Synopsis

Introduction

A biosensor can be defined as a device, which consists of a biological sensing element connected to a transducer to convert an observed biochemical response into a measurable signal, whose magnitude is proportional to the concentration of a specific chemical or set of chemicals. Biosensors can be classified as enzymatic biosensors, genosensors and immunosensors based on receptor. Biosensors can also be divided into several categories based on the transduction process, such as electrochemical, optical, piezoelectric and thermal/calorimetric biosensors. Among various kinds of biosensors, electrochemical biosensors are the most studied popular and successfully commercialized devices of biomolecular electronics. Electrochemical biosensors are the most useful in detection and monitoring for biological and chemical substances.

The fabrication of electrochemical biosensor involves four important steps. These are selection of biomolecules, matrix for immobilization, method of immobilization and transducer. Various immobilization methods have been reported. In the present work, LBL technique has been focused for enzymes immobilization.

Layers-by-Layer Technique

The control of specific properties at the molecular level has been achieved for a number of supramolecular systems through self-organization concepts. The self-assembly of organic and layered heterostructures was pioneered by Sagiv and coworkers, who produced layered films via covalent adsorption of molecules onto a solid substrate. This strategy requires chemical affinity between the molecules to be adsorbed and the previously deposited layer. The choice of molecules is limited due to the need for
Chemical affinity. Later, a novel method for the fabrication of ultrathin film assembly based on adsorption of polycations and polyanions on solid electrodes was reported. This method is popularly known as layer-by-layer technique. Decher and coworkers introduced this technique in 1991. The fundamental concept of LBL technique in most cases was the electrostatic interaction between oppositely charged species. The LBL technique has become the prime choice for fabrication of nano-structured films that can be achieved in a straightforward, low-cost manner. With this technique, a wide variety of materials may be employed and film fabrication is performed under mild conditions, which is particularly important for preserving activity of biomolecules. LBL technique could be used for various applications, such as electrochromism, catalysis, electrochemical sensors and biosensors.

Polycations such as poly(diallyldimethylammonium chloride), poly(ethylene imine), poly(allylamine) hydrochloride, polypyrrole etc. and polyanions such as poly(styrene sulfonate), poly(acrylic acid), poly(vinyl sulfonate), poly(anilinepropane sulfonic acid) etc. are some of the frequently used poly electrolytes to prepare self-assembled thin films by LBL technique. The roughness, thickness and porosity of the thin films can be tuned at the molecular level by varying experimental parameters such as pH, ionic strength and poly electrolyte concentration.

In the present thesis, the work is based on the development of multilayer thin films via LBL technique for the electrochemical detection of biomolecules. A cholesterol biosensor was constructed based on LBL technique and covalent binding method was employed using a cross linkers to construct cholesterol biosensor. For practical applications, cholesterol in blood serum samples was determined. Simultaneous
electrochemical determination of biomolecules such as AA, DA, UA and ACT were also carried out by means of LBL technique. Determination of ACT in pharmaceutical samples was carried out for the end user applications. The modified electrodes were characterized by electrochemical techniques such as CV and EIS. Similarly some of the physical characterization techniques like AFM, SEM have also been employed. During modification procedure for the determination of some of the biomolecules, functionalized carbon nanotubes or graphene have been used. Functionalization on these materials is confirmed by using FT-IR spectroscopy, Raman spectroscopy and TEM techniques. Preparations of nanomaterials such as Fe₃O₄, Fe₃O₄/Pd and AgNPs, have also been carried out for modification of electrodes. These nanomaterials were characterized by XRD, TEM, HRTEM and EDX. Furthermore, antioxidant activity of some of the medicinal plants was examined by electrochemical technique.

The work carried out in this thesis is divided and described in eleven chapters.

Chapter-1

General Introduction

This chapter describes briefly some information about chemical sensors, biosensors, electrochemical biosensors and LBL technique. The chapter gives the electrochemistry and importance of AA, DA, UA, ACT and antioxidants. In addition, the importance of CNTs and graphene was described in present work. It also covers a general introduction on the various electroanalytical techniques, their applications and finally scope of the present investigation.
Chapter-2

Literature Review

This chapter deals with the literature survey for the steps involved during fabrication of cholesterol biosensors like, different approaches, several matrices for enzyme immobilization and various methods for enzyme immobilization. This chapter also gives various strategies adopted for electrochemical detection of AA, DA, UA and ACT. Also, this chapter includes the literature survey on the procedure adopted for the determination of antioxidants.

Chapter-3

Experimental section

This chapter describes materials, equipments and procedures followed for the fabrication of modified electrodes and for the determination of various biomolecules.

