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Fabrication of bioanode by using electrically conducting polythiophene via entrapment technique

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Abstract−A glassy carbon electrode (GCE) was tailored with conducting polymer polythiophene and further immobilized by an enzyme glucose oxidase (GOx). A thin film of polymer was developed by electrochemical polymerization of thiophene monomer. During electrochemical polymerization of the monomer the enzyme GOx and the redox active mediator ferritin (Frt) were entrapped within this polymer matrix. In this novel approach, the entrapment of enzyme and mediators within a polymer matrix occurs without chemical reaction that could affect their activity. The entrapment of enzyme and mediator within the conducting polymer matrices increases the surface area of the electrode. The tailored GCE/Ptp/Frt/GOx electrode showed a high catalytic activity. The increased surface area causes a high rate of electron transfer between the electrode and Frt engaged as an electron transfer mediator. The electrochemical properties of the electrode were determined by cyclic voltammetry (CV) and linear sweep voltammetry (LSV). The fabricated bioanode showed a current density of 3.9 mA cm^{-2} at 1.0 V vs. Ag/AgCl in a 45 mM glucose solution and suggests proficient chances in biofuel cells (BFCs) applications.

Keywords: Optimization, Conducting Polymer, Polythiophene, Entrapment, Glucose Oxidase

INTRODUCTION

The energy demand is increasing every year, globally leading to the excessive discharge of global warming gas (CO_2). Nowadays, the energy demand is being fulfilled by current petroleum supply. However, the steep decrease in the renewable source of petroleum supply and associated problems accountable for global warming are motivating research in the area of alternative renewable energy technologies. [1]. Biofuel cells (BFCs) are acting as such enormous renewable energy alternatives. BFCs are electrochemical devices that can convert chemical energy of fuel into electrical currents by using biological catalysts that are highly specific in catalyzing a reaction; moreover, they can also speed up the rate of reaction [2-8]. The most important application of BFCs is that electrical currents generated from such cells can be used to power implanted devices. Scientists and engineers are very enthusiastic about using miniature BFCs in the human body to power implanted devices such as glucose sensors [9,10], blood pressure, temperature and metabolite concentration measuring instruments, pacemakers and bladder control valves [11-13], cochlear implants as hearing aids and robots. Thus, BFCs may act as a lifeline for those individuals who are suffering from fatal diseases. BFCs are considered as an option against conventional fuel cells to reduce green house gas emissions and to provide energy supply for urban as well as rural development. The BFCs technology has several advantages over the traditional fuel cells based on noble metal catalysts, such as the possibility to operate at ambient temperatures and in physiological conditions. The first enzyme based BFCs were developed in 1964 by using enzyme anode catalyst and glucose as fuel [14,15]. Power density and short lifetimes are two main barriers encountered in BFCs. To overcome these obstacles much efforts and considerable amount of development have been made during the last decade. Research in the BFCs is nowadays focused on the electrode structure improvement as well as on modern immobilization techniques [16]. This could be improved by using conducting polymers [17-21], redox polymers [22-25], and self assembled monolayer [26-28], or by making use of the nanotechnology [29-31]. The immobilization of enzyme on the electrode surface is solely responsible for the efficient functioning of the enzyme electrode assembly. Various chemical, biochemical and physical processes of enzyme immobilization techniques have been used such as adsorption, cross linking, covalent bonding and entrapment or encapsulation within polymers [32-35]. Among them the entrapment of enzyme in conducting polymer backbone during electrochemical polymerization is of great significance due to many advantages such as its simplicity, reliability and it is inexpensive as well as minimized enzyme leaching.

