Chapter 4

Electrospun Polyaniline/Polyvinyl alcohol/Multiwalled Carbon Nanotubes Nano Fibers as Promising Bioanode Material for Biofuel Cell Applications
Chapter 4

4.1 INTRODUCTION

Sharp rise in oil prices due to finite life of fossil fuels and ever growing environmental concerns especially related to green house gas emissions, respiratory disorders and other environmental hazards are suggesting the need for producing renewable energy sources to diminish the concerning issues of global warming. Keeping all these issues in mind, many research organizations have been ardent to develop biofuel cells (BFCs). Biofuel cell is a collective term of two parent technologies i.e. fuel cells and biotechnology. The eco-friendly fuel cells are believed to be the next generation energy devices bearing immense potential for generating electricity from renewable fuels such as sugars, alcohols and organic acids leveraging on enzymes or micro organisms as catalysts [1-5]. Principle of biofuel cells is based on the redox reaction between enzyme and substrate. Advantages of BFCs include the utilization of enzymes or micro organisms over precious metal based fuel cells in addition to their ability of operation at mild temperatures and physiological conditions [6-8]. Based on these specifications BFCs can be used in power implantable devices that can operate in biological fluids [9-12]. High power density and excellent operational stability are the two most important requirements for efficient functioning of BFCs to raise its applications. Both these challenges can somewhat be met from efficient immobilization of biocatalyst to the substrate and use of biocompatible redox active mediators that work by transferring electrons from the deeply located enzyme active site to the conductive matrix. These two processes help to improve the lifetime of biofuel cell. Various enzymes immobilization techniques have been carried out such as adsorption, covalent bonding, cross linking and entrapment in gels or polymer matrices and nanofibres [13-16]. Among them nanofibrous membranes are widely scrutinized as immobilization supports, with large surface area-to-volume ratio, highly porous nature and interconnectivity. High surface area provides enormous number of active sites that enhance the capability to bind more enzymes and interconnected pores provide interaction between reactants and enzymes which is valuable for efficient functioning of biological processes. These features make them supreme candidates as support materials in enzyme immobilization [16-18]. Compared to other immobilizing supports electrospun nanofibres possess several advantages such as light weight, long-lasting and can be easily recuperated from a
reaction solution when used as adhering supports for biocatalysts [19]. However, electrospun conducting fibers are steadily produced by combining a conducting polymer and a non-conducting polymer [20–23]. They bear the disadvantage of low electrical properties as compared to pure conducting nanofibres fabricated by using electro-polymerization and electro-deposition [24,25]. In order to enhance the electrical properties of these electrospun nanofibres, an effective solution is to incorporate carbon nanotubes which have superior electrical conductivity [26]. In addition to their high electrical conductivity, introduction of carbon nanotubes can enhance current densities outstandingly which is due to their strong carbon–carbon bonding. However, all the existing research studies have focused on designing carbon nanotubes/non conducting polymer composite fibers [27,28], in spite of many advantages of conducting polymers. In this research work, electrospun composite nanofibres were fabricated by mixing a suspension of multiwalled carbon nanotubes (MWCNTs) with a composite of polyaniline (PANI)/poly vinyl alcohol (PVA) using an electrospinning process.

Conducting polymers such as polypyrrole, polythiophene and polyaniline have been studied extensively in the past two decades. Among electrically conducting polymers polyaniline (PANI) has attracted great interest due to its several amazing characteristics including its improved environmental stability, facile synthetic route, excellent electrical and electrochemical properties [29,30]. The processability of PANI to make fibers is rather difficult due to difficulty in solubility in most of the common solvents. So, attempts have been made to overcome these difficulties such as blending with other soluble polymers e.g. polyvinyl alcohol (PVA) [31], polymethyl methacrylate (PMMA) [32], polyethylene oxide (PEO) [33] and polysulphonated styrene (PSS) [34]. In this work, PVA was chosen as a known soluble polymer in the preparation of electrospun fibers. PVA is semicrystalline polyhydroxy polymer that has been studied extensively and used as a material for electrospinning owing to its good film forming properties, good thermal stability, good physical properties, excellent biocompatibility, biodegradability and inexpensiveness [35]. BFCs work on the principle of electron transfer between the immobilized enzyme and the electrode which is a key factor for the performance of BFCs. In order to further intensify the shift of electrons, several electron mediators have been employed during the past
decade. Based on several characteristics, ferritin (Frt) has been employed as an electron transfer mediator due to many reasons. One and the most important reason is that this mediator is eco-friendly and biocompatible which is prime concern for the efficient functioning of BFCs in biological fluids [36-38]. Major idea of the work presented here was to apply environmental friendly and biodegradable materials and to maximize glucose oxidation.

