CHAPTER-III

EXPERIMENTAL

The present chapter describes the various experimental procedures and techniques that have been employed to prepare the Poly(3,4-ethylenedioxythiophene)(PEDOT) based nanocomposite electrodes. The parent materials and their properties have also been elucidated. The chapter embodies the details as to how the PEDOT based nanocomposites have been functionalized with biomolecules. The principles of various characterization techniques used to study the properties of PEDOT based nanocomposites and bioelectrodes have been described.

3.1 Parent materials

3, 4-ethylenedioxythiophene (EDOT ≥ 97%), monomer was procured from Sigma Aldrich which is used for the synthesis of the parent conducting polymer, Poly (3, 4-ethylenedioxythiophene) (PEDOT). The gold nanoparticles used to functionalize the PEDOT matrix were synthesized by reducing Gold (III) chloride hydrate ($HAuCl_4.3H_2O$) (99.9%) which purchased from Aldrich Chemical Inc. (USA). Other dopants used during the synthesis of the PEDOT based matrix, viz. Polystyrene Sulfonate (PSS), Graphene Oxide (GO) and Multiwalled Carbon Nanotubes (MWCNTs) were all procured from Sigma Aldrich. Lithium per chlorate ($LiClO_4$) and potassium chloride salt used as electrolytes during the polymerization of EDOT and synthesis of AuNPs were received from Merck. Acetonitrile and methanol used for dissolving EDOT monomer and pesticides were purchased from SRL. Sodium Ferrocyanide, Potassium Ferricyanide , Sodium Phosphate Dibasic and Sodium Phosphate Monobasic were used to make redox solution ($[Fe(CN)_6]^{3-/4-}$) and phosphate buffer, respectively were procured from Sigma Aldrich, USA. 1-Ethyl-3-(3-dimethylaminopropyl) Carbodiimide (EDC) and N-hydroxysuccinimide (NHS) used for activation of functional groups during immobilization of Proteins were received from Merck, Germany.

The monoclonal anti-aflatoxin B_1 (anti-AFB_1), Acetylcholinesterase (AChE) from Electrophorus electricus (electric eel), Aflatoxin B_1 (AFB_1) from Aspergillus flavus, methyl parathion and carbofuran have been procured from sigma Aldrich.

Table 3.1: Physical properties of the materials used:

Physical properties of monomer:

Monomer	**Molecular formula**	**Molecular weight (gm/mol)**	**Melting point (°C)**	**Boiling point (°C)**	**Density at 25°C (gm/ml)**	**Oxidation potential (Volt)**
EDOT	$C_6H_6O_2S$	142.18	10-11	193	1.331	1.2 vs. Ag/AgCl

Physical Properties of the analytes used:

Solvent	**Molecular formula**	**Molecular weight (gm/mol)**	**Melting point (°C)**	**Boiling point (°C)**	**Density (gm/ml)**
Acetonitrile	CH_3CN	41.05	-44	81.23	0.787
Methanol	CH_4O	32.04	-97.6	64.7	0.791
Ethanol	C_2H_6O	46.06	-114	78.37	0.789

Physical properties of the functionalizing agents used:

Functionalizing Agent	**Molecular formula**	**Molecular Weight (gm/mol)**	**Melting point (°C)**	**Boiling point (°C)**	**Density (gm/ml)**
Gold (III) chloride hydrate	$HAuCl_4.3H_2O$	339.79	254	298	3.9
Graphene oxide	$C_xH_yO_z$	364.45	--	--	0.981
Poly(sodium 4-styrenesulfonate)	$(C_8H_7NaO_3S)_n$	~70,000	460	--	4.35

Physical properties of the solvents used:

Analyte	Molecular formula	Molecular weight (gm/mol)	Soluble in
Aflatoxin B_1	$C_{17}H_{12}O_6$	312.277	Organic solvents
Acetylthiocholine chloride	$CH_3COSCH_2CH_2N(CH_3)_3Cl$	197.73	Water
Methyl parathion	$C_8H_{10}NO_5PS$	263.21	Acetonitrile
Carbofuran	$C_{12}H_{15}NO_3$	221.25	Acetonitrile

3.2 Synthesis of PEDOT matrix functionalized with AuNPs:

3.2.1 Synthesis of PEDOT/GC electrode:

In this work electrochemical method [76] is used to synthesize PEDOT based film over GCE by cycling the potential of the GCE. The monomer solution was prepared by adding 0.01 M EDOT in 10 ml vial containing acetonitrile based 0.01 M $LiClO_4$ and the solution was sonicated for 30 min. Electro-polymerization was carried using Cyclic Voltammetry by adjusting the potential from -0.5 V to 1.5 V [273]. The potential was cycled 3 times at a scan rate of 10 mV/s vs. Ag/AgCl as shown in Figure 3.2.

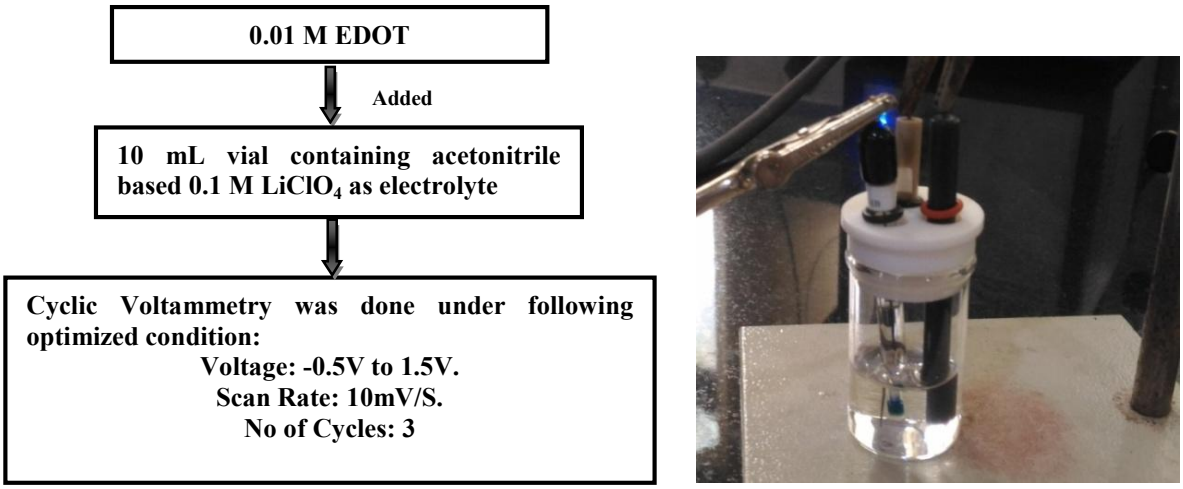


Figure 3.1: Block diagram of synthesis of PEDOT/GC electrode and experimental set-up.

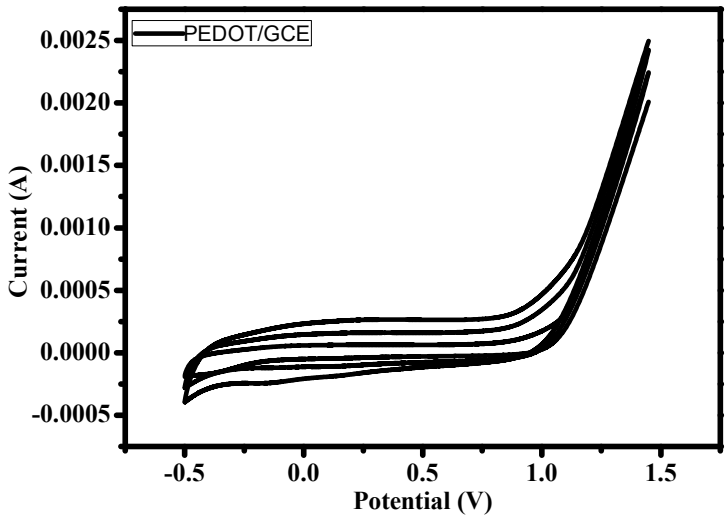


Figure 3.2: Cyclic voltammogram of polymerization of EDOT on GCE.