In this context, various conducting polymers have been used for the immobilization of enzymes and as electrical contacts in biosensors and BFCs [2,36,37]. Conducting polymers are optimistic materials for the components of the enzyme electrodes. These conducting polymers enhance the rate of electron transfer. The thin films of conducting polymers can be readily synthesized by electrochemical polymerization of heterocyclic compounds. Conducting polymers are excellent platforms for the immobilization of biomolecules at the electrode surface because they provide enhanced sensitivity, selectivity, biocompatibility [38-40], direct electrochemi-

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cal synthesis and flexibility for the immobilization of biomolecules. Electrically conducting polythiophene (Ptp) is considered to be a viable candidate in BFCs due to its high chemical stability, structural tailorability and biocompatibility. Ptp compounds are stable in both doped and undoped states, which makes these compounds multifaceted ranging from bioelectronics to biotechnology. Ptp is a conjugated polymer with excellent electronic and reasonably good mechanical properties, and is stable towards oxygen and moisture at ambient temperature. Mediators are generally redox active compounds with reversible electrochemistry and are capable to transfer electrons between the buried redox active sites of enzyme and the electrode [41]. The majority of the enzymes have a redox center deeply buried into the protein shell that interrupts the electrical communication occurring between the enzyme and the electrode. The obstacle in electron transfer can overcome by using a redox moiety known as mediator to shuttle electrons between the enzyme and the electrode. These mediators are acting as carriers of electrons from the redox center of the enzyme to the electrode. Literature reports a wealth of redox mediators which are non biocompatible, non-biodegradable abusing the environment [42-46]; on the other hand, the redox protein ferritin (Frt) is biocompatible, degradable and environmentally inert. Frt works close to the oxidation potential of enzymes [47,48]. Therefore, Frt is utilized as an electron transfer mediator [49-51]. It is an iron storage protein that accommodates up to 4500 Fe atoms as $Fe(OH)_3$ in the cavity. Soluble Fe(II) ions are oxidized to insoluble Fe(III) ions for incorporation into the mineral core, and the stored insoluble Fe(III) ions are released as Fe(II) by biological redox processes. Due to the redox reaction Frt can be used as an electrochemically active material, which can help to promote electron transfer from the redox active site of the enzyme to the electrode interface thereby serves as an efficient mediator.

The present work studied the bio electrochemical responses of Ptp/Frt/GOx modified anode developed *via* entrapment technique, where Ptp is used as a novel conductor while Frt is used as a redox mediator, which shortens electron tunneling distance and electrically communicates the electrons generated at the enzyme redox site with electrode surface. In entrapment, enzymes are entrapped inside the polymer matrix. Basically, entrapment occurs during the polymerization of polymer moiety containing biocatalyst and electron transfer mediator in polymer monomer solution. Entrapment is carried out by mixing the biocatalyst into a monomer solution, followed by polymerization.

EXPERIMENTAL

1. Chemicals and Reagents

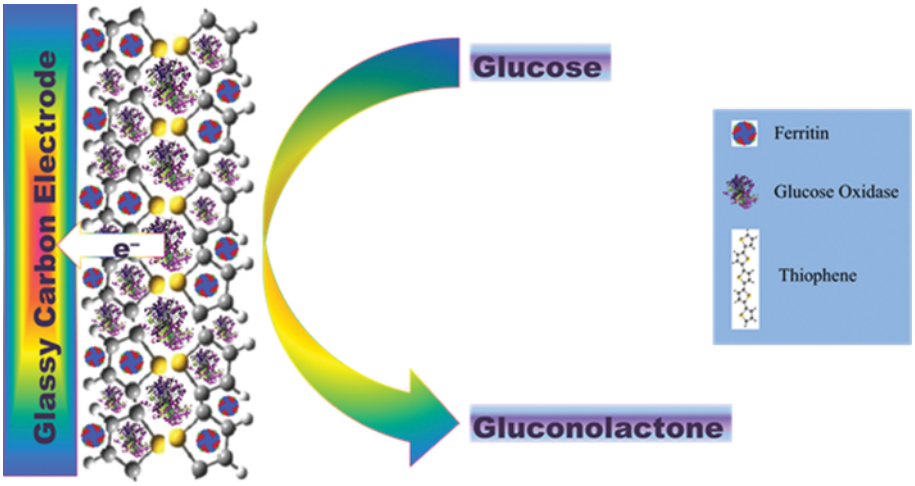
The ferritin (Frt) (10 mg ml^{-1} in 0.15 M NaCl) from horse spleen, thiophene and glutaraldehyde (Sigma Chemicals, India), phthalate buffer solution of pH 4.0 and phosphate buffer solution of pH 7.0 (Otto Pvt. Ltd. India), glucose oxidase (GOx) from *Aspergillus niger* lyophilized powder containing 100,000-150,000 units/g (Central Drug House, India) and D-(+)-glucose anhydrous (Himedia Laboratories Pvt. Ltd. India), were used as received.