In this work, glucose oxidase was covalently immobilized on to the surface of glassy carbon electrode (GCE) modified with electrospun nanofibres of PANI/PVA/MWCNTs, wherein Frt is used as a redox active mediator which reduces electron tunneling distance as well as electrically communicates the electrons generated at the enzyme redox site with electrode surface.
4.2 EXPERIMENTAL

4.2.1 Reagents and instruments

The ferritin (FrI) (10 mg mL\(^{-1}\) in 0.15 M NaCl) from horse spleen and carbon nanotubes, multiwalled (O.D × L, 6-9 nm × 5 µm) 95% carbon (Sigma Chemicals, India), glucose oxidase (GOx) from Aspergillus niger lyophilized powder containing 100,000-150,000 units g\(^{-1}\) and polyvinyl alcohol (cold) (Central Drug House, India), D(+)glucose anhydrous (Himedia Laboratories Pvt. Ltd. India), aniline and N,N dimethyl formamide (DMF) (Thermo Fisher Scientific Pvt. Ltd., India), potassium peroxodisulphate extra pure (Merck Specialties Pvt. Ltd., India), N-hydroxy succinimide (NHS) 98%, phosphate buffer solutions (PBS) of pH 7.0, phthalate buffer solution of pH 4.0 and 1-ethyl 3-3 dimethyl aminopropyl carbodiimide hydrochloride (EDC) 99% (Otto Pvt. Ltd. India) were used as received. Glucose standard solutions of different concentrations were prepared in a 0.1 M phosphate buffer solution (PBS, pH 7.0) one day prior to use and stored at 4 °C. The morphology of the PANI/PVA/MWCNTs fibers was evaluated by scanning electron microscopy (JEOL, JSM-6510 LV, Japan), electrochemical measurements were performed using a computer controlled Potentiostat/Galvanostat (302N Autolab, Switzerland). A conventional three electrode system including a working electrode of as prepared glassy carbon electrode (GCE), an Ag/AgCl reference electrode and a platinum wire counter electrode was used for all electrochemical characterization. The electrode was ultrasonicated with digital ultrasonic cleaner LMUC series Labman, India. Electrospun fibers of PANI/PVA/MWCNTs were synthesized using electrospinning system (Nano NC, NNC-ESP200, Korea).

4.2.2 Synthesis of PANI/PVA/MWCNTs electrospun fibers

Aqueous solution of PVA polymer was prepared by dissolving polymer in double distilled water (DMW) under continuous stirring by using a magnetic stirrer with hot plate maintained at 80 °C for 6 h. The synthesis of PANI was conducted via chemical route as follows: 0.1 M aniline monomer (10 mL) was dissolved in 1 M HCl (3 mL in 100 mL DMW) and kept in an ice bath. Potassium peroxodisulphate solution (4.6 g in 50 mL DMW) was then added to the mixture. After running the polymerization
for 6 h in an ice bath (0–5 °C) dark green color appears indicating the formation of proton-doped PANI precipitate which was obtained after rinsing with DMW repeatedly. Electrospinning solution was prepared by dispersing the synthesized PANI powder, processed PVA solution and MWCNTs in DMF solvent. The 1.0, 3.0 and 5.0 wt.% MWCNTs solutions with 0.3 wt.% PANI and 7.0 wt.% PVA were mixed thoroughly by continuous stirring on a magnetic stirrer for 24 h. The nanocomposite fibers were fabricated by electrospinning. The viscous polymer solutions were loaded into hypodermic syringes (10 mL). Electrospinning was carried out under dc voltage supply of 16 kV over an aluminium foil static collector. The flow rate of the solution was kept 0.001 mL min⁻¹ dropping from a stainless steel needle with inner and outer diameters of 0.5 and 0.8 mm, respectively. The needle tip-to-collector distance (TCD) was maintained 20 cm. The obtained electrospun fibers on aluminium foil were kept in a sealed jar to avoid contact from environment. The Scheme 4.1 illustrates the procedure for the synthesis of electrospun fibers.