Chapter-4

Direct electrochemistry of cholesterol oxidase on MWCNTs

This chapter describes the DET of ChOx on the surface of graphite electrode by immobilizing positively charged ChOx and negatively charged multi walled carbon nanotubes through electrostatic interaction using LBL technique. Two sets of well defined redox peaks were observed in cyclic voltammogram of the modified graphite electrode in phosphate buffer solution of pH 7. These peaks were corresponding to direct electron transfer of FAD/FADH$_2$ of ChOx and carboxylic groups of multi walled nanotubes. ChOx modified electrode was used for the determination of cholesterol.
Chapter-5

**Electrochemical detection of cholesterol on screen printed electrode**

This chapter deals with a simple and facile microwave method to prepare Fe₃O₄ and Fe₃O₄/Pd nanoparticles, which possess the mean particle diameter of 6 nM and 10 nM respectively. Formation of Fe₃O₄ and Fe₃O₄/Pd nanoparticles were confirmed from powder X-ray diffraction and FT-IR techniques. Negatively charged multiwalled carbon nanotubes (COO⁻-MWCNTs) were wrapped with positively charged poly(diallyldimethylammonium chloride) followed by coating with Fe₃O₄/Pd nanoparticles to get (Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs) composite. This composite was used for the determination of cholesterol by using ChOx enzyme on SPE. (Fe₃O₄/Pd/PDDA/COO⁻-MWCNTs) composite provides biocompatible microenvironment for the ChOx to exhibit DET on electrode surface. The linear range of the enzyme modified SPE was found to be 10-80 μM with a detection limit of 1 μM. The sensitivity of the enzyme modified SPE was found to be 10.45 μA μM⁻¹ cm⁻².

Chapter-6

**An amperometric bienzymatic cholesterol biosensor**

This chapter describes about an amperometric bienzymatic cholesterol biosensor construction based on ChOx and ChEt covalently immobilized onto FG modified graphite electrode. FG accelerates the electron transfer from electrode surface to the immobilized ChOx, achieving the direct electrochemistry of ChOx. The electron transfer coefficient (α) and electron transfer rate constant (Ks) were calculated and their values are found to be 0.31 and 0.78 s⁻¹ respectively. Some common interferents like glucose, ascorbic acid and uric acid did not cause any interference, due to the use of a low operating potential.
The proposed method gave satisfactory results in the determination of free and total cholesterol in serum samples.

Chapter-7

Simultaneous electrochemical detection of ascorbic acid, dopamine and uric acid

This chapter deals with the simultaneous electrochemical determination of AA, DA and UA using LBL technique on graphite electrode, by positively charged PDDA and negatively charged MWCNTs wrapped with PSS through electrostatic interaction. The modified electrode was characterized by EIS and CV techniques. The modified electrode exhibits superior electrocatalytic activity towards AA, DA and UA than the bare graphite electrode. No electrode fouling was observed during all the experiments and good stability and reproducibility was obtained for simultaneous determination of AA, DA and UA.

Chapter-8

Electrochemical determination of acetaminophen in presence of dopamine and uric acid

This chapter describes about electrochemical sensor fabrication via LBL method using both positively and negatively charged MWCNTs on PDDA/PSS modified graphite electrode, for the determination of ACT in the presence of DA and high concentration of AA. The modified electrode was characterized by AFM, SEM, CV and EIS. Experimental conditions such as pH, accumulation potential and time, effect of potential sweep rates and interferents were studied. The diffusion coefficient was calculated by chronocoulometric technique. The proposed method gave satisfactory results in the determination of ACT in pharmaceutical and human serum samples.
Chapter-9

Evaluation of antioxidant activity of Rotula aquatica herb extract and its application

This chapter deals with evaluation of antioxidant activity of Rotula aquatic, a species of aromatic flowering shrub, by using electrochemical method. This involves cyclic voltammetric technique by which superoxide (O$_2^-$) was generated at glassy carbon electrode by the reduction of molecular oxygen in aprotic solvent. Green synthesis of silver nanoparticles was achieved using the same herb extract. The prepared silver nanoparticles were characterized by cyclic voltammetry, pXRD, TEM, HR-TEM and EDX spectroscopy. From pXRD and TEM, average particle size of silver nanoparticles was found to be 14 nm.

Chapter-10

Electrochemical behavior and their antioxidant activity of some medicinal plants

In the present chapter, electrochemical behavior of Rotula aquatic, Abroma augusta and Coscinium fenestratum herb extracts were examined at glassy carbon electrode by cyclic voltammetry and differential pulse voltammetry techniques. DNA modified screen printed electrodes were used to evaluate antioxidant activity of above mentioned herb extracts.

Chapter-11

General conclusions

This chapter includes the summary and important conclusions of the work carried out.