2. Instruments

A computer controlled potentiostat/galvanostat (302N Autolab, Switzerland) was used for electrochemical measurements. A working electrode of as-prepared glassy carbon biocomposite electrode (GCE), an Ag/AgCl reference electrode and a platinum wire counter electrode were used for all electrochemical measurements in phosphate buffer saline (PBS) at pH 7.0 in the presence and in the absence of glucose at room temperature (25±3 °C) in air. The electrode was ultrasonicated with digital ultrasonic cleaner LMUC series Labman, India.

3. Preparation of Polythiophene/Ferritin/Glucose Oxidase Electrodes

A 3 mm diameter GCE was polished with 0.05 μm alumina slurry by using a velvet pad. The electrode was ultrasonicated for 20 min and washed with distilled water and allowed to dry at room temperature (25±3 °C). The concentrations of the electro active components of the electrodes are optimized (results are not shown). Before electrochemical polymerization, high purity nitrogen gas was passed into the buffer solution for 10 min. Thiophene modified GCE was prepared by electrochemical polymerization of thiophene (6 μl of thiophene dissolved in 30 ml of phthalate buffer of pH 4.0) using cyclic voltammetry by applying potential in the range of −1.5 V to +1.5 V upto 20 cycles at the scan rate of 100 mVs^{-1}.



Scheme 1. The entrapment of bioactive component on GCE.

Ptp/Frt modified GCE was prepared by entrapment of Frt in the matrices of polymer by electrochemical polymerization using cyclic voltammetry containing 6 µl of thiophene and 6 µl of Frt in 30 ml of phthalate buffer of pH.4.0 in a similar manner. Similarly, Ptp/Frt/GOx modified GCE was prepared by CV containing 9 µl of GOx [(10 mg/ml) in a phthalate buffer of pH 4.0], 6 µl of thiophene and 6 µl of Frt in 30 ml of phthalate buffer of pH 4.0 to achieve entrapment by electrochemical polymerization. The entrapment of the bioactive component of the electrode during electrochemical polymerization of thiophene along with the direction of electron transfer is as shown in Scheme 1.

RESULTS AND DISCUSSION

Electrochemical polymerization of thiophene was carried out by the entrapment of enzyme and redox active mediator simultaneously. The close contact of the enzyme with the conducting polymer leads to the enhancement in the transfer rate of electrochemical signal from enzyme to the electrode surface. The electrochemical properties of the modified bioanode were investigated by cyclic voltammetry. All experiments were performed by nitrogen purging.