4.2.3 Electrode preparation

A 3 mm diameter GCE was glazed with 0.05 μm alumina slurry with the help of a velvet pad. The electrode was then put into ultrasonicator for a period of 20 min to remove contamination, washed with distilled water and left to dry at room temperature (25±3 °C). After drying of the electrode, GOx was covalently bonded onto the fibrous membrane employing EDC/NHS activation procedure. After rinsing with PBS (pH 7.0), the pretreated electrode surface coated by the fibrous membranes was immersed into an EDC/NHS solution (10 mg mL⁻¹ in PBS, the molar ratio of EDC to NHS = 1:1) and stirred gently for 2 h at 4 °C. Then, the activated surface of the electrode was washed several times with PBS and dipped into GOx solution (10 mg mL⁻¹ in a phthalate buffer of pH 4.0). GOx immobilization was carried out at 4 °C for 24 h in which the -NH₂ groups of the nanofibres would react with the activated -COOH of GOx. This reaction brings about covalent linkage between GOx and the nanofibres. The modified bioanode was washed with PBS to discard the unadhered enzyme and then was kept for drying at room temperature. Finally, Frt was adsorbed on to the surface of the modified bioanode to form PANI/PVA/MWCNTs/GOx/Frt modified biocomposite nanostructured anode.
Scheme 4.1: The procedure for the synthesis of electrospun fibers.
The electrode was dried at room temperature and stored at 4 °C until the measurements were performed. The proposed design for the immobilization of biomolecules and their working involving the flow of electron is shown in Scheme 4.2.
Scheme 4.2: The proposed design for the immobilization of biomolecules and their working involving the flow of electron.
Chapter 4

4.3 RESULTS AND DISCUSSION

4.3.1 Surface morphology

Figure 4.1 shows the SEM images of electrospun nanofibres prepared from a PANI/PVA composite solution containing 1 wt.% MWCNTs. It was observed from Figure 4.1 that the composite nanofibres exhibited smooth surfaces and were randomly oriented. Average diameter of as prepared fibers was measured as 60-100 nm. According to the morphology, the beads had a rough surface. Beads indicate that MWCNTs and PANI scattered inside the PVA nanofibres. SEM image shows homogenous area of the composite nanofibres at high magnification and the area was surrounded with smooth and beadless composite nanofibres at high magnifications. Inconsistency of fiber shape may arise due to the result of agglomeration of carbon nanotubes.

4.3.2 Electrochemical properties of synthesized electrode materials

All the experiments were performed with nitrogen purging. The electrochemical properties of the following three electrodes viz. PANI/ PVA/ MWCNTs, PANI/PVA/MWCNTs/GOx and PANI/PVA/MWCNTs/GOx/Frt were analyzed by cyclic voltammetry (CV). The CVs were recorded in the potential range from -1.5 V to +1.5 V in the presence of 0.1 M PBS (pH 7.0) as the electrochemical marker at a sweep rate of 100 mV s⁻¹ as shown in Figure 4.2. The redox peak current values and the peak to peak separation values of different modified electrodes were compared. CV of PANI/PVA/MWCNTs composite nanofibres show slight protrusion of cathodic and anodic peaks at around 0.21 V and 0.09 V, respectively [39]. This peak is due to the transition of PANI backbone from the reduced leucoemaraldine state to half oxidized form of the polymer, emaraldine state and vice-versa. Slight shifting of redox peaks may arise due to the integration of nanotubes into the PANI matrix helps in the oxidation and reduction of PANI moieties in the composite. Thus, it can be well said that the nanotubes and PANI collaborate to the electrocatalytic process. CV of PANI/PVA/MWCNTs/GOx electrode shows a pair of redox peaks with the anodic and cathodic peak potential at -0.57 V and -0.42 V at 100 mV s⁻¹, respectively. The value of $E^\circ$ is -0.49 mV (at 100 mV s⁻¹) and an enhanced anodic current as compared to
Figure 4.1: SEM micrograph of PANI/PVA/MWCNTs electrode at (a) Low magnification and (b) High magnification.
Figure 4.2: CVs of (a) PANI/PVA/MWCNTs, (b) PANI/PVA/MWCNTs/GOx and (c) PANI/PVA/MWCNTs/GOx/Frt, confined onto GCEs in PBS (pH.7.0) at room temperature with a potential scan rate of 100 mV s\(^{-1}\).
PANI/PVA/MWCNTs electrode. The pair of peaks in this modified GCE can be ascribed to the conversion of FAD/FADH$_2$ of GOx. The separation of peak potential $\Delta$Ep is 150 mV indicating that GOx on PANI/PVA/MWCNTs fibers displays a quasi reversible behavior regardless of its bulky molecular arrangement. CV of PANI/PVA/MWCNTs/GOx/Frt modified GCE exhibited utmost current as compared to other electrodes. The enhanced peak current of this modified electrode reveals that electron transfer was speedy at the electrode. Reason behind this enhancement is due to the mediated electron transfer by Frt that works by enhancing GOx efficiency without affecting enzyme active sites. The increment in the enzyme activity can be attributed to the reduction of the electron transfer distances between the redox centre of the enzyme and the electrode. Hence, increases the speed of electron transfer. It is also due to the enlarged surface area of electrospun composite nanofibres leading to the higher amount of GOx immobilized on the electrospun polymer matrix resulting in the faster rate of redox reactions and hence higher redox current generation.

