Abstract

Conducting polymers are recently being considered for different biomedical applications such as artificial muscles, drug delivery, tissue engineering etc. Conducting polymers like Polypyrrole (PPy), Polyaniline (PAni) and Poly (3,4-ethylenedioxythiophene) (PEDOT) are extensively used for the development of biosensors due to their unique and tuneable electronic and optical properties properties. They have extended π-conjugation along the polymer backbone. The ability of conducting polymers to incorporate biomolecules gives them a strategy for optimization of biological properties and also detection of binding events. The highly conjugated polymer backbone chain can be assigned reversible chemical, electrochemical and physical properties controlled by a doping/de-doping process, which makes these polymers very attractive as transducer materials as well as ideal matrix for immobilization in various sensing devices. Conducting polymers and their derivatives have been used as active layers of gas sensors since early 1980s. The sensors made of conducting polymers have many improved characteristics such as high sensitivity, selectivity and short response time and workability at room temperature. To further improve their sensing characteristics and tenability, novel nanomaterials such as graphene, gold nanoparticles, graphene and other carbon nanomaterials based conducting polymer nanocomposites have been developed. PEDOT is one of the most promising conducting polymers that have been used in biological and biomedical areas such as biosensors and bio-interface as the deposition of PEDOT on electrode surface would effectively improve the electrochemical performance of the modified electrodes. It can be electropolymerized from 3,4-ethylene dioxythiophene (EDOT) monomer and the oxidative polymerization of EDOT results in positive charges on the polymer backbone, this allows for the incorporation of negatively charged doping agents, such as graphene oxide (GO), AuNPs, polystyrene sulfonate (PSS), carbon nanotubes (CNT) for charge neutrality.

Gold nanoparticles and carbon nanomaterials based immunological sensors, using electrochemical, optical and piezoelectric detection methods have drawn particular interest in the bioanalytical field due to their superior physical and chemical properties such as easy fabrication, compatibility and high catalytic activity. They modify the electrode substrate by creating nanostructured surfaces with improved

electrochemical response and also act as a carrier for the immobilization of biomolecules such as enzymes, antibodies and protein conjugates, for electrochemical signal transduction and amplification.

In the present thesis three different systems viz. AuNPs/PEDOT, AuNPs/PEDOT-GO and PEDOT-PSS-*f*MWCNTs have been studied and their electro-catalytic and biosensing properties have been investigated. All the systems, before and after functionalization have been investigated using sophisticated analytical tools. Field emission Scanning Electron Microscope (FESEM) (JEOL-JSM-6390LV) to study the morphology of the synthesized nanocomposites. The surface characteristics have been studied using contact angle measurements made by the Degree of Hydrophilicity measurement set up (Model: DSA 15B). The conformational changes in the materials have been studied using vibrational spectroscopy employing Fourier Transform Infrared (FTIR) spectroscopy. The electrochemical impedance spectroscopy (EIS) has been employed to investigate the resistance enocountered in the charge transfer mechanism at the interface of the electrode and the electrolyte solution. The electro-catalytic properties and the electron transfer mechanism have been studied using cyclic voltammetry (CV). The sensitivity of the synthesized composites towards analytes has been investigated and the real sample analysis of spiked and unspiked samples has been done using DPV. The real time analysis of the spiked and unspiked samples has been carried out using differential pulse voltammetry techniques.

The present thesis embodies seven chapters. **Chapter 1** begins with the fundamentals of conducting polymers with an emphasis on band formation in the conducting polymers, doping of conducting polymers and formation of charge carriers in doped form of conducting polymers. The general principles of biosensors and their classification are highlighted. The mechanisms of different bioreceptor and transducer based biosensors have been discussed. The chapter also describes different synthesis methods and applications of conducting polymers in diverse fields including biomedical applications. The chapter ends with the outlines of the scope of the thesis and statement of the thesis problem.

The different theoretical models and mechanisms used to explain experimental findings have been reviewed in **Chapter 2**. Different circuit modelling of charge transfer that are employed for the calculation of electron transfer resistance (R_{et}), capacitance (C_{dl}) and phase angle (Φ) using electrochemical impedance

spectroscopy have been discussed. The theory of enzyme kinetics has also been included in this chapter.