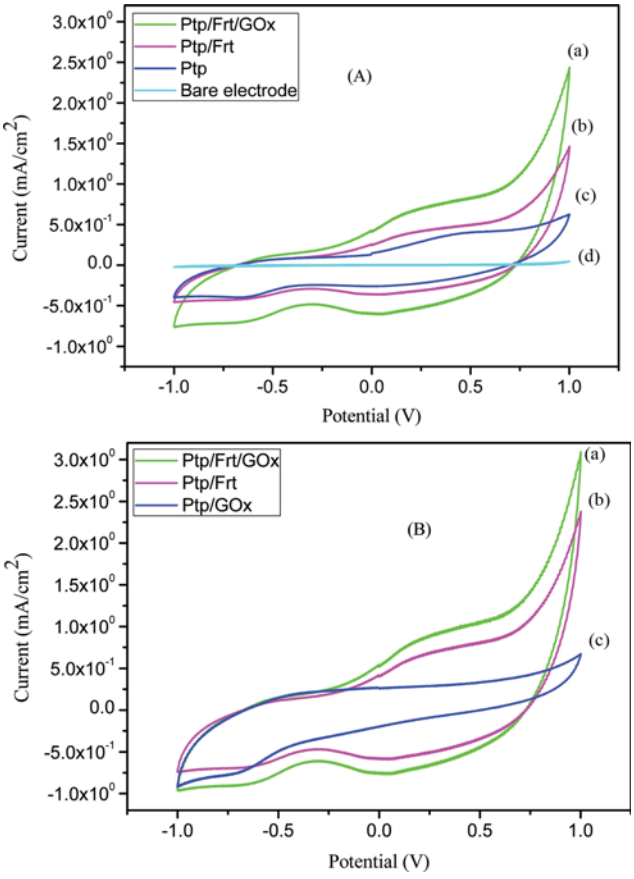


Fig. 1. (A) CVs of (a) Ptp/ferritin/GOx, (b) Ptp/ferritin, (c) Ptp, (d) Bare electrode confined onto GCEs in PBS (pH.7.0) at room temperature with a potential scan rate of 100 mVs^{-1}. (B) CVs of (a) Ptp/Ferritin/GOx in 45 mM glucose, (b) Ptp/Ferritin in 45 mM glucose, (c) Ptp/GOx in 45 mM glucose in PBS (pH.7.0) at scan rate of 100 mVs^{-1}.

As shown in Fig. 1(A) the glassy carbon electrode (GCE) generated a large oxidation current in the presence of both enzyme glucose oxidase (GOx) and redox active mediator ferritin (Frt). It is generally accepted that the redox site of GOx is buried in the interior of its protein shell and the direct electron transfer from GOx to the electrode surface is very difficult. Therefore, the large current shown in case of Ptp/Frt/GOx was considered to demonstrate the important role of Frt in electron transfer from GOx to the electrode surface. The performance of all the three electrodes, Ptp/GOx, Ptp/Frt and Ptp/Frt/GOx, were characterized by cyclic voltammetry in the presence of 45 mM glucose as shown in Fig. 1(B) In case of Ptp/GOx, current was hardly observed, whereas in case of Ptp/Frt the area under the curve increases as well as the oxidation and reduction peaks become visible with the considerable enhancement in the oxidation current, suggesting that Frt acted as redox mediator. However, Ptp/Frt/GOx acquired the highest current, and the oxidation and reduction peaks of the conducting polymer thiophene became more prominent.

The reason behind this enhancement of the current is due to the mediated electron transfer by Frt from the active sites of GOx at an electrode electrolyte interface. Thus, Frt shortens the electron transfer distance by entering in the clefts of enzyme and shuttle between the redox sites of enzyme GOx and electrode interface. Conducting polymer increases the efficiency of electron transfer and hence is responsible for fast electrochemical kinetics. The biocatalytic activity of the modified GCE (Ptp/Frt/GOx) for the oxidation of glucose was studied in 45 mM of glucose in PBS of pH 7.0 and in the absence of glucose in PBS of pH 7.0; as shown in Fig. 2 the biocomposite modified electrode generated a large glucose oxidation current in the presence of 45 mM of glucose. Thus, it is apparent that the modified bioanode showed catalytic activity in the presence of glucose.

Fig. 3(A) presents the effect of five different scan rates (20, 40, 60, 80 and 100 mVs^{-1}) of CV on the anodic currents of the modified biocomposite electrode in PBS of pH 7.0. The results show that the anodic current is increasing linearly with the increase in scan

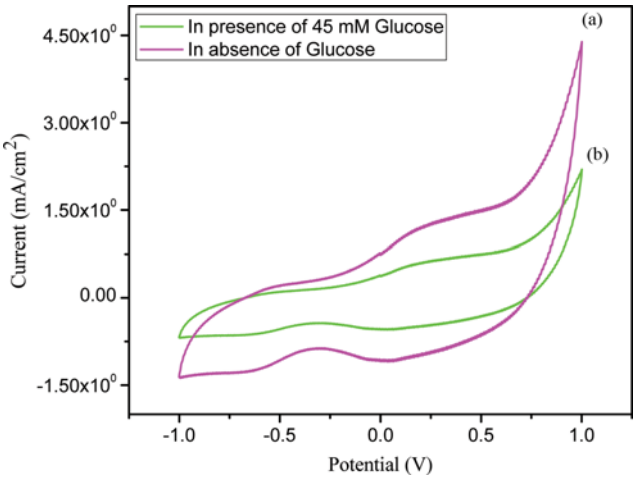


Fig. 2. CVs of (a) Ptp/ferritin/GOx in 45 mM glucose in PBS (pH 7.0), (b) Ptp/ferritin/GOx in absence of glucose in PBS (pH 7.0) at scan rate of 100 mVs^{-1}.