Electrochemical properties of the two electrodes before electrospinning simply by casting and after electrospinning were compared. As can be clearly seen from Figure 4.3 PANI/PVA/MWCNTs electrode fabricated by using electrospinning technique shows a higher peak current response for both oxidation and reduction. The peak to peak separation is also smaller. This indicates that the electrospun fibers have faster electron transfer kinetics than the cast film. The improved performance of electrode after electrospinning can be explained by two important factors one is that fibers increase the total surface area and secondly provides broad electrode electrolyte interface that causes fast electron transport. In addition, the porous structure of the fibers facilitates high enzyme loading leading to high enzyme activity thus improving electrochemical properties.

CVs were recorded for modified biocomposite electrode for different scan rates (20, 40, 60, 80 and 100 mV s$^{-1}$) as shown in Figure 4.4 (A). It can be clearly seen that the peak potentials and peak current (anodic as well as cathodic) are scan rate dependent. Both the anodic and cathodic peak currents increases linearly with increase in the scan rates with the correlation coefficient $R^2$ values of 0.96 and 0.977
Figure 4.3: CVs of PANI/PVA/MWCNTs modified GCE (a) Before electrospinning and (b) After electrospinning at room temperature with a potential scan rate of 100 mV s$^{-1}$. 
Figure 4.4: (A) CVs of PANI/PVA/MWCNTs/GOx/Frt modified GCE in 20 mM glucose in PBS (pH 7.0) at scan rate (a) 20, (b) 40, (c) 60, (d) 80 and (e) 100 mV s\(^{-1}\) and (B) The plots of peak currents vs. scan rate.
for the anodic and cathodic peaks, respectively. This result confirms that the redox process is restricted to redox peak currents (ipc) vs. scan rate are shown in Figure 4.4 (B) Thus, we can say that the scan rate influences catalytic current significantly.

The apparent heterogeneous electron transfer rate constant $K_s$ is estimated to be 3.09 s$^{-1}$ from the dependence of $\Delta E_p$ on the scan rates using the equation developed by Laviron [40].

$$\log K_s = \alpha \log(1 - \alpha) + (1 - \alpha)\log\alpha - \log(RT/nFV) - \alpha(1 - \alpha)(nF\Delta E_p/2.3RT)$$

where $\alpha$ is the charge transfer coefficient, the constants $R$, $T$ and $F$ express their usual meanings [$R = 8.314 \text{ J K}^{-1} \text{ mol}^{-1}$, $T = 298 \text{ K}$, $F = 96485 \text{ C mol}^{-1}$, $n =$ no. of electron transfer (2), $V =$ scan rate (100 mV s$^{-1}$) and $E_p = E_{pa} - E_{pc}$].

The high value of $K_s$ indicates that PANI/PVA/MWCNTs/Frt enhances the electron transfer ability between the deeply located redox active sites of GOx and the electrode surface and hence increases the rate of electron transfer. High surface area porous electrode modified with electrospun nanofibres provides enough active sites for heterogeneous reactions, which is essential for electrochemical power generation.

