ELECTROPOLYMERIZATION OF ANILINEAND ITS COMPOSITES: APPLICATION FOR HYDROGEN PEROXIDE SENSING

A DISSERTATION SUBMITTED TOWARDS THE PARTIAL FULFILLMENT OF
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SUBMITTED BY

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CERTIFICATE-II

This is to certify that **Ms. Prashansa Dalal** (2K11/NST/09) has carried out the major project titled “**Electrochemical Polymerization of Aniline and its Composites: Application for Hydrogen Peroxide Sensing**” as a partial requirement for the award of Master of Technology degree in Nanoscience and Technology by Delhi Technological University. The matter contained in this report has not been submitted elsewhere for the award of any other degree.

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DECLARATION BY THE CANDIDATE

I hereby declare that the work presented in this dissertation entitled "**Electrochemical Polymerization of Aniline and its Composites: Application for Hydrogen Peroxide Sensing**" has been carried out by me under the guidance of external supervisors Dr.A.M. Biradar and Dr. G.Sumanna, National Physical Laboratory and internal supervisor Dr. Pawan Kumar Tyagi, Assistant Professor, Department of Applied Physics, Delhi Technological University, Delhi and is hereby submitted for the partial fulfillment for the award of degree of Master of Technology in Nanoscience and Technology at Applied Physics Department, Delhi Technological University, Delhi.

I further undertake that the work embodied in this major project has not been submitted for the award of any other degree elsewhere.

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ABSTRACT

Titanium is a biocompatible and inexpensive metal and has been explored in many fields but here I have tried to explore its sensing properties .As titanium is less electroactive so it was a challenging job to use it as an electrochemical sensor but it was successfully achieved by depositing Polyaniline, which is a conducting polymer.

Here in, we have used galvanostatic technique for deposition and then PANI/Ti electrode's electrochemical properties were studied .Sensing properties of PANI/Ti electrode were confirmed by its impidometric response towards Hydrogen Peroxide.This electrode was used without any enzyme and thus it can be used for detection of many similar chemical species.

Keywords: Polyaniline, Titanium, PANI/Ti, Electrode, Galvanostatic, Impidometric, Hydrogen Peroxide

Chapter 1

Introduction

Biosensors have attracted much attention in recent times because of potential applications of these devices in clinical diagnostics, environmental monitoring, pharmaceuticals and food processing industries due to fast response and the ease of operation [1]. Direct electron transfer between an electrode and a redox enzyme is very important for biosensors fabrication. The chemical modification of enzymes with redox relay groups or the immobilization of enzymes in redox active polymers has been used to establish electrical communication between the enzyme and electrodes. [1] However, because of its unfavorable orientation on the electrode surface or the adsorption of impurities to make it denature, the enzyme often exhibits sluggish electron transfer at conventional electrodes. Some efforts have been reported to enhance its electron transfer. In some cases mediators have been employed to facilitate the electron transfers of enzymes [2], but the low molecular weight soluble mediator have shown the leaching problem as they can leach out from the electrode and diffused into the bulk solution. Such leaching of mediator leads to a significant signal loss and affect the stability of the biosensor. Hence, research for new materials and methods for immobilizing enzyme is still a very important subject to get more active and stable biosensors [3].A variety of matrices have been used for the immobilization of enzymes to improve the enzymatic activity and stability [4].

PANI has attracted much attention due to its desirable electrical, electrochemical and optical properties, as well as its excellent environmental stability [5]. H. H. Zhou et al.[48] deposited Polyaniline films on Titanium electrodes electrochemically using Pulse Potientiostatic method[6]. Deposition of Polyaniline Composites on titanium electrode has been reported by Mir Ghasem Hosseini, Mohamad Mohsen Momeni and Solmaz Zeynali[7]. Polyanilne films on titanium along with silver and gold Nanoparticles have been done for the studies of electro-oxidation of ascorbic acid and hydrazine. Similarly Renu Stngh et.al [8-12] has done a great work on deposited Polyaniline and composites on ITO and utilized them for fabrication of biosensor for Neisseria Gonorrheoa.

Graziella Scandurra et al synthesized enzymeless hydrogen peroxide sensor.Horseadish peroxidase enzyme that is used for detection has a limitation that enzymes do not work at high pH and in extreme temperature conditions, that's why enzymeless sensors have been synthesized [13-17] .Few groups have done work on enzymeless system of detectin H_2O_2. Electrochemical sensing, being essentially based on the electro-reduction/oxidation of the analyte, allows fast and sensitive detection of H2O2, with the advantage of requiring simpler experimental set-up, and without the need of expensive instrumentation [18].

Therefore, most of the research activity on electrochemical hydrogen peroxide sensors is currently addressed to the development and characterization of modified electrodes onto the surface of which reduction/oxidation of hydrogen peroxide is achievable by operating at lower voltage, thus improving the sensor's selectivity [19-21].According to the way the electrode is modified, the H2O2 electrochemical sensors can be distinguished [22-23].

1.1 Conducting Polymers

1.1.1 Introduction of Conducting Polymers

Conducting polymers are organic polymers that conduct electricity and such compounds may be true metallic conductors or semiconductors. The biggest advantage of conducting polymers is their processibility [24].

1.1.2 Historical Background

The electrochemical synthesis of conducting polymers has its roots in the early attempts of Dall'olio et al. in 1968. They prepared "pyrrole black", a polymer in the form of a powdery insoluble precipitate, by electrochemical oxidation of pyrrole monomer in aqueous sulfuric acid on a platinum electrode [24], the resulting polymer had a conductivity of approximately 8 Scm^{-1}.

In 1979, Diaz et al. revived the electrochemical approach for the synthesis of conducting polymers. They synthesized continuous and free standing films of conducting polypyrrole (PPY) using platinum electrodes [27]. These films showed much higher electrical conductivity (100 Scm^{-1}) and excellent air stability.

Polyparaphenylene was synthesized by Ivory et al. (1979). It forms highly conducting charge transfer complexes with both n and p type dopants. Doping with AsF_5 increases its conductivity to its values from 10^{-5} to 500 cm^{-1}. Its discovery was particularly exciting, since its property of solution processsability opened the door for potentially obtaining commercially viable conducting plastic [28]. Many conducting polymers such as polyfuran, polyindole, polycarbazole and polythiophene have also been synthesized.

1.1.3 Synthesis of Conducting Polymers

Many methods for the synthesis of conducting polymers have been developed. Most conducting polymers are prepared by oxidative coupling of monocyclic precursors. Such reactions entail dehydrogenation:

$$n\ H\text{-}[X]\text{-}H \rightarrow H\text{-}[X]_n\text{-}H + 2(n\text{-}1)\ H^+ + 2(n\text{-}1)\ e^-$$

Conducting polymers can be subjected to oxidation or reduction by electron acceptors or electron donors, resulting in considerable increase in electron mobility thus, enhanced electrical conductivity. Conducting polymers contain π-electron backbone responsible for their unusual electronic properties such as-

- Electrical conductivity
- Low energy optical transition
- Low ionization potential

The higher values of the electrical conductivity obtained in such organic polymers have led to the name “synthetic metals” [24-27] .It represents an important research with diverse scientific problems of fundamental significance and the potential for commercial applications. Conducting polymer has widened the possibility of modification of surface of the conventional electrodes providing new and interesting properties. They also create possibility in design of chemical and biochemical sensors.

1.1.4 Stability of Conducting Polymers

`There are two distinct types of stability

- Extrinsic stability

- Intrinsic stability

Extrinsic stability is related to vulnerability to external environmental agent such as oxygen, water which is determined by the polymer susceptibility of charged sites to attack the nucleophiles, electrophiles and free radicals. If it is extrinsic unstable then it should be protected by a stable coating [28].

Intrinsic stability is thermodynamic in origin which is likely to be caused by irreversible chemical reaction between charged sites of polymer and the dopant. The charge site can also become unstable due to conformational changes in the polymer backbone as observed in alkyl substituted polythiophenes.

1.1.5 Properties of Conducting Polymers:

- Conducting polymers act as suitable matrices for immobilization of biomolecules and can be used to enhance stability, speed, and sensitivity and are finding increasing use in medical diagnostics.

- Conducting polymers act as transducers in biosensors. A transducer converts a biochemical signal resulting through the interaction of a biological component into an electronic signal [29].

- Electrically conducting polymer has considerably flexibility in chemical structures, which can be modified as required electronic signal [29].

- The advantages offered by conducting polymers are that the electrochemical syntheses allow direct deposition of a polymer on the electrode surface while simultaneously trapping the protein molecules [29].

- It is possible to control the spatial distribution of the immobilized enzymes, the film thickness and modulation of enzyme activity by changing the state of the polymer [29].

- Conducting polymers can be reversibly doped and undoped using electrochemical techniques accompanied by significant changes in conductivity [29].

- The electrical conductivity of conducting polymers changes over several orders of magnitude in response to change in pH, redox potential or their environment.

- Conducting polymers have the ability to efficiently transfer electric charge produced by a chemical reaction.

- Conducting polymers can be deposited over defined areas of electrode. This unique property of conducting polymers along with the possibility to entrap enzymes during electrochemical polymerization has been exploited for fabrication of amperometric biosensors.

- Conducting polymers exhibit exchange and size exclusion properties due to which these are highly sensitive and specific towards desired substrates [29] .

1.1.6 Advantages

- They are biocompatible
- Their conductivity and structure can be modified
- Involves simple preparations
- Cost effective
- Light weight
- Corrosion resistant, high chemical inertness [30].

1.1.7 Applications of Conducting Polymers

- Conducting organic molecular electronic materials have attracted much attention largely because of their many projected applications in solar cells, light weight batteries, electrochromic devices, sensors and molecular electronic devices (diodes, field effect transistor, light emitting diodes)[31] .

- Polymeric hetero junctions, solar cells have been fabricated by electrochemical deposition of polypyrrole on n-silicon.

- Many conducting polymers such as polyacetylene, polythiophene, polyindole, polypyrrole, polyaniline etc. have been reported as electrode materials for rechargeable batteries.

- It has been reported that the conducting polyheterocycles are good candidates for electrochromic displays and thermal smart windows[33]
- Scientists have been used polypyrrole films in a neurotransmitter as a drug release system in to the brain.
- Polyaniline (PANI) is being used by Hitachi-Maxwell for anti-static coating of 4MB barium ferrite floppy disk [34] .
- Health care: in medical diagnosis (glucose, fructose, lactate, ethanol, cholesterol, urea etc.)
- Immunosensors: can be used in medical diagnostics and environmental sensors.
- Environmental monitoring: for control of pollution and detection of hazardous chemicals in biosensors (polyphenols, sulfites, peroxides, formaldehyde etc.)
- Food analysis: for detection of glucose, fructose, ethanol, sucrose, lactate, malate, galactose, citrate, lactose, urea, starch etc in food industries [35].

1.1.8 Conducting Polymer Based Biosensor

The use of conducting electroactive polymers (CEP) as active templates in biosensors represents an exciting area of sensor research. They possess the ability to combine the role of matrices and as signal transducers. It has been used to immobilize several biological species including enzymes, antibodies, haptens, DNA, receptors, whole cell, etc. [36]. However an excellent knowledge of the underlying mechanism of CEP is required to predict the sensor parameters such as-

- Sensitivity
- Selectivity
- Limit of detection

Conducting polymers based biosensors have been used for rapid and reliable measurements of analytes such as DNA, Phenols, IgG, digoxin and urea. The advantages of CEP loaded biosensors are-

- High protein loading and stability
- Direct and intimate contact with the bio-affinity reagents
- Modulation of analytic signals through the applications of electrical potential
- Direct incorporation of reagents, simplifies fabrication of sensing device.

1.2 Polyaniline

1.2.1 Introduction of Polyaniline

Polyaniline (PANI) is a conducting polymer of the semi-flexible rod polymer family [37]. Amongst the family of conducting polymers, polyaniline is unique due to its ease of synthesis, environmental stability, and simple doping/dedoping chemistry.

It is one of the so-called doped polymers, in which conductivity results from a process of partial oxidation or reduction. Polyaniline compounds can be designed to achieve the required conductivity for a given application. The resultant blends can be as conducting as silicon and germanium or as insulating as glass. Polyaniline compounds can be easily disposed of without environmental risk.

1.2.2 Oxidation states

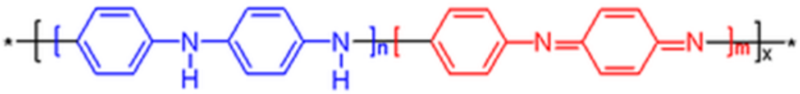

Figure 1.1 Chemical Structure of Polyaniline Monomer [38]

In Figure 1.1, n+m = 1, x = degree of polymerization. Polymerized from the aniline monomer, polyaniline can be found in one of three idealized oxidation states

- leucoemeraldine - white/clear
- emeraldine - green or blue
- pernigraniline - blue/violet

In above Figure, x equals half the degree of polymerization (DP).

