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Development of PANI-PVS-GOD electrode by potentiometric method for determination of glucose

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The polyaniline-polyvinyl sulfonate-glucose oxidase (PANI-PVS-GOD) electrode has been investigated in the present work. Indium tin oxide (ITO) coated glass electrode was used for the synthesis of polyaniline-polyvinyl sulfonate (PANI-PVS) film using potentiometric method with 0.2 M aniline, 0.5 M PVS solution, 1.0 pH and 1 mA/cm^2 current density. The synthesized PANI-PVS 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-PVS film by cross-linking via Glutaraldehyde in phosphate and acetate buffer. The sensitivity of PANI-PVS-GOD electrode in phosphate and acetate buffer has been studied. It was found that the phosphate buffer gives fast response as compared to acetate buffer in potentiometric measurements.

Keywords: Immobilization; composite film; glucose oxidase; cross-linking

1. INTRODUCTION

The determination of glucose is one of the most popular and well-known biosensor applications. The glucose is of special importance because of its involvement in human metabolic process. Thus in the past couple of decades numerous efforts have devoted to develop glucose biosensor with fast and accurate response. The immobilization technique for localizing enzyme at the surface of various electrodes plays a very important role in the research of glucose biosensor. The conducting polymers are being widely used in biosensor applications because it provides stable and porous matrix for the immobilization of biocomponent and it also facilitate the electron transfer process. The widely used conducting polymers for immobilization of enzyme are polyaniline,

polypyrrole, polythiophene etc [1, 5]. Since polyaniline is compatible to most enzymes and can be easily synthesized from aniline monomer in aqueous solution, the polyaniline is more suitable for biosensor applications. Electrochemically polymerized conducting polymers had received considerable attention over the last two decades [6, 10]. The remarkable switching capability of these electroactive materials between the conducting oxidized (doped) and the insulating-reduced (undoped) state is the basis of many applications. The poly-conjugated conducting polymers have recently been proposed for biosensing applications because of a number of useful features such as (1) direct and easy deposition on the sensor electrode by electrochemical oxidation of monomer, (2) control of thickness and (3) redox conductivity and polyelectrolyte characteristics of the polymer useful for sensor application [11]. PANI fulfills above requirements together with having the characteristics of easy oxidation, high chemical stability etc. The porosity is an important factor for the facile immobilization of enzyme. The development of enzyme based biosensor can be carried out by immobilization of biorecognition element using electrochemical technique [12, 13]. This method is simple and can be used to localize the biocomponent. However, as the biological component is randomly oriented within the polymer matrix it is often in accessible to the target analyte [14, 15]. Therefore, in the present investigation, we have initially electrochemically synthesized PANI-PVS composite film and then GOD was immobilized. We have described the results of our systematic studies relating to the electrochemical synthesis and characterization of the PANI-PVS film and the development of PANI-PVS-GOD electrode for determination of glucose. The advantage of using the composite PANI-PVS film lies in the electrostatic rejection of anions [16]. Sulfonate ion of the PANI-PVS composite film provides a charged surface for electrostatic interaction between the enzyme and the surface [17]. Cross-linking via Glutaraldehyde is likely to lead to greater stability of the enzyme in PANI-PVS film. In the present investigation we have immobilized GOD on (PVS)-doped porous PANI film by cross-linking via Glutaraldehyde for the development of glucose biosensor. We have also studied the influence of pH on the activity of the PANI-PVS-GOD electrode.

2. EXPERIMENTAL PART

2.1. Preparation of polyaniline-polyvinyl sulphonate (PANI-PVS) composite film

PANI-PVS films were synthesized in an aqueous solution of distilled 0.2 M aniline (S.D. Fine. Chem) and 0.5 M of polyvinyl sulfonate (Aldrich) using electrochemical deposition method. It was carried out by potentiometric technique at 27 °C in one compartment, three-electrode glass cell. The ITO coated glass plate was used a working electrode, platinum foil as counter electrode and Ag/AgCl was used as reference electrode. The electrolyte solution was prepared in distilled water. The applied current density 1 mA/cm^2 and the 1.0 pH were kept constant during synthesis of composite film. After synthesis the polymer coated electrodes were rinsed thoroughly in distilled water, dried in cold air and then use for subsequent characterization.