3.2.2 Deposition of AuNPs on PEDOT/GC electrode:

The AuNPs/PEDOT/GCE was synthesized by layer by layer deposition method. It has been achieved by depositing layers of AuNPs over PEDOT film by electrochemically reducing auric acid [273]. The synthesized PEDOT/GCE was immersed in a solution containing 0.1 M KCl and 500 µL of 3 mM $HAuCl_4$ in a 10 mL vial. The Au-NPs were electrochemically deposited onto the PEDOT/GCE using CV (Figure 3.4) by applying potential from -0.5 V to 1.2 V vs. Ag/AgCl keeping the scan rate of 10 mV/s [273].

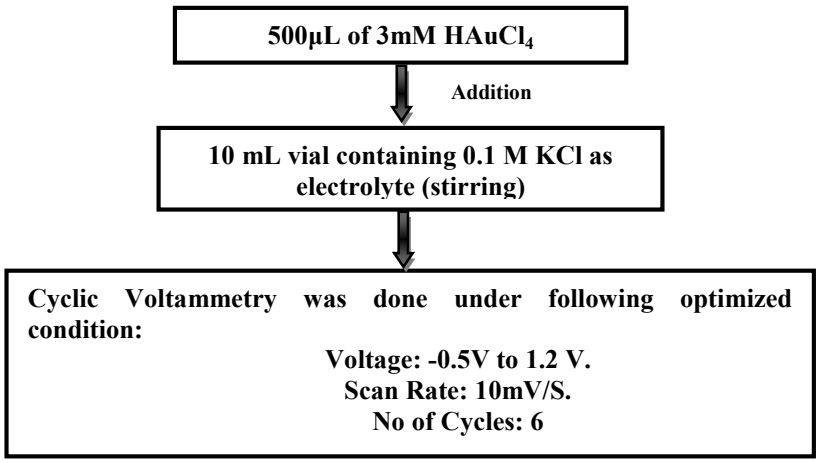


Figure 3.3: Block chart representation of synthesis of AuNPs/PEDOT/GCE.

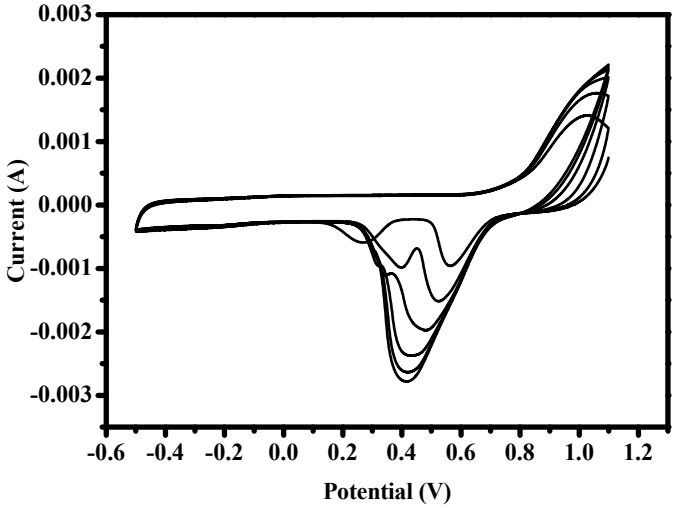


Figure 3.4: Cyclic Voltammogram pattern of deposition of Au-NPs onto PEDOT/GCE.

3.2.3 Immobilization of anti-AFB_1 and AChE onto the matrix of AuNPs/PEDOT/GCE:

The immobilization of anti-AFB_1 and AChE was done by using Carbodiimide coupling [271]. After the GCE was modified with AuNPs/PEDOT composite, the AuNPs/PEDOT was consequently immersed in 0.01 mol L^{-1} thioglycolic acid solutions for 6 h to introduce the carboxyl group onto the electrode surface [274]. After rinsing thoroughly with redistilled water, the electrode was soaked in EDC (0.4 M) and NHS (0.1 M) for 1 h to activate the carboxyl group [275]. Subsequently 1 mg/mL stock solution of anti-AFB_1 was prepared by adding 1 mg antibody in 1ml of Phosphate buffer solution. 5µL of the solution was dropped onto the AuNPs/PEDOT electrode and limited within the working electrode pool incubating for 10 h in 4 °C to form anti-AFB_1/AuNPs/PEDOT/GCE. After rinsing with PBS, 10 µL of 5 mg/mL BSA solution was used to block the unspecified sites to prevent non-specific adsorption.

Similarly 5 µL of 50 u stock solution of AChE in EDC/NHS and 100 mM phosphate buffer (pH 7.0) was limited within the working electrode area of AuNPs/PEDOT/GCE and incubated for 12 h at 4 °C to form AChE/AuNPs/PEDOT/GCE. Finally, the BSA/anti-AFB_1/AuNPs/PEDOT/GCE and AChE/ AuNPs/PEDOT/GCE was rinsed with PBS and stored at 4 °C when not in use.