The current response of modified biocomposite electrode has been characterized using linear sweep voltammetry (LSV) in presence of different concentrations of glucose. It was observed from the LSV that the electro-oxidation of glucose started around 0.2 V (E onset) with the peak potential shifted from 0.67 V at low concentrations of glucose to 0.78 V with the increasing concentrations of glucose. As can be clearly seen from Figure 4.5 (A) that the anodic current density increases linearly with the increase in the concentration of glucose up to 20 mM and then the current becomes stable. Thus, the remarkable enhancement in the peak current provides a clear evidence of the electro catalytic effect of PANI/PVA/MWCNTs/GOx/Frt bioanode towards glucose oxidation. The calibration for the glucose concentration vs oxidation current density generated using this bioanode is shown in Figure 4.5 (B). It is observed from the figure that the oxidation current density increases with increase in the glucose concentration and a maximum current density of 7.5 mACm$^{-2}$ at a scan rate of 100 mV s$^{-1}$ vs Ag/AgCl for the oxidation of glucose in 20 mM glucose concentration was attained.
Figure 4.5: (A) LSVs of PANI/PVA/MWCNTs/GOx/Frt modified GCE in PBS (pH 7.0) and different concentrations of glucose (a) 5 mM (b) 10 mM (c) 15 mM (d) 20 mM and (e) 25 mM at room temperature with a potential scan rate of 100 mV s\(^{-1}\) and (B) The calibration curve corresponding to the electro catalytic current against variable concentration of glucose.
This result clearly suggests that the current generation was due to oxidation of glucose on the modified bioanode. The biocatalytic activity of the modified GCE for the oxidation of glucose was studied in presence of 20 mM of glucose in PBS of pH 7.0 and in the absence of glucose in PBS of pH 7.0 as shown in Figure 4.6. The biocomposite modified electrode generated a large glucose oxidation current density in the presence of 20 mM of glucose as compared to the oxidation current density in the absence of glucose. Thus, it is visible that the modified bioanode unveil its catalytic activity only in the presence of glucose.

The surface concentration of the PANI/PVA/MWCNTs/GOx/Frt biocomposite on GCE was estimated using CV based on the following equation given by Brown-Anson.

\[
I = \frac{nF\varepsilon AV}{4RT}
\]

where \( n \) is the numbers of electrons to be transferred (in the present case \( n=2 \)), \( F \) is the Faraday constant \( (96,500 \text{ C mol}^{-1}) \), \( I^* \) is the surface concentration of the PANI/PVA/MWCNTs/GOx/Frt biocomposite (in mol cm\(^{-2}\)) to be determined, \( A \) is the surface area of the GCE \( (0.07 \text{ cm}^2) \), \( V \) is the scan rate \( (100 \text{ mV s}^{-1}) \), \( T \) is the absolute temperature in Kelvin and \( R \) is the gas constant \( (8.314 \text{ J mol}^{-1} \text{ K}^{-1}) \). The surface concentration of the bioelectrode confined by PANI/PVA/MWCNTs/GOx/Frt was found to be \( 2.85 \times 10^{-10} \text{ mol cm}^2 \).

### 4.3.3 Stability and reproducibility

The stability of two modified electrode that is one in which glucose oxidase was immobilized over the surface of GCE containing PANI/PVA/MWCNTs composites before electrospinning and the other in which glucose oxidase was immobilized over the GCE containing PANI/PVA/MWCNTs nanofibres was also studied electrochemically. It was observed that before electrospinning, the modified electrode when stored at 4 °C and current density periodically measured over several days, it was found that it retained less than half of its original current density after one month. On the other hand the enzyme immobilized on the nanofibres retained about 78% of its original current density after one month. Reason behind this long term stability was mainly the covalent binding and efficient interaction between the electrosprun fibers and enzyme. Covalent bonding is unaffected by changes in the surrounding environment.
**Figure 4.6:** CVs of (a) PANI/PVA/MWCNTs/GOx/Frt in 20 mM glucose in PBS (pH 7.0) and (b) PANI/PVA/MWCNTs/GOx/Frt in absence of glucose in PBS (pH 7.0) at scan rate of 100 mV s$^{-1}$. 


Moreover, covalent binding bind enzyme and prevent its leaching from the electrode surface during operation and storage. Increased surface area and porosity of the electrospun fibers provide enormous number of active sites to bind more enzymes and hence high loading of enzymes was attained in case of electrospun fibers.

4.4 CONCLUSION

In summary the nanostructured electrodes prepared via electrospinning techniques were applied to immobilize glucose oxidase for the fabrication of bioanode. The reactive groups possessed by the membrane after reaction with EDC/NHS were used to covalently immobilize GOx. GCE modified by the resultant nano composites showed excellent redox properties and exhibited good bioelectrocatalytic activity against the oxidation of glucose and also possesses long stability as well as reversibility. All these characteristics arise mainly due to extremely high surface area and porosity which makes them viable substrates for enzyme immobilization. Large surface area provides enormous number of active sites enhancing capability to bind more enzymes. Thus, PANI/PVA/MWCNTs nanofibres have remarkable potential to be used for novel BFC device.
REFERENCES