# SYNTHESIS OF NANO - POLYANILINE FILM BY ELECTROCHEMICAL POLYMERIZATION AND ITS APPPLICATION IN GLUCOSE SENSING

A DISSERTATION THESIS SUBMITTED FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE

OF

**MASTERS OF SCIENCE**

IN

**BIOTECHNOLOGY**

SUBMITTED BY

**VARSHA YADAV**

**2K20/MSCBIO/35**

UNDER THE SUPERVISION OF

**Prof. JAIGOPAL SHARMA**

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

**Dr. GAJJALA SUMANA**

SENIOR PRINCIPAL SCIENTIST

CSIR - NPL

SUBMITTED TO

**DELHI TECHNOLOGICAL UNIVERSITY**

**(FORMERLY DELHI COLLEGE OF ENGINEERING)**

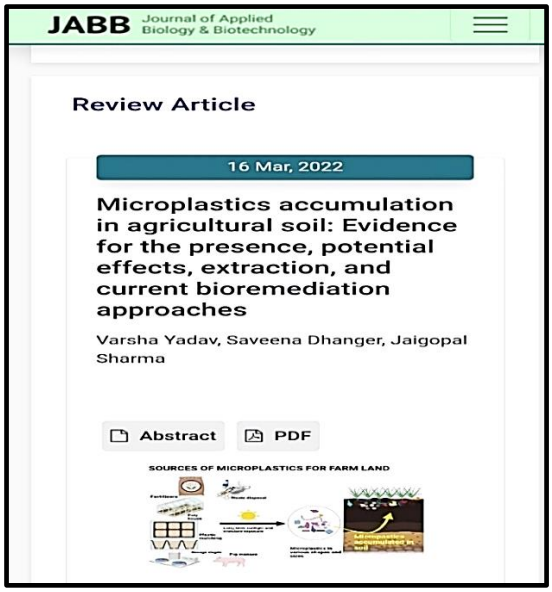
**BAWANA ROAD, DELHI-110042**

**(2020 – 2022)**

## CANDIDATE'S DECLARATION

I, hereby declare that the work which is presented in the Major Project entitled "**SYNTHESIS OF NANO - POLYANILINE FILM BY ELECTROCHEMICAL POLYMERIZATION AND ITS APPPLICATION IN GLUCOSE SENSING** "in fulfillment of the requirement for the award of Degree of Masters in Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my own work, carried during a period from 03-Jan-2022 to 06-May-2022, under the supervision of internal supervisor **Prof. Jaigopal Sharma, Delhi Technological University and external supervisor Dr. Gajjala Sumana, CSIR-NPL.**

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other university. Furthermore, for the partial fulfillment of project work, I communicated a review paper entitled "**Microplastics accumulation in agricultural soil: Evidence for the presence, potential effects, extraction and current bioremediation approaches**" in Scopus indexed journal "Journal of applied biology and biotechnology" that got accepted and will be published soon. Below is the insight for the same:




**VARSHA YADAV (2K20/MSCBIO/35)**

**DELHI TECHNOLOGICAL UNIVERSITY (FORMERLY DCE)**

SHAHABAD DAULATPUR, MAIN BAWANA ROAD, DELHI - 110042

## CERTIFICATE - I

This is to certify that **Ms. VARSHA YADAV** (ENROLLMENT No. 2K20/MSCBIO/35) has carried out the major project contained in the thesis entitled "**SYNTHESIS OF NANO - POLYANILINE FILM BY ELECTROCHEMICAL POLYMERIZATION AND ITS APPPLICATION IN GLUCOSE SENSING**" as a partial fulfillment for the award Masters in Science degree in Biotechnology by Delhi Technological University. The assistance and help received during the course of investigation has been fully acknowledged.

Date: 06- 05- 2022

Place: DTU, New Delhi

**Prof. Jaigopal Sharma (Internal Supervisor)**

This is certified that the Thesis Viva-Voice examination of **Ms. Varsha Yadav** (2K20/MSCBIO/35) has been held on dated **06-may-2022** and the thesis has been accepted for the award of the degree.

**Prof. Pravir Kumar**

**(Head of the Department)**

Department of Biotechnology

DTU, Delhi-110042

**Prof. Jaigopal Sharma**

**(Supervisor)**

Department of Biotechnology

DTU, Delhi -110042


**CSIR - NPL, Dr. KS Krishnan Marg, Delhi -110012**

## CERTIFICATE - II

This is to endorse that the Project entitled **"SYNTHESIS OF NANO - POLYANILINE FILM BY ELECTROCHEMICAL POLYMERIZATION AND ITS APPPLICATION IN GLUCOSE SENSING"** submitted by **Ms. VARSHA YADAV**, Enrollment no. **2K20/MSCBIO/35** in partial fulfilment of the requirements for the award of degree of **Masters in Biotechnology** from **Delhi Technological University** has done her dissertation work from **NPL(CSIR)** and it is an authentic work carried out by her under my supervision and guidance.

**Dr. Gajjala Sumana**

**Senior Principal scientist**

**(External supervisor)**

**(National Physical Laboratory)**

**CSIR**

## ACKNOWLEDGEMENT

I would also like to express my hearty felicitation to my internal supervisor, **Prof**. **Jaigopal Sharma**, DTU for his continuous motivation and guidance while writing the review entitled "**Microplastics accumulation in agricultural soil: Evidence for the presence, potential effects, extraction and bioremediation approaches**" for the partial fulfillment of the project work required during masters. Also, I would thank **Ms**. **Neha Tiwari** (Senior research scholar) at DTU for guiding and helping regarding review submission and revision process. I am highly grateful towards my internal supervisor for allowing me to pursue my dissertation at **CSIR-NPL.**

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Most importantly none of this would have been possible without the love and patience of my **Parents**. They have been a constant source of love, concern and support.

**Varsha Yadav (2K20/MSCBIO/35)**

## ABSTRACT

Biosensors have ubiquitous biomedical applications that cover a wide range of areas such as diagnosis, point-of-care monitoring of treatment and disease progression, environmental monitoring, food quality control, drug delivery, drug discovery, forensics and other biomedical research. Biosensors may indeed be developed using a diverse range of methodologies. Their interactions with high-affinity biomolecules facilitates sensitive and selective detection of a plethora of analytes. The use of conducting polymers in sensing is intriguing since they are known to improve the sensitivity of a biosensor. Polyaniline (PANI) is a notable conductive polymer, and it has gained considerable attention from researchers in the field of nanotechnology for the enhancement of sensors. Because of its ease of synthesis and outstanding environmental stability, PANI is readily doped by various acids and dopants. PANI is a highly conductive polymer with distinct qualities such as easy synthesis, low cost, and good environmental resilience. PANI's unique features have led to its widespread use in a variety of applications such as drug delivery, anti-corrosion materials, scaffold and sensing devices manufacturing. In this present thesis, polyaniline (PANI) was fabricated over ITO coated glass slides and further composite electrodes were fabricated by immobilization of Glucose oxidase (GOx) into electrochemically polymerized polyaniline thin films for biosensor application. The electrochemical polymerization of PANI on ITO was performed in aqueous solution of 0.1M HCl and 0.3 M aniline at constant current of 0.15 A. Furthermore, GOx enzyme combined with HRP was immobilized on the Polyaniline fabricated electrode. Analytical characterization of GOx modified polyaniline electrode in comparison to polyaniline electrode and bare ITO were carried out by Cyclic voltammetry and Amperometric curves for different glucose concentrations ranging from 50mM to 200mM were obtained.



**KEYWORDS:** Aniline**,** Biosensor, Conducting polymers, Cyclic voltammetry, Electrochemical polymerization, Galvanostatic, Glucose sensing, ITO, Polyaniline, Potentiostatic, Nanomaterials, Thin films

## LIST OF CONTENTS

.

## LIST OF FIGURES

## LIST OF TABLES

## LIST OF ABBREVIATIONS

**AB –** Antibody

**AFM –** Atomic Force Microscope

**Ag/AgCl** – Silver/Silver chloride

**BMI** – Brain-Machine Interface

**BCI** – Brain Computer Edge

**1-D** – One Dimensional

**2-D –** 2 Dimensional

**CV –** Cyclic Voltammetry

**CSD** – Chemical Solution Deposition

**CVD** – Chemical Vapor Deposition

**DI** – Distilled Water

**EB** – Emeraldine Base

**EBE** – Electron Beam Evaporation

**EC** -Electrochemical Cell

**FAD** – Flavin Adenine Dinucleotide

**FTIR** – Fourier Transform Infrared Spectroscopy

**GC** – Gas Chromatography

**GOx** – Glucose oxidase

**GPES** – General Purpose Electrochemical System Software

**HPLC** – High Performance Liquid Chromatography

**HRP** – Horseradish Peroxidase

**ITO** – Indium Tin-Oxide

**LB** – Leucoemeraldine Base

**NaCl** – Sodium Chloride

**NP** – Nanoparticles

**PANI** – Polyaniline

**PB** – Pernigraniline Base

**PBS** – Phosphate Buffer Saline

**PU -** Polyurethane

**RNA** – Ribonucleic Acid

**SEM** – Scanning electron Microscopy

**TEM** – Transmission Electron Microscopy

**XRD** – X-Ray Diffraction

# CHAPTER 1

# INTRODUCTION

This chapter gives insights on biosensors and their classification. It also focuses on importance of thin films in biosensing, techniques for deposition and characterization of thin films. Furthermore, this chapter includes the structure and objective of the thesis work.

## 1.1. BIOSENSOR

A biosensor is typically known as an analysis tool that transforms biological responses into quantifiable and processable outputs (Kawamura & Miyata, 2016). Because of their high sensitivities and specificities, biosensors and chemical sensors are gaining popularity in analytical chemistry research. Figure 1.1 represents a schematic diagram of a biosensor and its components.

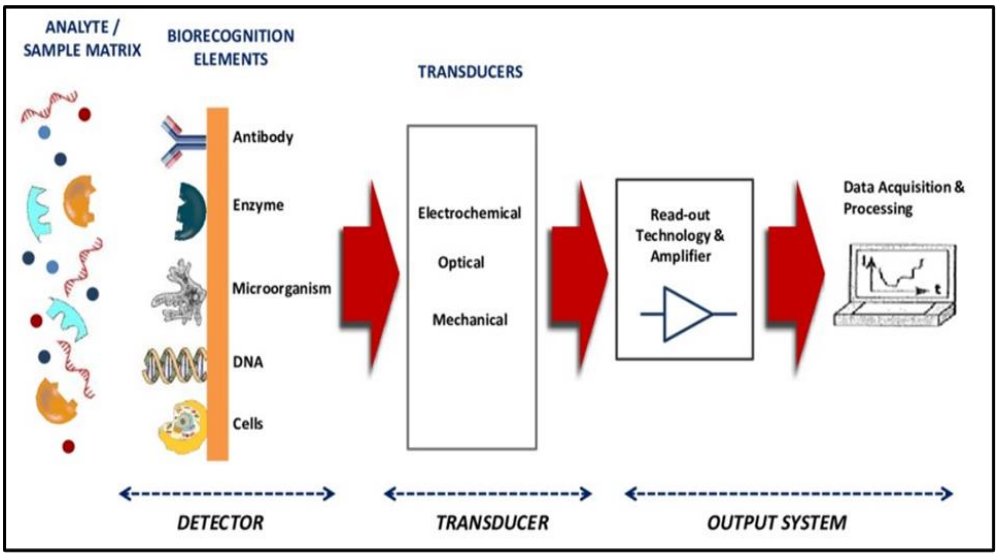



**Figure 1.1:** Schematic diagram of Biosensor and its components

## 1.2.Working principle and elements of a biosensor

The basic working principle of a biosensor is the conversion of biochemical signals produced upon analyte binding to the biorecognition element into an amplified electrical /digitally detectable signal. Elements of a biosensor comprises of bioreceptor, detector, transducer and the output system (amplification and output data).