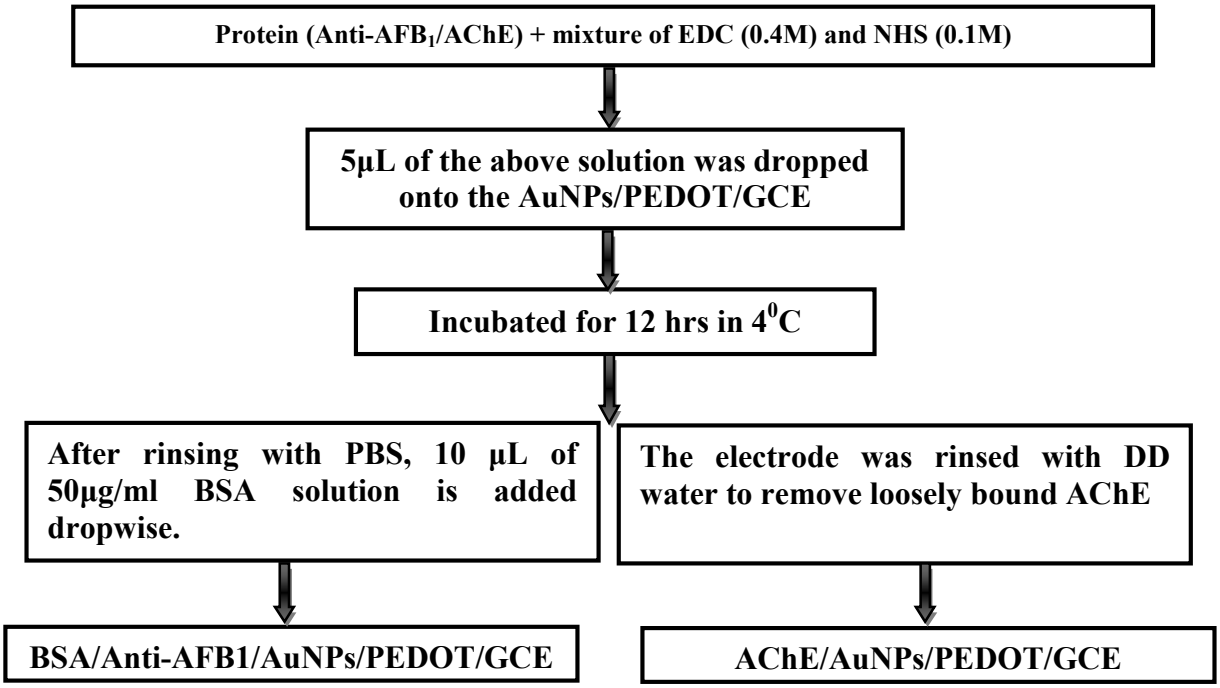
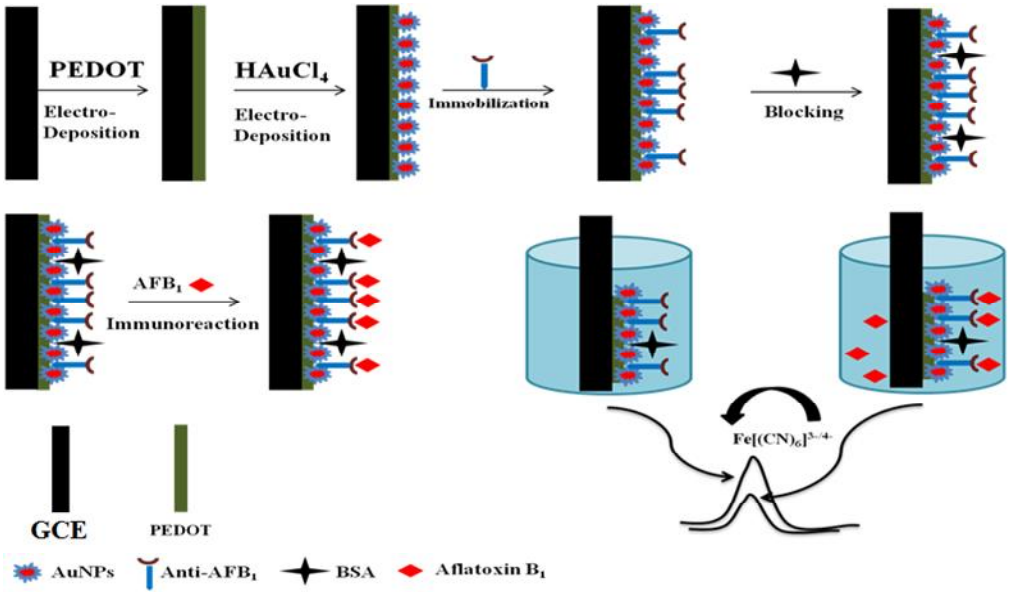


Figure 3.5: Block diagram of synthesis of BSA/anti-AFB_1/AuNPs/PEDOT/GCE and AChE/AuNPs/PEDOT/GCE.



Scheme 3.1: The schematic representation of the fabrication and sensing process of AuNPs/PEDOT/GCE based Immunosensor for AFB_1 detection.

3.2.4 Synthesis mechanism and Biosensor fabrication:

The cyclic voltammogram for polymerization of EDOT on to the GCE electrode for three cycle process at potential window of −0.5 V to +1.5 V vs. Ag/AgCl with a scan rate of 10 mV s^{-1} is presented in Figure 3.2. Successive three polymerization cycles with increase in area of cycle corresponds to enhance electrode property of GCE as the thickness of the film gradually increases during deposition [276]. The voltammetric peak current and number of cycles have shown positive relationship up to three cycles of electro-polymerization and beyond that the current saturates and the uniformity of the film decreases. Figure 3.4 displays the cyclic voltammogram for deposition of Au-NPs onto PEDOT/GCE for six cycles at potential range of -0.5 V to 1.2 V vs. Ag/AgCl. The peak at 0.38 V is attributed to the reduction of Au^{3+} which rapidly reduces to Au^{+} and then finally to Au^{0} [277]. The oxidative polymerization of PEDOT results in positive charges on the polymer backbone; this allows the incorporation of negatively charged doping agent Au-NPs [278]. The PEDOT surface can act as a good stabilizer and control the aggregation and nucleation of Au-NPs.

The presence of -COOH groups in the modified electrode and its activation using EDC/NHS can bind to the free NH_2 group of the antibodies as a result of which a covalent immobilization of anti-AFB_1 and AChE takes place [275]. The schematic representation of fabrication process of the AuNPs/PEDOT/GCE based bioelectrode (considering antibody) is shown in Scheme 3.1.

3.3 Synthesis of PEDOT-GO composite functionalized with AuNPs:

3.3.1 Electrochemical Synthesis of PEDOT-GO/GCE:

PEDOT-GO composite has been prepared by electrochemical polymerization of EDOT in presence of GO as dopant [279, 280]. 50 μL of GO (2 mg/mL) was added to 10ml d-H_2O in a vial containing 0.1 M EDOT and the mixture was stirred for 6h at a temperature below 5^0C. Prior to deposition, the solution was sonicated for 15 min for exfoliation of GO nanosheets. Electro-polymerization was carried out using cyclic voltammetry by adjusting the potential from -0.2 V to 1.2 V [279]. The potential was cycled 13 times at a scan rate of 10 mV/s vs. Ag/AgCl and the result is shown in Figure 3.8(A).

