# CHAPTER-VII

# Conclusions and Future Prospects

*This chapter outlines the main conclusions drawn from the present work, which deals with the synthesis, characterization and investigation of physico-chemical and biological properties of poly(3,4-ethylenedioxythiophene) based electrodes viz., AuNPs/PEDOT/GCE, AuNPs/PEDOT-Go/GCE and PEDOT-PSS-fMWCNTs towards detection of $AFB_1$ and OPs. The chapter also provides the future prospects of research in this area.*

### *7.1 Conclusions:*

The present thesis deals with the synthesis, characterization and electrochemical studies of poly(3,4-ethylenedioxythiophene) (PEDOT) and its composites for biosensing applications. The major conclusions drawn from the three systems synthesized and investigated in the present work are summarized below:

**AuNPs functionalized PEDOT system**

1. PEDOT films over GCE have been synthesized by electrochemical polymerization of EDOT monomer by applying standard oxidation potential vs. Ag/AgCl. Layers of spherical AuNPs have been deposited over PEDOT film by reducing gold chloride. The FESEM of AuNPs/PEDOT film confirms uniform deposition of AuNPs of average size 230 nm to 300 nm. The contact angle measurement for PEDOT/GCE and AuNPs/PEDOT/GCE are found to be $71^0$ and $44^0$ respectively indicating that the AuNPs modified surface is more hydrophilic in nature. The covalent bonding of anti-$AFB_1$ and AChE onto the matrix of PEDOT based composite films have been done using carbodiimide coupling.
2. The AuNPs/PEDOT/GC electrode showed a better electro-catalytic performance in redox active solution which can be concluded by decreased value of $\Delta E_p$ (176mV) and increased current values ($I_{pa}$ and $I_{pc}$) of 94.17 μA and 87 μA, respectively. The increase in area of GCE after deposition of PEDOT and AuNPs composite film implies that nano gold provides a large surface area which is

more suitable for the protein immobilization. Similarly the surface coverage of the ionic species (AChE and anti-$AFB_1$) is found to be in order $10^{-6}$ mol $cm^{-2}$ which is more than that for PEDOT/GCE. Therefore AuNPs not only help in better immobilization but also improved the charge transfer within the matrix which can be concluded by decreased value of $R_{et}$ (67 Ω) and enhanced value of $k_s$ ($1.91 \times 10^{-1}$ $cms^{-1}$).

3. A stability of 98% is obtained for over 50 consecutive CV cycles of the immunoelectrode BSA/anti-$AFB_1$/AuNPs/PEDOT/GCE implying that there is no degradation in the electrode and its performance towards redox probe indicating high stability of the immunoelectrode. The sensitivity of the BSA/anti-$AFB_1$/AuNPs/PEDOT/GCE immunoelectrode towards aflatoxin $B_1$ is found to be 0.725 μAng-$^1$mL and 0.431μAng-$^1$mL in two linear linear regions of 0.1-20 ng/mL and 20-40 ng/mL of $AFB_1$ concentration, respectively. The calculated values of corresponding LOD and LOQ for the fabricated immunosensor are0.0348 ng/mL and 0.102 ng/mL, respectively. The synthesized electrode have shown a recovery are 94.89 % and 98.15 %, respectively towards maize sample spiked with 20 ng/mL and 30 ng/mL of $AFB_1$.
4. The enzyme (AChE) immobilized electrode, AChE/AuNPs/PEDOT/GCE showed an oxidation peak at around 0.82 V vs. Ag/AgCl in cyclic voltammogram corresponding to oxidation of thiocholine. Therefore AuNPs/PEDOT/GCE can be considered as biocompatible electrode that provides ideal environment to keep the activity of immobilized AChE. The $K_m$ and $I_{max}$ of AChE/AuNPs/PEDOT/GCE towards the hydrolysis of acetylthiocholine are found to be 0.823 mM and 171 μA, respectively. Small value of $K_m$ depicts good enzymatic behaviour of AChE towards its analyte which represents an efficient transfer of electrons between the electrode surface and active site of AChE. In presence of methyl parathion the electrode showed a linearity un the range of 0.1 to 35 ng/mL with sensitivity (I% per unit change in concentration) of 1.07 ng$^{-1}$mL with LOD and LOQ of 0.708 ng/mL and 2.36 ng/mL, respectively.