Leucoemeraldine with n = 1, m = 0 is the fully reduced state.

Pernigraniline is the fully oxidized state (n = 0, m = 1) with imine links instead of amine links.

The emeraldine (n = m = 0.5) form of polyaniline, often referred to as emeraldine base (EB), is either neutral or doped, with the imine nitrogens protonated by an acid. Emeraldine base is regarded as the most useful form of polyaniline due to its high stability at room temperature and the fact that upon doping the emeraldine salt form of polyaniline is electrically conducting. Leucoemeraldine and pernigraniline are poor conductors, even when doped with an acid.

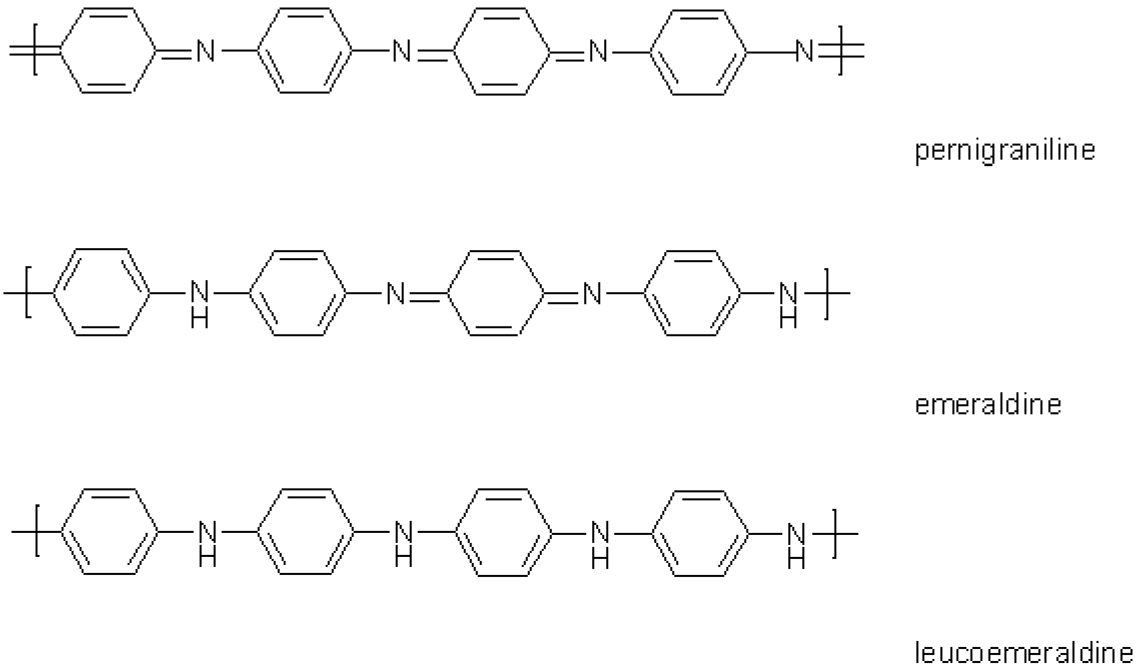

Figure 1.2: **Chemical structure of oxidation of states of, Leucoemeraldine (LEB) Emeraldine(ES) And pernigraniline(PN)**[39]

The color change associated with polyaniline in different oxidation states can be used in sensors and electrochromic devices. Though color is useful, the best method for making a polyaniline sensor is arguably to take advantage of the dramatic conductivity changes between the different oxidation states or doping levels.

1.2.3 Synthesis of Polyaniline

The most common synthesis of polyaniline is by oxidative polymerization with ammonium persulfate as an oxidant [40].The components are both dissolved in 1 M hydrochloric acid and slowly (the reaction is very exothermic) added to each other. The polymer precipitates as small particles and the reaction product is an unstable dispersion with micronscale particulates [41].

A three stage model for the formation of emeraldine base is proposed.

- In the first stage of the reaction the pernigraniline salt PS oxidation state is formed.
- In the second stage pernigraniline is reduced to the emeraldine salt as aniline monomer gets oxidized to the radical cation.
- In the third stage this radical cation couples with ES salt. This process can be followed by light scattering analysis which allows the determination of the absolute molar mass [41].

1.2.4 Properties of Polyaniline

Polyaniline exists as bulk films or as dispersions. A recurring problem with these dispersions is particle aggregation which limits possible applications.A 2006 study proposes a strategy to prevent aggregation based on a model for nucleation and aggregate formation.

The model identifies two nucleation modes for particle formation, one by so-called homogeneous nucleation forming long elongated polyaniline nanofibers and very stable dispersions that can last for at least months [42]. The other nucleation mode is by heterogeneous nucleation taking place on any alien body available in the reactor such as the surface of the reactor wall forming not elongated fiber but granular coral-like material.[42] With polyaniline, formation by secondary nucleation also takes place on the nanofibers itself. In the study, heterogeneous nucleation is predominant when the reaction medium is stirred or when the reaction temperature is lowered. With both reaction conditions SEM imagery display nanofibers covered in a layer of coral like granules. The granules act as suppression of homogeneous nucleation is that this requires a local concentration gradient prior to the onset of nucleation which is destroyed by stirring or by low temperature. An important property of polyaniline is its electric conductivity, which makes it suitable for e.g. manufacture of electrically conducting yarns, antistatic coatings, electromagnetic shielding and flexible electrodes contact points for a nanoscale glue to link the particles together, causing aggregation.

Wide and controllable Range of Conductivity

The electrical property of Polyaniline based compositions can be closely controlled over a wide range and for neat Polyaniline compositions conductivity levels as high as 100 S/cm can be achieved [43].The full of range of conductivity levels from less than 10^{-10} to 10^{-1} S/cm (melt processing);can be achieved for polymer blends containing polyaniline compositions .An important advantage is that the classical high percolation threshold for the onset of electrical conductivity observed in globular carbon black filled plastics does not exist.

Melt and solution Processable Material

Polyaniline based compositions can be processed using conventional techniques such as blow and injection moulding, extrusion film casting and fibre spinning [44]. These compositions withstand temperatures as high as 230-240° C for short periods of time (5-10 minutes) without significant change in electrical properties; can be melting blended with many commodity polymers. Selected industrial solvents can be used for solution processing neat Polyaniline compositions.

Conductive Blends with many Commodity Polymers

Electrically conductive Polyaniline based blends with commodity polymers can be produced by using common solution and melt processing techniques, examples of commodity polymers are polyethylene ,polypropylene, polystyrene, PVC, phenols-formaldehyde resins and different types of thermoplastic elastomers [45] .Unlike conventional filled materials, the mechanical properties of these blends are close to those of the insulating matrix polymer.

Function as Processing aids in addition to providing Conductivity

Plasticized Polyaniline compositions improve melt processing performance by lowering the melt viscosity, lowering the processing temperature and shortening the processing time. The improved melt flow properties makes moulding of complex shapes easier.

Colored & Transparent Electrically Conductive Products

Electrically conductive, colored and transparent thin films and coatings,which would otherwise be difficult to achieve with conventional filled materials, can be made using Polyaniline based compositions[43-46] .

Applications

Polyaniline and the other conducting polymers such as polythiophene, polypyrrole, and PEDOT/PSS have potential for applications due to their light weight, conductivity, mechanical flexibility and low cost. Polyaniline is especially attractive because it is relatively inexpensive, has three distinct oxidation states with different colors and has an acid/base doping response [47].This latter property makes polyaniline an attractive for acid/base chemical vapor sensors. The different colors, charges and conformations of the multiple oxidation states also make the material promising for applications such as actuators, supercapacitors and electrochromics. They are suitable for manufacture of electrically conducting yarns, antistatic coatings, electromagnetic shielding, and flexible electrodes.

1.3 Biosensors

1.3.1 Introduction of Biosensors

A biosensor is an analytical device which converts a biological response into an electrical signal. According to IUPAC the term ‘biosensor’ has been defined as “**a compact analytical device incorporating a biologically derived sensing element either; integrated with in or intimately associated with a physicochemical transducer”** [48] .

- The usual aim of a biosensor is to produce either discrete or continuous digital electronic signals, which are proportional to a single analyte or a related group of anlayte.
- This biochemical interaction produces some detectable physical or chemical change i.e. measured and converted into an electrical signal by a transducer.
- Finally, the electrical signal is amplified, interpreted and displayed as analyte concentration.
- The biological materials used are usually enzymes, nucleic acids, antibodies, lectins, whole cells, entire organs or tissue slices. The term 'biosensor' is often used to cover sensor devices used in order to determine the concentration of substances and other parameters of biological interest even where they do not utilize a biological system directly.
- Biosensors represent a rapidly expanding field, at the present time, with an estimated 60% annual growth rate; the major impetus coming from the health-care industry, but with some pressure from other areas, such as food quality appraisal and environmental monitoring.
- The estimated world analytical market is about 12,000,000,000/year^{-}of which 30% is in the health care area.

1.3.2 Evolution of Biosensor

Over the past 20 years, the field of biosensor research has had a significant impact in both laboratory research and the commercial sector. Over that period, biosensors have revolutionized the care and management of diabetes and have had important impacts in several other areas of clinical diagnostics. Europe, North America and Asia-Pacific have all seen the rise of small and medium sized companies seeking technical and application niches in the manufacture or use of biosensors [49].The current activity in both gene and protein 'biochips' can be seen as the latest set of tools that allow users who are not analytical science practitioners to make technically complex and reliable biological weapons and the need for their rapid and reliable detection will need to be met by devices that have many characteristics in common with biosensors .

1.3.3 Components of Biosensor

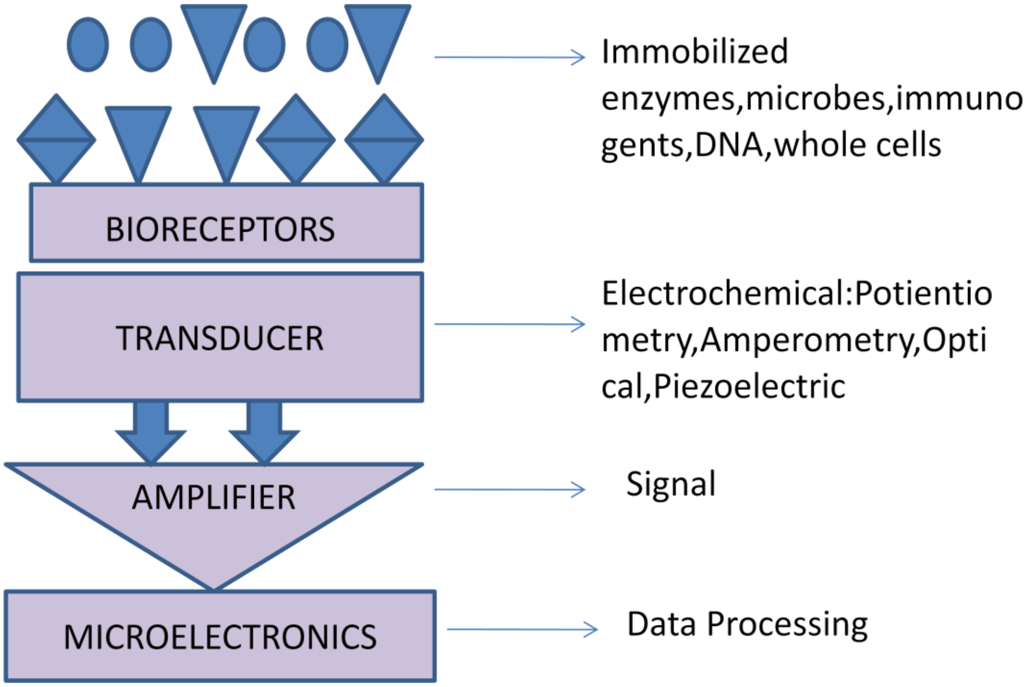

Figure 1.3: Schematic diagram showing the main components of a biosensor

The biocatalyst converts the substrate to product. This reaction is determined by the transducer which converts it to an electrical signal. The output from the transducer is amplified, processed and displayed.

1. Biological component

The biological components perform the following two key functions:

- It specifically recognizes an analyte.
- It interacts with analyte, which produces some physical change detectable by the transducer. (e.g. Enzymes, antibodies, nucleic acids etc)

2. Physical Component

The biological component interacts specifically to the **analyte**, which produces a physical change close to the **transducer** surface [50] (Transducer, amplifier) .

Transducer or the detector element: The key part of a biosensor is the **transducer** (shown as the 'black box') which makes use of a physical change accompanying the reaction. This may be

- Calorimetric biosensors the heat output (or absorbed) by the reaction
- Potentiometric biosensors changes in the distribution of charges causing an electrical potential to be produced
- Amperometric biosensors movement of electrons produced in a redox reaction
- Optical biosensors light output during the reaction or a light absorbance difference between the reactants and products.
- Piezo-electric biosensors effects due to the mass of the reactants or products.