### 1.1.1. Bioreceptors

Bioreceptors are considered the key component for fabrication of biosensors because they possess the ability to recognize and bind to the specific analyte or sample. Bioreceptors are generally classified into five major categories that include:

**i) Antibodies ii) Enzymes iii) Cells iv) Tissues and v) Nucleic acids.**

**Antibody Bioreceptors:** Presence of a highly ordered and specific sequence of amino acids, makes antibody a highly complex biomolecule that specifically binds to its target, called antigen this unique feature of antibody makes its perfect for use in immunosensor fabrication, where the analyte/ sample of interest is detected by the antigen-antibody interaction (Sharma et al., 2016). However, the dependence of AB binding capacity on assay conditions (e.g., pH and temperature) and irreversible interaction of AB–antigen complex present major limitations with their use in bioassays.

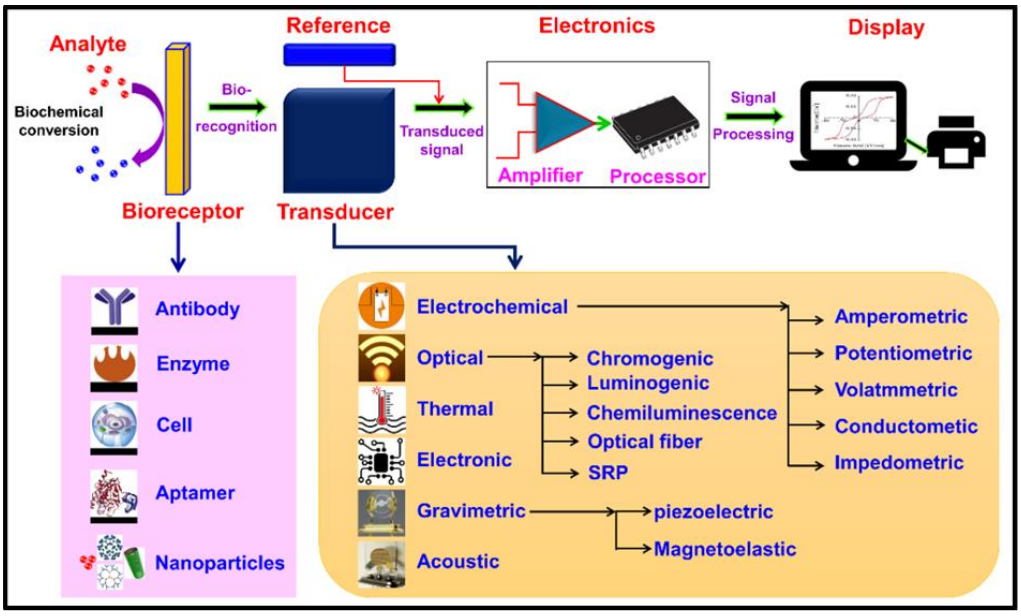



**Figure 1.2:** Graphical representation of a distinctive biosensor with its components (bioreceptor, transducer, electronic system, and display)

**Enzyme Bioreceptors:** other than their specific binding capabilities, enzymes also exhibit catalytic activity, which makes them potential bioreceptors for sensing assays. All the enzymes, except some catalytic RNA molecules, are proteins. The catalytic activity of antibodies relies on the integrity of their native protein conformation and allows for much lower limits of detection compared to it obtained with other class of bioreceptors (Nguyen et al., 2019). Except

for their ability to catalyze biochemical reactions, the foremost merits with enzymes as receptor molecule include their potential towards the detection of a gaggle of analytes and suitability with several different transduction methods. Notably, since enzymes aren't consumed in reactions, the biosensor can repeatedly be used.

**Cellular Bioreceptors**: Cellular structures present another class of bioreceptors that recognize either cell or a microorganism or cellular binding species. These tend to connect to the surface and are thus easily immobilized, sensitive to surrounding environment, and may answer nearly all types of stimulants. However, the cell-based biosensors have limitations with reference to the slow response resulting in poor specificity in comparison to pure enzymatic biosensors. this can be because of the undesired side reactions catalyzed by other enzymes in a very cell. Poor stability of the cellular receptors also limits the sensor period of time.

**Tissue Bioreceptors:** Tissues are used as bioreceptors for the abundance of enzymes in them. Their advantages over cellular bioreceptor include easier immobilization onto substrate, better ability to retain enzymes activity in natural environment, easier availability, and relatively low price. Nevertheless, major disadvantages are the shortage of specificity because of the interference of other enzymes and longer interval thanks to transport barrier.

**Nucleic Acid Bioreceptors:** Biosensors employing macromolecule interactions are stated as genosensors. In recent years, the interest for genosensors has tremendously grown because these biosensors can enable the detection and diagnosis of diseases even before the emergence of physical signs and symptoms in the body.

**Immobilization Matrix**

The immobilization of a bioreceptor is a vital think about the event of a biosensor. The control of this step is paramount to confirm high reactivity, orientation, accessibility and stability of the surface–confined probe and to avoid nonspecific binding. Adsorption, electrochemical entrapment, biotin–avidin coupling, and covalent binding are a number of the methods that are used for the biomolecular immobilization, (Fig 1.3) and therefore the selection of those methods relies on the selection of matrix.

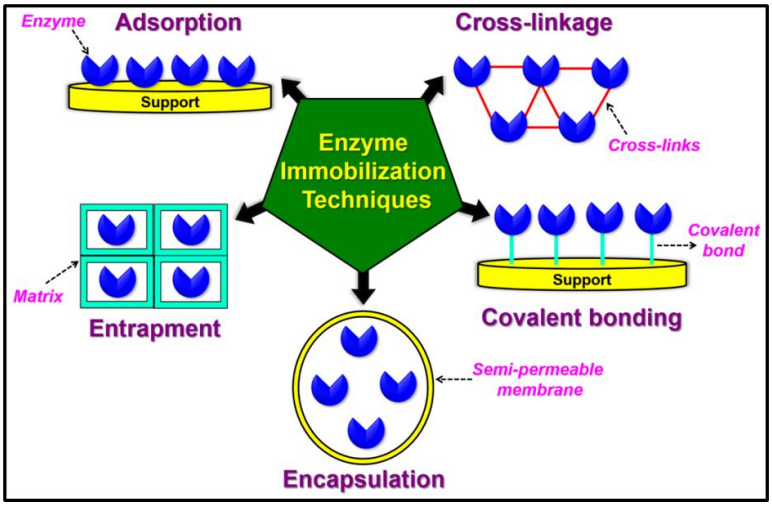



**Figure1.3** Biomolecule Immobilization methods

Optimization of the immobilization matrix on the sensing chip could be a pre–requisite to keep up the bioactivity of biomolecule and improve its contact with the transducer surface so on obtain accurate sign. so as to achieve highly stable and efficient biosensor, the matrix must provide same local aqueous micro–environment to the bioreceptor as in biological media, so on prevent self–aggregation and microbial attack and enhance accessibility towards the target analyte.

### 1.2.2. Transducer

Transducer functions to observe the physiochemical change produced by specific interactions between the target analyte and bioreceptor. It converts a biochemical signal into an electrical signal that's processed into an analogue or digital signal. The concentration of an analyte is proportional to the number of signal generated, allowing the transducer to perform both: the qualitative and quantitative measurements.

Transducers can perform optical (i.e., luminescence, absorption, surface plasmon resonance, etc.), electrochemical, and mass–sensitive measurements (i.e., surface sound wave, microbalance, etc.) for the specified biosensing assay (Purohit et al., 2020). In optical biosensing, fluorescence– based detection and label–free detection is studied. Fluorescence–based detection necessitates labelling of either target or bioreceptor and also the variation in intensity is monitored as a function of target concentration. Though, this detection protocol is sensitive enough to detect the target right down to one molecule, yet the laborious labelling processes, un–predictable number of attached fluorophores, and interreference of label with the biomolecule function are major shortcomings. In label–free protocol, detection is

comparatively easy and cheap to perform as biomolecules don't seem to be modified and therefore the target is detected in its natural forms, but the sensing parameters are relatively poor.

### 1.3. Classification of biosensors

Major characteristics of a biosensor include sensitivity, reliability, reproducibility, speed, stability and linearity in the sensing results for any analyte. Biosensors have been classified into several categories (Fig 1.4) on the based upon –

- Type of bioreceptor
- Type of transducer
- Technology
- Type of detection system

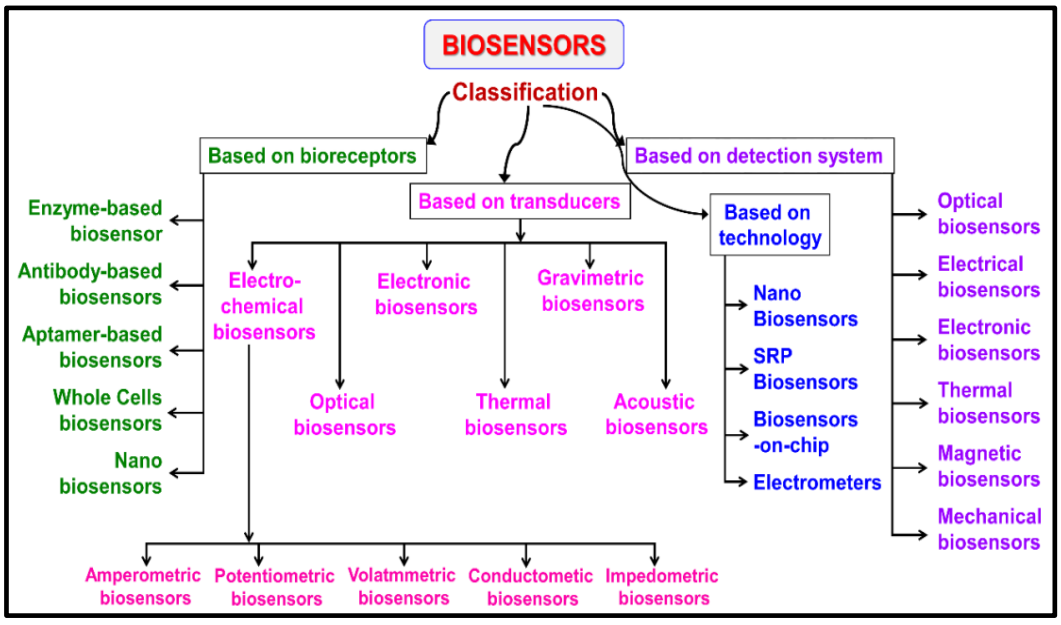



**Figure 1.4** Classification of biosensors based on bioreceptors, transducers, technology used and detection system.

This thesis work involves use of both enzymatic and electrochemical biosensors because glucose oxidase and horseradish peroxidase enzymes have been used for immobilization and the fabrication of material was performed using electrochemical method.

