 0.4 to 2.0 mmol L−1 with good repetability and reproducibility.

***Keywords*—**Nanocomposite, Nanoparticles, Modified SPE, Ferrocenecarboxaldehyde.

I. Introduction

OVER the past years, the intensive development of biosensing system based on nanotechnology has shown significant progress. The development of biosensors, a new generation of analytical devices, is one of today's most promising directions of investigation in the field of analytical biology. Biosensors are divided into optic, acoustic and electrochemical types, depending on how the signal is transformed. Electrochemical sensors, in their turn, can be amperometric, conductometric and potentiometric [1]. Electrochemical method is one of the most frequently used methods for fabrication of conducting polymer electrodes. However, some general considerations such as choice of counter ions (organic/inorganic) determine the mechanical behavior of the conducting polymer films for obtaining high operational stability, sensitivity and fast response time [2].

Many nanoparticles have been used for immobilization of biomolecules due to their unique optical, electrical and molecular properties which recently have led to increased interest as immobilization matrices for biosensor application.

For examples, the nano-titaniumoxide (TiO_2) with diameters of 10-20 nm was employed to speed up the electron transfers of protein. In the other work, silver nanoparticles were used to facilitate the direct electrochemistry of cytochrome [3]. Carbon nanotubes are also able to provide good biological affinity and compatibility for some proteins, such as myglobin, horseradish peroxidase and glucose oxidase enzymes. These reported examples indicate that nanoparticles are good materials for protein immobilization with a favored orientation and also facilitate and accelerate electron transfer between proteins and electrodes [4].

The specific gravity of ZrO_2 is 5.49. It has the properties of high hardness, high melting point (2700°C), and small thermal expansion coefficient. It is also insoluble in an acid or an alkali except H_2SO_4 and HF. In fact, ZrO_2 particle itself has been used as support for enzyme immobilization. Zirconia particle could immobilize enzyme with good retention of activity due to its high specific gravity mechanical and chemical robustness, as well as its high thermal and pH stabilities [4]. Zirconium oxide nano-materials, which possess good adsorption properties and biocompatibility, were also employed to immobilize enzymes for the investigations of their bioactivity and catalysis [3]. It is an organic oxide with the thermal stability, chemical inertness, lack of toxicity and affinity for the groups containing oxygen, so it is an ideal candidate of materials for immobilization of biomolecules with oxygen groups [5]. In recent years there is a growing interest in the development of glucose biosensor based on immobilized enzyme since rapid, accurate and in situ determination of glucose in food industry, medical and bioanalysis fields is of paramount importance. Considerable attention has been focused on immobilizing glucose oxidase (GOx) on different solid matrices which are subsequently positioned on a transducer surface to produce workable biosensors. This enzyme immobilization process is crucial as it can determine the analytical performance of a glucose biosensor [7].

Among the various methods available for determination of glucose, biosensors are comparatively more simple, sensitive and rapid analysis. Nowadays, several methods can be used for determination of glucose, including high-performance liquid chromatography (HPLC), gas chromatography, high-performance thin layer chromatography, flow injection analysis and spectrophotometry. However, these methods require expensive laboratory equipment and chemicals,

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sample pre treatment and time consuming for the analysis. Electrochemical and optical biosensor can represent an alternative method to overcome the disadvantage mentioned.

Herein the biosensing application of ZrO_2 was exploited for the development of glucose biosensor using self assembly method. Self-assembly (SA) is now popular for it provides a simple, versatile method to prepare modified surface at high-level order. The good homogeneity and organization, together with better controlling the microenvironment of the recognition molecules, make SA attractive in biomolecules electronics and biosensor development [12]. In this current research, the biosensor was constructed by immobilizing GOx in CTAB/PEG/ZrO_2 nanocomposite film. The CTAB/PEG/ZrO_2/GOx nanocomposite film modified screen printed electrode showed an amperometric response to glucose when ferrocene carboxaldehyde was used as electron-transfer mediator.

II. Procedure

A. Chemicals and Reagents

Glucose oxidase (from Aspergillus niger) and Chitosan were purchased from Sigma. ZrO_2 nanoparticle sized below 50 nm was from Aldrich. Cetyl Trimethyl Ammonium Bromide from Sigma. Glucose stock solution, sodium dihydrogen phosphate, di-sodium hydrogen phosphate, acetic acid, chitosan flakes, Ferrocenecarboxaldehyde and ethanol were of analytical reagent grade and used as received without further purification. All solutions were prepared with doubly distilled water..

B. Apparatus

Cyclic voltammetric were performed using a Potentiostat (Autolab). Amperometric measurements were carried out using the same model. The three-electrode system consisted of a CTAB/PEG/ZrO_2/GOx on carbon electrode as working electrode, Ag/AgCl as a reference electrode and a Pt wire as counter electrode. All electrochemical experiments were carried out in 5ml phosphate buffer (pH7.0).