Analyte: That transforms the signal resulting from the interaction of the analyte with the biological element into another signal (i.e., transducers) that can be more easily measured and quantified.

1.3.4 History of Biosensors

Prof. Clark Jr. has been known as the father of the biosensor concept since the publication of his first definitive work on the oxygen electrode in 1956 [51].

However, general interest in biosensor grew considerably since the description by Updike and Hicks who described the functional enzyme electrode based on glucose oxidase deposited on an oxygen sensor in 1967. There are three so-called 'generations'[52] of biosensors

First generation biosensor - The normal product of the reaction diffuses to the transducer and causes electrical response.

Second-generation biosensors - They involve specific mediators between the reaction and the transducer in order to generate improved response. The second-generation biosensor involves two steps; first there is a redox reaction between the enzyme and substrate i.e. reoxidized by the mediator, and in second step eventually the electrode oxidizes the mediator.

Third generation biosensors - Arise from the self-contained nature of the sensor where the reaction itself causes the response and no product or mediator diffusion is directly involved. Third generation sensors are accompanied by co-immobilization of enzyme and mediator at an electrode surface making the biorecognition component an integral part of the electrode transducer i.e. direct electrical contact of the enzyme to electrode since neither the mediator nor enzyme must be added, third design facilitated repeated measurement [52] .

1.3.5 Functioning of Biosensor

Biosensors convert biological information of analyte in to electrical information, [53] which involves the following steps:

- The analyte diffuses from the solution to the surface of biosensor.
- The analyte reacts specifically and efficiently with the biological component of the biosensor, which is immobilized onto the transducer.
- The transducer detects and measures this change and converts it into an electrical signal.
- The electrical signal produce by transducer is very low so, an amplifier amplifies it.
- Microprocessor - the amplified signal is fed into the microprocessor. The signal is then processed and interpreted.
- Display Unit - The calculated value is displayed in a suitable unit which is human readable.

1.3.6 Features of Biosensor

A successful biosensor must possess at least some of the following beneficial features [54]:

- The biocatalyst must be **highly specific** for the purpose of the analyses, should be **stable** under normal storage conditions and, except in the case of colorimetric enzyme strips and dipsticks, show good stability over a large number of assays (i.e. much greater than 100).
- The reaction should be as **independent of such physical parameters** as stirring, pH and temperature as is manageable. This would allow the analysis of samples with minimal pre-treatment. If the reaction involves cofactors or coenzymes these should, preferably, also be co-immobilised with the enzyme.
- The response should be **accurate, precise, reproducible and linear** over the useful analytical range, without dilution or concentration. It should also be **free from electrical noise**.
- If the biosensor is to be used for invasive monitoring in clinical situations, the probe must be **tiny and biocompatible**, having no toxic or antigenic effects. If it is to be used in fermenters it should be sterilisable. This is preferably performed by autoclaving but no biosensor enzymes can presently withstand such drastic wet-heat treatment. In either case, the biosensor should not be prone to fouling or proteolysis.
- It should be **durable** i.e. should be capable of repeated use.
- The assay **cost** should be lower than that of conventional tests.
- It should required small **sample volume**.
- It should be **robust, stable and utilizable**.
- There should be a **market** for the biosensor. There is clearly little purpose developing a biosensor if other factors (e.g. government subsidies, the continued employment of skilled analysts, or poor customer perception) encourage the use of traditional methods and discourage the decentralisation of laboratory testing.

1.3.7 Classification of Biosensor

Biosensors are classified according to two systems

1) **Based on transducer system**

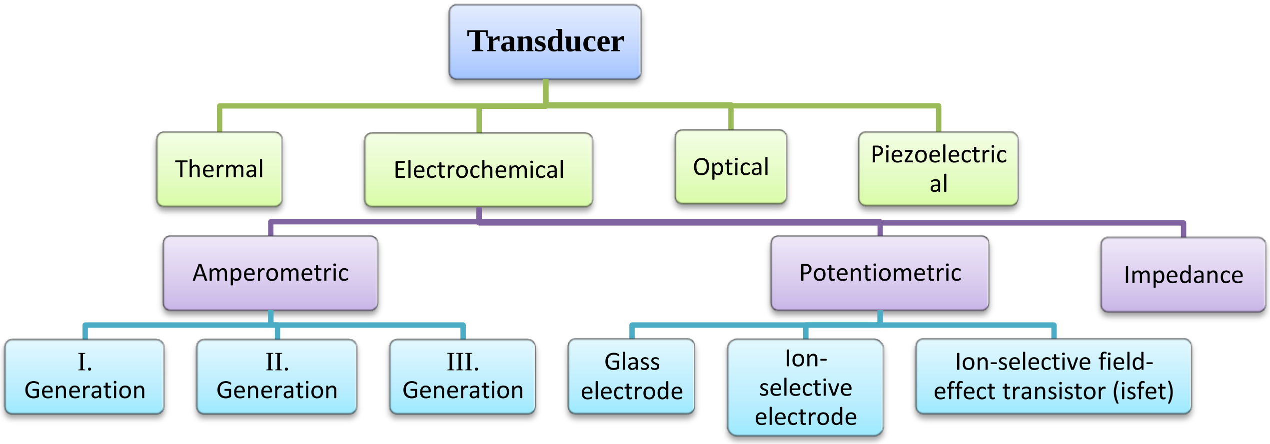

Figure 1.4: Classification of Biosensors

Optical biosensor

These are based on the measurement of light absorbed or emitted as a consequence of a biochemical reaction. They can be used for measurement of pH, O_2, H_2O_2, and CO_2 etc. Though very sensitive but it cannot be used in turbid media.

Thermal Biosensors

Predominantly measures the change in temperature of the fluids following the reaction of a suitable substrate with immobilized enzyme molecules. Measurements based on heat evolved or absorbed during a biochemical reaction. Used successfully in clinical monitoring of blood glucose, cholesterol, heavy metals, TELISA (Thermometric biosensor to Enzyme Linked immunosorbent Assay). Limitations - its stability and fluid handling.

Piezoelectric biosensor

They are also called 'acoustic wave biosensor'. These biosensors operate on the principal of generation of electrical dipoles on subjecting anisotropic natural crystals to mechanical stress. They use the measurement of ammonia, nitrous oxide, CO_2, hydrogen, methane and certain organophosphorus compounds.

Calorimetric biosensor

The biosensors detect an analyte on the basis of heat evolved due to biochemical reaction of the analyte with a suitable enzyme. Different substrates, enzymes, vitamins and antigens have been determined using the thermometric biosensors. It has a drawback that it cannot be utilized with systems, which has very little heat exchange. Based on principle “biochemical reactions involve change in enthalpy”.Thermal signals generated by redox reactions were measured.

The thermistors, used to detect the temperature change, function by changing their electrical resistance with the temperature, obeying the relationship

$$Ln\left(\frac{R_1}{R_2}\right) = B\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$$

Therefore

$$\frac{R_1}{R_2} = e^{B\left(\frac{1}{T_1} - \frac{1}{T_2}\right)}$$

Where R_1 and R_2 are the resistances of the thermistors at absolute temperatures T_1 and T_2 [55] respectively and B is a characteristic temperature constant for the thermistors.

Electrochemical Biosensor

Electrochemical biosensors have emerged as the most commonly used biosensors. They have been found to overcome most of the disadvantages, which inhibit the use of the other types of biosensors. These are based on the fact that during bio-interaction process, electrochemical species are consumed or generated producing an electrochemical signal, which can in turn be measured by an electrochemical detector. Depending upon the electrochemical property to be measured by a detector system, electrochemical biosensor may be further divided into conductometric, potentiometric and amperometric biosensors.

Amperometric Biosensor

Amperometric biosensors measure the changes in current on working electrode during the oxidation or reduction of a product or reactant at a constant applied potential. The most important factor affecting the functioning of amperometric biosensor is the electron transfer between catalytic molecules. The simplest biosensor uses the Clark oxygen electrode, which determines the reduction of oxygen present in sample solution. These biosensors are used to measure redox reactions.

Potentiometric Biosensor

Potentiometric biosensors measure the changes in voltage on working electrode during the oxidation or reduction of a product or reactant at a constant applied current.

Conductometric Biosensor

Conductometric biosensor measures the changes in the conductance between a pair of electrodes as a consequence of the biological component. They measure the change in conductance of the biological component arising between a pair of metal electrodes. They have been constructed for estimation of glucose, urea, neutral ChOx, pepsin, Haemoglobin by monitoring the change in electroconductivity arising as a change in redox potential and pH of the micro-environment [55] .

2) Based on sensing elements:

It is classified on the basis of interaction between analyte and the biological material.

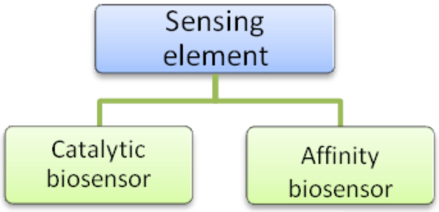

Figure 1.5: **Classification of sensing elements**

Catalytic Biosensor

Enzyme and microbial cells based biosensors come in this category. The immobilized sensing biomolecule act as a catalyst and catalyze the biochemical reaction. In this sensor an analyte may be converted into a new chemical molecule usually by enzymes. The example of a catalytic biosensor is the glucose biosensor based on glucose oxidase, cholesterol biosensor based on cholesterol oxidase, urea biosensor based on urease.

Affinity Biosensor

This type of biosensor work on the affinity between the analyte and bio-component immobilized onto the censor surface. Biomolecules such as antibodies, nucleic acids, lectins and hormone receptors are used to bind complementary molecules irreversibly and non-covalently. The analyte may simply binds to the biological materials present on the biosensors (e.g. to antibodies, nucleic acids etc.). Immunosensors and DNA biosensors are examples for affinity biosensors [56].

1.3.8 Applications of Biosensor:

There are many potential applications of biosensors [57]. The main requirement for a biosensors approach in terms of research and commercial applications are the identifications of a target molecule, availability of a suitable biological recognition element, and the potential for disposable portable detection systems to be preferred to sensitive laboratory-based techniques in some situations. Some examples are given below:

- In food and drink analysis.
- In Industrial effluents analysis.
- In military applications.
- In bacterial and viral analysis.
- In pharmaceutical and drug analysis.
- In environmental pollution control and monitoring e.g. the detection of pesticides and river water contaminants.
- In clinical diagnosis and biomedicine.
- In veterinary analysis [58-62].

1.4 Hydrogen Peroxide Sensing

Hydrogen peroxide (H_2O_2) is a chemical miscible with water and able to cross cell membranes, widely employed in many industrial processes including textile and paper bleaching, sterilization, food and pharmaceutical industry [63].The fast and reliable detection of hydrogen peroxide at concentrations ranging from micromolar to tens of millimolar is therefore of great importance, because it is demonstrated that exposure to H_2O_2 at levels ≥ 50 μM is cytotoxic to a wide range of animals, plants and bacterial cells in culture. In addition, the quantitative determination of the H_2O_2 finds increasing biological applications, as hydrogen peroxide is a by-product in a variety of enzymatic reactions. The analytical techniques commonly employed to detect hydrogen peroxide include spectrophotometry[64-67]cromathography, chemiluminescence, fluorescene, titrimetry and electrochemistry Compared to the other techniques, electrochemical sensing, being essentially based on the electro-reduction/oxidation of the analyte, allows fast and sensitive detection of H_2O_2, with the advantage of requiring simpler experimental set up, and without the need of expensive instrumentation. As the direct electro reduction/oxidation of H_2O_2 on commercially available bare gold or carbon electrodes requires high overpotential, electrochemical hydrogen peroxide sensors using such kind of electrodes require to be operated over a potential window where interference problems arising from common electroactive species cannot be avoided. Therefore, most of the research activity on electrochemical hydrogen peroxide sensors is currently addressed to the development and characterization of modified electrodes onto the surface of which reduction/oxidation of hydrogen peroxide is achievable by operating at lower voltage, thus improving the sensor's selectivity. According to the way the electrode is modified, the H_2O_2 electrochemical sensors can be distinguished in enzymatic and nonenzymatic. The former ones owe their high sensitivity, selectivity and fast response to the presence of redox active enzymes immobilized onto the top of the electrode surface [67]. Their disadvantage is a lack of stability arising from the alteration that the immobilized enzymes, strongly affected by temperature, pH, humidity and exposure to chemicals, may undergo during sensors fabrication, use and storage. To overcome such problems, electrochemical detection of hydrogen peroxide can be achieved by using non-enzymatic electrodes, modified with nanostructured metal oxides [68], nanocomposites based on carbon nanotubes [69], and conjugated polymers [70]. Once the enzymatic or the enzymeless electrodes have been prepared, their response towards hydrogen peroxide is tested in electrolytic solutions, and the sensing performances are usually found to be affected by the nature and concentration of the supporting electrolyte, and by the pH of the solution [71].

