tra Chemistry Vol. 7(2), 161-166 (2011).

Development of PANI-EPOXYRESIN – GOD electrode by potentiometric method for determination of Glucose

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(Acceptance Date 25th April, 2011)

Abstract

The polyaniline-epoxy resin-glucose oxidase (PANI-EPOXY RESIN-GOD) electrode has been investigated in the present work. The synthesized PANI-EPOXY RESIN composite films were characterized by electrochemical technique, electrical conductivity, UV-visible spectroscopy, Fourier transform infrared (FTIR) spectroscopy and scanning electron microscope (SEM). The GOD was immobilized on synthesized PANI-Araldite film by cross-linking via Glutaraldehyde in phosphate buffer.

Key words: Immobilization, composite film, glucose oxidase, cross-linking.

Introduction

Use of glucose as an energy source cells is via aerobic or anaerobic respiration. is critical in the production of proteins and in pid metabolism. In plants & most animals it a precursor for vitamin-C production. So the termination of glucose is of special importance bio-sensor application. Therefore numerous forts have devoted to develop glucose biosensor th fast and accurate response. The first neral method of immobilization, cross-linking zyme molecules is often brought about by action of glutaraldehyde. The conducting lymers are being widely used in bio-sensor plication because it provides stable and porous matrix for the immobilization of biocomponent and it also facilitate the electron transfer process. The most widely used conducting polymers for immobilization of enzyme are polyaniline[1-5]. It can be easily synthesized from aniline monomer in aqueous solution. In the present study we have immobilized GOD on epoxy resin PANI film by cross linking via glutaraldehyde for the development of glucose bio-sensor.

2. Experimental Part

Preparation of polyaniline-epoxyresin composite film

Polyaniline is prepared from 1.6 ...

distilled aniline (Beachem) and 100 ml of aqueous hydrochloric acid (1M) is taken in 250 ml beaker[6]. About 0.2g of powered precipitate was thoroughly mixed with Araldite (Huntsman advanced materials, India Pvt. Ltd) and the paste was applied on Whatmann filter paper No. 42. This was spread uniformly over the filter paper to obtain 0.9mm thickness of the electro active material with matrix. This was left in air to dry for 48 hours to get an electro active membrane. The layers of the membranes thus obtained were dipped in distilled water to remove the paper from the membrane surface.

Immobilization of GOD on polyaniline (PANI) composite film :

The enzyme GOD (SIGMA) was immobilized by cross-linking via Glutaraldehyde (Loba Chemie) on composite PANI-EPOXY RESIN film, thus restricting the leaching of the enzyme from the film. The stock solution of GOD (1 mg/ml) prepared in 0.1 M phosphate buffer and was adsorbed onto the surface of PANI film. This film was subsequently dipped in 0.1% Glutaraldehyde solution, left for 30 min and washed with respective buffer. The enzymatic incorporation was done in Glutaraldehyde media. This kind of immobilization results in a greater physical and chemical stability of the catalytic material due to the cross-linking formed with the Glutaraldehyde and enzyme. In this case, the active sites of the enzyme could be more accessible for the enzymatic reaction. The lifetime of the biosensor was studied when it was kept at (4°C) in phosphate buffer.

3. Results and Discussion

The amount of glucose can be determined by measuring the anodic potential of oxidation of hydrogen peroxide, produced in the reaction given below

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

Formation of hydrogen peroxide is detected by the potentiometric method during electro Oxidation