**AuNPs functionalized PEDOT-GO system**

5. PEDOT-GO and PEDOT-GO functionalized with Au-NPs have been synthesized electrochemically and their morphological, surface properties and electrochemical activities have been investigated. FESEM micrograph confirms that the thickness of graphene oxide sheets is 12 nm to 15 nm and the spherical AuNPs of average size 45-56 nm are uniformly distributed on the petal like surface of PEDOT-GO onto which anti-$AFB_1$ antibody and AChE enzyme have been immobilized using covalent coupling. The contact angle of $22^0$ of AuNPs/PEDOT-GO film implies the hydrophilic nature which is considered more suitable for anti-$AFB_1$ and AChE immobilization.
6. The $R_{et}$ value of the functionalized electrode AuNP/PEDOT-GO/GCE which has been used for the immobilization of anti-$AFB_1$ and AChE is found to be 105 Ω. The value of $C_{dl}$ is maximum for BSA/anti-$AF_{B1}$/PEDOT-GO/GCE and AChE/AuNPs/PEDOT-GO/GCE with phase angle of $78.4^0$ and $60^0$ indicating that the AuNPs/PEDOT-GO electrode has became more capacitive after immobilization of anti-$AFB_1$ and AChE which are non-conducting in nature.
7. The appearance of two reduction peaks at 0.45 V and 0.546 V, corresponding to the reduction reactions of Au(3+)→Au(+) and Au(+)→Au(0), respectively implies that the Au nanoparticles are successfully deposited on the surface of the PEDOT-GO film. The electro active surface area of AuNPs/PEDOT-GO/GCE is found to be 0.23 $cm^2$ which is higher than that of PEDOT-GO/GCE and GCE. The surface coverage of anti-$AFB_1$ and AChE over AuNPs/PEDOT-GO/GCE was found to be of order $10^{-5}$ mol $cm^{-2}$ which is higher than the value obtained AuNPs/PEDOT/GCE. This may be attributed to the synergistic effect of spherical AuNPs and GO nanocomposite. The presence of carboxyl groups on the planes of GO and dative binding of AuNPs were involved in forming chemisorption covalent bond with the amine group of proteins.
8. There is no decrease in the values of $I_{pa}$ & $I_{pc}$ and the difference in peak potential ($\Delta E_p$) remains constant of the BSA/anti-AFB1/AuNPs/PEDOT-GO/GCE indicating the stability of the electrode towards redox reaction of Fe(III)/Fe(IV) and confirms the decrease in current during immune-reaction is due to antigen-antibody interaction. A high sensitivity of 0.989 $\mu Ang^{-1}mL$ towards the detection of antigen $AFB_1$ has been achieved by the synthesized immunosensor

BSA/anti-$AFB_1$/AuNPs/PEDOT-GO/GCE. The applicability of BSA/anti-AFB1/AuNPs/PEDOT-GO/GC electrode towards real maize sample has been studied and the electrode shows a linearity of 0.1-1.81 ng/mL with an excellent sensitivity of 11.81 $\mu Ang^{-1}mL$. The fabricated immunoelectrode BSA/anti-AFB1/AuNPs/PEDOT-GO/GCE shows a recovery of 92.25% and 95.79 % towards real maize sample spiked with $AFB_1$ of concentration 20 $ngmL^{-1}$ and 40 $ngmL^{-1}$, respectively.