Chapter 2

Experimental details

2.1Synthesis Techniques

2.1.1 Electrochemical Cells

An electrochemical cell is used in electrochemical sensor studies. The electrodes themselves play an important role in the performance of electrochemical biosensors. The electrode material, its surface modification or its dimensions affects the detection ability of the electrochemical biosensor [72]. There are three kinds of electrodes in the electrochemical cell:

- Working electrode
- Reference electrode
- Auxiliary (counter) electrode

Reference electrode:

The other electrodes in the cell are referred to this electrode. Reference electrode types:

Type 1: the hydrogen electrode
Type 2: the calomel electrode
Type 3: glass electrodes

Reference electrode is a kind of standard hydrogen electrode. Hydrogen is potentially explosive and is not very suitable using an electrode with hydrogen gas for routine measurements. So there are two common use and commercially available reference electrode types:Ag/AgCl Electrode: There is a Ag wire that coated with AgCl and dipped into NaCl solution.Saturated-Calomel Electrode: Calomel is the other name of mercurous chloride (Hg_2Cl_2).

Auxiliary (Counter) Electrode:

In a two-electrode system, when a known current or potential is applied between the working and auxiliary electrodes, the other variables may be measured. The auxiliary electrode functions as a cathode whenever the working electrode is operating as an anode and vice versa. The auxiliary electrode often has a surface area much larger than that of the working electrode. The half-reaction occurring at the auxiliary electrode should occur fast enough not to limit the process at the working electrode. The potential of the auxiliary electrode is not measured against the reference electrode but adjusted to balance the reaction occurring at the working electrode. This configuration allows the potential of the working electrode to be measured against a known reference electrode. Auxiliary electrode is often fabricated from electrochemically inert materials such as gold, platinum or carbon[73].

Working Electrode:

It is the electrode on which the reaction occurs in an electrochemical system [17-19]. In an electrochemical system with three electrodes, the working electrode can be referred as either cathodic or anodic depending on the reaction on the working electrode is a reduction or an oxidation. There are many kind of working electrodes. Glassy carbon electrode, screen printed electrode, Pt electrode, gold electrode, silver electrode, Indium Tin Oxide coated glass electrode, carbon paste electrode, carbon nanotube paste electrode etc. Screen printed electrodes are prepared with depositing inks on the electrode substrate (glass, plastic or ceramic) in the form of thin films. Different inks can be used to get different dimensions and shapes of biosensors. Screen-printed electrochemical cells are widely used for developing amperometric biosensors because these biosensors are cheap and can be produced at large scales. This could be potentially used as disposable sensor that decreases the chances of contamination and prevents loss of sensitivity. Performance factors of an electrochemical biosensor are: Selectivity, response time, sensitivity range, accuracy, recovery time, solution conditions and the life time of the sensor.

2.1.2 Cyclic voltammetry (CV)

Cyclic voltammetry is a type of potentiodynamic electrochemical measurement. In a cyclic voltammetry experiment, the working electrode potential is changed linearly versus time. Cyclic voltammetry experiment ends when it reaches a set potential value. When cyclic voltammetry reaches the set potential, potential ramp of the working electrode is inverted back. This inversion can happen multiple times during a single experiment until a set cycle number is obtained. The plot of the current at the working electrode vs. the applied voltage gives the cyclic voltammogram of the reaction. Cyclic voltammetry is a general way to study the electrochemical properties of an analyte in a solution [74].

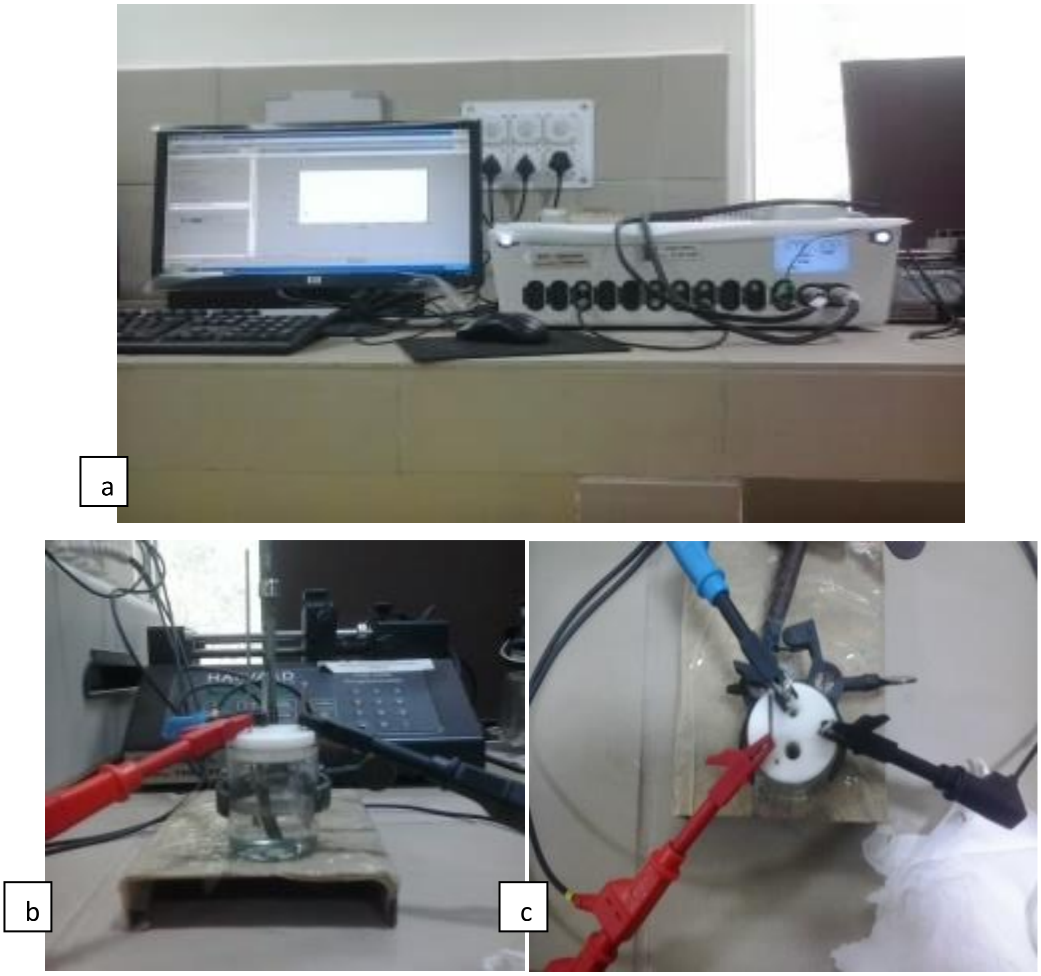

Figure 2.1: Photograph of (a)Potientiostat-Galvanostat (b) Electrochemical cell (c) top view of electrochemical cell,at BECPRL-NPL

2.1.3 Chronoamperometry and Chronopotentiometry

A potential is applied to the working electrode and steady state current is measured as a function of time for chronoamperometric measurement. There is a diffusion layer between solution media and electrode surface. The concept of a diffusion layer was introduced by Nernst. Diffusion controls the transfer of analyte from the bulk solution of higher concentration to the electrode. Thus there is a concentration gradient from solution media to the electrode surface. Cottrell equation can indicate this situation better: It defines the current-time dependence for linear diffusion control at an electrode [75].

2.1.4 Potentiostatic method

A potentiostat is a control and measuring device that, in an electrolytic cell, keeps the potential of the working electrode at a constant level respect to the reference electrode. It consists of an electric circuit which controls the potential across the cell by sensing changes in its resistance, varying accordingly the current supplied to the system: a higher resistance will result in a decreased current, while a lower resistance will result in an increased current, in order to keep the voltage constant[76].It is a simple application of Ohm's law $R = U / I$As a result, the variable system resistance and the controlled current are inversely proportional

$I_o = U_c / R_v$

Where, I_o is the output electrical current of the potentiostat, U_c is the voltage that is kept constant R_v is the electrical resistance that varies.

2.1.5 Galvanostatic method:

A galvanostatic is a control and measuring device capable to keep constant the current flowing through an electrolytic cell in coulometric titrations [76], disregarding changes in the load itself. The simple galvanostat consists of a high-voltage source producing a constant voltage U with a resistor R_x connected in series: in order to force the flow of an almost constant current through a load, this resistor shall be much higher than the load resistor R_{load} As a matter of fact, the current I flowing through the load is given by $I = U / R_x + R_{load}$, Where the current I is approximately determined by R_x as follows $I = U/R_x$ if $R_x >> R_{load}$.

2.1.6 Deposition Using ExpEYES

Till date people have used conventional deposition systems but there is a limitation that we cannot control the deposition rate, for that we use Potentiostat-Galvanostat system but it is quiet expensive. So, ExpEyes can be used as a substitute for a inexpensive device for a controlled deposition. I have tested it for the same and it is successful and can be optimized further[77].

ExpEYES is a software hardware interface developed by Inter University Accelerator Centre (a Research Centre of UGC). ExpEYES is interfaced and powered by the USB port of the computer. For connecting external signals, it has 32 Input/output terminals, arranged on both sides, as shown in Figure below. It can monitor and control the voltages at the terminals. In order to measure other parameters (like temperature, pressure etc.), we need to convert them in to electrical signals by using appropriate sensor elements.

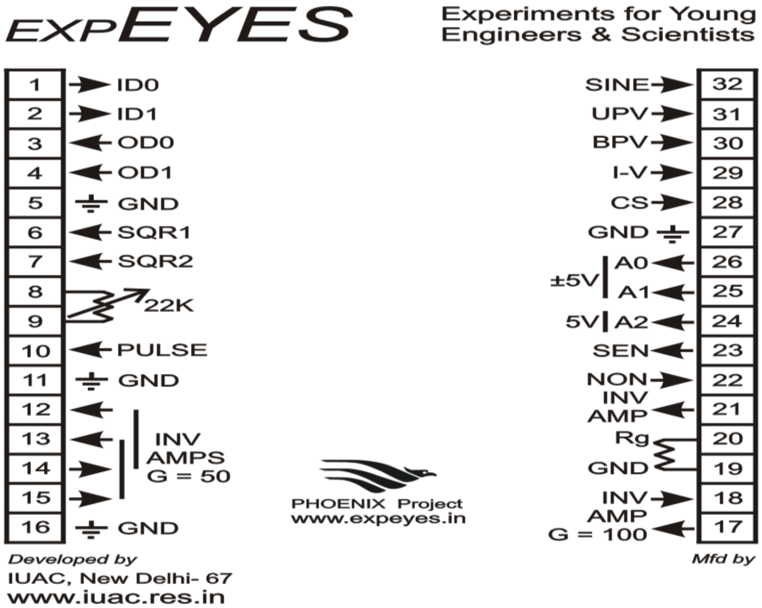

Figure.2.2: The ExpEYES top panel showing the external connections on both sides. The arrows indicates the direction of the signals

This device is inexpensive and is easily available and thats'why it has been used for deposition using two electrode system. One can electrodeposit any material using this device in an improved control way than the conventional two electrode deposition system. I have tried to deposit Polyaniline, Silver and some other materials on ITO and successfully did it.

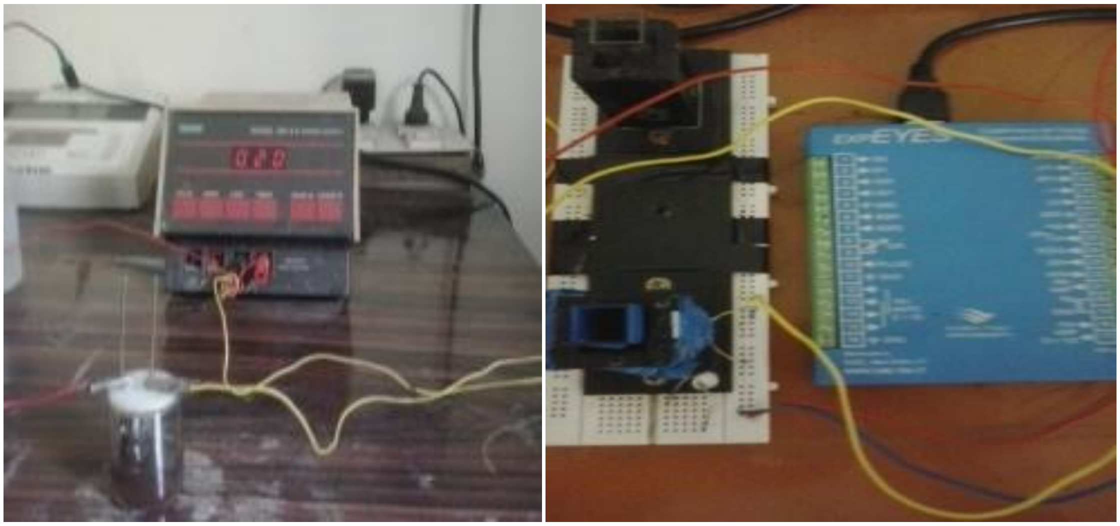

Figure2.3: Photograph of Left- conventional two electrode system, Right: ExpEYES system

2.2 Characterization Techniques

2.2.1 Electrochemical Impedance Spectroscopy (EIS)

EIS is a powerful technique for the characterization of electrochemical systems. The promise of EIS is that, with a single experimental procedure encompassing a sufficiently broad range of frequencies, the influence of the governing physical and chemical phenomena may be isolated and distinguished at a given applied potential. EIS has found widespread applications in the field of characterization of materials. It is routinely used in the characterization of coatings, batteries, fuel cells, and corrosion phenomena. It has also been used extensively as a tool for investigating mechanisms in electro-deposition, electrodissolution, passivity, and corrosion studies [78] .It is gaining popularity in the investigation of diffusion of ions across membranes and in the study of semiconductor interfaces. The fundamental approach of all impedance methods is to apply a small amplitude sinusoidal excitation signal to the system under investigation and measure the response (current or voltage or another signal of interest).