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

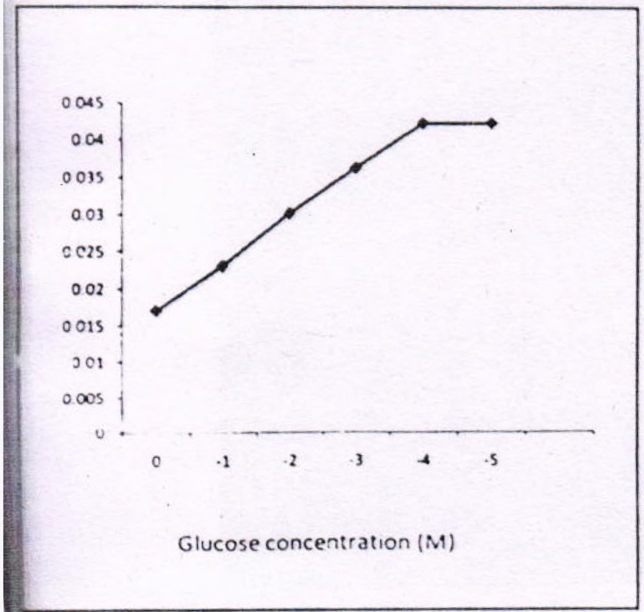
The enzyme electrode formed by glucose oxidase with Glutaraldehyde is used for potentiometric measurement of glucose with an reference electrode. The Glutaraldehyde plays a significant role in potential response. In order to construct the potentiometric enzyme sensor, GOD is used as an example of a redox protein. The enzyme catalyzes in the presence of molecular oxygen which lead to the oxidation of glucose to gluconic acid and hydrogen peroxide. The conversion of glucose to gluconic acid involves the transfer of two protons and two electrons from the substrate to the flavin moiety of the enzyme. The electron transfer from the redox cofactor to the sensing electrode is also facilitating the presence of a polymeric conducting material.

UV-Vis studies of PANI film :

The UV-Visible spectrum of synthesized PANI film recorded using UV-Visible spectrometer 2500 is shown in fig. 1. A black colour film showed one absorption peaks for PANI composite film. The peak at 310 nm is because of Π - Π * transition is corresponds to conducting phase for PANI.

FTIR studies of PANI-Araldite Composite

ELECTRODE RESPONSE



Plot of cell EMF (volts) versus Glucose concentration

UV-VIS STUDIES OF PANI-ARALDLITE FILM

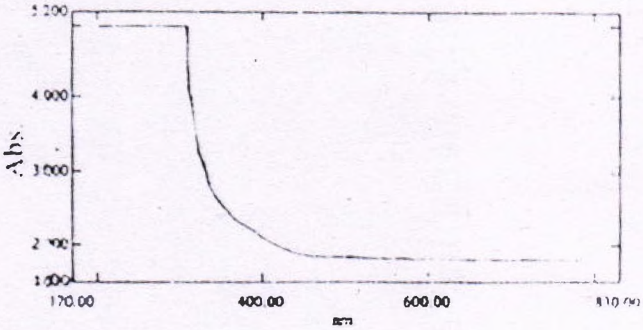


Fig. 1.