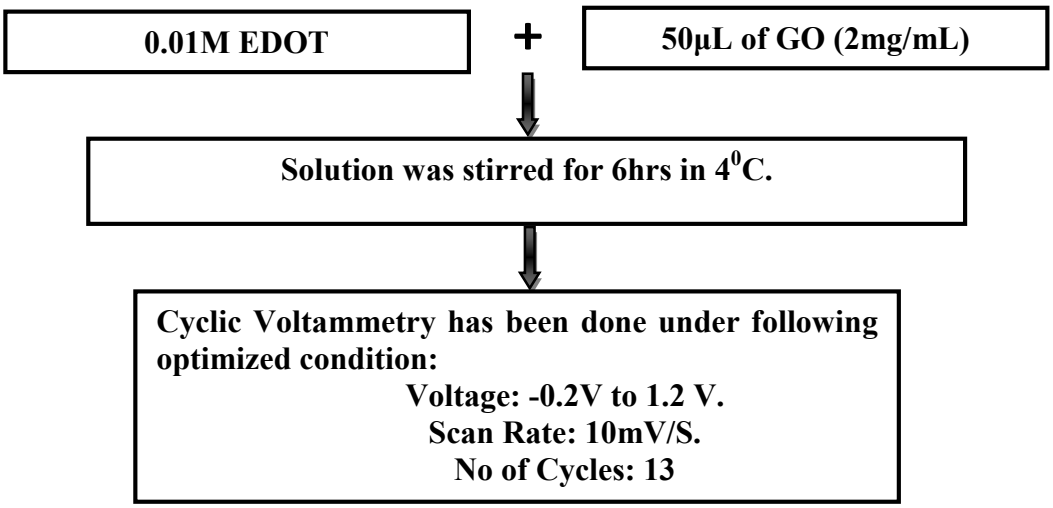


Figure 3.6: Flow chart representation of synthesis of PEDOT-GO/GCE electrode

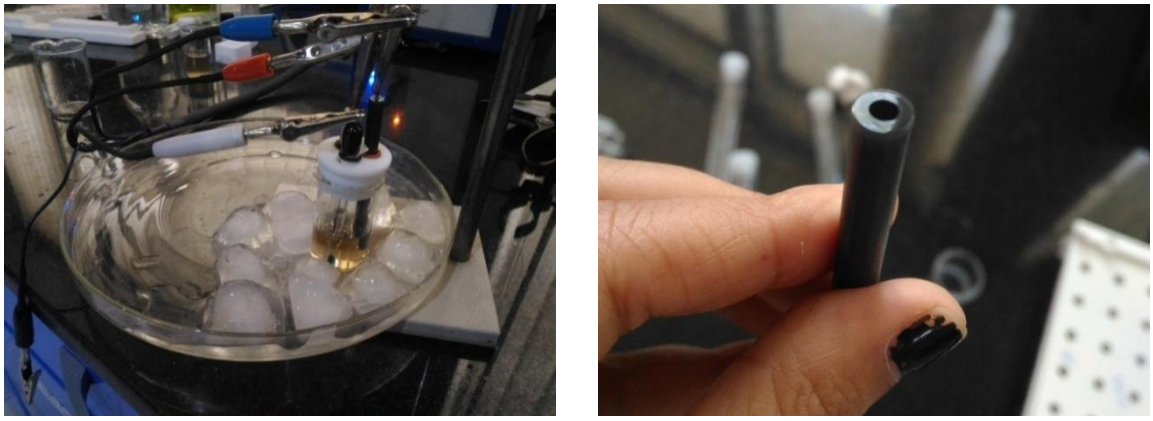


Figure 3.7: The experimental set up representing polymerization of PEDOT-GO composite and the GCE after deposition of PEDOT-GO film.

3.3.2 Electrochemical deposition of AuNPs over PEDOT-GO/GCE:

The Layers of AuNPs over PEDOT-GO composite film was deposited by reducing auric acid by applying reduction potential using cyclic voltammtery. The synthesized PEDOT-GO/GCE was immersed in a solution containing 500 µL of 3 mM $HAuCl_4$ and 0.1 M KCl in a 10 mL vial. The Au-NPs were electrochemically deposited onto the PEDOT-GO electrode using CV by applying potential from -0.2 V to 1.2 V vs. Ag/AgCl at a scan rate of 10 mV/s and it shown in Figure 3.8 (B) [281].

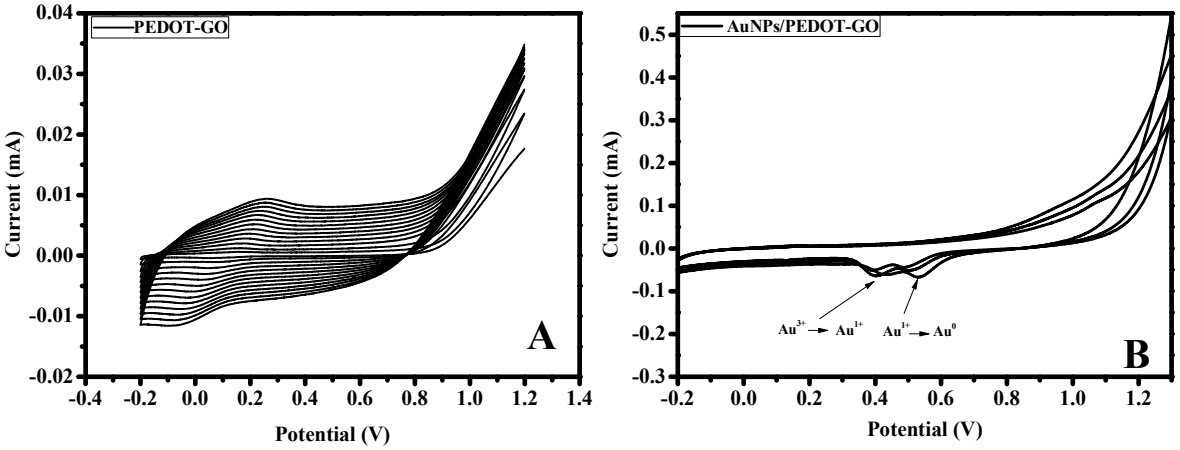


Figure 3.8: Cyclic Voltammogram pattern of electrochemical polymerization of (A) EDOT in presence of GO as dopant (B) Deposition of Au-NPs onto PEDOT-GO/GCE.

3.3.3 Immobilization of monoclonal anti-Aflatoxin B_1 and Acetylcholine esterase on to AuNPs/PEDOT-GO/GCE:

The synthesized AuNPs/PEDOT-GO/GCE, it was soaked in EDC (0.4 M) and NHS (0.1 M) for 30 min to activate the carboxyl group [275]. 10 µL of 5 µg mL^{-1} stock solution of monoclonal anti-AFB_1 antibody in 100 mM phosphate buffer (pH 7.4) was limited within the working electrode area and incubated for 5 h at 25 °C to form anti-AFB_1/AuNPs/PEDOT-GO/GCE [281]. After rinsing with PBS, 10µL of 5 $mgmL^{-1}$ BSA solution was used to block the unspecified sites. Similarly 10 µL 50 u of AChE was dropped on the surface of AuNPs/PEDOT-GO/GCE and incubated for 10 h at 25^0C.

3.3.4 Mechanism of Electrochemical deposition and electrode fabrication:

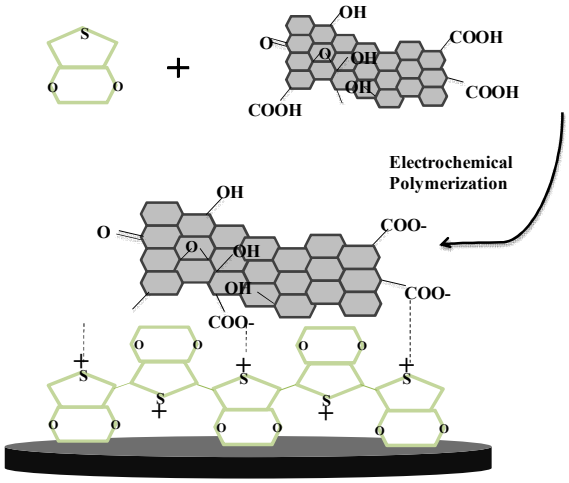
On application of optimal potential in the range of -0.2 V to 1.2 V vs. Ag/AgCl, the electro- polymerization of EDOT (EDt) on the electrode in presence of GO can be described as [281]:

$$EDt - e' \leftrightarrow EDt^{0+} \quad (3.1)$$

$$EDt^{0+} + A^- \rightarrow EDt^{0+}A^- \quad (3.2)$$

$$2EDt^{0+}A^{-} \rightarrow EDtEDt + 2A^{-} + 2H^{+} \quad (3.3)$$

where EDt stands for the EDOT monomer molecule and A^{-} for the anion, EDt^{0+} represents the cation radical of EDOT; two H^{+}s lost in Eq. (3.3) are the two α-H of EDOT, $EDt^{0+}A^{-}$ is the intermediate complex of the cation radical and anion. Due to the presence of rich negatively charged functional groups at the GO surface [280], it is predictable that the positively charged cation (EDt^{0+}) radicals of EDOT could be firstly attracted to the basal planes and edges of GO sheets followed by polymerization of EDOT at the surface of GO sheets. The schematic representation of the formation of PEDOT and GO composite is shown in Scheme 3.2. The composite is formed by π-π stacking of polymer layers and GO sheets interactions and hydrogen bonding between the GO layers and thiophene rings [282, 283].