9. The enzyme electrode AChE/AuNPs/PEDOT-GO/GCE shows good enzymatic activity and the oxidation of thiocholine occurs at 0.75 V vs. Ag/AgCl. An excellent affinity of the immobilized AChE towards AThCl was found with $K_m$ value of 0.2 mM and $I_{max}$ of 255µA. The AChE/AuNPs/PEDOT-GO/GCE shows linearity in two ranges 0.1-20 ng/mL and 30-40ng/mL towards the inhibition of methyl parathion. After inhibition, the residual activity of the enzyme in AuNPs/PEDOT-GO/GCE decreases as the pesticides sample concentration increases with time. The immobilized enzyme retains 54.8% of its activity after the pesticides inhibition. The AChE/AuNPs/PEDOT-GO/GCE showed a good activity and recovery of 91% after weekly usage till 140 days when storage in phosphate buffer at $5^0$C. The electrochemical deposition of AuNPs/PEDOT-GO/GCE biocompatible film not only simplified the fabrication but also made the bioelectrode more sensitive and stable.

**CNTs functionalized PEDOT-PSS system**

10. A nanohybrid composite of PEDOT-PSS-*f*MWCNTs has been synthesized by electrochemical oxidation of EDOT in presence of PSS and *f*MWCNTs. The anti-$AFB_1$ and AChE was immobilized over the matrix of PEDOT-PSS-*f*MWCNTs probe using EDC/NHS linker between the COOH group of PEDOT-PSS-*f*MWCNTs and $NH_2$ group of the proteins. The surface morphology of the synthesized films showed a rough surface and a porous network of PEDOT-PSS film. In PEDOT-PSS-*f*MWCNTs the nanotubes of thickness ca. 5 nm are found to be wrapped in the growing polymer and an interconnected network of incorporated nanotubes may have formed. The increase in surface hydrophilicity (CA~$44^0$) is due to the presence of hydrophilic carboxyl groups in PEDOT-PSS-*f*MWCNTs composite. Fundamental stretching vibrations of all the synthesized

composite films have been assigned using FTIR and the presence of primary amine band between 1600-1650 $cm^{-1}$ confirms that the proteins retain their native state after immobilization.

11. The activation barrier to the flow of electrons is found to be minimum for PEDOT-PSS-*f*MWCNTs/GCE with $R_{et}$~140 Ω, thus the presence of *f*MWCNTs may generate nano channels for successful electron transfer by increasing interfacial catalytic active sites between PEDOT-PSS-*f*MWCNTs/GCE electrode and the redox active solution ($Fe[(CN)_6]^{3-/4-}$). The EIS result are correlated to the cyclic voltammetry results as the PEDOT-PSS-*f*MWCNTs/GCE showed an improved electrochemical behavior with peak current intensity of 169 μA and -168 μA ($\Delta E_p$ ~ 69 mV) towards the redox species.
12. The large surface area of the nanotubes (1315 $m^2/g$ (theoretical value)) contributes a remarkable increase in electro active area of GCE which results in large embedding of protein molecules of order $10^{-5}$ $mol/cm^2$. The mean value of $k_s$ $1.8 \times 10^2$ $s^{-1}$ for PEDOT-PSS-*f*MWCNTs/GCE indicates fast kinetics of the redox couple which may be characteristic property of highly conducting carbon nanotubes network incorporated across polymer matrix. The value of $k_s$ after immobilization of proteins is found to be in order $10^{-2}$ which suggests that the electron transfer pathway of the protein behaved as a quasi-reversible system.
13. The immunosensing experiments of BSA/anti-$AFB_1$/PEDOT-PSS-*f*MWCNTs/GCE have been performed in optimized condition of operating pH 7.2 and incubation time of 5 min. The synthesized electrode showed linear response in two ranges of 0.1-25 ng/mL and 30-55 ng/mL towards the detection $AFB_1$ with a tremendous sensitivity of 2.36 μAng$^{-1}$mL within the linear range of 0.1-25 ng/mL and 0.307 μAngmL$^{-1}$ within linearity range of 30-55 ng/mL, respectively. The minimum concentration of $AFB_1$ that can be detected and quantified are found to be 0.481 ng/mL and 1.64 ng/mL, respectively. An excellent sensitivity of 8.89 μAngmL$^{-1}$ towards spiked maize sample has been found with LOD and LOQ of 0.123 ng/mL and 0.428 ng/mL, respectively. The interference of other component and cross reaction of BSA/anti-$AFB_1$/PEDOT-PSS-*f*MWCNTs/GCE can be considered minimum as the electrode showed a recovery of ca 98% in presence of real corn sample.