UV-V is spectrum of PANI synthesized from 1M aniline with Araldite

FTIR STUDIES OF PANI-ARALDLITE COMPOSITE FILM

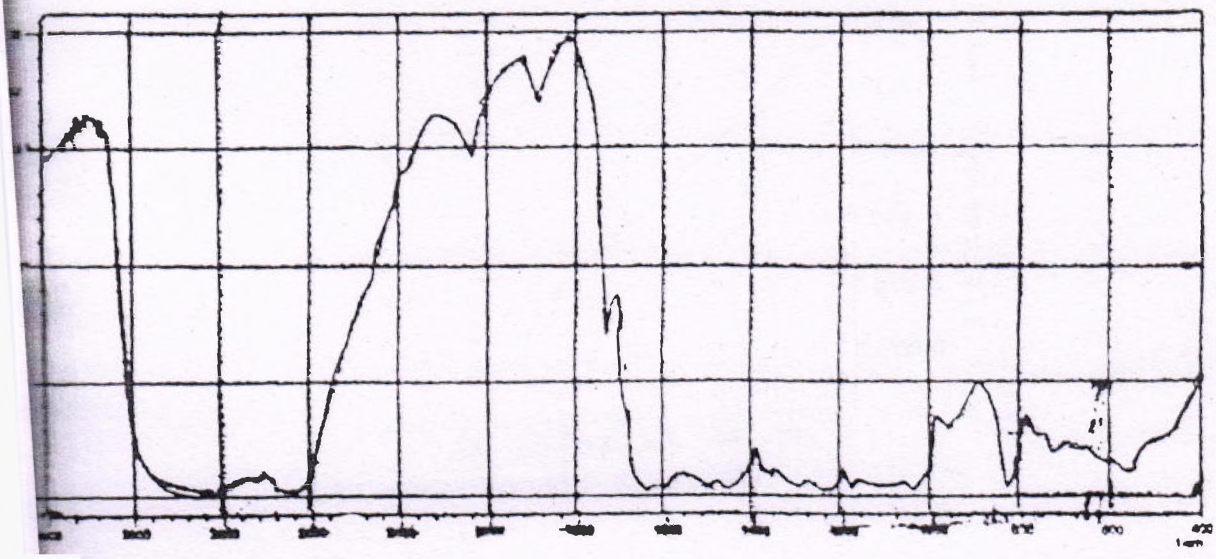


Fig. 2.

FTIR spectrum of PANI film [illegible]

SEM STUDIES OF PANI-ARALDLITE FILM

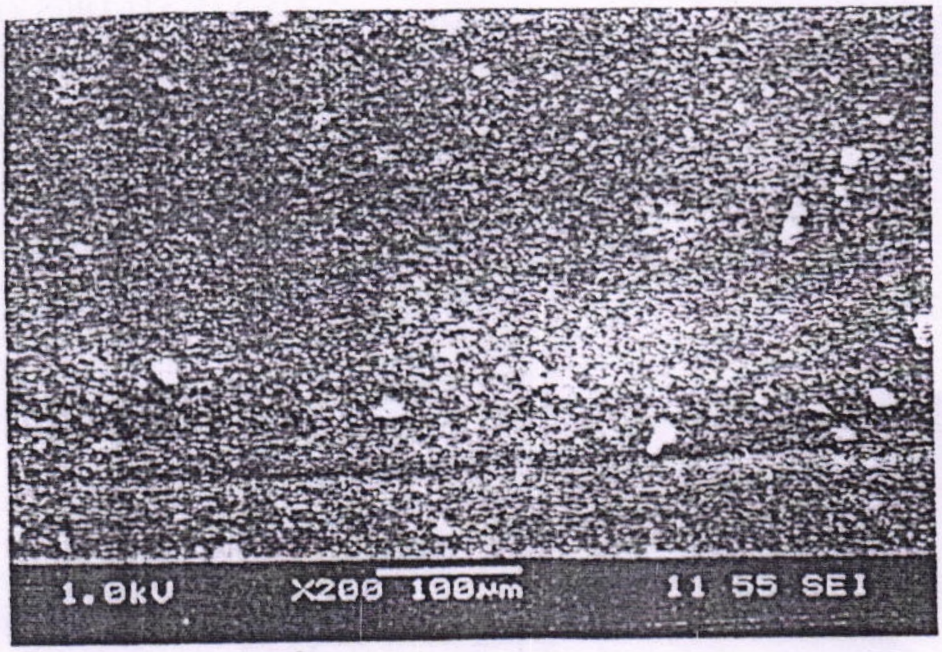


Fig. 3.
SEM spectrum of PANI film synthesized from 1M aniline with araldite matrix

MICHAELIS-MENTEN CONSTANT (KM)