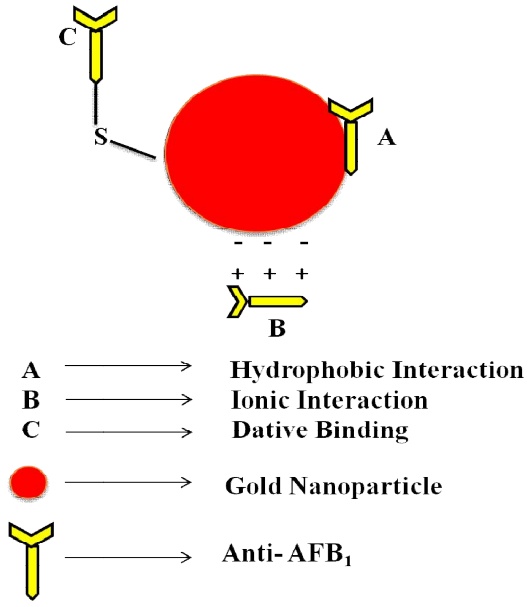
Scheme 3.2: Schematic representation of the formation of PEDOT and GO composite.

Figure 3.8 (B) shows the cyclic voltammogram of the deposition of AuNPs on PEDOT-GO/GCE electrode and during the process nanoparticles become negatively charged as their surface is bound with $AuCl^{4-}$ and $AuCl^{2-}$ ions and they are uniformly distributed onto the surface of PEDOT-GO/GCE [284]. The two peaks appearing at 0.45 V and 0.546 V during the process corresponds to the reduction reactions $Au^{3+} \rightarrow Au^{1+}$ and $Au^{1+} \rightarrow Au^{0}$ [284].

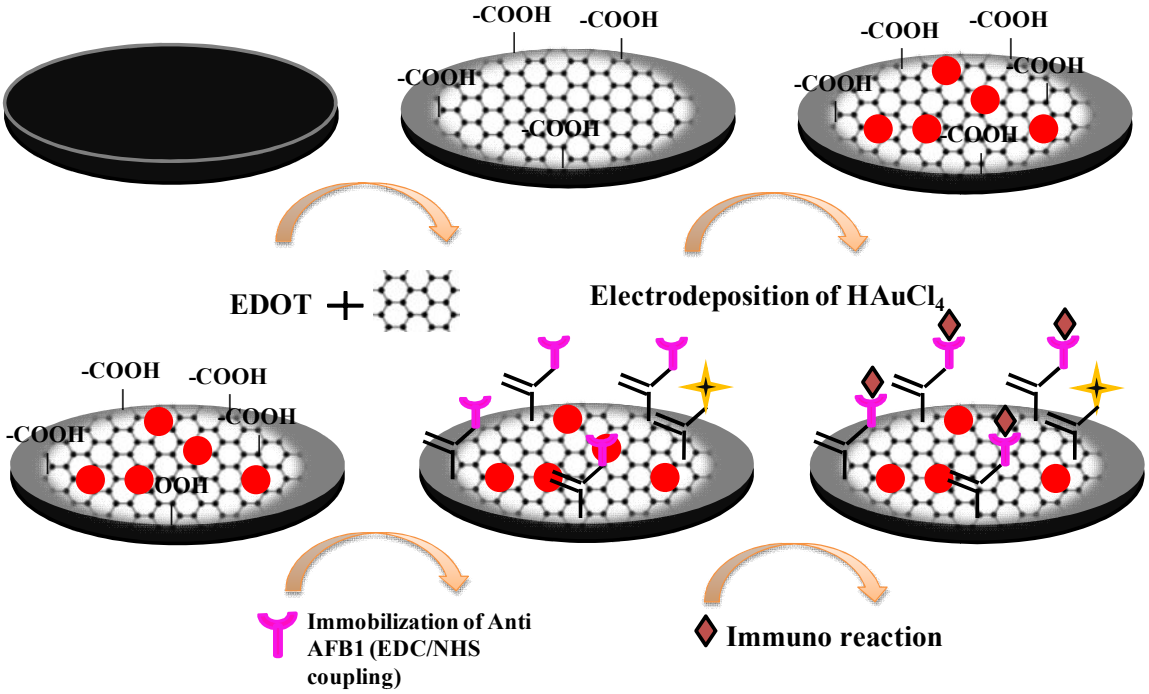
The immobilization of the AChE and anti-AFB_1 on the substrate of AuNPs/PEDOT-GO modified GCE has been done by a carbodiimide-assisted amidation reaction [285]. The activated carboxyl groups on the surface will covalently bind to the amine groups of the antibody and AChE. The AuNPs

modified surface can further helps in immobilization of anti-AFB_1 and AChE by providing a desirable support [286]. The binding between the AuNPs with the protein (taking anti-AFB_1) is shown in Scheme 3.3 [287]. Different types of interaction namely, ionic interaction and dative binding may occur. Further the presence of AuNPs helps in immobilization of proteins. Anti-AFB_1 and AChE becomes positively charged in PBS (pH 7.2) due to the presence of positively charged amino acids and N-terminal as a result of which ionic interactions are formed between these groups and the negatively charged surface of the AuNPs. Apart from NH_2 the other common reactive groups in antibodies are thiol residue from the sulfur-containing amino acid cystine and its reduction product cysteine. Dative binding can occur by formation of a chemisorption covalent bond between the gold nano particle and free sulfhydryl (thiol) groups of the Protein as shown in Scheme 3.3 (keeping anti-AFB_1 under consideration). The antibody molecules adsorb readily because they lower the surface free-energy of the substrate and are stable due to the strong chemisorption of the thiol groups [288-291].

The schematic diagram representing the step by step synthesis of AuNPs/PEDOT-GO/GCE and immobilization of AFB_1 has been displayed in Scheme 3.4 [292] (keeping anti-AFB_1 under consideration).



Scheme 3.3: The schematic diagram representing possible ways of immobilization in presence of AuNPs.



Scheme 3.4: The schematic representation of the fabrication process of BSA/anti-AFB1/AuNPs/PEDOT-GO/GCE based Immunosensor for AFB_1 detection.

3.4 Synthesis of PEDOT-PSS composite functionalized with fMWCNTs:

3.4.1 Electrochemical Synthesis of PEDOT-PSS/GCE:

PEDOT-PSS/GCE electrode has been prepared by electrochemical oxidation of EDOT in presence of PSS as a dopant. 100µL of PSS was added to 10ml d-H_2O in a vial containing 0.01M EDOT and the solution was sonicated for 30min. Electro-polymerization was carried out using cyclic voltammetry by adjusting the potential from -0.6 V to 1.2 V. The potential was cycled 6 times at a scan rate of 10 mV/s vs. Ag/AgCl and the result is shown in Figure 3.11 (A).

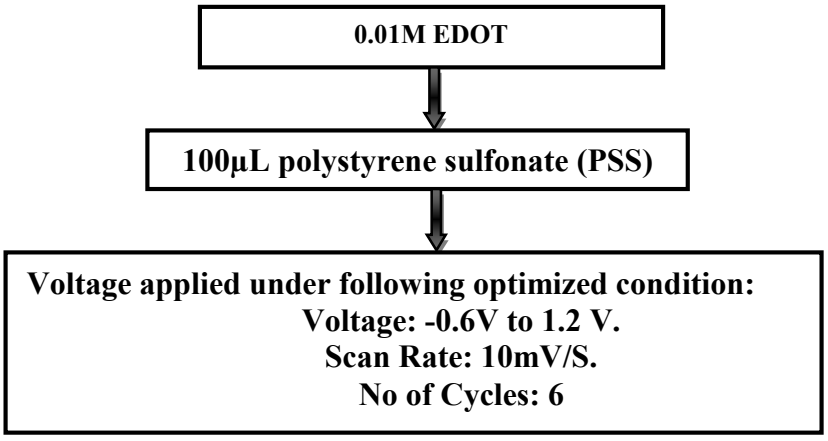


Figure 3.9: Flow chart representation of synthesis of PEDOT-PSS/GCE electrode.