**Enzyme based biosensors:** Biosensors employing enzymes as bioreceptor / biorecognition element are referred as enzymatic biosensors (Zhu et al., 2019). Most commonly used enzymes

for enzymatic biosensors in biomedical and healthcare include glucose oxidase, cholesterol oxidase, urease, uricase etc.

**Electrochemical biosensors:** Electrochemical biosensors, because of their simplicity, cost–effectiveness, accuracy and high sensitivity for target analyte detection, have received much attention in biosensor development. Amperometric and potentiometric transducers are most typical among electrochemical biosensors (Cho et al., 2020). Thanks to inherent miniaturization possibilities, the electrochemical biosensing devices are commercialized in clinical, environmental, industrial, and agricultural fields, permitting them to rival the foremost advanced optical protocols.

### 1.4. Nanomaterial in biosensing

Over the past decades, nanomaterials are a topic of enormous interest. The presence of unique properties that get accumulated when a particle changes from micro to nano scale level is huge as the physico-chemical properties of strength and stability are highly modified.

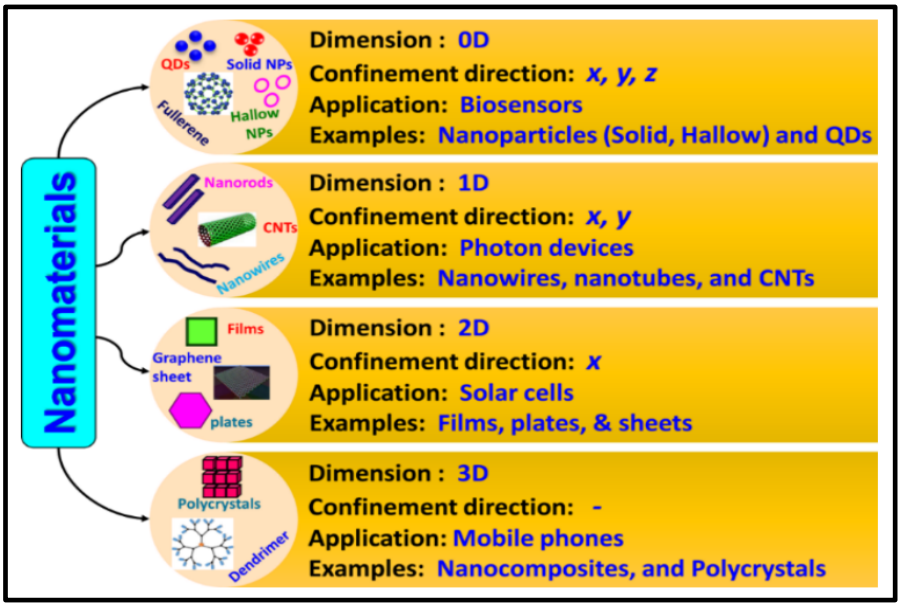



**Figure 1.5** Nanomaterials classification based on dimension and their applications

Figure 1.5 represents the broad classification of nanomaterials on the basis of their dimensions. can be broadly classified by the total number of their nanoscopic dimensions.

With the invention of novel nanomaterials and therefore the development of exquisite nanofabrication tools, there are an oversized development within the field of biosensors within the last 20 years. Particularly, researchers round the world are tailoring a large number of

nanomaterials–based biosensors and developing new strategies to use them in ultrasensitive biosensing.

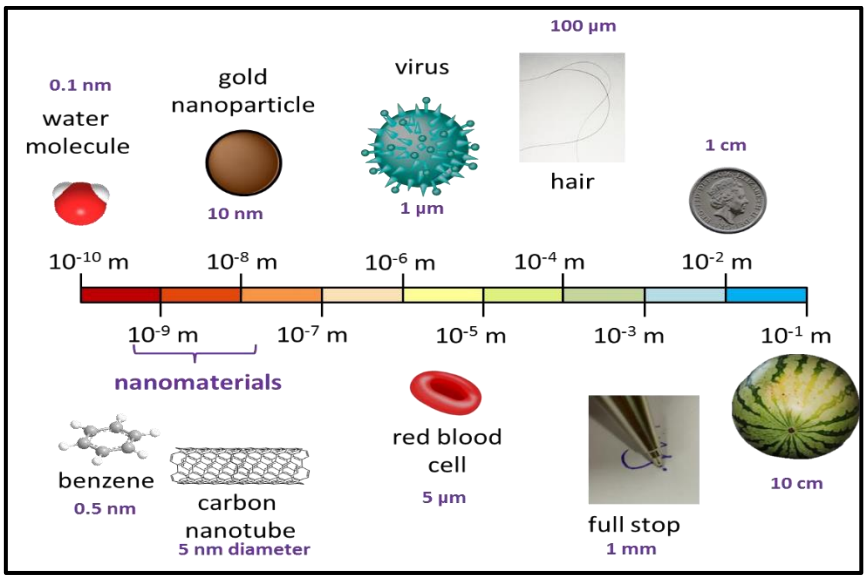



**Figure 1.6** Nanoscale (comparing sizes of various objects)

The NPs play distinct roles in several sensing by providing labelling of biorecognition elements and increasing electron transfer rates within the biosensor to make it sensitive and rapid. The high surface to volume ratio enhances their biomolecular immobilization capacity, making the devices very sensitive which will allow one cell detection which is extremely attractive in monitoring of the disease (Pandey et al., 2008).

## 1.5.STRUCTURE AND OBJECTIVE OF THESIS WORK

The present thesis entails 6 chapters:

**Chapter 1:** This first chapter gives an overview and introduction of biosensors, its elements and types. It also includes the use of nanomaterials, thin film deposition methods in biosensing technology.

**Chapter 2:** This chapter gives insights on properties of ITO and polyaniline and their applications in biosensing. Further it includes review of literature on importance of glucose sensing.

**Chapter 3:** It's the major experimental section that includes detailed procedures followed while synthesizing polyaniline films and immobilizing the PANI fabricated electrode with enzymes.

**Chapter 4:** The results and discussion chapter talk about the Amperometric curves and cyclic voltammogram obtained for comparison of electrodes and effect of glucose concentrations on current.

**Chapter 5:** This chapter has insights on the conclusions derived from the results and future perspectives on the experiments performed.

**Chapter 6:** This chapter includes all the references used for the thesis writing.

**Objectives of the thesis include:**

- Fabrication of polyaniline on ITO coated glass slide (ITO electrode)
- Electrodeposition of polyaniline on ITO electrode through galvanostatic method (at constant current flow)
- Immobilization of Glucose oxidase on Polyaniline fabricated electrode
- Sensing of glucose by using the bioelectrode

# CHAPTER 2

# REVIEW OF LITERATURE

## 2.1. APPLICATIONS AND IMPORTANCE OF ITO IN BIOSENSING

One of the most promising materials in biosensing technologies is ITO (indium tin oxide) electrode, which is colorless and transparent thin sheet. Indium tin oxide (ITO) is comprises of 9:1 solution of indium oxide and tin oxide and several procedures have been used to create ITO thin films, including direct current/radio frequency, magnetron bombardment, ion beam sputtering, electron beam evaporation (EBE), chemical vapor deposition (CVD), and chemical solution deposition (CSD) (Her & Chang, 2017). Because it is easy and inexpensive, the chemical solution deposition (CSD) process, which employs dip or spin-coating, is a popular approach for producing ITO thin films. Its unique qualities have made ITO is an outstanding material that has been extensively used in biosensor investigations, such as –

- Optically transparent nature
- Very High conductivity
- Ability to adhere the substrate
- Low capacitive current, and
- Stable electrochemical and physical features

Because of these distinguishing characteristics, it can be employed in electrochemical research (Khan, 2016). Because ITO sheet is less expensive than other typical electrodes such as gold, silver, and platinum, it is widely used. ITO sheets have been utilized in many investigations, although they have several limitations, such as the growing cost of indium and the need of high temperature during production process.

## 2.2. IMPORTANCE OF GLUCOSE SENSING

Measurement of glucose concentration is not only critical in clinical diagnosis but is also vital for food industry, fermentation reactors, pharmaceutical industries, the textile industry, environmental monitoring, pollution management and diagnostic centers. Glucose is an important carbohydrate and is the main source of energy for metabolic activities in the living organisms. Among the two forms of glucose, L-glucose and D-glucose (figure 2.1) the latter form is utilized by living organisms (Ouellette & Rawn, 2015). The normal glucose level in blood should be 80- 120mg/dl and change in this concentration has adverse effects on health.

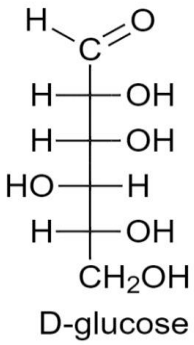



**Figure. 2.1** D - glucose

Additionally, glucose plays major role in fermentation industry as it's a growth limiting substrate which is required for microbial growth (Thakur & Ragavan, 2013). The variations in glucose concertation can affect the yield and quality of fermented products. Hence, glucose monitoring is very crucial. Glucose measurement techniques that is being used currently include "high-performance liquid chromatography (HPLC), polarimetry, capillary electrophoresis, gas chromatography (GC), colorimetry, electrochemiluminescence, and biosensors", but all these are quite expensive techniques (Gamessa et al., 2018). Glucose biosensors have recently gained popularity due to their inexpensive cost, dependability, quick and precise responsiveness. The amperometric glucose biosensor is a substantially superior approach that is quite simple to manufacture in the laboratory.

A glucose biosensor's processing and response is highly dependent on mode of based on enzyme immobilization used to fabricate the enzyme on the surface of the electrode (chip). The surface which is utilized for immobilization should be uniform enough to permit for an even and smoothly passage of an electron from the bioreceptor element to the electrode surface, hence the electrode surface should be highly biocompatible (Yoo & Lee, 2010). It should also improve response qualities and be responsive to stimulation so that a response can be captured appropriately. In this context, intrinsically conducting polymers having self-conjugated systems either with the double or a single bond alternating throughout the polymer chain and this property seems promising for application in biosensing. Continuous conjugation in conductive polymers provides extraordinary electrochemical features such as strong electrical conductivity, low ionization potential, higher electron affinities (K & Rout, 2021).