2.2. Immobilization of GOD on polyaniline-polyvinyl sulfonate (PANI-PVS) composite film

The enzyme GOD (SISCO) was immobilized by cross-linking via Glutaraldehyde (Loba Chemie) on composite PANI-PVS 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/or 0.1 M acetate buffer (pH 7.4) was adsorbed onto the surface of PANI-PVS 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 and acetate buffer. An adequate concentration of GOD and Glutaraldehyde in cross-linking mixture were chosen so that it ensure higher enzyme loading and provide excellent potentiometric response with an efficient retention of the enzyme.

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 electrode 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 Ag/AgCl electrode [18]. The Glutaraldehyde plays a significant role in potential response [19]. 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 into 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 [20]**.** The electron transfer from the redox cofactor to the sensing electrode is also facilitating by the presence of a polymeric conducting material.

3.1. Potentiometric studies of PANI-PVS composite film

The potential curves of the potentiometricaly synthesized PANI-PVS composite is shown in Fig. 1. The PANI-PVS film was synthesized on ITO coated glass from 0.2 M concentration of aniline

and 0.5 M of PVS with 1 mA/cm^2 current density at 1.0 pH and temp 27 ºC. This has resulted conducting, with uniform and porous surface morphology of synthesized PANI-PVS film. The behavior of the potentiometric synthesis overshoot during first few second probably indicates difficult formation of dimmers and oligomers. After this, potential remain constant suggesting that building up of the film proceeds according to the same reaction along the full thickness of the polymer. The electrical conductivity of synthesized composite PANI-PVS was measured by four probe technique and it was 1.6 S/cm.

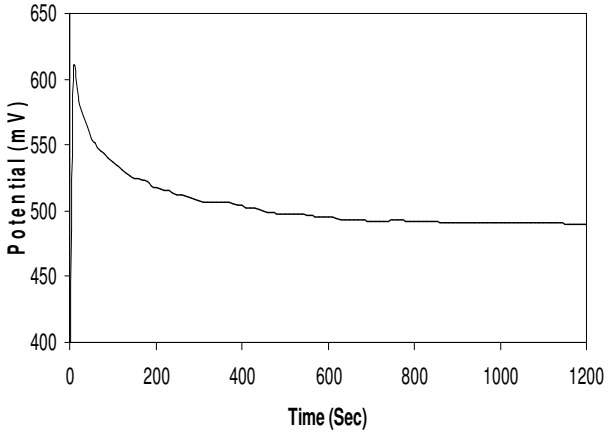

Figure 1. Chronopotentiogram of PANI-PVS film synthesized at 1.0 pH, 0.2 M aniline, 0.5 M PVS, 1 mA/cm^2 current density and temp 27 ºC.

3.2. UV-Vis studies of PANI-PVS composite film

The UV-visible spectrum of synthesized PANI-PVS film recorded using UV-visible spectrophotometer 1601 is shown in Fig. 2. A green colored film showed two absorption peaks for PANI-PVS composite film. The peak at 493 nm is because of π-π^* transition and a broad peak at 810 nm is corresponds to the conducting phase for PANI-PVS.

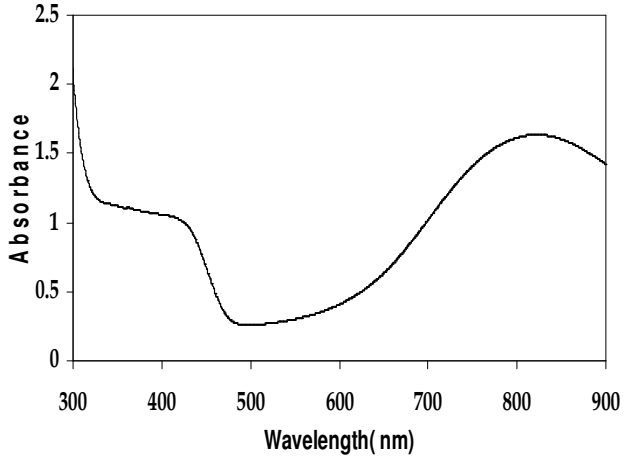

Figure 2. UV-visible spectra of PANI-PVS film synthesized at 1.0 pH, 0.2 M aniline, 0.5 M PVS, 1 mA/cm^2 current density and temp 27 ºC

3.3. SEM studies of PANI-PVS Composite film

The scanning electron micrograph of synthesized composite PANI-PVS 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.