Scanning electron micrographs of CTAB/PEG/ZrO_2/GOx nanocomposite film were taken with scanning electron microscopy (Carl Zeiss SMT-NanoTechnology System Division FESEM SUPRA 40VP).

C. Preparation of CTAB Solution

Appropriate amount of Cetyl Trimethyl Ammonium Bromide powder was dispersed in 0.5 M NaCl with pH 8.0. The mixture was vortex for 5 min to get homogeneous solution.

D. Construction of CTAB/PEG/ZrO_2/GOx Electrode

A solution of glucose oxidase at 80mg/mL was prepared in 0.050 M phosphate buffer at pH 7.0. An appropriate volume of mixture of CTAB solution was pipetted onto the surface of a working electrode. The casting solution was allowed to dry at room temperature for 1 hour. Then 2.0uL of ZrO_2/PEG was dropped onto the electrode surface and dried at 4^0C. Finally, 5uL of GOx was pipette onto the electrode and allowed to dry at 4^0C overnight.

III. Result and Discussions

A. Morphology of Surface-Treated ZrO_2/CTAB Composite Film

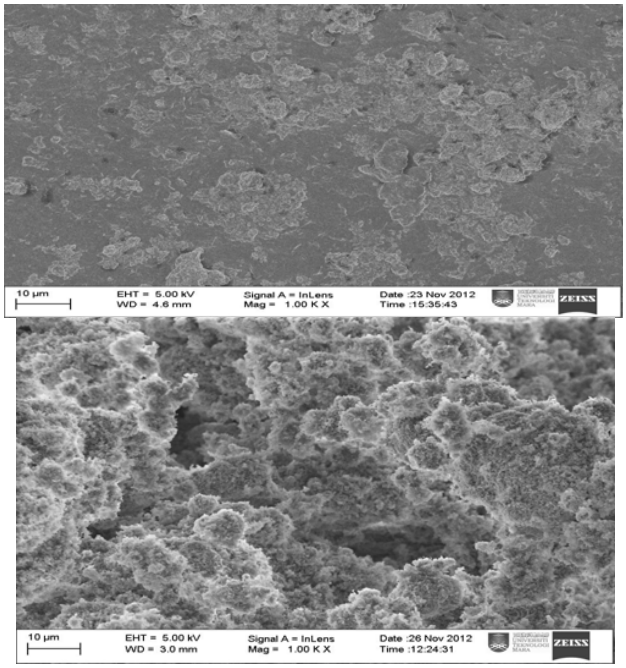

Fig. 1 SEM images of (A) CTAB/PEG/ZrO_2 and (B) CTAB/PEG/ZrO_2/GOx

In order to have an understanding about the conformation of ZrO_2 on the composite film-modified SPE surface, scanning electron microscopy (SEM) experiments were also performed. The results of SEM studies carried out on CTAB/PEG/ZrO_2 and CTAB/PEG/ZrO_2/GOx are shown in Fig. 1. Uniform distribution of zirconium oxide nanoparticles with regular and patterned morphology is observed in the case of CTAB/PEG/ZrO_2/GOx (Fig. 1B). After the $\{PEG/ZrO_2\}_2$ films were mixed with glucose oxidase, the particles on the surface film become larger. At the same time, the morphology changes into another aggregated structure can be attributed to covalently bound enzymes molecules and revealing presence of enzyme onto ZrO_2 electrode [5], [9], [10].

B. Cyclic Voltammetric Characterization

Cyclic voltammetric (CV) studies have been carried out in phosphate buffer solution (PBS,50mM, pH 7.0) containing 0.2mM ferrocenecarboxaldehyde and in the potential range -1.0 to 1.0 V.

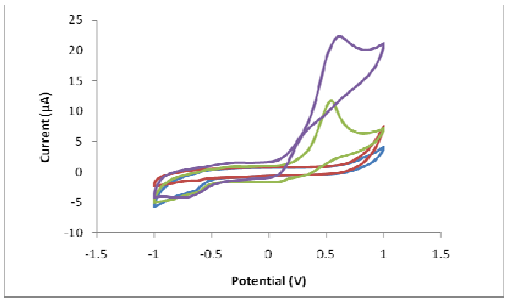

Fig. 2 Cyclic voltammograms of CTAB/PEG/ZrO2/GOx modified electrodes electropolymerized in the absence (a) and presence (b) of glucose. Experimental conditions: 0.05 M PBS pH 7 containing 0.2 mM Ferrocene at scan rate 100 mVs-1.