2.2.2 Fourier Transform Infrared Spectroscopy(FT-IR)

FT-IR is most useful for identifying chemicals. It can be applied to the analysis of solids, liquids, and gasses. The term FT-IR refers to a manner in which the data is collected and converted from an interference pattern to a spectrum. FT-IR instruments are computerized which makes them faster and more sensitive than the older dispersive instruments. FT-IR is perhaps the most powerful tool for identifying types of chemical bonds (functional groups). The wavelength of light absorbed is characteristic of the chemical bond as can be seen in this annotated spectrum. Molecular bonds vibrate at various frequencies depending on the elements and the type of bonds. According to quantum mechanics, these frequencies correspond to the ground state (lowest frequency) and several excited states (higher frequencies). One way to cause the frequency of a molecular vibration to increase is to excite the bond by having it absorb light energy [79].

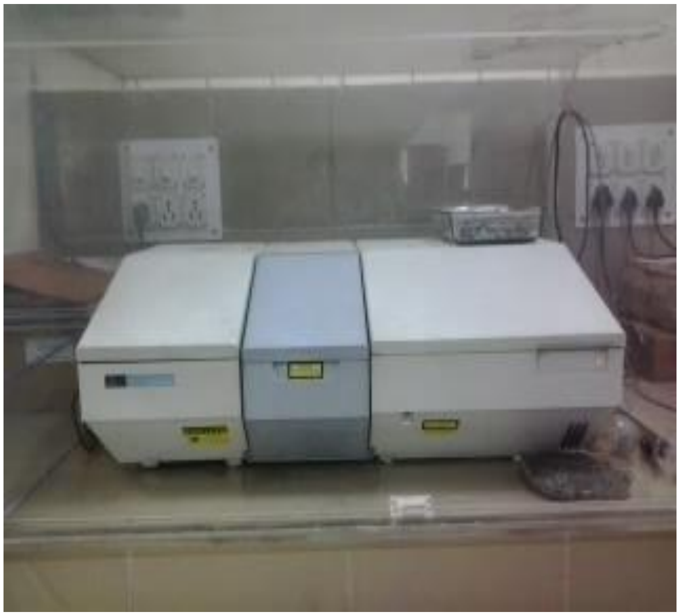

Figure 2.4: **Photograph of Fourier Transform Infrared Spectroscopy Instrument at NPL**

2.2.3 Scanning Electron Microscope (SEM)

The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron-sample interactions reveal information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample[80].In most applications, data are collected over a selected area of the surface of the sample, and a 2-dimensional image is generated that displays spatial variations in these properties. Areas ranging from approximately 1 cm to 5 microns in width can be imaged in a scanning mode using conventional SEM techniques (magnification ranging from 20X to approximately 30,000X, spatial resolution of 50 to 100 nm). The SEM is also capable of performing analyses of selected point locations on the sample; this approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions (using EDS), crystalline structure, and crystal orientations (using EBSD). The design and function of the SEM is very similar to the EPMA and considerable overlap in capabilities exists between the two instruments.

2.2.4 Dynamic Light Scattering (DLS)

Dynamic light scattering (also known as photon correlation spectroscopy or quasi-elastic light scattering) is a technique that can be used to determine the size distribution profile of small particles in suspension or polymers in solution[81].It can also be used to probe the behavior of complex fluids such as concentrated polymer solutions.

Figure 2.5: Photograph of Dynamic Light Scattering instrument (Zetasizer) at BECPRL-NPL

2.3 Materials Used:

- Aniline ($C_6H_5NH_2$) has been distilled prior to polymerization.
- All other chemicals are of analytical grade and have been used without purification.
- Deionized water (18MΩcm) is from the Millipore water purification system.
- Pre-cleaned Titanium glass plates have been used as substrates for deposition of PANI.

- Electrochemical impedance spectroscopy (EIS), Cyclic Voltammetry (CV) and Differential Pulse Voltammetry (DPV) measurements have been conducted on an Autolab Potentiostat/Galvanostat (Eco Chemie, Netherlands) using a three-electrodes system in H_2SO_4 (0.5M,pH 1).
- FT-IR spectra have been recorded with Perkin-Elmer instrument. Julabo F-10 chiller system has been in distillation unit.
- Dynamic Light scattering (Malvern-Zetasizer)
- Hydrogen Peroxide (Sigma –Aldrich)

2.4 Experimental: Synthesis

The preparation of titanium thin film over glass substrates need cleaning of glass substrates and cleaning procedure should be done with lot of care. The cleaning procedure is discussed below:

2.4.1 Cleaning of substrates

Cleaning is a very basic but very important step as it decides the quality of a film as if cleaning is not done properly then the impurity on glass substrate will not allow formation of a uniform film. Cleaning consists of mainly three steps [82].Three steps are discussed below in detail:

1. Cleaning with soap

- Take soapy water in a beaker and using lint free wipe and cotton swap gently and then using this soap water rubbing of glass substrates was carefully done.
- After rubbing with soap solution, glass substrates were rinsed with distilled water.
- Washed substrates were then dried using nitrogen blow drier.

2: Cleaning with Solvent

First of all, before cleaning wear eye protection and gloves for your safety. This process is very important step as it can help in removal of oil and any other organic residues present on glass surface.

- Acetone was taken in glass container and was heated at 55ºC and then was ultrasonicated for 10 minutes.
- Glass substrates were placed in warm acetone for 10 minutes.
- Now the glass substrates were placed in methanol for 2-5 minutes and then the beaker containing methanol and glass substrates were ultrasonicated for 10 minutes.
- Glass substrates were thoroughly rinsed with distilled water and then were dried with nitrogen.

3: Acid Treatment

- A solution of 30% HNO_3 or 30% HCl was prepared and then was mixed with 200 ml of distilled water.
- Glass substrates were soaked in the above solution for 30 minutes.
- Glass substrates were rinsed with distilled water and were dried with nitrogen.

2.4.2 Synthesis of Titanium Thin Films

Titanium electrodes of active surface were prepared by using the commercial glass slides were produced in a MILMAN DC-magnetron sputtering. [83].Deposition time of titanium thin film is two minute through argon plasma.

2.4.3 Preparation of Aniline Solution

Aniline was distilled by boiling at its boiling point (161°C) and then cooling it by at 4° C and we collected it[84].This distilled aniline is stored in dark place to avoid its photo degradation. The solution of aniline for deposition is prepared in acidic condition and here it is prepared in hydrochloric acid (9 ml water + 1 ml acid).

2.4.4 Synthesis of Gold Nanoparticles (Citrate Reduction Method)

- All glassware was rinsed with pure water before starting. 20 mL of 1.0 mM $HAuCl_4$ was added to a 50 mL beaker or Erlenmeyer flask on a stirring hot plate. A magnetic stir bar was added and the solution was brought to a rolling boil.
- To the rapidly-stirred boiling solution, 2mL of a 1% solution of trisodium citrate dihydrate, $Na_3C_6H_5O_7.2H_2O$ was quickly added. Gradually the gold sol was formed as the citrate reduces the gold (III)[85].
- Solution was removed from heat as the solution turned deep red or 10 minutes has elapsed

2.4.5 Synthesis of Silver Nanoparticles (Citrate Reduction Method)

- All glassware was rinsed with pure water before starting.50 mL of 0.001M Silver nitrate ($AgNO_3$) solution was added to a 100 mL beaker or Erlenmeyer flask on a stirring hot plate .A magnetic stir bar was added and the solution was brought to a rolling boil.
- To the rapidly-stirred boiling solution, 5 mL of a 1% solution of trisodium citrate dihydrate, $Na_3C_6H_5O_7.2H_2O$ was quickly added. Gradually the silver sol was formed as the citrate reduces the silver (III)[85].
- Solution was removed from heat as the solution turned deep red or 10 minutes has elapsed.

2.4.6 Synthesis of Iron oxide nanoparticle

Iron oxide nanoparticles of Sigma Aldrich were dissolved in water.10 mg of presynthesized iron oxide nanoparticles were dissolved in 50ml of distilled water [85].

2.4.7 Deposition of Polyaniline on Titanium Films

Deposition of polyaniline was done using chronogalvanostatic method .The deposition was carried out using an Electrochemical Analyzer (Potentiostat-Galvanostat) of AUTOLAB. For deposition a three electrode system was used. Titanium (Ti) Electrode was used as a working electrode, Platinum (Pt) as Counter Electrode and Silver (Ag/AgCl) as Reference Electrode.

Deposition was carried in a acidic solution of Hydrochloric acid and distilled polyaniline. With the help of software known as General Purpose Electrochemical System Manager (GPES Manager) a suitable method (galvanostatic in this case) is first selected and then particular parameters is entered .After entering the particular values the cycle is run. Deopos---ition of PANI was carried out at 150 Amperes for 900 seconds.

After every 300 seconds the titanium substrate is taken out and was gently washed with distilled water and then was again put as working electrode foe next cycle. Three cycles of each 300 seconds were provided for a uniform and proper deposition. An Emerald greenish thin film was observed on titanium electrode. Figure 2.6 shows the steps followed in the synthesis of PANI/Ti electrodes.

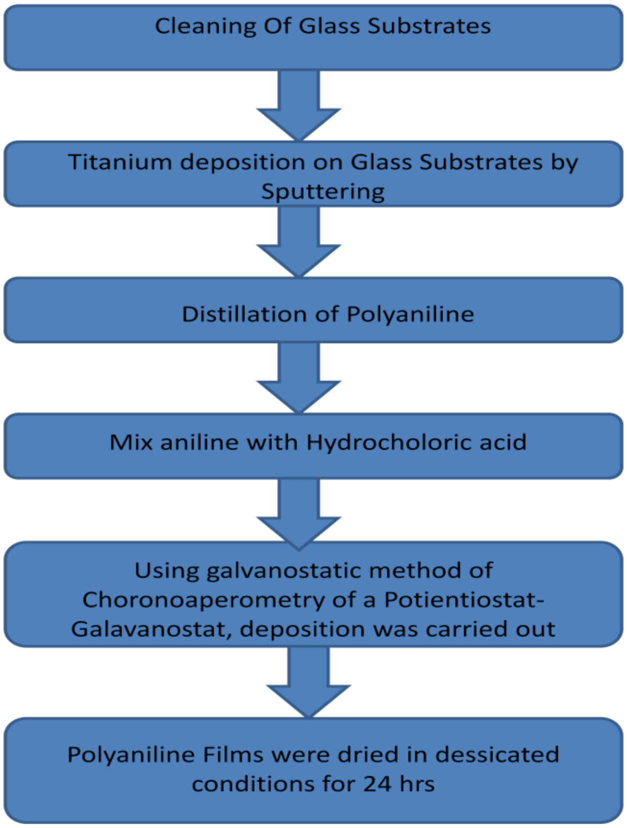

Figure 2.6: Flow chart of Steps followed in synthesizing Polyaniline Films

Figure 2.7: Photograph of Titanium film before and after deposition

2.4.8 Synthesis of Polyaniline Nanocomposite Film on Titanium

Polyaniline Nanocomposite films with Gold, Silver and Iron oxide nanoparticles have been deposited by mixing a ratio of 2:1 Nanoparticles: Polyaniline in an acidic medium and then deposition has been carried out on Titanium in different conditions; the data of different conditions is given below in Table 2.8:

Electrode Number	**Materials to be Deposited**	**Current given**	**Time Provided for Deposition**	**Number Of Cycles**
1	Polyaniline	150 microamperes	300 seconds	3
2	Polyaniline+ Au Nanoparticles	150 microamperes	300 seconds	2
3	Polyaniline+ Ag Nanoparticles	150 microamperes	300 seconds	2
4	Polyaniline+Fe_2O_3Nanoparticles	150 microamperes	300 seconds	2

2.4.9 Sensing

PANI/Ti Films were utilized to fabricate Hydrogen Peroxide sensor using electrochemical studies. For sensing different concentrations of H_2O_2 [65] were prepared and an enzymeless sensing using cyclic voltammetry and Impedence Spectroscopy was done.