## 2.3. POLYANILINE AND ITS FORMS

Polyaniline (PANI) is an organic polymer with conductive properties. It belongs to the "semi-flexible rod family of polymers". The presence of unique properties of easy synthesis procedure, stable morphology in environmental conditions and easy doping techniques makes polyaniline extremely important amongst the polymers. During doping and de-doping of polymers , conductivity is gained by the polymer either through oxidation or by reduction process and by using this technique polyaniline can be utilized in any desired application by mixing it with other materials (Prabhakar et al., 2011). Upon making the composites or blends the resultant products exhibit a conductivity range which is comparable to semi-conductors, or they might exhibit a insulation power which is almost equivalent to strong insulating materials. The biggest advantage of using polyaniline is its eco-friendly nature and biodegradability. Polyaniline exists as 3 different oxidation/reduction states (Fig 2.2) when the polymerization process is conducted using aniline as monomer, the three state are mentioned below:

- **Leucoemeraldine** – is also called the full reduced from of polyaniline which exists as a clear or whitish color. This form is known to have quite low conductivity and hence is not very useful in sensing applications.
- **Emeraldine** is referred to as the "emerald base" which is the half-oxidized form of polyaniline and has highest conductivity and strong stability as compared to all the three forms. Hence, it is regarded as the most useful and widely applied form of PANI. It has green to bluish colored appearance.
- **Pernigraniline** is the fully oxidized form of polyaniline which appears blue to violet in color and also referred as "pernigraniline base" (PB).

Conductivity of PB and LB cannot be raised even when subjected to furious doping with acids and this makes them not so useful for the sensing devices (Bhandari, 2018). Additionally, the level of conductivity of PANI is determined and greatly influenced by the technique an methodology used for the synthesis.

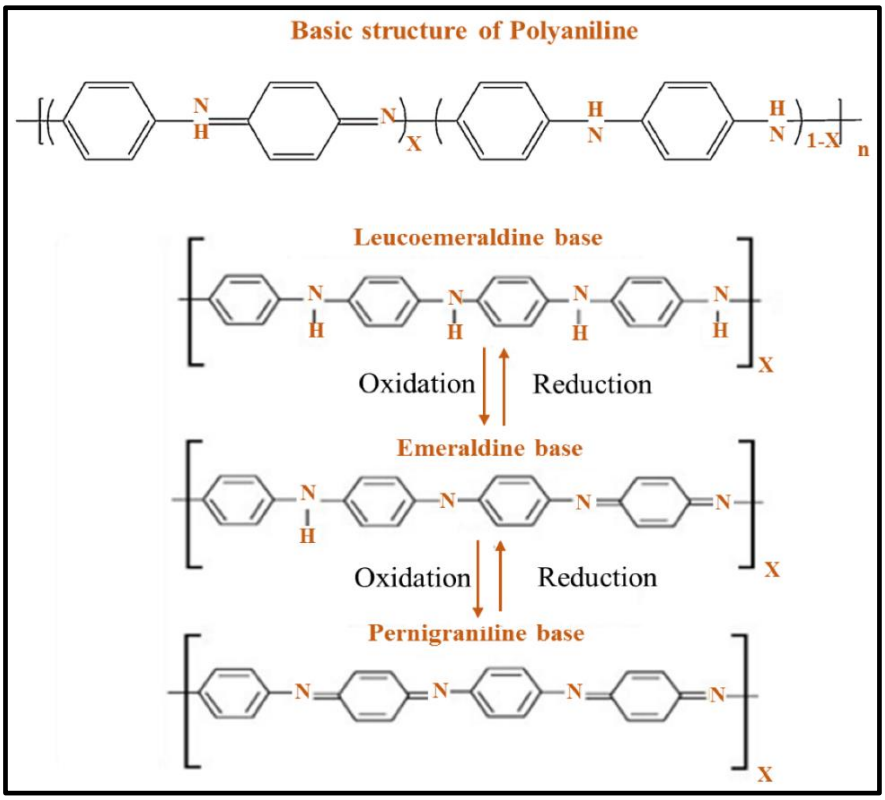



**Figure. 2.2** Basic structure of polyaniline and its 3 forms.

## 2.4. POTENTIAL APPLICATIONS OF PANI

Conductive polymers, as the name suggests exhibit electrically conductive nature and activity and this provides them with inherent unique properties that has made it possible to use them in wide areas of applications (Gerard, 2002). Figure 2.3 shows the biomedicals, sensing and environmental pollution controlling applications of polyaniline due to its highly conductive nature and inexpensive synthesis and biodegradability in environment.

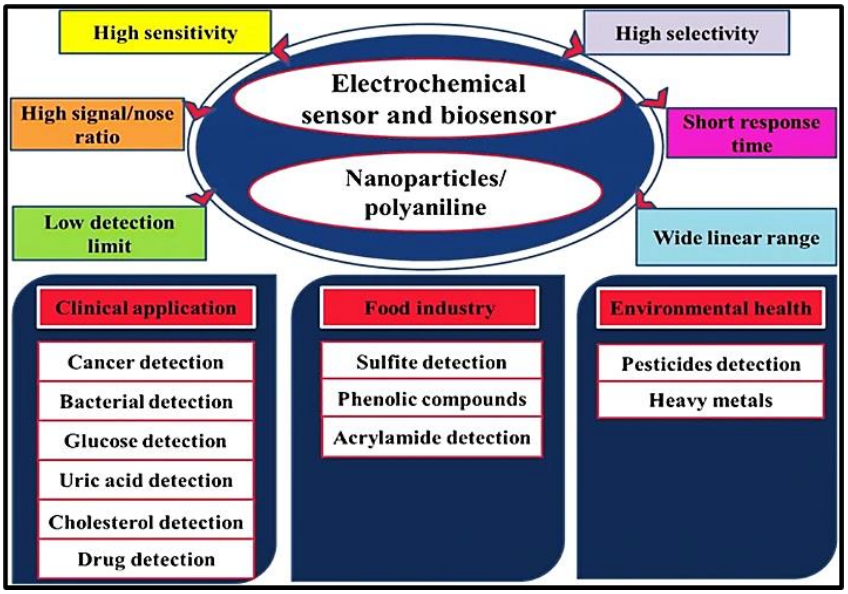



**Figure. 2.3** Potential applications of polyaniline films in biosensing

Presently, medicine is about engineering, and improvements in this field necessitate the development of new intellectual technologies. Neuroscientists seek technologies that compensate for nerve weakening while also contributing to neuroscience progress. Scaffolding has been used to treat organ problems, and biocompatibility conductive scaffolding provides excellent bio-counterfeit qualities. Furthermore, PANI applications have gained a great deal of interest in delivery systems; as a consequence, novel delivery architectures including electro-drug delivery systems are being investigated (Zare et al., 2020). The use of PANI as an anti-corrosion barrier has shown positive results.

Table 2.1. depicts several biological applications of polyaniline and PANI composites along with their method of manufacturing and features.

**Table 2.1**. Biological applications of PANI and its nanocomposites

| Materials used | Method of fabrication | Characteristics | Uses | References |
|---|---|---|---|---|
| Polyaniline (PANI) / Titanium dioxide for ammonia gas sensing | Polymerization, spin-coating | Nanowire features | Sensors | (Pawar et al., 2012) |
| PANI for ammonia sensing | Chemical oxidation polymerization | High sensitivity and reliability | Gas Sensor | (Liu et al., 2021) |
| Cyanide detection by PANI /catalase | Electropolymerization | Storage ability and reproducibility | Sensor | (Özcan & Aydin, 2016) |
| PU-PANI nanofibrous scaffolds | Electrospinning method | Electrical conductivity, biocompatibility | Scaffolds | (Ghorbani et al., 2020) |
| Cholesterol oxidase and polyaniline for cholesterol sensing | Electropolymerization | High conductivity and easy synthesis | Enzymatic biosensor | (Shukla et al., 2015) |

### 2.4.1. Sensors

In recent years PANI has got a huge amount of interest for use in sensors. It exhibits remarkable architectures with varied morphologies that includes nanowires, nanoflowers and nanotubes. Majorly being employed in gas-based and volatile compound-based biosensors like H2S, ammonia, hydrochloric acid, carbon monoxide, methanol, and chloroform and others (Patni et al., 2017). PANI gets easily swelled up and shows doping with additional features of getting easily reduced to change its structure when it comes in contact with sch chemicals and this property of polyaniline is being exploited while manufacturing the sensors for detection of these compounds.

**Electronic tongue:** Sensing has reached a farther stage where PANI can be used as sensors which becomes an electronic tongue. This electronic tongue involves a combination of approximately six to seven sensors which have the same efficacy to detect the different types of tastes as human tongue receptors. This is a huge breakthrough for the food industry. This electronic tongue is highly beneficial for the food business and can be utilized to improve food value, taste and quality without the need of human testing (Riul et al., 2003). The procedure of the taste detection is equivalent to signal generation in sensors (for instance potentiometric changes) and the integration of the taste information with the sensor is used to form a fingerprint of a particular taste, such as:

- Presence of hydrogen ions will indicate citric acid
- Sodium chloride can indicate salty taste
- Presence of sugar designates sugariness
- Magnesium chloride recognizes the bitterness of chemical composites (such as caffeine).

### 2.4.2. Medical Applications

Polyaniline is most significant conductive polymer with a great potential for application in biomedical field due to the unique properties of higher conductivity and biocompatibility which is achieved by the hydrophilic nature, less toxicity, higher stability and nanostructred morphology of PANI (Stejskal et al., 2014). Major applications of PANI-based nanocomposites in the medical field consist of neural prosthetics, engineered tissue/scaffolds, and drug-delivery vehicles (Fig 2.4). Here, the foremost biomedical applications of PANI are described.

**Neural Prosthesis/Biotic–Abiotic Interfaces**: People with disorders related to the central nervous system are unable to speak, move, or regulate their surroundings. To compensate for such deficiencies, brain–computer edge (BCI)/brain–machine interface (BMI) through neural prosthesis might be proposed. The neural prosthesis uses PANI and its composites and converts neural electrodes, neural tissue, and bioelectric impulses to electrical signals (Zhou et al., 2009).

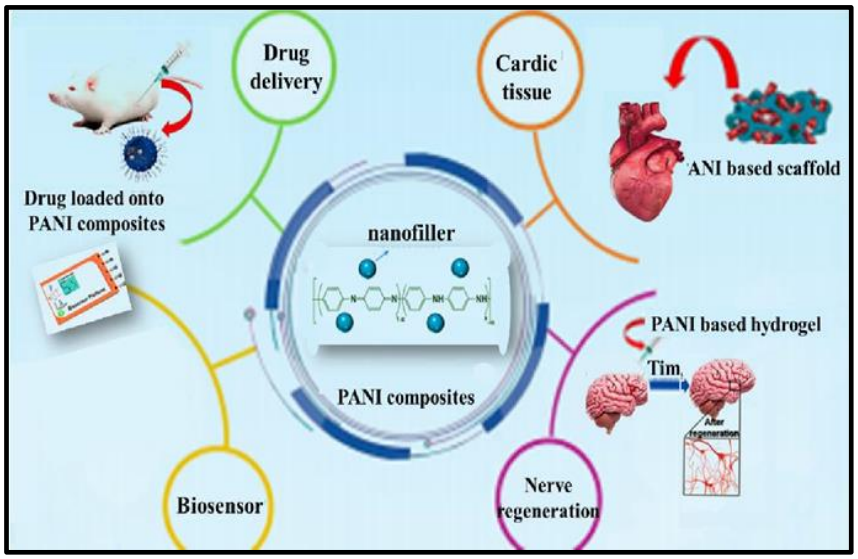



**Figure. 2.4** Schematic of PANI in medical applications

**Scaffolds**: Discovery of synthetic and prosthetic structures have indeed moved the global life quality towards an upward trajectory. Tissue engineering can play substantial role in the formulation of novel materials that actually can mimic the functions of real organs (Fortunato et al., 2018). To lessen the danger of rejection in the body, implanted scaffolds should be biocompatible and polyaniline and its composites with other materials exhibits unique properties of being antioxidant. Additionally they posse some, antibacterial properties and exhibit biocompatibility, and hemostatic characteristics which are vital for all the engineered tissues and scaffolds (Qazi et al., 2014).