14. The AChE based biosensors AChE/PEDOT-PSS-*f*MWCNTs/GCE can be considered as an efficient platform for thiocholine oxidation as the oxidation potential of thiocholine decreases from 0.8 V to 0.55V vs. Ag/AgCl. The enhancement of amperometric signal and the lowering of oxidation potential are attributed to the presence of *f*MWCNTs on the surface of GCE, which possessed a relatively large specific surface area and an inherent, high electricity conducting ability, thus they could enhance the rates of reactions as well as the electron transfer rate at a lower potential. The enhanced enzymatic activity of the immobilized AChE can be further explained by the small $K_m$ value of 0.14 mM. A high value of $I_{max}$ depicts efficient affinity of AChE towards AThCl.
15. In presence of carbofuran, the enzymatic bioelectrode AChE/PEDOT-PSS-*f*MWCNTs/GCE showed linearity in two ranges of 0.1-30 ng/mL and 30-50 ng/mL with LOD and LOQ of 0.073 ng/mL and 0.245 ng/mL, respectively. Similarly the analytical performance of the biosensor towards different concentration of methyl parathion was studied and bioelectrode showed a linearity of 0.1 to 20 ng/mL and 20 to 40 ng/mL towards the inhibition of Methyl Parathion. A small detection and quantification limit of 0.212 ng/mL and 0.707 ng/mL, respectively signifies an efficient detection of pesticides. The shelf life and operational stability of the AChE/PEDOT-PSS-*f*MWCNTs/GCE electrode have been studied and the biosensor showed a consistent behavior towards its hydrolysis of its analyte and there is 90 % recovery in the amperometric signal till 210 days when stored in phosphate buffer at $15^0$C.

## *7.2 Future Prospects:*

With the intervention of nanoscience and technology a new horizon has opened up in the field of device architecture. ELISA technique requires time, cost, skilled man power and sophisticated instruments. Furthermore, these methods sometime may not reach the very low limit of detection. In this context, biosensors are very promising for mycotoxin assay provided the challenges pertaining to technology and thought can be addressed judiciously such as development of a synthetic receptor like aptamer, MIP etc in place of costly unstable monoclonal antibody, adaptation of label free easy scheme for detection, integration of modern technologies e.g, microfluidics and sensing design etc, finally a determined step for the conversion of

innovation to application. In future, other novel approaches like use of intrinsic peroxidase activity of NPs like AuNPs to estimate sensitive and quick electrochemical detection of OPs can be envisaged.

It has been observed from the present thesis work that we can improve the performance of conducting polymer based bioelectrode by synthesizing nanocomposites with other novel materials.

1. In the first chapter, we have synthesized electrode system based on AuNPs functionalized PEDOT for detection of $AFB_1$ and OPs. This work can be further extended for different PEDOT nanostructures (nanowires, nanotubes. etc) as well as more real sample analysis can be done.
2. In the second system, we have investigated AuNPs/PEDOT-GO nanocomposite bioelectrodes and observed the enhancement in sensing properties. Further studies can be carried out on similar types of conducting polymer with other metal nanostructures such as Ag, Pt etc. in order to get a deeper understanding of the physico-chemical and biological properties of conducting polymer-metal nanocomposites. Composites of conducting polymer with novel material like Metal organic framework (MOF), Molybdenum disulfide ($MoS_2$) can be prepared for biosensing applications
3. In the last system we have explored the multi-component matrix based on PEDOT, PSS and *f*MWCNTs biosensor probe. This work can be further extended by functionalizing PEDOT-PSS with aligned carbon nanoutubes. Moreover, immobilization of different biomolecules such as cholesterol oxidase, HRP, uricase, DNA, Lactate oxidase etc. on conducting polymer-biopolymer nanocomposites for biosensor applications is an emerging field of research.