Chapter 3

Results and Discussion

3.1 Scanning Electron Microscope (SEM) Studies

SEM Images of PANI/Ti reveals uniform distribution of Polyaniline that provides fibrous morphology to electrochemically deposited PANI on titanium substrate. Figure 3.1 shows deposition of Polyaniline with nanostructure.

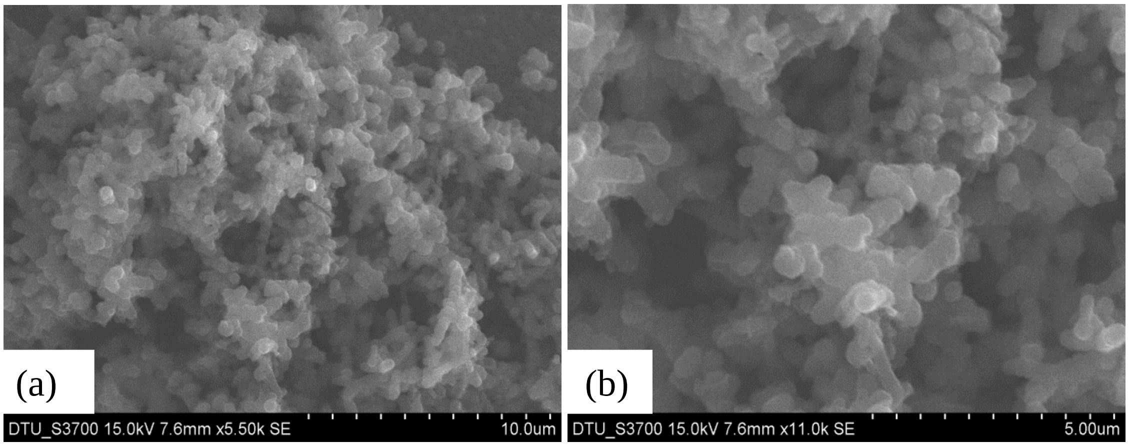

Figure 3.1: (a) and (b) SEM Micrograph of Electrochemically deposited PANI/Ti Electrode at 10µm and 5µm resolution respectively.

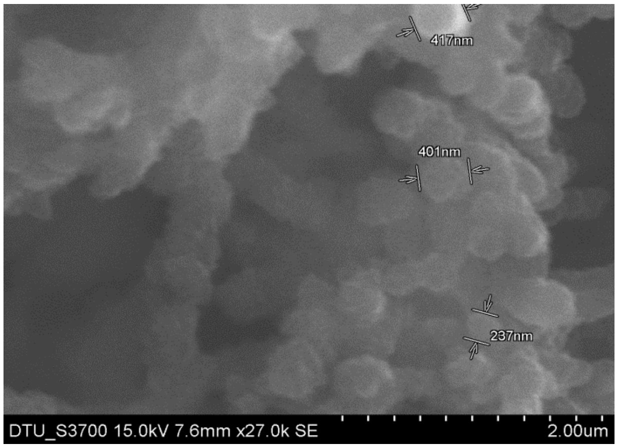

Figure 3.2: SEM Micrograph of PANI/Ti Electrode at 2 µm resolution.

3.2 Energy Dispersive X-ray Spectroscopy (EDX) Studies

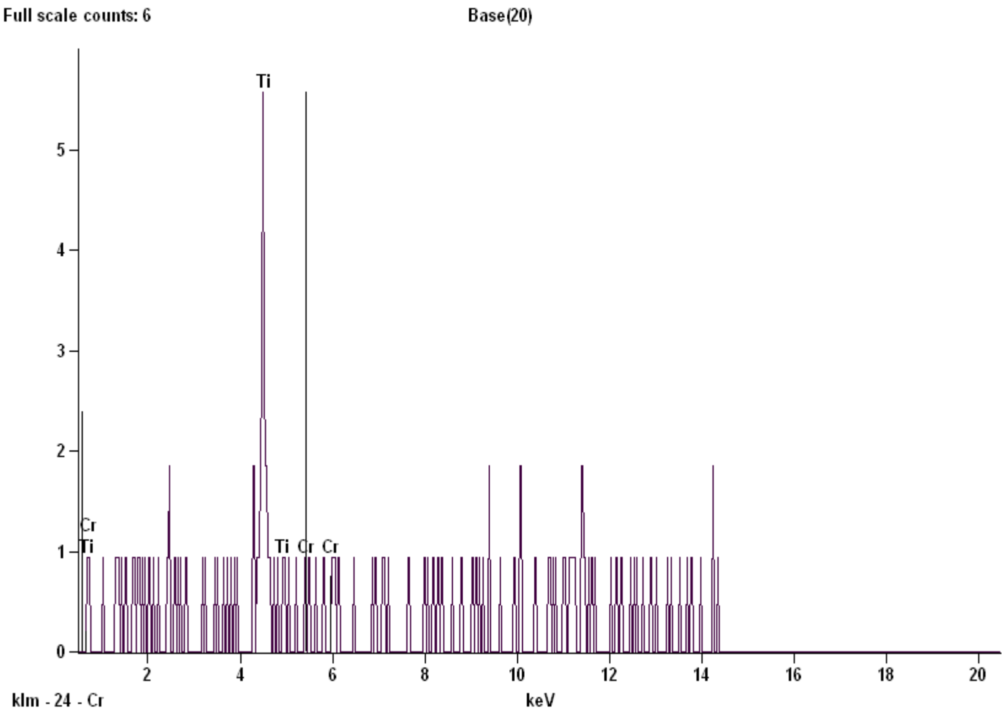

Figure 3.3: EDX graph showing presence of Titanium metal

Elemental Analysis showed that there is 93.6(weight %) of Titanium in it .Titanium metal was deposited on glass slide by sputtering and a peak of titanium in EDAX was obtained a little amount of chromium was also observed.

Element Line	Net Counts	Int. Cps/nA	Weight %	Weight % Error	Atom %	Atom % Error	Formula	Standard Name
Ti K	28	0.000	93.65	+/-20.07	94.12	+/-20.17	Ti	
Ti L	10	0.000	---	---	---	---		
Cr K	1	0.000	6.35	+/-12.71	5.88	+/-11.77	Cr	
Cr L	0	0.000	---	---	---	---		
Total			100.00		100.00			

Table 3.4: Quantitative result of EDX

3.3 Fourier Transform Infrared Spectroscopy (FTIR) Studies

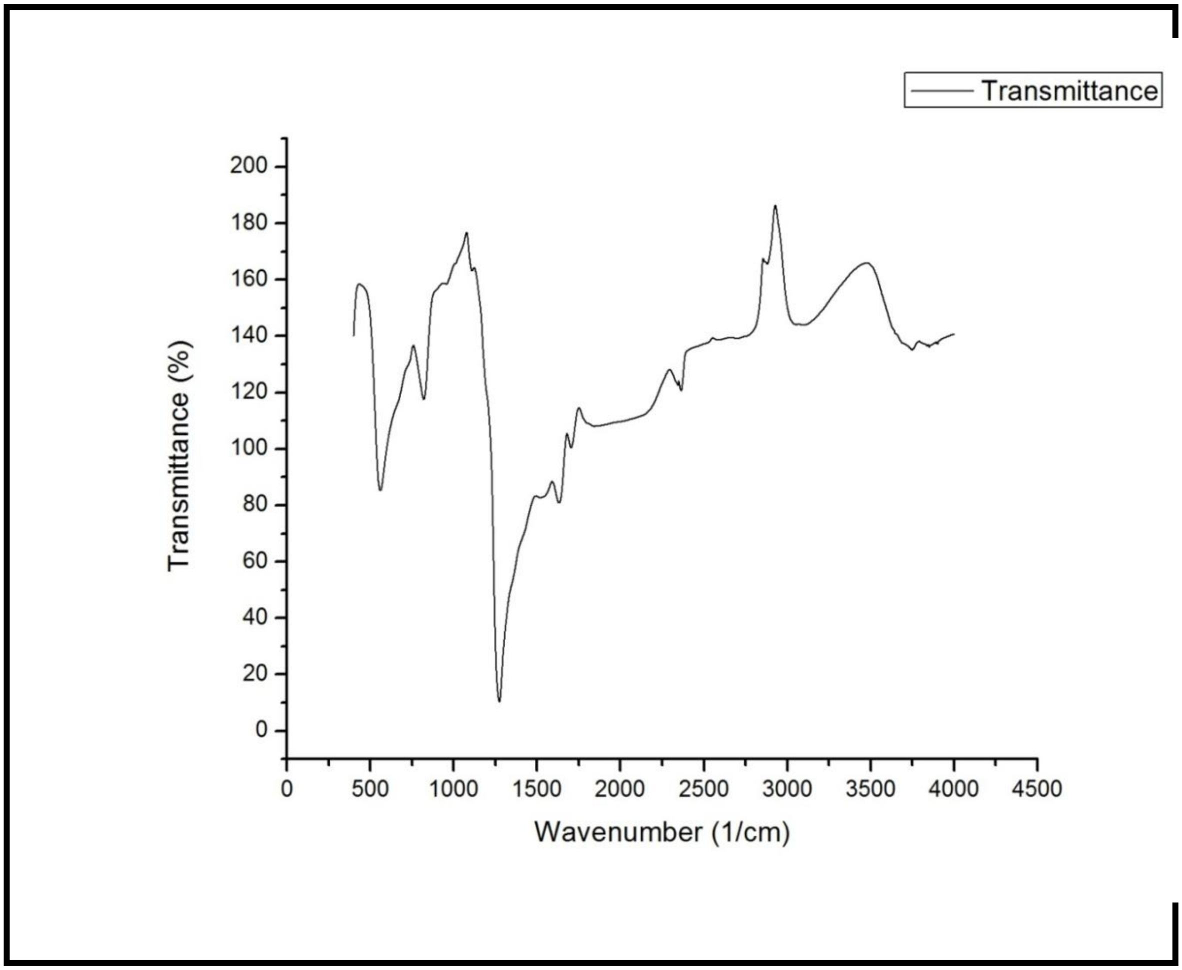

Figure 3.5: FT-IR spectra obtained for PANI/Ti Electrode

Figure 3.5 shows the FT-IR spectra obtained of PANI/Ti and electrode respectively, in the region of 500 to 4500 cm^{-1}. In the FT-IR spectra of PANI-NT, the peak around 1493 cm^{-1} and 1574 cm^{-1} are attributed to C=C benzenoid and quinoid ring vibrations, respectively. The peaks seen at 1306 cm^{-1} and 1141 cm^{-1} are assigned to C-N stretching of secondary aromatic amines and to aromatic C–H in-plane bending respectively.

3.4 Electrochemical Studies

Electrochemical analysis of PANI/Ti electrode has been carried out using **cyclic voltammetry** (CV).

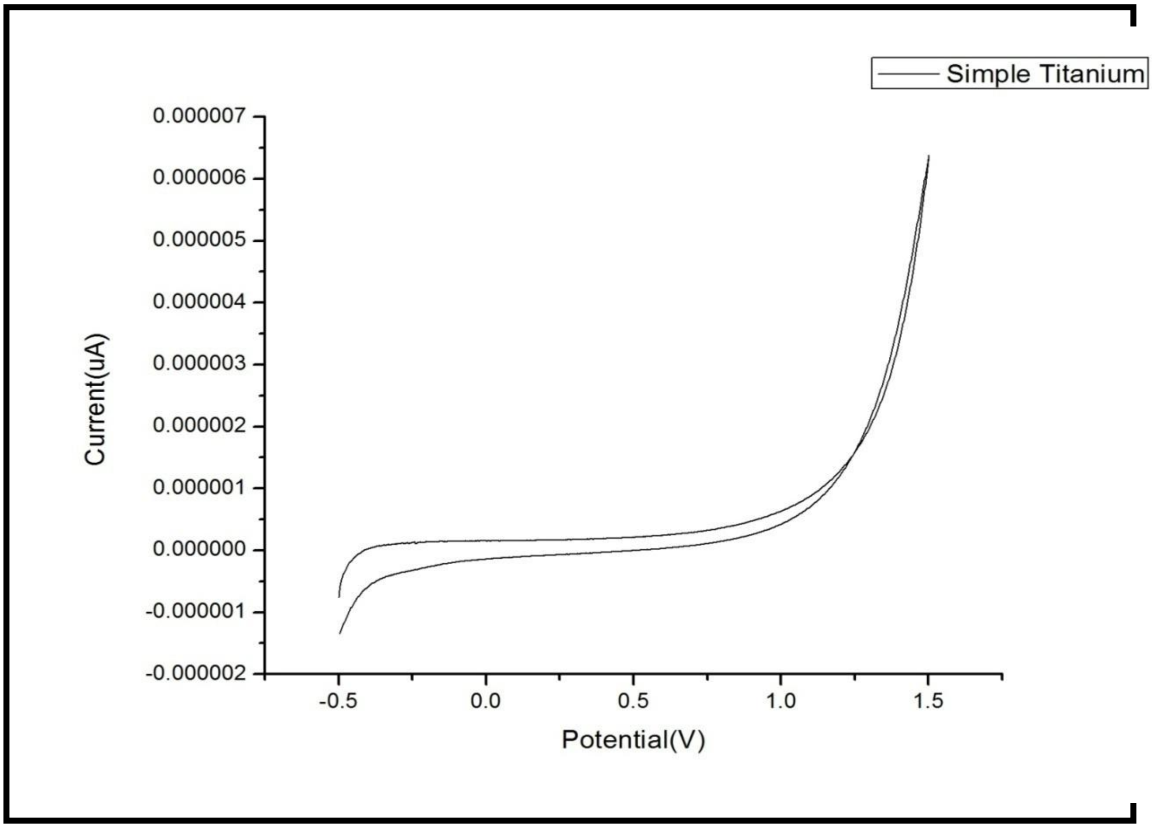

Figure 3.6: Cyclic voltammetry plot between current and potential for Bare Titanium Film

Titanium is a less electroactive species and thus does not show a redox peak. This graph shows the Current against potential curve of bare titanium film taken at a potential range of - 0.5 to + 1.5 V at a scan rate of 100mV/s in 0.5M H_2SO_4.This graph shows that it was a challenging job to use Titanium and make it a an electroactive species, which was done by electrochemically depositing PANI on it, which can be seen in the next graph.