Fig. 2 shows the cyclic voltammograms of CTAB/PEG/ZrO_2/GOx modified SPE response to glucose when ferrocene carboxaldehyde is used as an artificial redox mediator. The CV spectra of the CTAB/PEG/ZrO_2/GOx shows increased peak current to 10mmolL^{-1} glucose compared to that has no glucose. This result is attributed to the redox characteristics of the electroactive centers of the immobilized GOx, indicating enhanced electron transfer between GOx and CTAB/PEG/ZrO_2.

C. Effect of Applied Potential

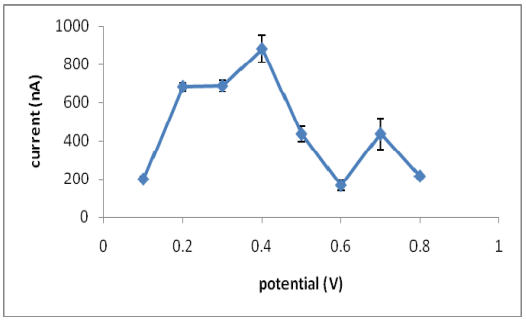

Fig. 3 Effect of working potential on the amperometric response of the biosensor to 1.0 mmolL−1 glucose in 50 mmolL−1 PBS (pH 7.0) containing 0.2mmolL−1 ferrocene carboxaldehyde, 80mg/mL GOx modified on the electrode. Error bars = ±standard deviation and n =3

Fig. 3 displays the effect of the working potential of biosensor on the amperometric response of 1mmolL^{-1} glucose. The response current increased rapidly as the applied potential shifted from 0.1 to 0.4 V, which was due to the increased driving force for the fast reduction of glucose at low potential. When the applied potential was more positive than 0.4 V, the currents decrease visibly. So applied potential of 0.4V was selected throughout the study. This present work is slightly better than studies in [11] since higher applied potential could lead to other interferences. This selected applied potential is considered big enough for H_2O_2 oxidation.

D. Effect of pH

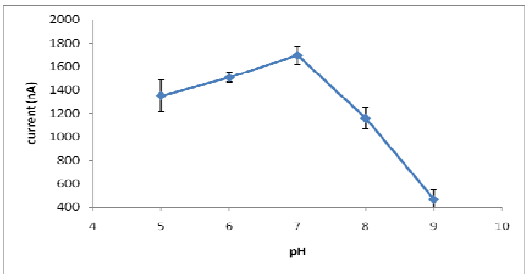

Fig. 4 Effect of pH on the amperometric response of the biosensor to 1.0 mmolL^{-1} glucose in 50 mmolL^{-1} PBS (pH 7.0) containing 0.2mmolL^{-1} ferrocene carboxaldehyde, 80mg/mL GOx modified on the electrode. Error bars = ± standard deviation and n = 3

Fig. 4 shows the effect of the pH value of the detection solution on the response behaviour of the CTAB/PEG/ZrO_2/GOx electrode. According to literature [14], Poly(diallyldimethylammonium chloride) (PDDA) which has same characteristic as CTAB is a highly positively charged species, which can absorb on the surface of electrode, while GOx can be used as a negatively charged material to absorb the polycationic PDDA at pH 6.8. In this figure, the maximum response current can be observed at pH 7.0 very close to pH 6.8.

E. Effect of ZrO_2/PEG Layer

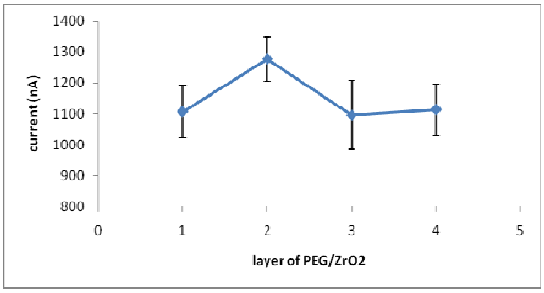

Fig. 5 Effect of ZrO2/PEG layer on the amperometric response of the biosensor to 1.0 mmolL−1 glucose in 50 mmolL−1 PBS (pH 7.0) containing 0.2mmolL−1 ferrocene carboxaldehyde, 80mg/mL GOx modified on the electrode. Error bars = ±standard deviation and n =3

Zirconia is a technological important material that has recently attracted considerable interest in practical applications. It is a unique material of excellent chemical inertness and biocompatibility feature; moreover its surface has both oxidizing and reducing properties, as well as acidic and basic properties [13]. Polyethylene glycol (PEG) is a neutral non-ionic polymer with no charges on its backbone, while ZrO_2 nanoparticle carries negative charges in it aqueous suspension. Therefore, the driving force of the assembly of