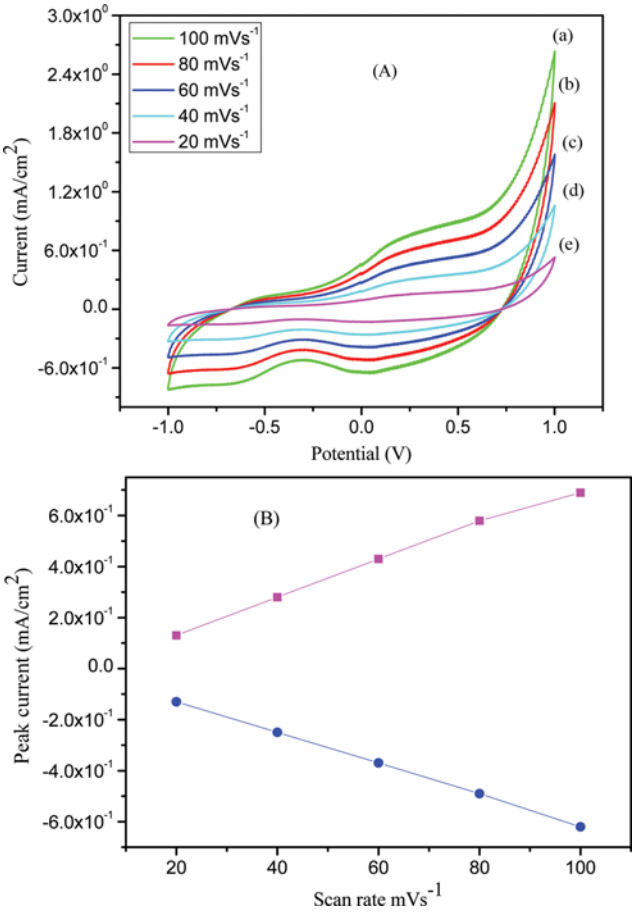


Fig. 3. (A) CVs of Ptp/ferritin/GOx modified GCE in 45 mM glucose in PBS (pH.7.0) at scan rate (a) 100, (b) 80, (c) 60, (d) 40 and (e) 20 mVs^{-1}. (B) The plots of peak currents vs. scan rate.