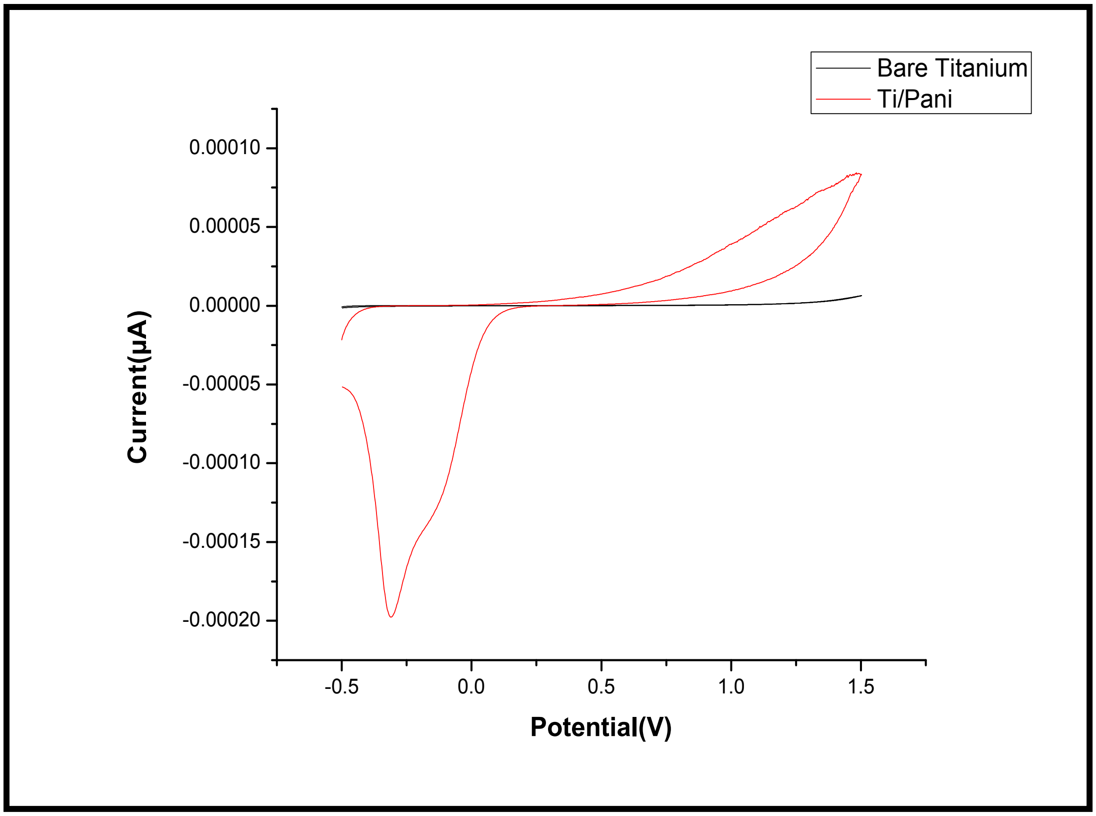

Figure 3.7: Cyclic voltammetry graphs of Bare Titanium Film and PANI/Ti Electrode

The graph above shows that there is an increase in current on titanium substrate after deposition of Polyaniline at same range of potential -0.5 t0 1.5 V, at a scan rate of 100 mV/s in 0.5M H_2SO_4(pH 1). A sharp reduction peak at -0.25 V can be clearly observed.

Electrochemical Studies of Polyaniline **Nanocomposites** Films Using **Cyclic Voltammetry.**Titanium based polyanilne films were successful with cyclic voltammetry, so experiments of synthesis of composites of polyaniline films with gold(Au), silver(Ag) and iron oxide(Fe_2O_3) Nanoparticles have been performed.

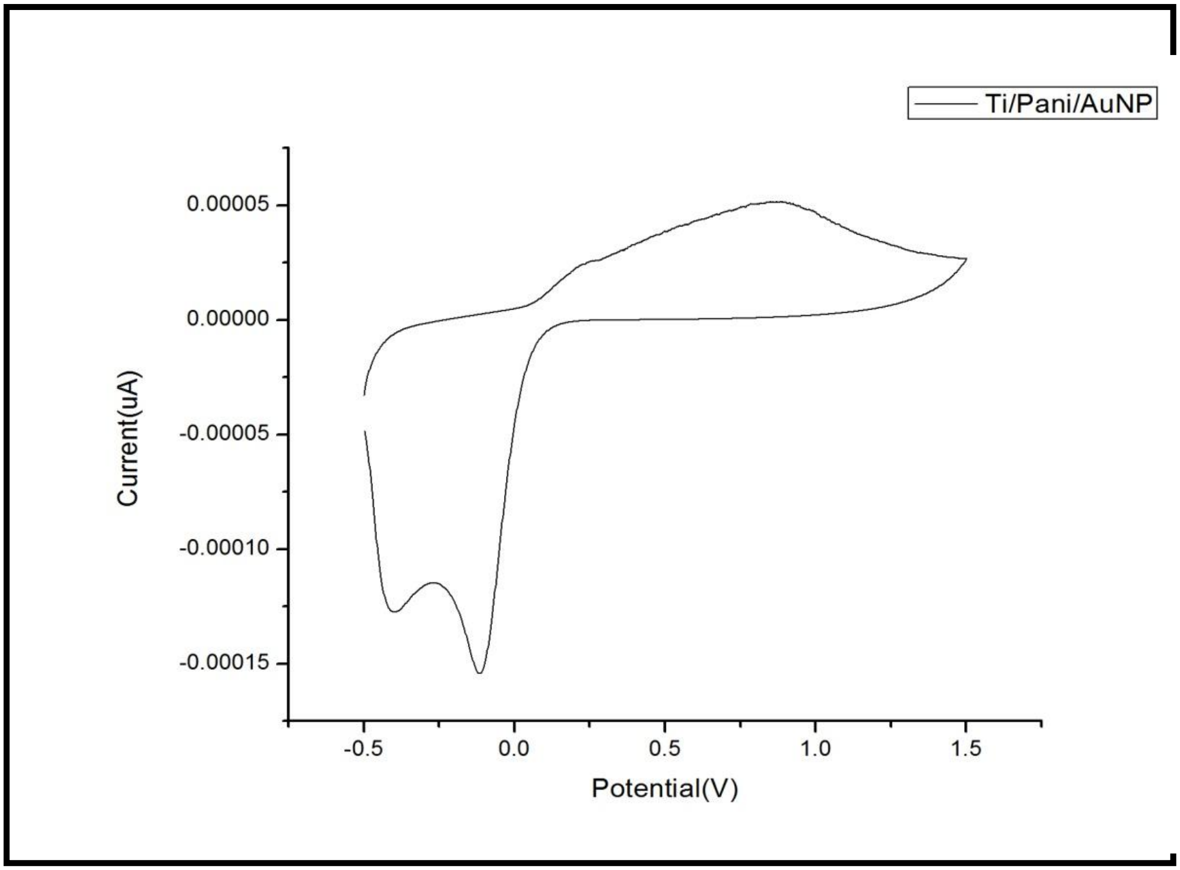

Figure 3.8: Cyclic Voltammetry graph of PANI-AuNP/Ti Electrode

The above graph shows the current versus voltage curve of Gold / Polyaniline composite on titanium substrate. Two reduction peaks at -0.4 and -0.10 V can be clearly seen and an oxidation peak at +0.75V can be observed, showing change in the redox process due to addition of gold.

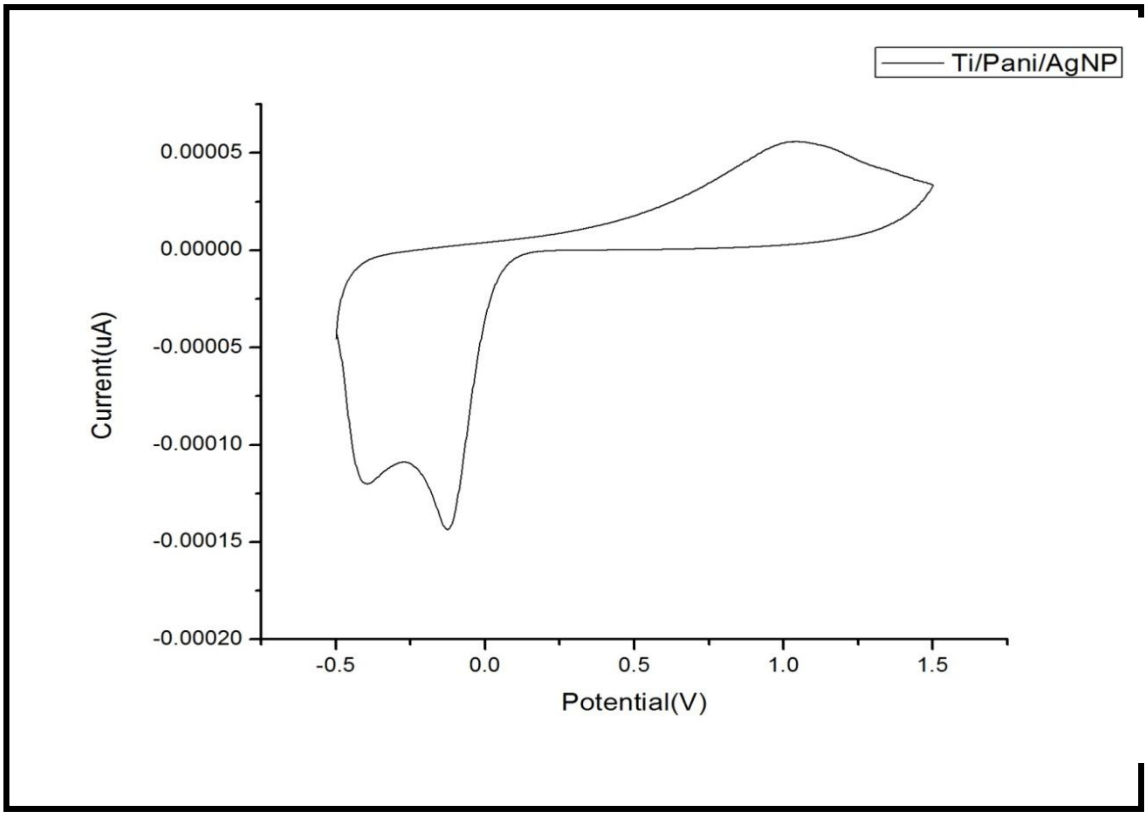

Figure 3.9: Cyclic Voltammetry graph of PANI-AgNP/Ti Electrode

The above graph shows the current versus voltage curve of Silver/polyaniline composite on titanium substrate. Two reduction peaks at -0.4 and -0.25 V can be clearly seen and an oxidation peak at +1V can be observed, showing change in the redox process due to addition of silver.