**Delivery Systems**: During drug delivery the most difficult task is to deliver the drug in a smartly organized and specifically targeted manner. Release of drug in the body is highly dependent upon the location and surrounding conditions , however the electroactive behavior and biocompatilbity of conducting polymers like polyaniline can make it possible to release the drug based upon appropriately chosen conditions and locations inside body (Guo et al., 2013).

### 2.4.3. Applications of PANI in pollutant removal

Adsorption using polyaniline composites is emerging as a potential method for removal of synthetic or organic dyes as pollutants from aqueous solutions. The adsorption of adsorbate onto polyaniline is indeed a unique process since it is dependent on its cationic property. Polyaniline's amino group undergoes protonation, allowing it to adsorb dye molecules via several types of contact mechanisms and demonstrating increased anionic dye adsorption (Zarrini et al., 2017). Many polyaniline composites have demonstrated exceptional ability for dye removal, including methylene blue, direct red 23, acid green, congo red, reactive orange 16, reactive violet, reactive blue, diamond green dye, coomassie brilliant blue, acid red G, and remazol brilliant blue R. This dye removal is made possible by electrostatic interactions, hydrogen bonding, and π-π interactions, which enhance dye absorption on polyaniline composites (Bhadra et al., 2020).

# CHAPTER 3

# EXPERIMENTAL SECTION (MATERIALS AND METHOD)

## 3.1.MATERIALS USED

Sodium phosphate monobasic (NaH2PO4)( Acros organics),Sodium phosphate dibasic (Na2HPO4) (Merck),Sodium Chloride (NaCl) (Sisco research laboratories),Sodium hydroxide(NaOH), Hydrochloric acid (HCl) ( Merck), Ethanol (C2H5OH) (Omnis), Distilled water (Mili-Q, USA), Hydrogen Peroxide (Sigma Aldrich) , ITO coated glass substrate, Ammonia (NH3), Aniline (C6H5NH2) (Fisher scientific), Sodium sulfate, PBS buffer, Potassium ferrocyanide (K4[Fe(CN)6]), Potassium ferricyanide (K3[Fe(CN)6), Horseradish peroxidase enzyme, D-Glucose, Glucose oxidase enzyme (*Aspergillus niger)* (Sigma Aldrich) etc.

## 3.2. EQUIPMENT USED

Round bottom flask, Condenser, Burner, Glass Funnel, Whatman filer paper, measuring cylinders, Micropipette, Standard flasks, Beakers, Electrochemical cells for CV (cyclic voltammetry), Petri dishes of various sizes, Diamond cutter, ITO, Hot air oven, contact angle, Multimeter, Weighing balance, pH meter, Origin Pro 8.5 software.

## 3.3. METHODOLOGY

### 3.3.1. Preparation of buffer solutions

Preparation of all the buffers was done at pH 7.4 because it's the pH of human blood and during the in vitro experiments it was intended to maintain the pH similar to human blood (Constable, 2009). All chemicals used were of analytical grade.

**Preparation of PBS buffer**

- To make PBS buffer 2 solutions are needed that are – Sodium phosphate monobasic and sodium phosphate dibasic.
- Concentration calculations were done and Monobasic concentration was prepared by dissolving sodium phosphate monobasic 1.2 g in 50 ml distilled water.
- Dibasic concentration was prepared by adding 1.4 g of Sodium phosphate dibasic in 100ml distilled water.
- 9.5 ml volume was taken from Monobasic stock and 40.5 ml form dibasic stock solution and 1.6 g NaCl were added to 50ml water to make a 0.1M PBS buffer.

- pH meter was used to check the pH and adjusted to 7.4.

**Preparation of ferro-ferri buffer**

- Calculations were done for the required molar mass of Potassium ferricyanide and Potassium ferrocyanide by using the molarity equation.
- 184 mg of Potassium ferrocyanide and 164 mg of Potassium ferricyanide were added to 100ml PBS budder to make a PBS-ferro-ferri buffer of 5mM concentration.
- This buffer was used in the electrochemical cell while recording CV responses**.**

### 3.3.2. Overall Procedure

The ITO coated glass substrate of 2 cm x 1 cm was used as a working electrode. Prior to film deposition, the ITO substrate was made contamination free by hydrolysis method and further cleaning was done with ethanol and distilled water (Bermudez et al., 2006). After washing ITO were dried and subjected to polyaniline film deposition through electro-polymerization of aniline using Potentiostatic approach. The deposited PANI/ITO films were further immobilized with 5 microliter glucose oxidase enzymes through physical adsorption along with 10 microliter HRP. After immobilization, glucose stock solution was prepared in PBS buffer and left for muta-rotation for 24 hours (Ouellette & Rawn, 2018). Sensing of glucose was done at varying concentrations i.e. from range 50 mM to 20 mM. Figure 3.1 shows the block diagram of overall procedure followed for the experiments.

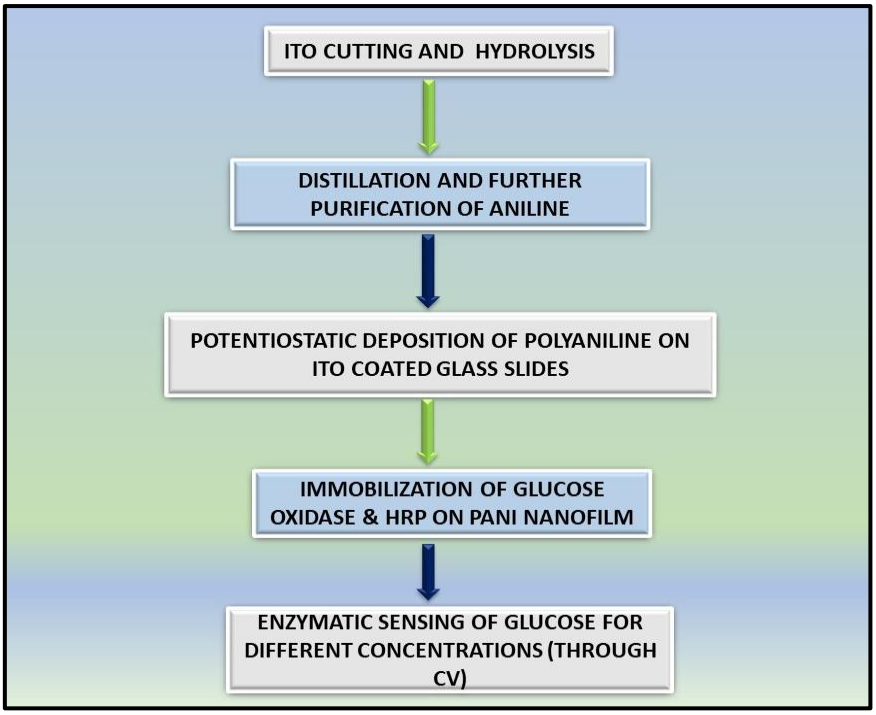



**Figure 3.1** Overall procedure summary followed for the experimental section

### 3.4.Cutting and Hydrolysis of ITO coated glass

- Large ITO slide was taken and marked with desired dimensions. The Cutting of ITO (2x1 dimensions) was done using a diamond cutter pen.
- ITO glass slides were cleaned with ethanol by dipping the films in ethanol containing petri dish and after cleaning they were dipped in distilled water.
- Solution for ITO hydrolysis was prepared by mixing distilled water. Hydrogen peroxide and ammonia (5:1:1= Di water:H2O2: Ammonia)
- ITO coated glass slides were dipped in the prepared solution in petri dish and the petri dish was covered properly.
- The petri dish was placed in incubator at 80degrees for 30 minutes. After 30 minutes, the petri dish coating ITO coated glass slides were taken out with precaution and were allowed to cool.
- After cooling, all the slides were washed with distilled water.
- Slides were taken out and the water from the films was soaked with the help of a tissue paper.
- The slides were left for drying overnight (The slides could have been dried by keeping in oven for again half an hour at 80 degrees Celsius.)

Hydrolysis of ITO is done so that the ITO can become active with the OH- group and can become more hydrophilic for the films to be deposited on it. The figure 3.2 below shows ITO coated glass slides in a petri dish after hydrolysis and drying.

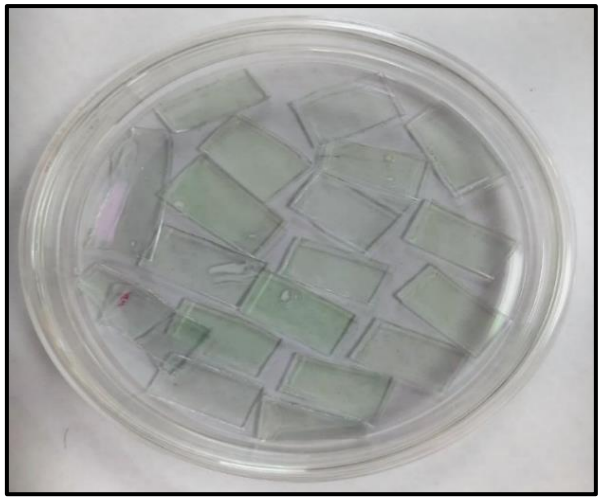


**Figure 3.2** ITO films after hydrolysis and drying in oven

### 3.5.DISTILLATION OF ANILINE

Distillation is a separation technique that is used in chemistry, industry, and food science which involves the separation of all the components of a mixture depending on their boiling points and condensing abilities. Distillation is used for a variety of purposes and in various industries like alcohol purification, salt removal and oil refining etc. Figure 3.3 depicts the distillation apparatus and the way it should be arranged to obtain the distillate properly.

