Abstract

Sensors based on electroanalytical techniques can be portable, miniaturized and easy to handle. Label free, electrochemical immunosensors are affordable and highly suitable for on-site monitoring. Several nanostructured materials and composites are being investigated for affordable and reliable electrochemical biosensors; however, commercialization is still lacking. Regarding this aspect, conducting polymers and 2D layered nanostructures have attracted considerable attention due to their inherent physico-chemical properties and their ability to respond external stimuli. The unique properties of conducting polymers make them well-suited for use in various applications, including solar cells, supercapacitors, biosensors, and flexible electronics. Amongst various flexible conducting polymers, PEDOT-PSS (poly (3, 4-ethylene-dioxythiophene) -polystyrene sulfonic acid) has been recognized as a suitable candidate for biosensing due to its enhanced electrical conductivity, chemical stability, mechanical flexibility and the presence of functional groups in its polymer backbone can facilitate the attachment of biomolecules of interest. Graphene oxide (GO), one of the widely explored 2D materials, comes with abundant oxygen functional groups, such as hydroxyl, epoxides, carboxyl etc. which could make it hydrophilic in nature and easily dispersible in aqueous solutions. Additionally, existence of these functional moieties facilitates immobilization of biomolecules through covalent linkages making it suitable for a reliable biosensor electrode with PEDOT-PSS. Moreover, transition metal dichalcogenides (TMDCs) offer similar potential advantages, including their layered structure that enhances specific surface area, stability, low cytotoxicity and appropriate conductivity. The presence of gold nanoparticle (AuNP) layers over a polymer-2D composite improves charge transport at the electrode-electrolyte interface and enhances the conductivity of the system. Again, polyaniline (PANI) is widely used in electrochemical application due to its electrochemical stability and electroactivity. PANI and its derivatives have been recognized as an excellent immunosensor material due to their high biocompatibility, easy modification, and excellent electrochemical responses. The biocompatibility of PANI leads to the preservation of enzyme active sites while allowing analytes to permeate to enzyme catalytic sites.

In the present thesis, the fabrication of electrochemical sensors has been carried out for the detection of two distinct analytes—glucose and aflatoxin B_1 (AF-B_1). These analytes address two important sectors: glucose monitoring is crucial in the health sector for managing deadly diseases such as diabetes, while AF-B_1 detection is significant in the food sector for ensuring food safety, as AF-B_1 is a potent toxin produced by certain molds. The research involves the development of three different composite systems based on conducting polymer PEDOT-PSS and PANI, that form the basis of these electrochemical sensors. Each system is explored in two sections: A and B, where section A focuses on glucose sensor development, and section B is dedicated to the development of an AF-B_1 sensor. Overall, the thesis presents a thorough investigation into the fabrication and optimization of electrochemical sensors for glucose and AF-B_1. By utilizing advanced material systems and techniques our research demonstrates significant improvements in sensor performance, contributing profoundly to health and food safety measures.

Chapter 1

Offering an overview of scientific background and motivation, it lays the foundation of the thesis in hand. It includes the basics of electrochemical sensing mechanism and scope of the materials for development of electrochemical biosensor. The importance of glucose and AF-B_1 detection have also been included in this chapter.

Chapter 2

This chapter explains materials and methodologies employed in synthesizing conducting polymer poly (3, 4-ethylene-dioxythiophene)-polystyrene sulfonic acid (PEDOT-PSS) and their respective nanocomposites with 2D materials like graphene oxide (GO) and tungsten disulfide (WS_2) and metal nanoparticles (AuNP). Moreover, the synthesis procedure for another conducting polymer, polyaniline (PANI) and its matrix with polyvinyl alcohol (PVA) is described. This chapter also presents the details of how conducting polymer-based nanocomposites have been functionalized with biomolecules (Antibody/Enzyme). It also explained the principles of various characterization techniques that are used to study different properties of PEDOT-PSS and PANI based nanocomposites. Necessary theoretical principles behind different electrochemical techniques used for distinct during the experiments are also described.

Chapter 3

This chapter focuses the physical and electrochemical properties of PEDOT-PSS and PANI based nanocomposites. A systematic study of structural, morphological and vibrational properties of the synthesized conducting polymers and their nanocomposites were carried out through XRD, FESEM, FTIR; respectively. An investigation on the electrocatalytic behaviour and charge transfer kinetics of the prepared electrodes has been performed by employing cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS).