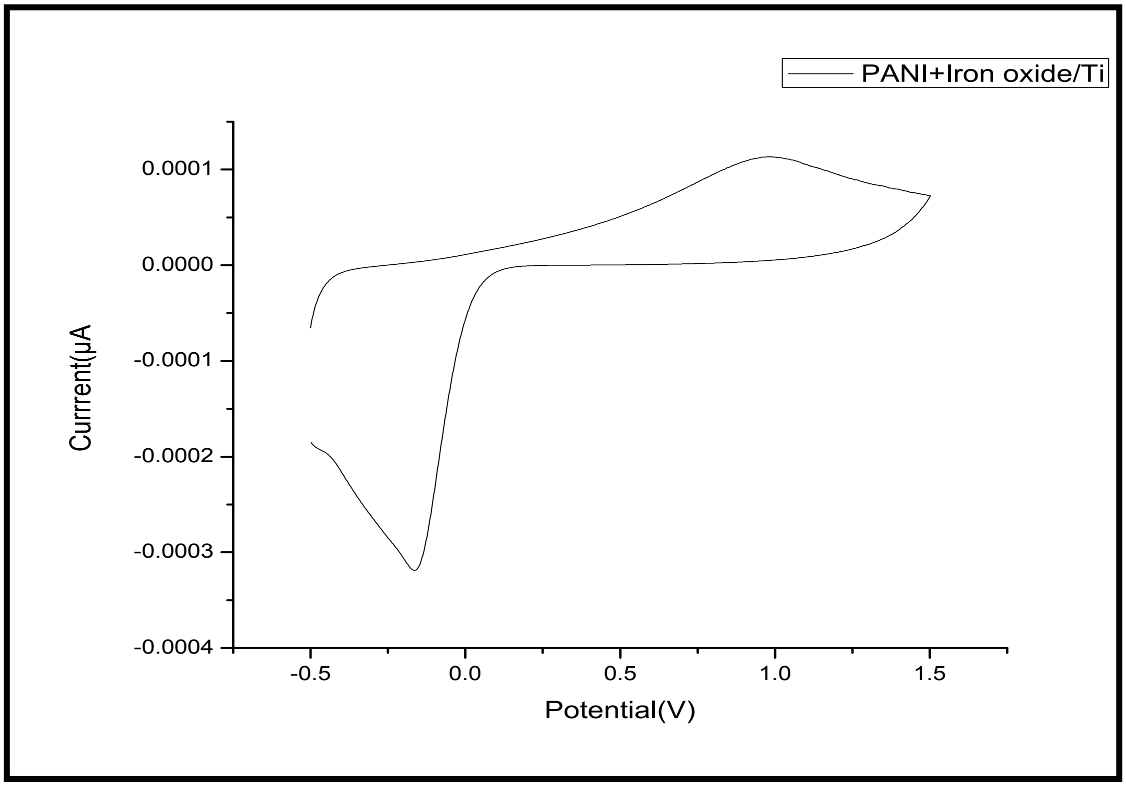

Figure 3.10: Cyclic Voltammetry graph of PANI-AgNP/Ti Electrode

The above graph shows the current versus voltage curve of Fe_2O_3/PANI composite on titanium substrate. Two reduction peaks at -0.25 V can be clearly seen and an oxidation peak at +1V can be observed, showing change in the redox process due to addition of iron oxide.

3.5 Effect of Hydrogen Peroxide Concentration on PANI/Ti Electrode

Electrochemical Impedance Studies were carried out to observe changes that electrode shows with increasing concentration of H_2O_2.Cyclic Voltammetry and Electrochemical Impedance Spectroscopy studies were done.

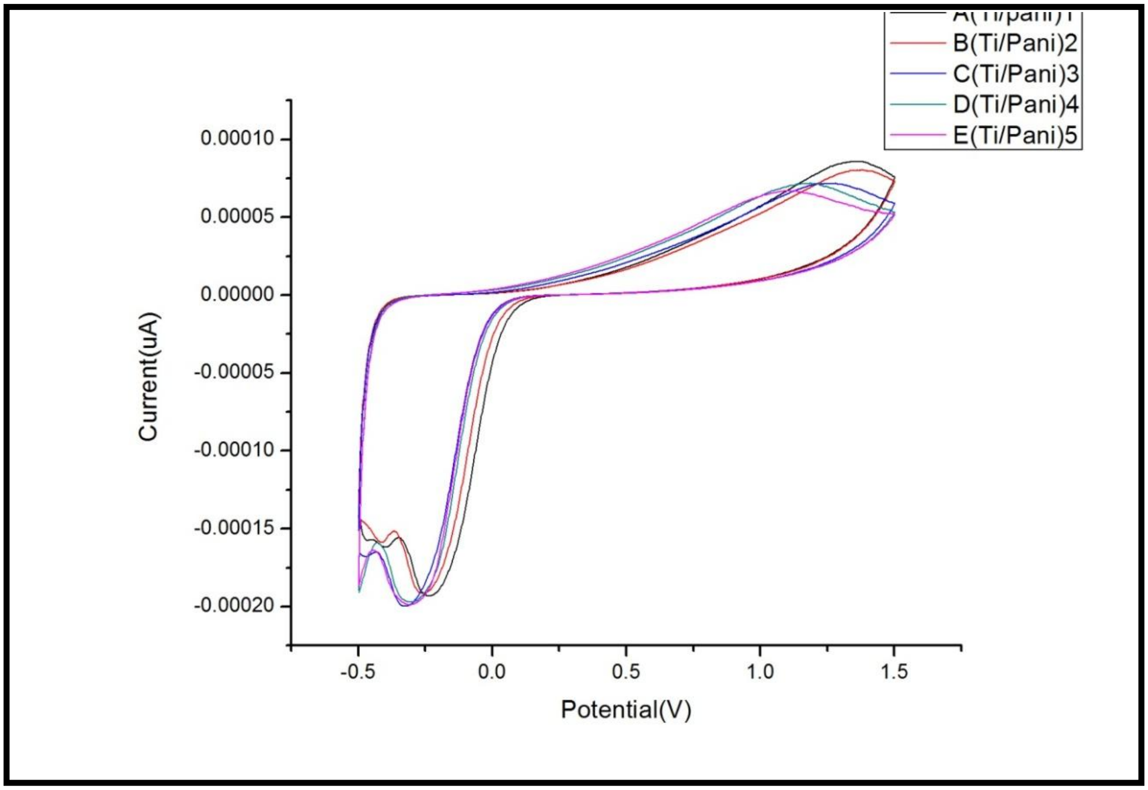

Figure 3.11: Cyclic Voltammetry Response of PANI/Ti Electrode as a function of H_2O_2 Concentration

With different concentrations of H_2O_2, change in current can be observed.H_2O_2 behaves as free charge carrier in the acid medium and try to damage the polymer and results in depolymerization and thus a decrease in current can be observed which can used as a sensing property of Hydrogen peroxide .

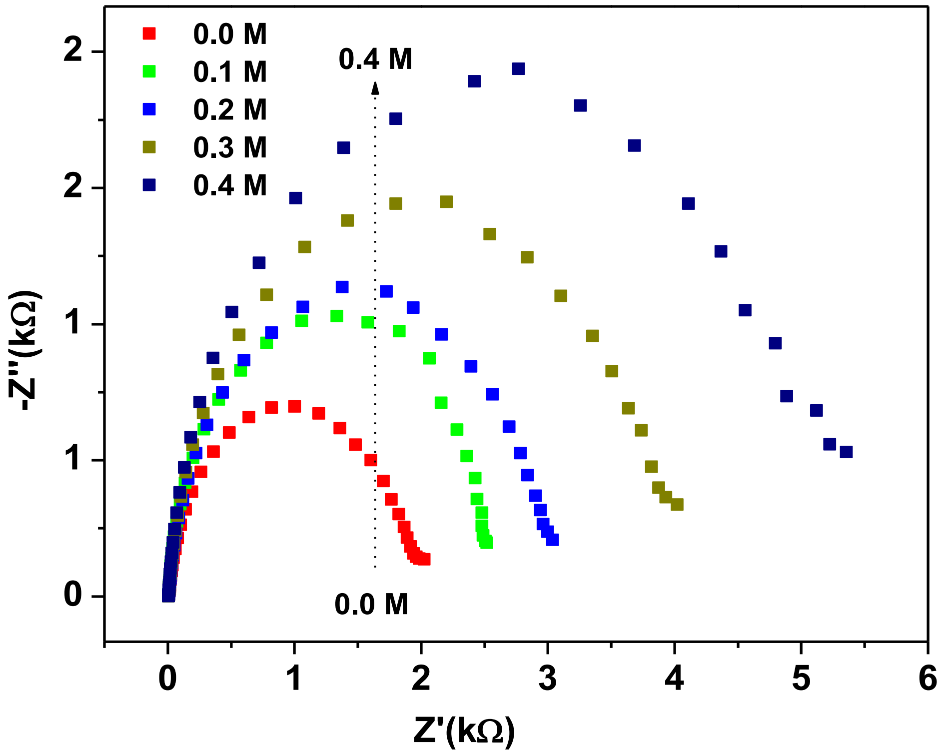

Figure 3.12: Change in Impedance with increase in H_2O_2 Concentration

Electrochemical impedance spectroscopy (EIS) provides an effective method to probe electronic features of surface-modified electrodes. Figure 3.12 shows Nyquist plots obtained for PANI/Ti electrodes, with increasing concentration of H_2O_2. Nyquist diameter (real axis value at lower frequency intercept) indicates the value of charge transfer resistance (R_{CT}) i.e. hindrance provided by the electrode material to transfer charge from solution to the electrode that can be correlated with the modification of the surface. It can be seen that R_{CT} Value for 0.4 M H_2O_2 is highest and for 0.1M is lowest, this shows that more concentration of Hydrogen peroxide provides hindrance to permeability of H_2SO_4 to the electrode surface .And thus PANI/Ti electrode proved to be successful in sensing H_2O_2 .

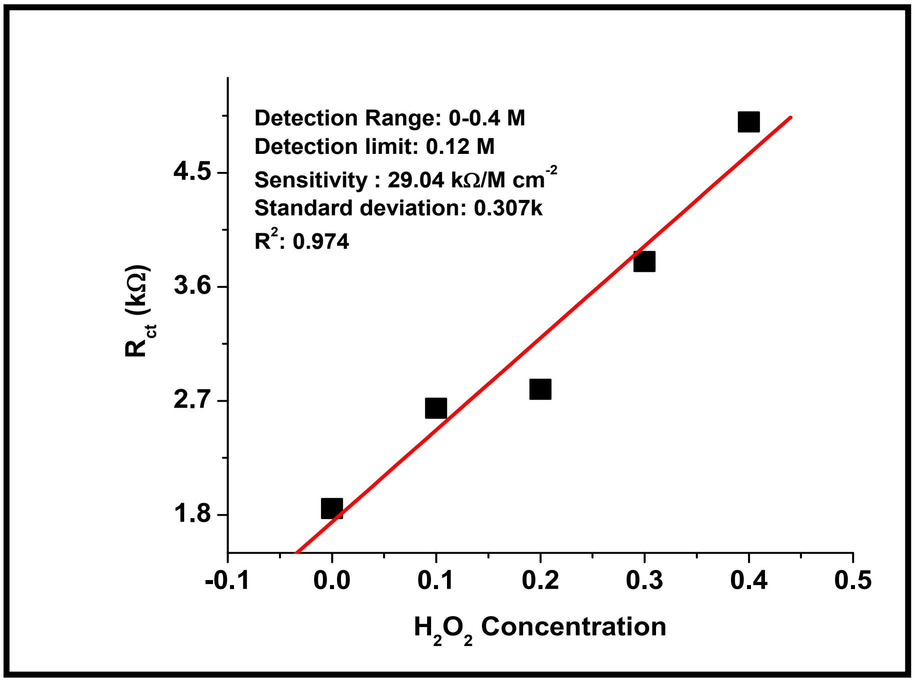

Figure 3.13: Impediometric Response of PANI/Ti Electrode as a function of H_2O_2 Concentration

Figure 3.13 show the amperometric response of PANI/Ti electrode as a function of hydrogen peroxide concentration. It can be seen that the value of resistance increases linearly with H_2O concentration in the range 0.1-0.4M. The value of sensitivity calculated from linear region of the calibration curve is 29.04kΩMcm^{-2} with standard deviation (SD) as 0.307k.

Chapter 4

Conclusion

Titanium is a biocompatible and inexpensive metal that has many applications in biomedical field [1] Inspite of being less electroactive species I have tried to explore its electrochemical properties for sensing and thus have used polyaniline for enhancing its electrochemical property

.Polyaniline Films on Titanium Substrate has been successfully synthesized using distilled aniline solution using Galvanostatic method (Chronoamperometry) using three electrode system. Stable films were obtained and these films can be used as sensors. Sensing of Hydrogen Peroxide without using any enzyme can be achieved successfully. There is an increase in resistance with every increasing concentration of Hydrogen Peroxide and this change can be used effectively as a sensing parameter.

There are many optimizations that can be done and sensing properties of Titanium and Polyaniline can be explored further for many analytes in near future. An experiment regarding same has been started with different composites of polyaniline with metal and metal oxides nanoparticles like silver, gold and iron oxide nanoparticles and sensing can also be further studied for some gases like carbondioxide, hydrogen, ammonia etc.

In future, a study of conversion of titanium into titanium oxide and some titanium nanostructures can also be persued and thus their sensing properties can also be explored.

An interesting topic in this regard is detection of blue-green algae also known as Hyphens,[86] present in agricultural fields effecting the growth of plants positively and also their presence in some food products can negatively affect the product. Hyphens live in acidic environment and their detection can help agriculturists and food technologists in their respective fields. Study in this area can be further preceded.

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