3.4.2 Electrochemical Synthesis of PEDOT-PSS-fMWCNTs/GCE:

5 mg of MWCNTs were dispersed in 10mL of 1:3 concentrated HNO_3 and H_2SO_4 solution for 2 h under ice bath sonication. The solution was incubated at 20 °C for 24hours and then washed with water and separated by centrifugation at 8000 rpm. The activation of carboxylic groups has been done by immersing the mixture in 1:1 solution of 100 mM EDC and 100 mM NHS for 30mins [293].The PEDOT-PSS-*f*MWCNTs/GCE electrode was prepared by cycling a potential of -0.2 V to 0.8 V vs, Ag/AgCl in a 10 mL solution containing 50 µL of *f*MWCNTs (5mg/mL) and 100 µL PSS. The potential was cycled 6 times at a scan rate of 10 mV/s vs. Ag/AgCl and the result is shown in Figure 3.11 (B).

3.4.3 Immobilization of monoclonal anti-Aflatoxin B_1 and Acetylcholine esterase on to PEDOT-PSS-fMWCNTs/GCE:

The immobilization of the anti-AFB_1 and AChE over PEDOT-PSS-*f*MWCNTs/GCE has been done following the methods as discussed in section 3.2.3.

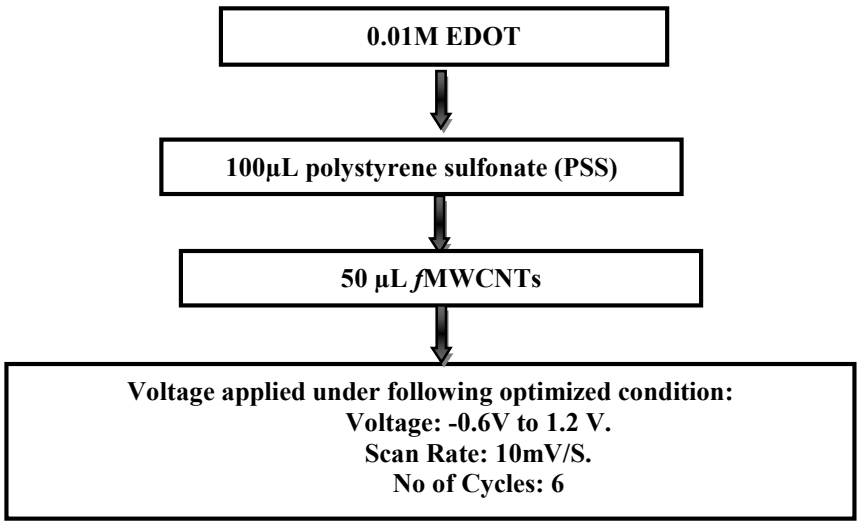


Figure 3.10: Flow chart representation of synthesis of PEDOT-PSS-*f*MWCNTs/GCE electrode.

3.4.4 Mechanism of Electrochemical deposition and electrode fabrication:

The PEDOT-PSS film was prepared following the concept that oxidative polymerization of EDOT results in positive polymeric backbone. The PSS which was dispersed with monomer solution is negatively charged due to the presence of deprotonated sulfonyl groups. During polymerization charge compensation of counter polyanion to the positive PEDOT backbone yields to PEDOT-PSS film [293].

PEDOT-PSS-*f*MWCNTs was synthesized by polymerizing EDOT in presence of two dopants PSS and *f*MWCNTs. The carbon nanotubes were functionalized by treating with concentrated HNO_3 and H_2SO_4 solution which generates COOH groups on the nanotube surface. In presence of acid either carboxylation or sulfonation may take place. The carbon atom of the nanotube may

undergo nitration by nitric acid and nitro group isomerizes to form isonitro group. The isonitro groups are susceptible to hydrolysis which results in formation of COOH groups [294]. The surface modification of carbon enhances their compatibility and solubility. The presence of oxygen containing group makes CNT negatively charged. Therefore it has been used as secondary dopant together with PSS.

The introduction of -COOH groups into the modified electrode and its activation using EDC/NHS can bind to the free NH_2 group of the antibodies as a result of which a covalent immobilization of anti-AFB_1 and AChE takes place [275]. The overall synthesis method of the PEDOT-PSS-*f*MWCNTs and the immobilization methods have been displayed in Scheme 3.5 (Keeping AChE under consideration).

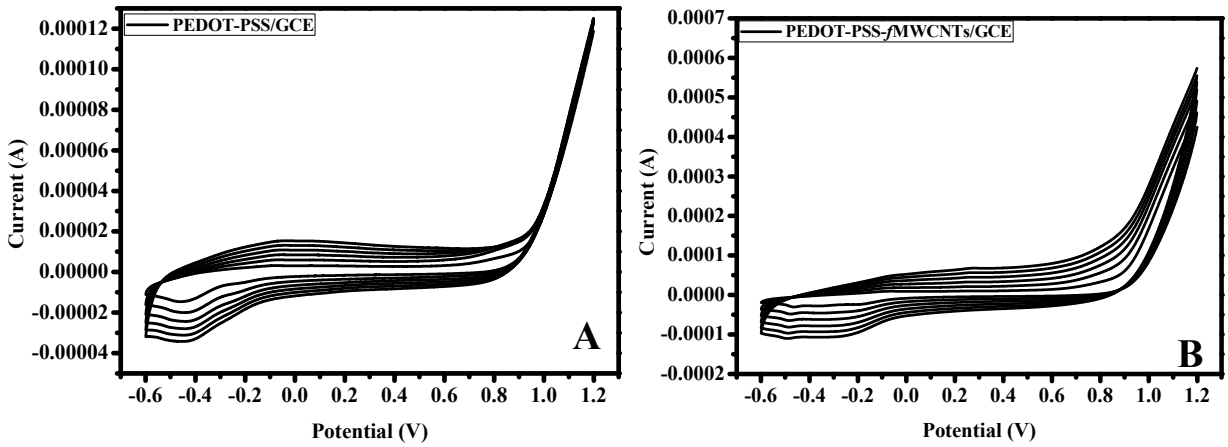
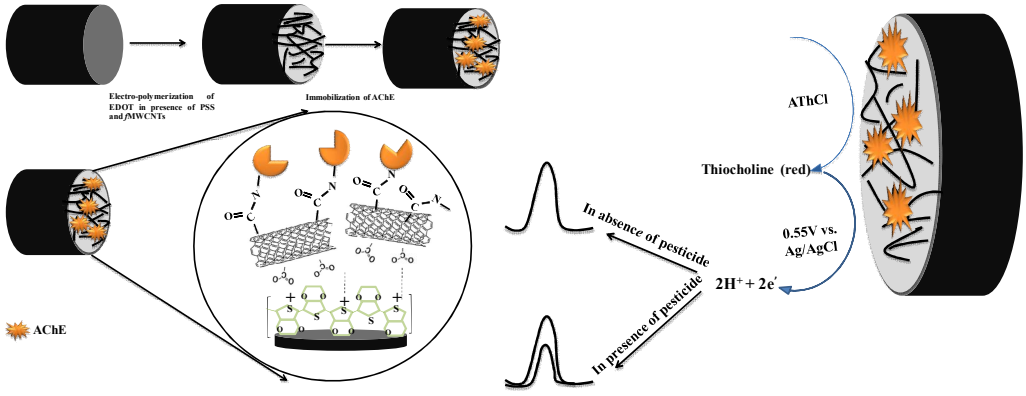


Figure 3.11: Cyclic Voltammogram pattern of electrochemical polymerization of EDOT in presence of (A) PSS and (B) PSS and *f*MWCNTs.