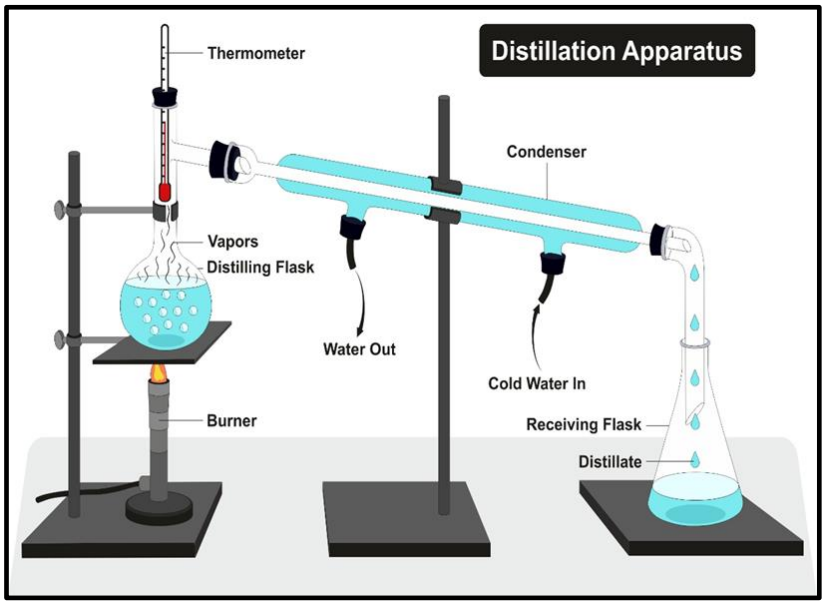



**Figure 3.3** Diagrammatic Representation of steam distillation

**Procedure for aniline distillation**

- Distillation apparatus was set up as shown in figure 3.4.
- 20ml DI water and 6 ml aniline were added to the round bottom flask and the burner was turned on.
- As the temperature raised the contents of the round bottom flask started boiling and via the condenser distilled aniline was collected into a flask eventually**.**
- The distilled aniline had certain amounts oof water along with it.
- To remove the water, the distillate was filtered by using sodium sulfate on Whatman filter paper. Thus, obtained aniline was pure and free from water.
- Distilled aniline was colorless and free from water.
- The flask containing freshly distilled aniline was covered with aluminum foil and kept in refrigerator at 4 degrees Celsius for further experiments**.**

**Figure 3.4** Distillation apparatus (Source: Biomedical section, NPL-CSIR)

### 3.6.CV (Cyclic voltammetry) (Potentiostat / Galvanostat)

Electrochemical deposition is a method of synthesis which involves the synthesis of a thiin filmof a material over an electrically conducting surface such as electrode (ITO). Three electrodes named working, counter and reference electrodes are major components of this technique (Rodriguez & Tremiliosi-Filho, 2013). Electrochemical deposition is done in a three-electrode, single compartment glass cell using a potentiostat and the electrodes are –

- **Working electrode –** at the working electrode reaction of the electrochemical system occurs. The working electrode can eb a cathode or anode subject to the reduction or the oxidation reaction. Different kinds of working electrodes can be employed in an electrochemical senor like – ITO (Indium tin oxide) glass electrode, glassy carbon electrode, multiwalled carbon nanotube, gold electrode, Platinum electrode.
- **Counter electrode** – Also known as auxiliary electrode. If the working electrode is cathode, then counter electrode behaves as anode and the opposite of this is also valid. The counter electrode has a larger surface area as compared to the working electrode and inert materials are chosen to make the counter electrode such as platinum.
- **Reference electrode –** This electrode has constantly maintained potential which is utilized by other electrodes in the system for measurement. Reference electrodes used in electrochemical cell includes Ag/AgCl electrode, hydrogen electrode and calomel electrode.

Electrochemical deposition and measurements are carried out usually at room temperature (25˚C). The growth of nanoparticles/polymers (or any other fabrication material) on the ITO coated glass substrates by using electro chemical deposition method are observed by cyclic voltammetry. A schematic diagram of a three-electrode, single compartment cell used for CV experiments is represented in the Figure 3.5 and 3.6.

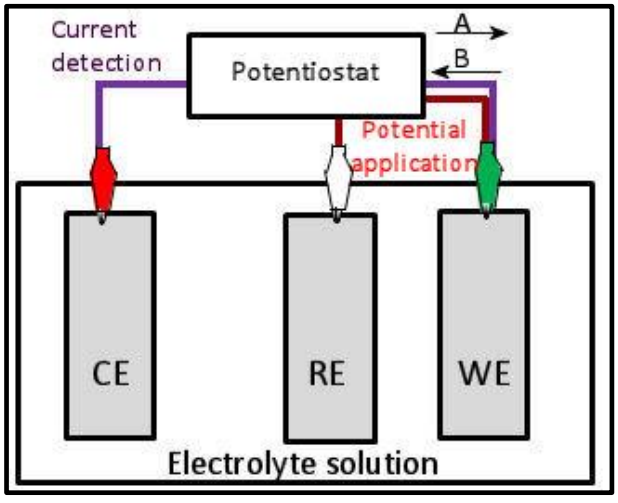
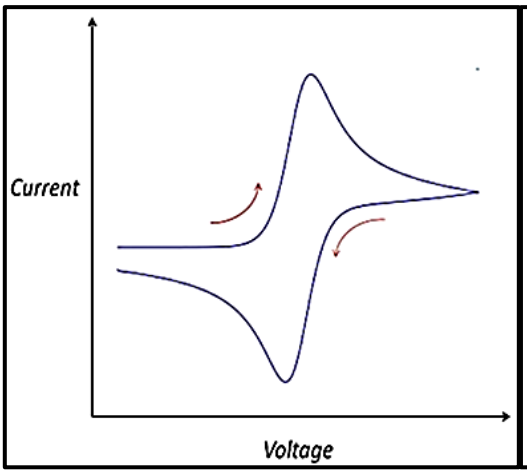



**Fig. 3.5.** Schematic representation of an electrochemical cell for CV experiments

(a) (b)

**Figure. 3.6** Represents a electrochemical cell (a) side view (b)top view

**Cyclic Voltammetry -** It is an immensely popular electro chemical technique due to its efficiency. Even though, CV is mostly used to explore the oxidation and reduction of molecules but it is also used to study catalysis and other chemical reactions 9majorly the reactions which are initiated due to electron transfers). The traces in Figure 3.7 are known as cyclic voltammogram. The x and y axes represent the applied potential (E) and the resulting current that is passed respectively (*EC lab manual*).



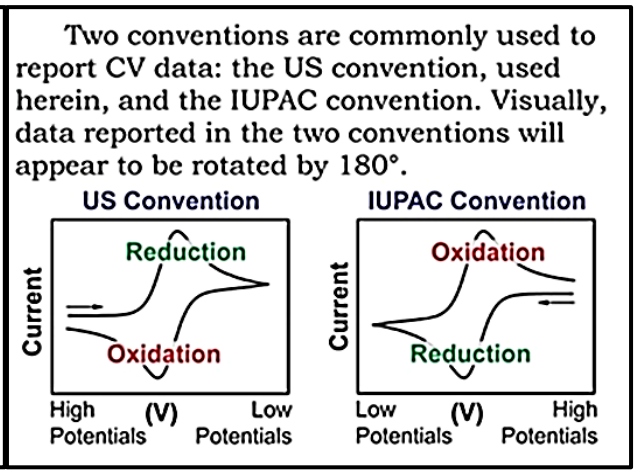




**Figure 3.7** Cyclic voltammogram **Figure 3.8** Two conventions of reporting CV data

Electro-polymerization has several advantages in polyaniline thin film synthesis such as- inexpensive way to make thin films, used to make thin multilayers of wide range of materials, with a provided ease of deposition at any temperature.

## 3.7.POLYANILINE FILM SYNTHESIS BY ELECTROPLYMERIZATION METHOD

Galvanostat is used to maintain constant current flow rate in the electrochemical cell. The current is kept constant and spontaneously changes in the voltage of the working electrode are measured with time. Due to the constant current application, the fabricating material monomers get polymerized on the working electrode surface. Prior to the thin film deposition of aniline, ITO substrates were hydrolyzed to increase their hydrophilicity and remove impurities. During the electrochemical synthesis of polyaniline, there is requirement of 3 reactants that includes aniline, oxidant and acidic medium.

**Working of electrochemical deposition (CV) -** Hydrolyzed ITO coated glass plates are taken and their conductive sides are detected with the use of a multi- meter. These ITO glass substrates are hydrolyzed with ammonia, DI water and hydrogen peroxide. Then these are cleaned with DI, acetone and left overnight for making them activated for use as working electrode in the EC. "Platinum is kept as the counter/auxiliary electrode while Ag| AgCl behaves as the reference electrode". Further, the deposition or growth of polyaniline on ITO coated glass electrode substrates with the application of electrochemical deposition method is observed by using cyclic voltammetry (Goswami & Mahanta, 2021). The cyclic voltammogram of these PANI- modified electrode is observed to have a characteristic oxidation and reduction peak as of PANI.

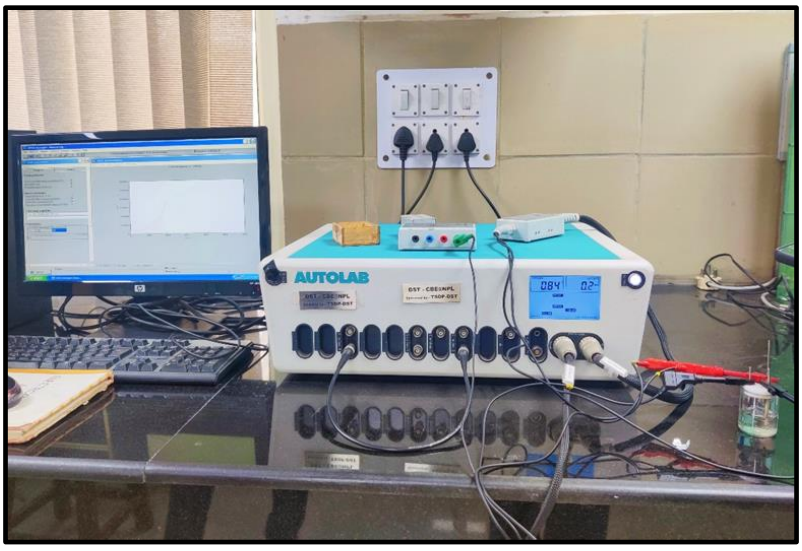



**Figure. 3.9** Potentiostat – Galvanostat (Autolab)
[Source: Biomedical instrumentation section, NPL-CSIR]

**Procedure for polyaniline nano-film deposition**

- ITO coated glass slide was added to the 3-electrode system and was placed in the cell containing a 100ml solution of 1M HCL and 0.3 M freshly distilled aniline.
- All the electrodes were connected with the potentiostat-galvanostat (Fig 3.9). Using the GPES software of the instrument the constant current was set to 0.15 A.
- 150mA of current was applied to deposit PANI on ITO conductive glass. This current was applied at time intervals of 1min, 2min, 3 min and then for 5 minutes.
- Different ITO glass slides were used for different time interval.