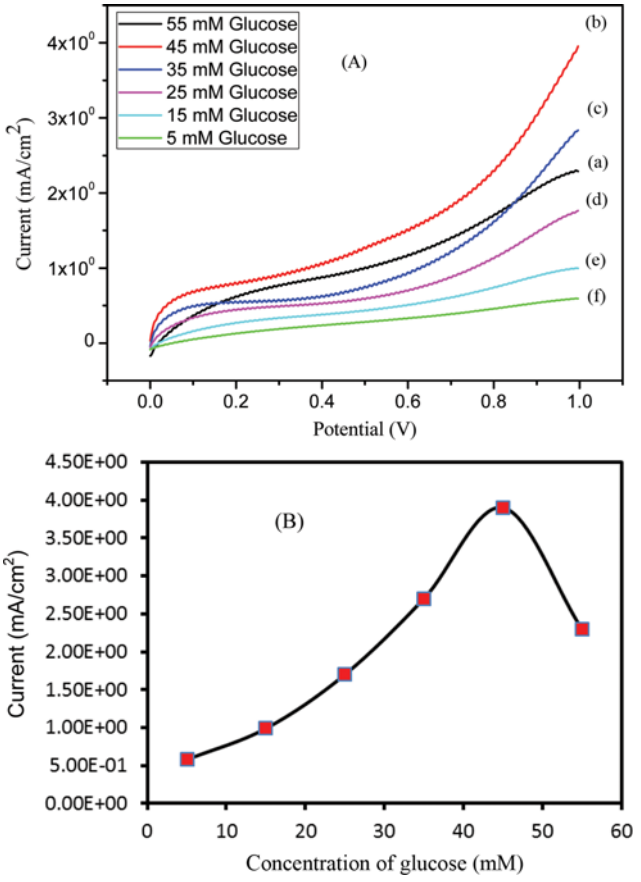


Fig. 4. (A) LSVs of Ptp/ferritin/GOx modified GCE in PBS (pH 7.0) and different concentrations of glucose (a) 45 mM (b) 35 mM (c) 25 mM (d) 15 mM and (e) 5 mM at room temperature with a potential scan rate of 100 mVs^{-1}, (B) the calibration curve corresponding to the electro catalytic current against variable concentration of glucose.

rates. The redox peak currents (ipc) vs. scan rate are shown in Fig. 3(B). The anodic and cathodic peak currents are linearly proportional to the scan rate ranging from 20-100 mVs^{-1}, indicating a surface-controlled reaction phenomenon. Thus, we can say that the scan rates have a significant influence on the catalytic current.

The surface concentration of the Ptp/Frt/GOx biocomposite on GCE was estimated by using cyclic voltammetry based on the following equation given by the Brown-Anson model:

$$I_p = \frac{n^2F^2I^*AV}{4RT}$$

where n is the number of electrons to be transferred (in the present case n=2), F is the Faraday constant (96,484 C mol^{-1}), **I*** is the surface concentration of the Ptp/Frt/GOx biocomposite (in mol cm^{-2}) to be determined, A is the surface area of the GCE (0.07 cm^2), V is the scan rate (100 mVs^{-1}), T is the absolute temperature in Kelvin and R is the gas constant (8.314 J mol^{-1} K^{-1}). The surface concentration of the bioelectrode confined by Ptp/Frt/GOx was found to be (1.4×10^{-10} mol cm^{-2}).

In Fig. 4 the current response of Ptp/Frt/GOx modified GCE was signalized by using LSV in the presence of different concentrations of glucose. Fig. 4(A) shows that the catalytic current of the biocomposite modified electrode is increasing with the increase in the glucose concentration up to 45 mM, and a further increase in glucose concentration results in lower cell performance. The reason for the lower performance at higher glucose concentration is not clear for now; further studies required. This is obvious as electrode is working for the catalytic oxidation of glucose by virtue of the electron transfer mechanism. The calibration for the glucose concentration versus oxidation current density generated by using this bioanode is shown in Fig. 4(B). The oxidation current density increases with increase in the glucose concentration and a maximum current density of 3.9 $mAcm^{-2}$ at 1.0 V vs. Ag/AgCl for the oxidation of glucose in 45 mM glucose concentration was attained. This result evidently means that the current generation was due to glucose oxidation on the modified bioanode. The electrode showed its performance in the presence of glucose and the behavior of oxidation current density increases with an increase in the concentration of glucose up to 45 mM. So, it can be well said that this bioanode can also be used as glucose biosensors. The lifetime of entrapped enzyme over the surface of Ptp/Frt/GOx was found to be approximately 38 days.

It is considered that the conducting polymers became a promi-

nent material for the immobilization of biomaterials during their polymerization due to their biocompatibility, bioavailability and conductivity. The conducting polymers due to their excellent electrical conductivity may provide electrical communication with the redox enzyme and the regeneration of biocatalysts with the help of redox mediators, which serves as an intermediate for the electron transfer.

CONCLUSIONS

The polymerization of thiophene has been successfully achieved along with the entrapment of enzyme and mediator. Entrapped redox mediator ferritin within polymer matrices abbreviates the electron transfer distance while the conducting polymer thiophene enhances the conduction of electron transfer, and in turn enhances the speed of electron transfer. The entrapment technique within polymer matrices offers many advantages such as enhanced biocatalytic stability, easy protection against the negative environmental impact, e.g., contamination by microbes and undesirable electrochemical interactions.

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