Scheme 3.5: The schematic representation of the fabrication process of AChE/PEDOT-PSS-*f*MWCNTs and the detection principle towards pesticide inhibition.

3.5 Preparation of analyte and Real samples:

3.5.1 Preparation of Aflatoxin B_1 solution:

Aflatoxin B_1 is crystalline substances which is insoluble in nonpolar solvents and slightly soluble in moderately polar solvents such as methanol, chloroform, acetonitrile and dimethyl sulfoxide [295]. Therefore in this work standard AFB_1 solution was prepared by dissolving it in 10 % methanol and 90 % PBS solution (1:9; v/v) and stored in 4^0C.

3.5.2 Preparation of spiked maize samples:

Real Sample matrix is prepared by adding 20 mg non infected corn powder with 10% methanol solution in PBS solution (80:20, v/v) and mixed with 0.1 g NaCl [296]. The mixture was shaken for 30 min and then centrifuged at 4000 rmp for 30 min. 1mL of supernatant was then diluted with 3 mL PBS and filtered with glass fiber. Finally, the extract was spiked with standard AFB_1 at different concentrations of 20 ng/mL and 40 ng/mL, respectively.

3.5.3 Preparation of Pesticides (methyl parathion and carbofuran) solution:

The pesticide solution is not soluble in polar solution like water [297] therefore the pesticide samples were prepared by dissolving it in 10 % methanol and 90 % PBS solution (1:9; v/v) and stored in 4^0C.

3.5.4 Preparation of Real samples spiked with methyl parathion and carbofuran:

The real samples have been prepared by taking 50 g of green tea and soil sample and spraying 0.2 mL of 20 ng/mL of carbofuran and methyl parathion. In order to reproduce the field condition the spiked samples were kept in air at room temperature for 24 hours. Later on the samples were mixed with 5 mL of 100 mM phosphate buffer and stirred for 2 hours and sonicated for 30 mins. The upper part of the liquid was used for analysis [298].

3.6 Preparation of solutions used during experiments:

3.6.1 Preparation of phosphate buffer saline:

0.01M Disodium phosphate (Na_2HPO_4) and 0.0018 M Sodium phosphate monobasic (KH_2PO_4) is added to 500 mL of DD water [299]. The two solutions have been stirred for 10 minutes and mixed properly and finally 0.01 M KCl is added to the above solution to make it saline. The pH of prepared solutions has been adjusted to desired value by adding NaOH or HCl.

3.6.2 Preparation of ferrocyanide/ferricyanide Redox Couple:

5mM sodium ferrocyanide and pottasium ferricyanide has been mixed to 100mM PBS and the standard solution of 100 mM PBS with 5 mM Fe $[(CN)_6]^{3-/4-}$ has been used for all the electrochemical experiments.

3.7 Characterization techniques:

3.7.1 Field Emission Scanning Microscope:

A field emission scanning microscope scanning the sample surface with high energy electron beam produced by a field emission source instead of light source. The liberated electrons interact with sample surface at atomic level to produce the signals containing surface topographic and compositional information. Researchers in

biology, chemistry and physics use FESEM to visualize very small topographic details on the sample surface. This technique may be employed for morphological study of structures that may be as small as 1 nanometer (= billion of a millimeter). The FESEM may be employed to study organelles and DNA material in cells, synthetic polymers, and coatings on microchips [300, 301].

Field emission gun produces highly accelerated primary electrons with high current density within the vacuum column. In standard electron microscopes electrons are mostly generated by tungsten single crystal with a needle-shaped tip by means of a current to a temperature of about 2800°C. Tungsten filament mount with crystal of lantanumhexaboride (LaB_6) is also used as electron source to achieved better resolution. In a field emission (FE) scanning electron microscope no heating but a so-called "cold" source is employed [302].

These focused electrons are deflected by electronic lenses to produce high intense beam that bombard the sample. The secondary electrons thus produced are catched by the detector and finally an electronic signal is released. The angle and the velocity of secondary electrons depend on the morphological structure of the sample [302].

FESEM imaging was performed using a JEOL JSM 6390 LV model scanning electron microscope installed at Institute of Advanced Study in Science and Technology, Assam, India. The micrographs were obtained at an accelerating voltage varying between 5-20 kV and magnification is fixed according to need from 3,000 X-20,000 X.

3.7.2 Scanning electron Microscope:

The most common form of electron microscope is the scanning electron microscope (SEM), which is most widely applied instruments in material sciences which provides information on the surface morphology, composition, phase distribution, crystal structure and presence defects. A focused electron beam of energy between ~ 0.2 keV to 40 keV is liberated by a typical tungsten filament is allowed to incident on the sample [303]. Before hitting the specimen the electron is demagnified using two or three condenser lenses to a spot of about 0.4 nm-5 nm in diameter. The interaction of the electrons with the sample results in formation of different signal comprising of secondary electrons, auger electrons, backscattered electrons,

characteristic X-rays and several other radiations. The secondary electrons are released by atoms near the surface of sample from the depth of 5 and 50 nm which provides the information of surface topography. The elastic scattering of the beam with the specimen surface results in back-scattered electrons which are used for elemental analysis. Back scattered electrons emerge from deeper locations and provides information about elemental compositions [304]. Modern SEM uses a PC to control the electron beam, to select the signals and to record and store the digital images. In the present work, the surface morphology of the PEDOT based nanocomposites were studied by JEOL JSM 6390 LV model scanning electron microscope installed at Dept. of Physics, Tezpur University, Assam, India.

3.7.3 Contact Angle Measurements:

Measurement of contact angle is a quantitative way of determining solid liquid and solid vapour interfacial tensions and helps in characterizing the average wettability of a surface of sample. Geometrically it is defined as the angle formed during the wetting of a solid by a liquid. The measurement of equilibrium of the drop under the action of interfacial forces has great possible usefulness in a wide range of problems in pure and applied science. It helps in studying the surface characteristic of the sample viz. hydrophilic or hydrophobic nature. The wettability can be measured by monitoring the contact angle after the spread of liquid drop over plane solid surface. If the contact angle is less than 90°, the wetting of the surface is thermodynamically favorable and the fluid spreads over a large area of the surface [305]. Those surfaces are hydrophilic in nature. Complete wetting is represented by zero contact angle and the surface is considered non wetting if it is greater than 90^0.

In the present work contact angle measurements were carried out applying sessile drop method using a contact angle measurement set up (Dataphysics OCA 15 EC, GmbH, Germany). To measure contact angle, a drop of double distilled water (4 μl) was dispensed on the electrolyte film whose contact angle was to be measured. After dispensing, the drop was allowed to stable and the shape of the drop was then observed with a digital camera and the contact angle was determined as the slope of the contour line at the three-phase contact point. The contact angle measurement set up installed at Materials Research Laboratory, Dept. of Physics, Tezpur University.