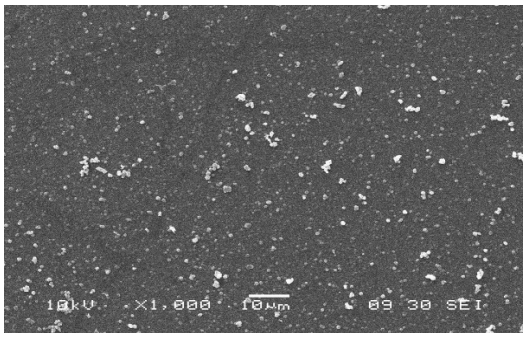

Figure 3. SEM micrograph of PANI-PVS film synthesized at 1.0 pH, 0.2 M aniline, 0.5 M PVS, 1 mA/cm^2 current density and temp 27 ºC.

3.4. FTIR studies of PANI-PVS Composite film

The FTIR spectra of synthesized PANI-PVS film were recorded by using Testscan Shimadzu FTIR-8400 series, in the region 2000-2100 cm^{-1} is shown in Fig. 4. The peak at 1533 cm^{-1} and 1497 cm^{-1} corresponds to the quinine and benzene ring stretching deformation respectively. The C-N stretching in the quinoid ring is observed at 1378 cm^{-1}, while C-N stretching of a secondary aromatic amine is observed at peak 1038 cm^{-1}. The peak at 1093 and 1099 cm^{-1} corresponds to the C-H in plane bending mode. C=N stretching of quinine di-imine unit is observed at 1565 cm^{-1}.

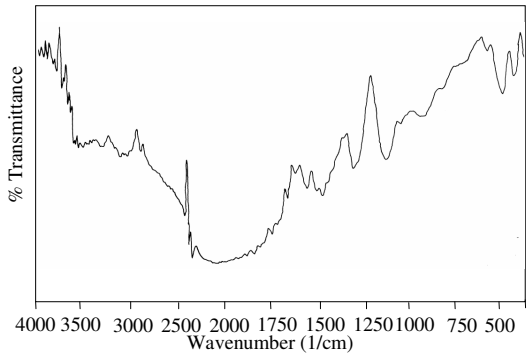

Figure 4. FTIR spectra of PANI-PVS film synthesized at 1.0 pH, 0.2 M aniline, 0.5 M PVS, 1 mA/cm^2 current density and temp 27 ºC

The peak observed at 1035 cm^{-1} and 694.3 cm^{-1} are due to the symmetric stretching of SO_3^- group. Thus, the FTIR spectral results confirm the formation of polyaniline film composite with PVS medium.

3.5. Potential response of PANI-PVS-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 as (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-PVS-GOD electrode is depends on several factors, such as analyte pH, temperature, polymer film potential, substrate concentration and enzyme loading. The GOD was immobilized on electrochemically synthesized PANI-PVS film by cross-linking via Glutaraldehyde. The potential-time relationship of PANI-PVS-GOD electrode when the applied current of the enzyme was set 0.5 mA in phosphate and acetate buffer is as shown in Fig. 5 and 6 respectively. It was found that 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 and acetate buffer at pH 7.4 is shown in Fig. 7 and 8 respectively. It was found that, potential increases with increasing glucose concentration in the range 1 mM - 50 mM. 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.