Chapter 4

This chapter outlines reliable, user-friendly, versatile and direct electrochemical sensors based on conducting polymer composite electrode, PEDOT-PSS and GO decorated with spherical AuNPs, for glucose sensing and label-free detection of AF-B_1. To demonstrate biosensing advantages of these electrodes, interaction between covalently immobilized glucose oxidase (*GlOx*) enzyme and D-glucose is monitored via chronoamperometry and CV. Validation of the glucose sensors has shown satisfactory results using real saliva samples (diabetic and non- diabetic). Two different electroanalytical techniques namely, transient capacitance and differential pulse voltammetry has been compared, to select the most prominent technique for analyzing the mycotoxin easily. For demonstration of application of this sensor directly using minimum sample preparation, AF-B_1 sensing has been confirmed using white button mushrooms (*Agaricus bisporus*) and okra (*Abelmoschus esculentus*) stored at ambient conditions.

(A) The fabricated Glucose sensor yielded good linearity within 3.84-373.33 µM. The sensor exhibits good selectivity toward glucose in presence of various interference species. The LOD and sensitivity of the sensor were calculated to be 2.33 µM and 10.59 µA mM^{-1}; respectively.

(B) For AF-B_1 sensor, the mouse IgG immobilized immunosensor exhibited good selectivity and sensitivity within the concentration range from 18.18-291.42 ng mL^{-1} with LOD 67.75 ng mL^{-1} at 77 Hz and 62.5 ng mL^{-1} at 1 kHz from transient capacitance measurements. The anti-AF-B_1 immobilized immunosensor exhibited high selectivity and sensitivity within the concentration range from 18.18-300 ng mL^{-1} at 77 Hz and 1 kHz from transient capacitance studies. The LOD of the sensor is estimated to be 55.41 ng mL^{-1} and 62.45 ng mL^{-1} at 77 Hz and 1 kHz; respectively. From DPV, the dynamic concentration range of the sensor is found to

be from 18.18-342.85 ng mL^{-1} with a LOD of 65.29 ng mL^{-1}. The sensor offers good selectivity in presence of non-specific protein molecule BSA and human serum IgG.

Chapter 5

This chapter discusses highly sensitive, selective, user-friendly and versatile electrochemical sensors for glucose and AF-B_1 based on AuNP decorated WS_2 incorporated PEDOT-PSS nanocomposite. The EIS and CV were used to examine the charge transfer kinetics and electroactive properties of the modified electrodes. The interaction between glucose oxidase (*Glox*) enzyme and D- glucose was monitored via Chronoamperometry. Both capacitive and amperometric sensing techniques were employed to evaluate sensing parameters for AF-B_1 detection. The feasibility of the synthesized biosensors has been tested by evaluating their performance with real samples.

(A) For sensing Glucose, the bioelectrode depicted good selectivity in presence of different interference species and giving a sensitivity of 13.1 μA mM^{-1}. The LOD of the sensor was calculated to be 1 μM within a linear concentration range 0.74-440.67 μM.

(B) From Transient capacitance measurements, the AF-B_1 sensor showed a dynamic concentration range within 7.69-313.72 ng mL^{-1} with a LOD of 30.97 ng mL^{-1} and 31.40 ng mL^{-1} at 77 Hz and 1 kHz; respectively. From DPV measurements, the sensor yielded a LOD of 37.87 ng mL^{-1} within a dynamic concentration range, 7.69-325.04 ng mL^{-1}. The selectivity of the sensor was thoroughly evaluated in the presence of the non-specific protein molecule human serum IgG, utilizing both sensing techniques namely, transient capacitance and DPV.

Chapter 6

This chapter demonstrates performance and versatility of PANI-PVA nanofiber based electrochemical biosensors for detection of glucose and AF-B_1; independently. Amperometric detection of glucose in standard and real human saliva samples was carried out using biofunctionalized modified electrodes. The sensing performance of the bioelectrodes towards the respective analytes has been evaluated, and various sensing parameters, including linearity, sensitivity, selectivity, LOD, and reliability, have been

determined. For AF-B_1 detection, a comparative study of the modified electrodes has been done using two detection techniques, transient capacitance and DPV. In order to implement the proof of concept on real samples, the DPV was carried out on BSA/anti-AF-B_1/GLU/PANI-PVA/ITO immunosensor with white button mushroom and okra.

(A) The PANI-PVA nanofiber derived Glucose sensor shows good linearity within 0.19-455.52 μM. The LOD and sensitivity of the sensor is calculated to be 1.65 μM and 11.7 μA mM^{-1}; respectively. A fairly good selectivity toward glucose sensing has been realized in presence of various interference species.

(B) The PANI-PVA nanofibers based AF-B_1 sensor exhibited a dynamic concentration range within 3.92-445.76 ng mL^{-1} at 77 Hz and 1 kHz in both Transient Capacitance and DPV. The sensor showed good selectivity in presence of non-specific protein molecule human serum IgG. The LOD of the sensor is estimated to be 12.82 ng mL^{-1} and 18.57 ng mL^{-1} at 77 Hz and 1 kHz; respectively with Transient capacitance studies and 17.85 ng mL^{-1} with DPV analysis.

Chapter 7

Summary of our research findings is highlighted in this chapter. Future scope of the work is also presented at last.