3.7.4 FTIR spectroscopy:

Fourier transform infrared spectroscopy (FTIR) is a technique used to determine components of unknown mixture by providing specific information regarding vibrational and rotational bonding of molecular structure. It is applied for the recognition of functional groups, chemical bonds and molecular structure of organic compounds. IR relies on the concept that the functional groups present in a molecule adsorb specific frequencies of vibration. When a molecule is exposed to an infrared radiation, it causes change in dipole moment as a result of which the molecule can undergo excitation to higher vibrational state. The characteristic energy of bending or stretching mode is displayed in terms of wave number. The infrared spectrum is considered as finger print of sample as the resultant absorption peaks corresponds to the specific frequencies of atomic vibrations of specific composition of the sample. Two different materials cannot produce identical infrared spectrum since each sample has a specific atomic configuration. Band positions are presented as wave numbers (in cm^{-1}). Band intensities are expressed either as transmittance 'T', the ratio of the radiant power transmitted by the sample or the absorbance 'A', logarithm to the base 10 of the reciprocal of the transmittance, $A = \log_{10}(1/T)$. There are three types of accessories namely Transmission, Attenuated Total Reflectance (ATR) and specular reflectance to measure FTIR. The surface properties of thin film polymer can be studied using ATR as it penetrates a depth of around 1 or 2 micro meters. ATR is based on principle of measuring the changes that occur in a totally internally reflected infrared beam when a sample surface is exposed to IR beam [306].

The FTIR spectroscopy study of the PEDOT based nanocomposites materials were recorded for understanding the bond structures, composition and doping of the conducting polymers. Scanning was performed in the range of 500-4000 cm^{-1} with a resolution of 1 cm^{-1} of standard potassium bromide (KBr) pressed pellets using a Perkin Elmer Spectrum 100 spectrophotometer installed at Tezpur University, Assam, India.

3.7.5 Potentiostat:

The basic components of a modern electro analytical system for voltammetry are a potentiostat, computer, and the electrochemical cell. The task of applying a known potential and monitoring the current falls to the potentiostat. The most widely used

potentiostats today are assembled from discrete integrated-circuit operational amplifiers and other digital modules [223].

The main component of potentiostat is the cell and the electrodes. A typical electrochemical cell consists of three (or sometimes two) electrodes and the sample dissolved in a solvent, an ionic electrolyte. The shape, size and materials of the cells (that is, sample holders) vary with the application, types of sample used and the analytical data to be obtained. For e.g. the material like Teflon, polyethylene, glass is selected to minimize the reaction of the cell with the sample. The other three components are:

(a) Reference Electrodes:

In a two electrode there will be flow of current between the electrodes but it is difficult to know at which potential the reaction occurs therefore a standard electrode is required. The reference electrode should provide a reversible half-reaction with Nernstian behavior, it remains constant over time, and is easy to assemble and maintain. The reference electrodes mostly used for aqueous solutions are the calomel electrode and the silver/silver chloride electrode (Ag/AgCl), with potential determined by the reactions Hg_2Cl_2 (s) + $2e^-$ = 2Hg (l) + $2Cl^-$ and AgCl(s) + e^- = Ag(s) + Cl^-. In most cases the reference electrode should be as close as possible to the working electrode; in some cases, to avoid contamination, it may be necessary to place the reference electrode in a separate compartment [223].

(b) Counter Electrodes:

This electrode is used to complete the circuit and to carry current. In most voltammetric techniques the analytical reactions at the electrode surfaces occur over very short time periods and thus isolation of the counter electrode from the sample species is not normally necessary. Most often the counter electrode consists of a thin Pt wire, although Au and sometimes graphite have also been used.

(c) Working Electrodes:

The working electrodes are of various geometries and materials, ranging from small Hg drops to flat Pt disks depending on the application. Glassy carbon electrode is widely used widely in electrochemistry as it permits low electrical resistance, low friction, low thermal resistance, extreme resistance to chemical attack and impermeability to gases and liquids. Other commonly used electrode materials are gold, platinum, and mercury electrodes. All the electrochemical measurements

were performed using a Potentiostat/Galvanostat/ZRA (Gamry Reference 3000, United States of America) with Gamry Echem Analyst Software. Glassy carbon electrode, Ag/AgCl (3 M KCl) and a platinum wire were used as working, reference and auxiliary electrode, respectively.

3.7.5.1 Electrochemical Impedance Spectroscopy:

Electrochemical impedance spectroscopy (EIS) is a powerful technique that utilizes small amplitude, alternating current (AC) signal to probe the impedance characteristics of a cell. The AC signal is scanned over a wide range of frequencies to generate an impedance spectrum for the electrochemical cell under test. EIS differs from direct current (DC) techniques in that it allows the study of capacitive, inductive, and diffusion processes taking place in the electrochemical cell. EIS has far reaching applications including coatings, batteries, fuel cells, photovoltaics, sensors, and biochemistry. EIS is most commonly run in 3 electrode mode. In this configuration there is a working electrode (material sample), counter electrode (graphite and platinum are commonly utilized), and an independent reference electrode, Saturated Calomel Electrodes (SCE) and Silver/Silver Chloride (Ag/AgCl) are most common. In this work the EIS techniques have been to study the membrane capacitance and resistance to investigate the resistance occurring in the charge transfer mechanism within the interface of the electrode and the electrolyte solution [221]. The experiment was carried out in an electrochemical cell comprising of three electrodes and a standard $[Fe(CN)_6]^{3-/4-}$ solution as the redox probe. The impedance spectra were measured in the frequency range from 10^6 Hz to 0.08 Hz at amplitude of 20 mV vs. Ag/AgCl. The techniques has been used to evaluate the value of parameters like Electron transfer resistance (R_{et}), solution resistance (R_s), double layer capacitance (C_{dl}) and phase degree (Φ) of the PEDOT based nanocomposites.

3.7.5.2 Cyclic Voltammetry:

Cyclic Voltammetry (CV) is arguably the most widely used electrochemical technique in the world as it allows studying a variety of electrochemical systems, revealing both fundamental and application oriented information [236]. It has been used for the study of redox processes, for understanding reaction intermediates, and for obtaining stability of reaction products. This technique is based on sweeping the

potential of the working electrode in both forward and reverse directions at a specific sweep rate called scan rate (in volts / second), and measures the resulting current vs. time curve. Usually the sweep is reversed at a specific switching potential, hence the name cyclic voltammetry. Since the sweep rate is constant and the initial and switching potentials are known, one can easily convert time to potential, and the usual protocol is to record current vs. applied potential. Depending on the analysis, one full cycle, a partial cycle, or a series of cycles can be performed.

In the present work cyclic voltammetry has been used to synthesize the modified electrodes using electro chemical polymerization. Further it has been used to study the details of electrocatalytic activity of all the synthesized electrodes and the measurements were performed in 100mM PBS containing 5mM $K_3[Fe(CN)_6]$ / $K_4[Fe(CN)_6]$ keeping 1:1 ratio as redox probe.

3.7.5.3 Differential Pulse Voltammetry:

Differential pulse voltammetry (DPV) (also differential pulse polarography, DPP) is a voltammetry method used to make electrochemical measurements particularly for quantitative analysis and a derivative of linear sweep voltammetry or staircase voltammetry, with a series of regular voltage pulses superimposed on the potential linear sweep or stairsteps. This technique is comparable to normal pulse voltammetry in that the potential is also scanned with a series of pulses. In the process each potential pulse is fixed, of small amplitude (10 to 100 mV) can be applied, and is superimposed on a slowly changing base potential. Current is measured at two points for each pulse, just before the application of the pulse (I_0) and at the end of the pulse (I_f) and the difference (I_f-I_0) is displayed with respect to the base potential. These sampling points are selected to allow for the decay of the nonfaradaic (charging) current. Therefore DPV is considered as a suitable technique for the sensing as it measures the faradaic (heterogeneous) current [307]. In the present work DPV technique has been used to study the activity of the synthesized bioelectrodes towards their respective analytes.