{PEG/ZrO_2}n, multilayer films could not be ascribed to electrostatic interaction between PEG and ZrO_2. One PEG molecule contains a great number of ether oxygen group in its ethylene oxide structure. This kind of oxygen has one active unshared lone pair of electrons, and thus has a high coordination capability as the electron donor. ZrO_2, on the other hand, has many unoccupied orbitals and can accept at most 8 lone pairs of electron, and thus shows good coordination capability as the electron acceptor [6]. In order to optimize the output current of the sensor, the number of layers of ZrO_2/PEG multilayer films on the screen printed electrodes was evaluated by electrochemical measurements. Fig. 5 shows the sensitivity of modified electrodes with different numbers of ZrO_2/PEG layers. The highest response was observed for the two layers. Then it decreased upon further increasing the number of layers. As too thick of ZrO_2/PEG films formed in CTAB/PEG/ZrO_2/GOx, CTAB would result in the loss of flexibility of the polymer chain in the film, thus glucose molecular cannot pass smoothly through the thicker films. So the sensitivity decreased along with the further increasing of the number of ZrO_2/PEG layers after reaching the maximum sensitivity. Thus, the two layers of ZrO_2/PEG multilayer films modified screen printed electrodes were used in this work.

F. Scan Rate Study

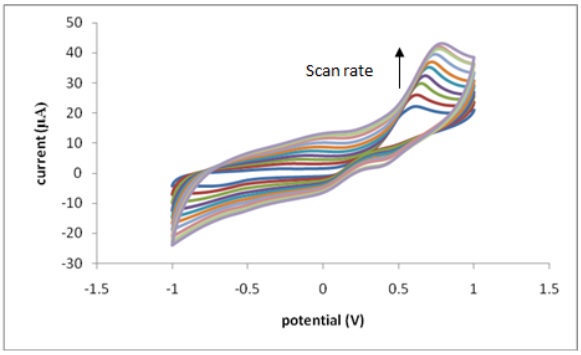

Fig. 6 Cyclic voltammograms of CTAB/PEG/ZrO_2/GOx of different scan rate from 100m V/s to 1000 mV/s, 10mmolL-1 glucose in 50 mmol L-1 PBS (pH7.0) containing 0.2mmolL-1 ferrocene carboxaldehyde

Fig. 6 shows cyclic voltammograms obtained with different scan rates at a CTAB/PEG/ZrO_2/GOx in a PBS (50mM). The increase of potential scan rate promoted an increase of current peak in anodic and cathodic reactions. At higher scan rates, the signal broadened considerably and peak separation increase.

G.Calibration of the Immobilised Electrode

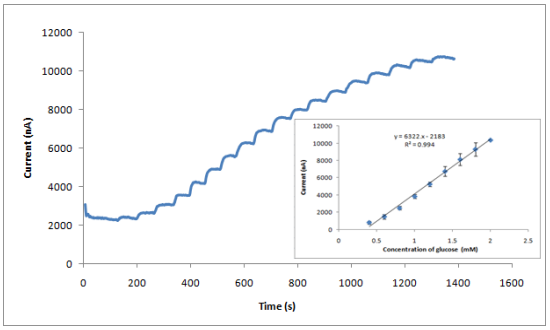

Fig. 7 Amperometric response of the two layers of CTAB/PEG/ZrO2/GOx films modified screen printed electrode upon successive addition of glucose at +400mV. The inset shows calibration curve. Error bars = ±standard deviation and n =3

Fig. 7 shows the steady-state calibration curve of seven layers of CTAB/PEG/ZrO2/GOx multilayer films modified screen printed electrode. The proposed biosensor presents a linear response to glucose concentration within the range from 0.4 mM to 2.0 mM. When glucose concentration was higher than 2.0mM, a plateau current was observed. The calibration curve for glucose (Fig. 7 inset) gave a linear range from 0.4mM to 2.0mM with correlation coefficient of 0.994. The repeatability study was measured at 1.0 mM glucose. The relative standard deviation (RSD) was 9.98% for eight successive assays. Four CTAB/PEG/ZrO2/GOx modified electrodes exhibited an acceptable reproducibility with RSD 5.01% for the measurement of 1.0 mM glucose.

IV. Conclusions

The glucose biosensor, CTAB/PEG/ZrO_2/GOx was fabricated by immobilize glucose oxidase on modified screen printed electrode with CTAB as cationic surfactant for ZrO_2 nanoparticles. The optimum condition of this glucose biosensor has been studied in detail. It has been exhibited that the SPE could be used for at least 8 measurements with a good repeatability. The CTAB/PEG/ZrO_2/GOx modified SPE shows great potential for the development of glucose biosensor. The proposed construction method is simple, cheap and promising for the development of glucose biosensors, especially in forming a modified electrode for routine monitoring of glucose in drinks sample in future.

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