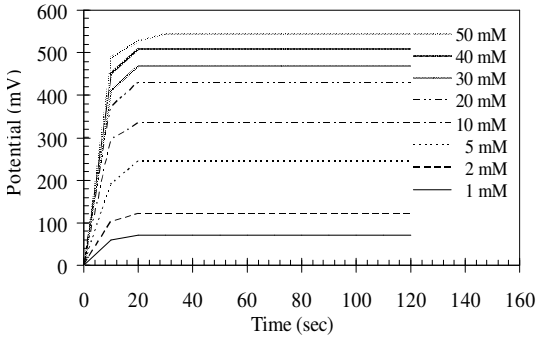

Figure 5. Potential-time curves for the PANI -PVS-GOD electrode for various glucose concentrations in 0.1M Phosphate buffer, pH 7.4

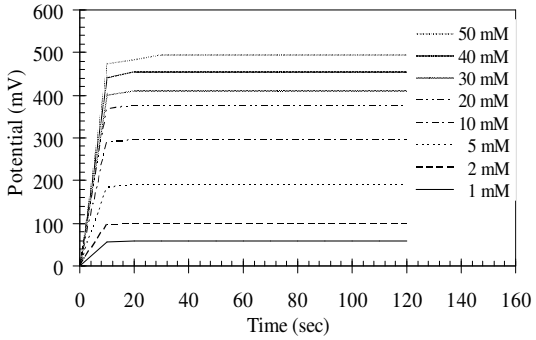

Figure 6. Potential-time curves for the PANI -PVS-GOD electrode for various glucose concentrations in 0.1M acetate buffer, pH 7.4

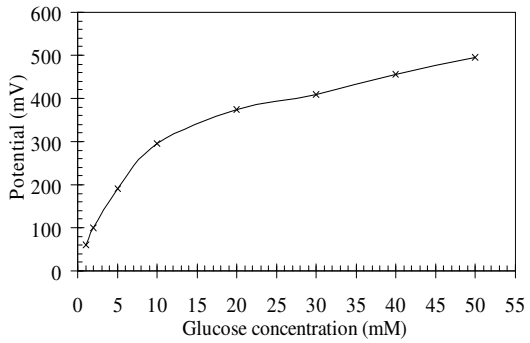

Figure 7. The relationship between response potential and glucose concentration for the PANI-PVS-GOD electrode in 0.1 M phosphate buffer, pH 7.4

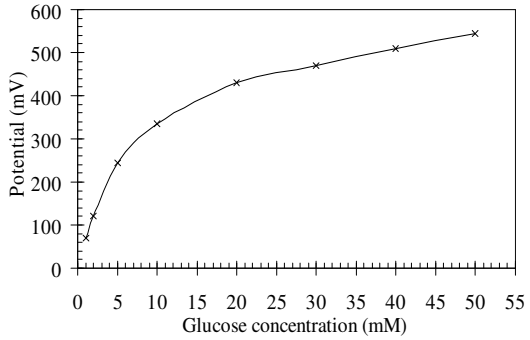

Figure 8. The relationship between response potential and glucose concentration for the PANI-PVS-GOD electrode in 0.1 M acetate buffer, pH 7.4

3.6. Michaelis-Menten Constant (K_m)

The apparent Michaelis-Menten constant *(K_m),* was calculated for the immobilized enzyme by potentiometric method [21]. The relationship between 1/potential against 1/Glucose concentration in 0.1 M phosphate and acetate buffer is shown in Fig 9 and 10. The maximum voltage (V_{max}) is 500 mV and Michaelis-Menten constant *(K_m)* is 7.14 *mM* for phosphate buffer and for acetate buffer, the maximum voltage is 540 mV and Michaelis-Menten constant (K_m) is 8.3 mM (Table 1).

Table 1. Comparison of the analytical performance of PANI-PVS-GOD electrode for phosphate and acetate buffer at pH 7.4.

Sr. No	Parameters	Buffers	
		Phosphate	Acetate
1	V_{max} (mV)	500	540
2	K_m (mM)	7.14	8.3
3	Linearity (mM)	1-5	1-5
4	Sensitivity (mV/mM)	50	30

The value of the K_m depends on the immobilization of enzyme; lesser K_m gives faster response to the glucose. The sensitivity of PANI-PVS-GOD electrode in phosphate buffer is 50 mV/mM and in acetate buffer, it is 30 mV/mM.