Chapter 3 highlights the properties of materials used for synthesis of Poly(3,4-ethylenedioxythiophene)(PEDOT) based nanocomposite electrodes. The immobilization of proteins onto the matrix of PEDOT based nanocomposites has been discussed. The principles of various characterization techniques viz., HRTEM, SEM, FTIR, Cyclic voltammetry, Electrochemical impedance spectroscopy and Differential pulse voltammetry employed in the present work have been briefly discussed.

Chapter 4 deals with the synthesis and characterization of composite AuNPs/PEDOT/GCE which has been synthesized by layer-by-layer deposition of spherical gold nanoparticles onto the matrix of PEDOT/GCE by reduction of gold chloride solution ($HAuCl_4$). The monoclonal anti-aflatoxin antibodies (anti-AFB_1) and acetylcholine esterase were immobilized over the surface of PEDOT/Au-NPs/GCE using physical adsorption. The surface morphology and characteristics of the modified electrodes were investigated by field emission scanning electron microscope (FESEM) and contact angle measurements, respectively.

The FESEM for PEDOT shows sponge like rough structure with slightly porous nature of the surface. After deposition of the Au-NPs the porosity of the surface increases and the nanoparticles are deposited onto the surface of PEDOT. The Au-NPs are less agglomerated as the PEDOT film provides a stable surface and resists the agglomeration of the nanoparticles. The average size of the Au-NPs is determined to be in the range of 230 nm to 300 nm. The contact angle measurement for PEDOT/GCE and AuNPs/PEDOT/GCE and the calculated values are 76^0 and 34^0, respectively. The functionalization of PEDOT matrix with Au-NPs results in decrease in contact angle making the film more hydrophilic.

The electrochemical analysis of the fabricated bio-electrode and the immobilization of the antibodies/enzyme have been evaluated and confirmed by performing Cyclic Voltammetry (CV), Electrochemical Impedance Spectroscopy (EIS) and Fourier Transform Infrared Spectroscopy (FTIR). Decrease in the value of electron transfer resistance (R_{et}) and increase in the peak current values after incorporation of Au-NPs signify the enhanced properties of PEDOT embedded Au-NPs. The heterogeneous rate constant (k_s) and transfer coefficient (α) have been determined by using Laviron's method.

The fabricated immunosensor anti-AFB_1/AuNPs/PEDOT/GCE exhibits highly sensitive amperometric response of 0.725 $\mu AngmL^{-1}$ towards AFB_1 concentration in a linear range of 0.5-20 ng/mL and 0.431 $\mu AngmL^{-1}$ in a linear range of 25-40 ng/mL with detection limit (LOD) of 0.0308 ng/mL. The fabricated immunoelectrode shows a reproducibility of 96.13% and 94.5% towards real maize sample spiked with AFB1 of concentration 20 ng/mL and 40 ng/mL, respectively.

The calculated values of K_m and I_{max} of the synthesized enzyme electrode AChE/AuNPs/PEDOT/GCE towards its enzymatic reaction were 0.823 mM and 171.2 μA, respectively. The biosensor showed a linearity of 0.1 to 35ng/mL towards the detection of Methyl parathion.