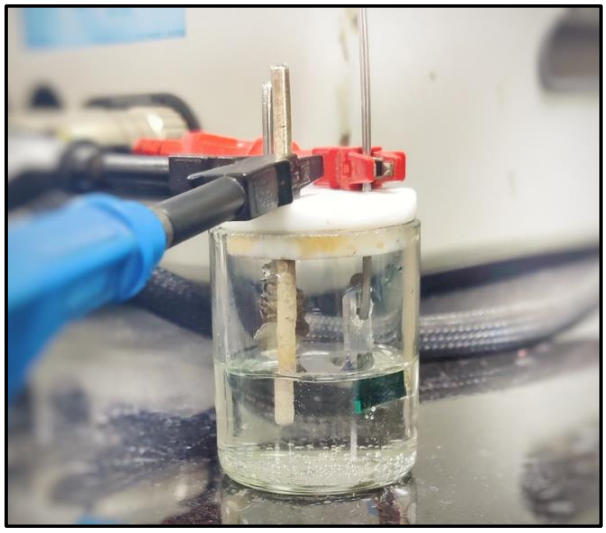


**Figure 3.10** Green colored polyaniline film deposited on the working electrode inside the electrochemical cell

**Table: 3.**1 Potentiometric deposition of aniline with time variations

| **Deposition time** | **Color of film** | **Polyaniline form** | **Constant current** |
|---|---|---|---|
| 1 min | Green | Emeraldine | 0.15 A |
| 2 min | Green | Emeraldine | 0.15 A |
| 3 min | Dark green | Emeraldine | 0.15 A |
| 5 min | Bluish - Violet | Pernigraniline | 0.15 A |

The initially deposited polyaniline films were green in color depicting the emeraldine base form of PANI while in later stages the color of films obtained was dark bluish to violet that indicated the presence of pernigraniline from of PANI as shown in Figure 3.11.

**a)** **b)** **c)**

**Figure 3.11** Showing comparison of a) Bare ITO with b) and c) Deposited PANI films at constant current and varying time interval

Since, the conductivity of pernigraniline is lower than emeraldine thus, for further experiments of enzyme immobilization and glucose sensing, PANI/ITO electrode with green color were utilized after washing with distilled water to remove the aniline monomers and oligomers from the electrode surface. The obtained PANI coated ITO slides were placed in a petri dish and stored at 4 degrees Celsius.

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1. IMMOBILIZATION OF ENZYME ON PANI FILMS

**Preparation of solutions –** 1grams of glucose was weighed and mixed with 1ml 0.1M PBS to make a stock solution of glucose for sensing. This stock solution was left for muta rotation for 24 hours at 4 degrees before use. Dilutions of varying glucose concentrations were prepared from the stock solution (50mM, 1000mM, 150mMand 200mM). PBS buffer (0.1M, 100ml, pH 7.4) was prepared and 5mg of glucose oxidase was added to 1ml PBS to make the stock solution of the enzyme**.**

**Enzyme Immobilization**

- The glucose oxidase enzyme was immobilized on PANI/ITO through physical adsorption method.
- For immobilization large petri dish was filled with water and the smaller petri dish was placed onto the water.
- The solution of enzyme mixture (10 microliter HRP and 5 microliter glucose oxidase) was added to an Eppendorf tube.
- PANI/ITO were placed on the smaller petri dish and the mixture of enzymes was immobilized over the material.
- The smaller petri plate was covered with glass funnel to set up the humid chamber and left undisturbed.
- After 4 hours the funnel was removed carefully and the immobilized slides (GOx/PANI/ITO) were stored in a cool place.
- Immobilized films were stored at 4 degrees Celsius for further use in sensing.

## 4.2.ELECTROCHEMICAL CHARACTERIZATION OF THE SYNTHESIZED FILMS

For the electrochemical characterization of the deposited PANI films, CV was performed for each film. For this the PANI-ITO slide was added to the 3-electrode system and was placed in the cell containing a solution of PBS buffer and Redox species (Potassium ferricyanide and potassium ferrocyanide). CV was taken at scan rate of 0.5Volt (50mv) and voltage was set from 7 to -7V.

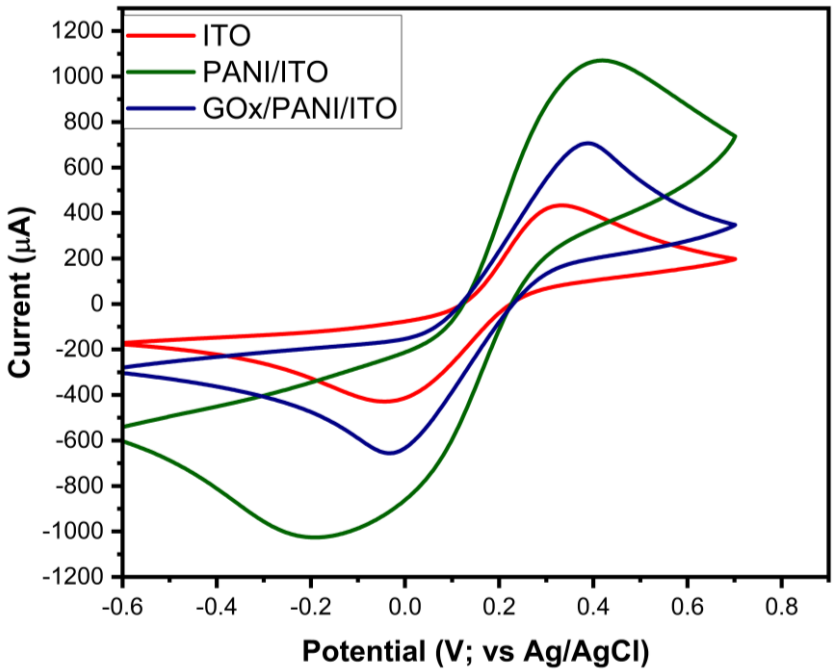



**Figure 4.1** Cyclic voltammogram of bare ITO, PANI/ITO and GOx/PANI/ITO

CV was performed using 3 working electrodes consecutively for 3 types of electrodes which are – ITO, material electrode and immobilized electrode**.** Cyclic voltammetric responses were recorded for all 3 electrodes as shown in figure 4.1. The red line represents ITO, green line shows the PANI/ITO electrode and the blue line shows the enzyme immobilized bioelectrode. The Potential v/s Current graph obtained in fig shows that the conductivity increased after Polyaniline fabrication and further increased upon enzyme immobilization. This increase in conductivity was because polyaniline is a conductive polymer and its nanofilm provided a high surface area to value ratio for enzyme immobilization. Upon enzyme immobilization the conductivity decreased because of overloading of proteins(enzymes) on the conductive surface. These proteins apparently responsible for the resistance that lowered the conductivity.

### 4.3.BIOSENSING OF GLUCOSE USING GOX/PANI/ITO

Glucose concentrations were diluted from stock of 1g/ml and following dilutions were prepared using PBS buffer pH 7.4– 50mM, 1000mM, 150mM and 200mM. The apparatus was set up for CV and PBS buffer (containing potassium ferrocyanide and potassium ferricyanide) was added too the cell. Working electrode (bioelectrode – GOx/PANI/ITO) was placed in the cell and all electrodes were connected carefully. Using the GPES software graphs were obtained for each different concentration of glucose. Figure 4.2 shows the cyclic voltammograms obtained for different glucose concentration.

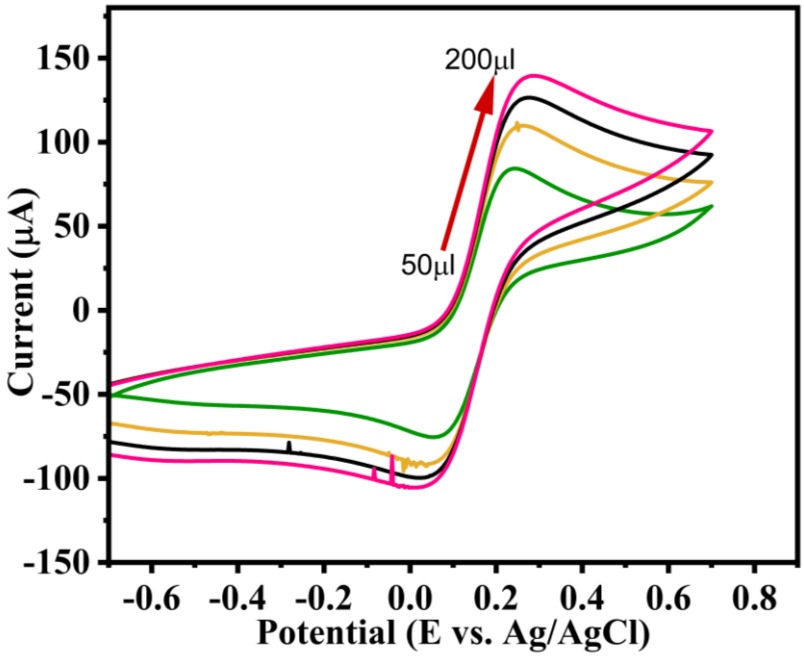



**Figure 4.2** Cyclic voltammogram of polyaniline/GOx film at various glucose concentrations

Cyclic voltammetric (CV) responses were obtained to evaluate the electron transfer phenomena of the fabricated PANI/ITO and PANI/GOx/ITO electrodes. As shown in figure 4.2, the scan rate was held constant at 0.5 V, and the glucose content was varied by dilution of the glucose stock solution in 0.1 M PBS with pH 7.4. The green line represents 50ul, the yellow line represents 100µl, the blue line represents 150µl, and the pink line shows the oxidation/reduction peaks for 200µl glucose. It is apparent that the current has grown greatly in parallel with the increase in glucose quantity. The direct electrochemistry of GOx immobilized in the PANI/GOx/ITO electrode revealed distinct glucose oxidation peaks. As a result, the glucose sensing process may be described as follows:

$$\boldsymbol{GOx(FAD) + Glucose \rightarrow GOx(FAD2) + Glucono - d\ lactone} \quad \textbf{(1)}$$

$$\boldsymbol{GOx(FADH2) + O2 \rightarrow GOx(FAD) \rightarrow H2O2} \quad \textbf{(2)}$$

The Glucose Oxidase (GOx) enzyme is made up of "two identical protein subunits and one flavin adenine dinucleotide (FAD) coenzyme molecule present in the GOx enzyme's active region". FAD is a cofactor which supports the electrochemical reversibility of this enzyme (Li et al., 2015). FAD may be converted to FADH2 via a two-electron, two-proton mechanism.

The biochemical interaction between GOx and glucose results in the "oxidation of glucose to glucono-d-lactone" and the reduction of "FAD to FADH2". Following that, FADH2 is oxidized by O2 present in the solution, creating H2O2 and transforming into FAD2 (Bauer et al., 2022). The following equation may be used to represent these processes:

$$\boldsymbol{Glucose + O2 \overset{GOx}{\rightarrow} Glucono - d - lactone + H2O2}$$

## 4.4.CURRENT RESPONSE OF BIOELECTRODE WITH CHANGING GLUCOSE AMOUNT

Current values for all the oxidation peaks were noted and plotted against the respective concentrations to obtain the linear graph (amperometric calibration curve).