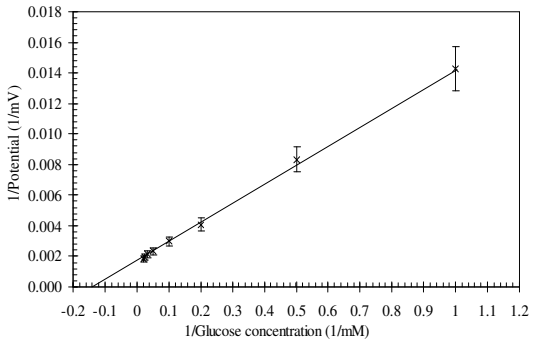

Figure 9. Determination of apparent Michaelis-Menten constant (K_m) for the PANI-PVS-GOD electrode, in 0.1 M Phosphate buffer, pH 7.4

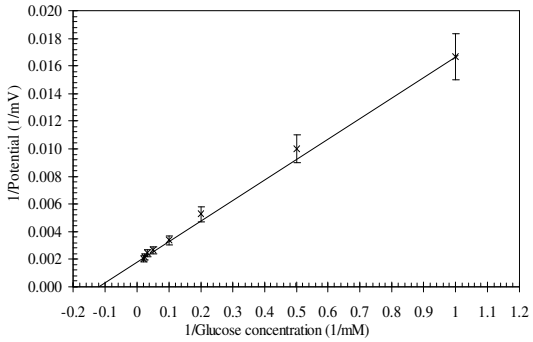

Figure 10. Determination of apparent Michaelis-Menten constant (K_m) for the PANI-PVS-GOD electrode, in 0.1 M acetate buffer, pH 7.4.

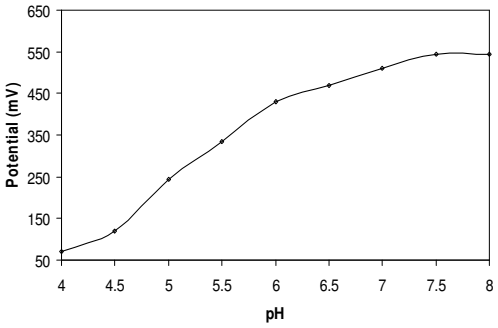

Figure 11. Effect of pH on the PANI-PVS-GOD electrode response of steady potential measured at 0.5 mA in 5 mM glucose solution in 0.1 M phosphate buffer.

3.7. Effect of pH

The value pH of the reaction medium allows an efficient entrapment of the enzyme. It also prevents the loss of the enzyme activity under polymerization conditions [21]. Therefore, enzyme

sensor response depends on the working pH of the sample solution. The effect of pH on the behavior of the enzyme electrode was studied with 0.1 M phosphate and acetate buffer solution with 5 mM glucose. The steady state potential at 0.5 mA as a function of the pH for phosphate and acetate buffer is shown in Fig 11 and 12. The electrochemical response is quite good at pH ranging from 4.0-8.0 and the maximum potential was observed at pH 7.4 in phosphate and acetate buffer [22, 23].

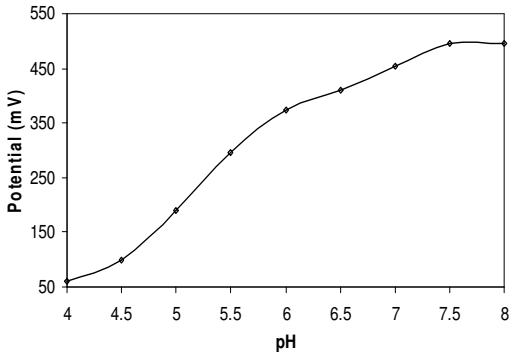

Figure 12. Effect of pH on the PANI-PVS-GOD electrode response of steady potential measured at 0.5 mA in 5 mM glucose solution in 0.1 M acetate buffer.

4. CONCLUSIONS

We have successfully developed of PANI-PVS-GOD biosensor for determination of glucose. It was found that the conducting PANI-PVS 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 porous PVS doped 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-PVS-GOD electrode is an additional advantage of this electrode.

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