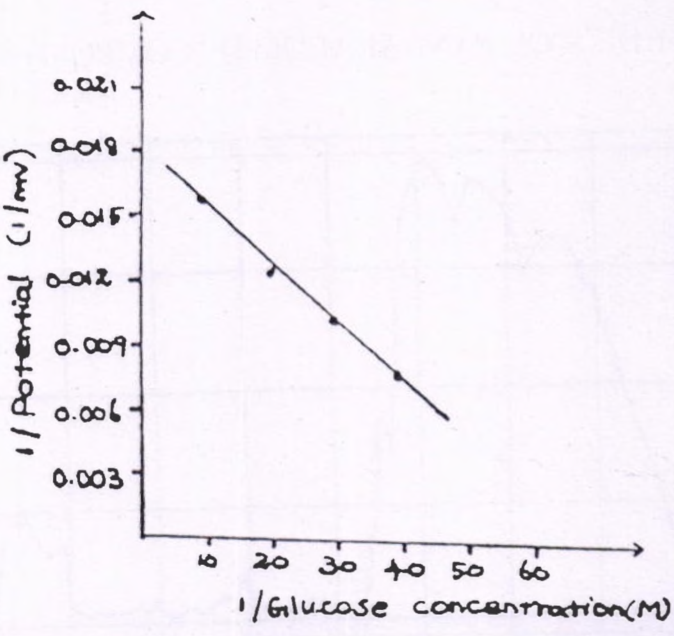


Fig. 4.
The relationship between 1/potential against 1/Glucose concentration in 0.1 M phosphate buffer

The FTIR spectra of synthesized PANI film were recorded by using Testscan Shimadzu Prestige-21 series. in the region 2000-2100 cm^{-1} is shown in Fig. 2. The peak at $1770cm^{-1}$ corresponds to the C = O. C - N stretching of a secondary aromatic amine is observed at peak $2210cm^{-1}$. The peak at 1400 and 909 cm^{-1} corresponds to the C-C stretching vibration and N-H wagging forprimary amine respectively.

SEM studies of PANI-Aradlite film :

The scanning electron micrograph of synthesized *composite PANI-ARALDLITE film* is *as shown in* Fig. 3. The scanning electron micrograph was recorded using JEOL. JSM-6360A SEM machine. It can be seen that the surface morphology is porous. uniform with granular like structure. which is suitable for immobilization of biocomponent.

Potential response of PAN-GOD electrodes:

The change in response potential of the active device is the parameter of interest for sensor applications. The response potential of the device depends on several factors such (1) the contact resistance between the metal electrode and the polymer film. (2) the geometric factor of the film and (3) the film conductivity. The conductivity of PANI-GOD electrode is depends on several factors. such as polymer film potential. substrate concentration and enzyme loading. The GOD was immobilized on electrochemically synthesized PANI film by cross-linking via Glutaraldehyde. It was found the response potential of the enzyme electrode easily reached to steady state. The relationship between response potential and glucose concentration in 0.1 M phosphate buffer shown in Table 1. It was found that. potential increases with increasing glucose concentration in the range 1M - $1x10^{-5}$ M. In the present case assuming that the enzyme is uniformly distributed throughout the film. the reaction takes place predominantly on the surface of the film in the lower concentration. However. at higher concentration the reaction on the surface of the film and the diffusion occurring simultaneously which delay the response time. with increasing concentration of glucose. the response potential also increases and finally reached to the steady state value.

Table 1. Enzyme electrode

Glucose concentration	EMF
(M)	(mv)
1	47
$1 \cdot 10^{-1}$	65
$1 \cdot 10^{-2}$	87
$1 \cdot 10^{-3}$	101
$1 \cdot 10^{-4}$	118
$1 \cdot 10^{-5}$	118

Michaelis-Menten Constant (Km) :

The apparent Michaelis-Menten constant *(Km)*. was calculated for the immobilized enzyme by Potentiometric method. The relationship between 1/potential against 1/Glucose concentration in0.1 M phosphate is shown in Fig. 4. The Michaelis-Menten constant *(Km)* is $2.538x10^{-4}$ for phosphate buffer.

Conclusions

We have successfully developed of PANI-GOD biosensor for determination of glucose. It was found that the conducting PANI having amine functional group can be utilized as a suitable matrix for the cross-linking of GOD via Glutaraldehyde. This efficient cross-linking via Glutaraldehyde on the functionalized PANI film, lead to the enzyme electrode to exhibit a good performance in terms of dynamic range of detection and short response time. The cost effectiveness and simple method of development of PANI-GOD electrode is an additional advantage of this electrode.

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