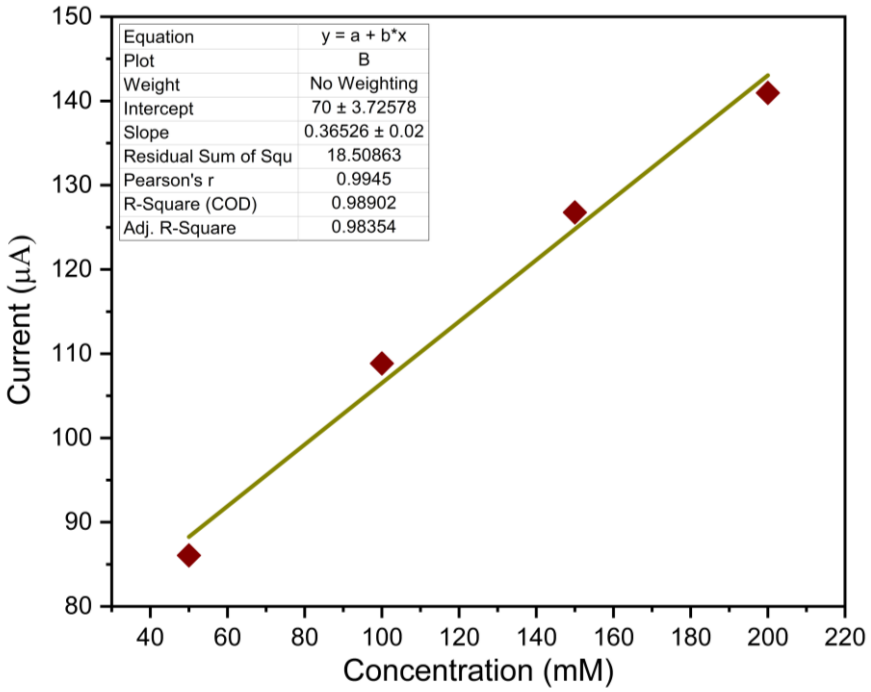



**Figure. 4.3** Amperometric response of polyaniline/GOX films obtained as a function of glucose concentration

Figure 4.3 demonstrates the calibration plot of the PANI/GOx/ITO electrode constructed PANI/GOx/ITO electrode towards varying concentrations of glucose from 10 mM to 50 mM in the supporting electrolyte 0.1 M PBS (pH 7.4). Amperometric i v/s t (current versus time

interval) curves for the PANI/GOx/ITO bioelectrode at a constant voltage of 0.5 V was plotted. The Amperometric response of the constructed PANI/GOx/ITO electrode toward the detection of glucose was noted and plotted. The concentration of glucose in the 0.1 M phosphate buffer (pH 7.4) solution was increased systematically (50mM, 100mM, 150mM and finally 200mM of glucose concentrations were used in the study). A general trend of increasing current with increasing glucose concentration clearly validates the sensitivity of the constructed PANI/GOx/ITO electrode to varying glucose concentration. The steady-state calibration curve for PANI/GOx/ITO electrode exhibits a linear range from 50 mM to 200 mM concentration of glucose.

# CHAPTER 5

# CONCLUSION AND FUTURE PERSPECTIVES

## 5.1. CONCLUSION

Major conclusions that can be derived from the experiments conducted for this thesis are:

Polyaniline films were successfully deposited using potentiometric method (by applying constant current at varying time intervals). The characterization of the deposited PANI films showed that the films have high conductivity as compared to bare ITO coated glass slides. The Glucose oxidase (GOx) enzyme gets adsorbed nicely on PANI by electrostatic interactions because it possesses negative charge. This kind of entrapment during the immobilization process not only maintains the accessibility of catalytic sites but also prevents the enzyme from getting detached from the electrode surface. CV results inferred that conductivity increased in the following order-

**ITO < PANI/ITO > GOx/PANI/ITO**

The decreased conductivity of bioelectrode can be due to resistance developed as a result of the protein overloading on the surface. While, higher conductivity of polyaniline fabricated electrode indicates the unique properties of PANI. Current value increased in following order -

**50mM < 150mM < 100mM < 200mM**

Apparently, there is increase in current with respect to the change in glucose concentration and it clearly signifies the sensitivity of the electrode towards changing glucose concentration**.** The fabricated bioelectrode exhibits a linear range of glucose concentration from 50mM to 200mM, when plotted in the calibration curve.

## 5.2. FUTURE PROSPECTS

In these experiments the sensitivity and reliability of the synthesized electrode could not be tested due to time boundations. Consequently, there is high scope for further analysis. Additionally, the characterization of the synthesized electrodes can be further done by various sophisticated techniques including SEM, TEM, XRD, AFM, FTIR and others. Following steps can be done for future studies:

- Conductivity and sensitivity of PANI can be amplified by doping and composite making.
- Glucose control is major issue while designing of PANI based glucose biosensors, so it should be tested thoroughly.
- Further analysis can be done on sensitivity and detection limits for the GOx/PANI/ITO electrode.

# CHAPTER 6

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Acceptance Letter for Review article " Microplastics accumulation in agricultural soil: Evidence for the presence, potential effects, extraction and current bioremediation approaches" in **Journal of Applied Biology and Biotechnology**

February 17, 2022

Dear Jai Gopal Sharma

I am pleased to inform you that your manuscript titled "Microplastics accumulation in agricultural soil: Evidence for the presence, potential effects, extraction, and current bioremediation approaches" (Manuscript Number: JABB-2021-12-803) is accepted for publication in the Journal of Applied Biology & Biotechnology.

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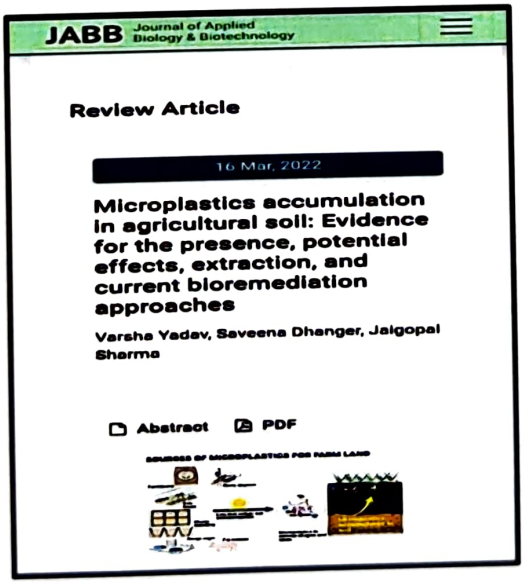
## CANDIDATE'S DECLARATION

I, hereby declare that the work which is presented in the Major Project entitled "**SYNTHESIS OF NANO - POLYANILINE FILM BY ELECTROCHEMICAL POLYMERIZATION AND ITS APPPLICATION IN GLUCOSE SENSING** "in fulfillment of the requirement for the award of Degree of Masters in Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my own work, carried during a period from 03-Jan-2022 to 06-May-2022, under the supervision of internal supervisor **Prof. Jaigopal Sharma, Delhi Technological University and external supervisor Dr. Gajjala Sumana, CSIR-NPL.**

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other university. Furthermore, for the partial fulfillment of project work, I communicated a review paper entitled "**Microplastics accumulation in agricultural soil: Evidence for the presence, potential effects, extraction and current bioremediation approaches**" in Scopus indexed journal "Journal of applied biology and biotechnology" that got accepted and will be published soon. Below is the insight for the same: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Review Article

16 Mar, 2022

Microplastics accumulation in agricultural soil: Evidence for the presence, potential effects, extraction, and current bioremediation approaches

Varsha Yadav, Saveena Dhanger, Jaigopal Sharma

Abstract PDF

**VARSHA YADAV (2K20/MSCBIO/35)**

**DELHI TECHNOLOGICAL UNIVERSITY (FORMERLY DCE)**

SHAHABAD DAULATPUR, MAIN BAWANA ROAD, DELHI - 110042

## CERTIFICATE - I

This is to certify that **Ms. VARSHA YADAV** (ENROLLMENT No. 2K20/MSCBIO/35) has carried out the major project contained in the thesis entitled **"SYNTHESIS OF NANO - POLYANILINE FILM BY ELECTROCHEMICAL POLYMERIZATION AND ITS APPPLICATION IN GLUCOSE SENSING"** as a partial fulfillment for the award Masters in Science degree in Biotechnology by Delhi Technological University. The assistance and help received during the course of investigation has been fully acknowledged.

Date: 06- 05- 2022

Place: DTU, New Delhi

**Prof. Jaigopal Sharma (Internal Supervisor)**

This is certified that the Thesis Viva-Voice examination of **Ms. Varsha Yadav** (2K20/MSCBIO/35) has been held on dated **06-may-2022** and the thesis has been accepted for the award of the degree.

06/05/2022

**Prof. Pravir Kumar**

**(Head of the Department)**

Department of Biotechnology

DTU, Delhi-110042

**Prof. Jaigopal Sharma**

**(Supervisor)**

Department of Biotechnology

DTU, Delhi -110042

Department of Biotechnology
Delhi Technological University
Bawana Road, Delhi-110042

CSIR - NPL, Dr. KS Krishnan Marg, Delhi -110012

## CERTIFICATE - II

This is to endorse that the Project entitled **"SYNTHESIS OF NANO - POLYANILINE FILM BY ELECTROCHEMICAL POLYMERIZATION AND ITS APPPLICATION IN GLUCOSE SENSING"** submitted by **Ms. VARSHA YADAV**, Enrollment no. **2K20/MSCBIO/35** in partial fulfilment of the requirements for the award of degree of **Masters in Biotechnology** from **Delhi Technological University** has done her dissertation work from **NPL(CSIR)** and it is an authentic work carried out by her under my supervision and guidance.

Duration 2/3/2022 to 5/5/2022

**Dr. Gajjala Sumana**

**Senior Principal scientist**

**(External supervisor)**

**(National Physical Laboratory)**

**CSIR**

NATIONAL PHYSICAL LABORATORY
(HRD GROUP)
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डॉ. रीना शर्मा / Dr. RINA SHARMA
प्रमुख, मानव संसाधन विकास समुह
Head, Human Resource Development Group
सी.एस.आई.आर. -राष्ट्रीय भौतिक प्रयोगशाला
CSIR - National Physical Laboratory
डॉ. के. एस. कृष्णन मार्ग, नई दिल्ली-110012
Dr. K. S. Krishnan Marg, New Delhi-110012

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I would also like to express my hearty felicitation to my internal supervisor, **Prof. Jaigopal Sharma**, DTU for his continuous motivation and guidance while writing the review entitled "**Microplastics accumulation in agricultural soil: Evidence for the presence, potential effects, extraction and bioremediation approaches**" for the partial fulfillment of the project work required during masters. Also, I would thank **Ms. Neha Tiwari** (Senior research scholar) at DTU for guiding and helping regarding review submission and revision process. I am highly grateful towards my internal supervisor for allowing me to pursue my dissertation at **CSIR-NPL.**

I would like to express my deepest gratitude to my External Supervisor, **Dr. Gajjala Sumana, Principal scientist** at **Biomedical Instrumentation section of CSIR-NPL** for her excellent guidance, patience, and for providing with a very supporting atmosphere for completion of the project work entitled "**Synthesis and characterization of nano- polyaniline film by electrochemical polymerization and its application in enzymatic glucose sensing**". She guided and motivated me in each and every step of this project. Her constructive criticism and insights were necessary without which the project would not have shaped as it has.

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Most importantly none of this would have been possible without the love and patience of my **Parents**. They have been a constant source of love, concern and support.

Varsha

**Varsha Yadav (2K20/MSCBIO/35)**