Synthesis of Gold nanoparticles (AuNPs) modified surface of PEDOT doped with graphene oxide (GO) and characterization of their electrochemical and biosensing application have been described in **Chapter 5**. The PEDOT and GO composite has been synthesized using *in situ* electrochemical polymerization of PEDOT in presence of GO using cyclic voltammetry at a potential range -0.5 V to 1.2 V vs. Ag/AgCl. 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. The incorporation of GO introduces the functional groups –COOH onto surface of PEDOT which helped in the covalent immobilization of antibody/enzyme. However the poor conductivity of PEDOT-GO matrix has been modified after the function of PEDOT-GO surface with spherical AuNPs. FESEM micrograph shows that the thickness of graphene oxide sheets is 12.43 nm to 17.08 nm and the spherical AuNPs of average size 45 nm to 56 nm are uniformly distributed on the petal like surface of PEDOT-GO onto which anti-AFB1 have been immobilized. The surface characteristics study carried out by contact angle measurements of PEDOT-GO/GCE and AuNPs/PEDOT-GO/GCE and the calculated values are 63^0 and 22^0, respectively. The hydrophilic nature of GO and functionalization of PEDOT-GO/GCE matrix with Au-NPs result in decrease in contact angle making the film more suitable for anti-AFB_1 immobilization. The electrochemical deposition of AuNPs/PEDOT-GO biocompatible film not only simplifies the fabrication but also makes the immunoelectrode more sensitive and stable. The deposition of Au-NPs on PEDOT-GO surface enhances the electro-catalytic properties of PEDOT-GO electrode by decreasing the R_{et} value and increasing the heterogeneous rate transfer constant k_s. The immunosensor anti-AFB_1/AuNPs/PEDOT-GO/GCE exhibits a very

high sensitivity of 0.998 μAngmL^{-1} and 0.378 μAngmL^{-1} in the linear range of 0.1 - 20 ng/mL and 20-60 ng/mL, respectively.

The limit of detection (LOD) and limit of quantification (LOQ) of the linear range 0.5-20 ng/mL are determined to be 0.109 ng/mL and 0.377 ng/mL, respectively. The fabricated immunoelectrode shows a reproducibility of 92.25% and 95.79 % towards real corn sample spiked with AFB_1 of concentration 20 ng/mL and 40 ng/mL, respectively. The immunoassay was also applied for analysis of maize samples spiked with AFB_1 and the sensitivity was found to be 11.81 μAngmL^{-1} in the linear range of 0.1-1.8 ng/mL.

The calculated values of K_m and I_{max} of the synthesized enzyme electrode AChE/ AuNPs/PEDOT-GO/GCE towards the detection of carbofuran were 0.2 mM and 254.15 μA, respectively. The biosensor shows linearity of 0.1-25 ng/mL and 35-50 ng/mL towards the detection of methyl parathion.

In **Chapter 6,** a platform of PEDOT doped with PSS and functionalized with multi walled carbon nanotubes (*f*MWCNTs has been discussed. The bio-hybrid has been synthesized by in situ electrochemical polymerization of the components on glassy carbon electrode (GCE) and AChE/anti-AFB_1 was covalently immobilized using EDC/NHS coupling. The morphological and surface studies have been performed using Field emission Scanning electron microscope (FESEM) and contact angle measurements. The biosensor shows good electro-catalytic behavior which has been studied using Cyclic Voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS).

The fabricated immunosensor anti-AFB_1/PEDOT-PSS-*f*MWCNTs/GCE exhibits high sensitive amperometric response of 2.31 μAng^{-1}mL towards AFB_1 concentration in the linear range of 0.5 - 20 ng/mL with a detection limit (LOD) of 0.212 ng/mL. The fabricated immunoelectrode shows a reproducibility of 99.83% and 92.89% towards real maize sample spiked with AFB_1 of concentration 20 ng/mL and 40 ng/mL, respectively.

The synthesized electrode has been applied as an enzyme based electrode AChE/PEDOT-PSS-*f*MWCNTs/GCE. The oxidation of thiocholine occurs at 0.5 V vs. Ag/AgCl and calculated value of K_m and I_{max} were 0.14 mM and 418 μA, respectively. The biosensor showed a linearity of 0.1-20 ng/mL and 20-40 ng/mL towards methy parathion inhibition. Similarly, a linear response of 0.1-30 ng/mL and 30-50 ng/mL towards inhibition of carbofuran has been found. The recoveries of the

synthesized electrode in real sample spiked with 10 ng/mL methyl parathion and carbofuran is 100.51% and 98.47%; and 98.48% and 96.44%, respectively.

Chapter 7 summarizes the main conclusions drawn from the present work.. Finally, the future scope of research in the field of conducting polymer nanostructures and their physico-chemical and biological aspects has been briefly mentioned towards the end of this chapter.