Synthesis and Characterization of Copper oxide nanowires for Application in Electrochemical Biosensing

To be submitted as the Major Project under the partial fulfillment of the requirement for the degree of

Master of Technology

In

Nanoscience and Technology

Submitted by

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CERTIFICATE

This is to certify that the dissertation entitled **"Synthesis and Characterization of Copper oxide nanowires for Application in Electrochemical Biosensing"** submitted by **Yogita Mehta (2K15/NST/11)** in the partial fulfillment of the requirement for the reward of the degree of Master of Technology in Nanoscience and Technology, Delhi Technological University (Formerly Delhi College of Engineering) is an authentic record of the candidate's own work carried out by her under my constant guidance. The information and data contained in this project is original and has not been submitted anywhere else for the award of any other degree.

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DECLARATION

I hereby declare that the project work entitled **"Synthesis and Characterization of Copper oxide nanowires for Application in Electrochemical Biosensing"** is a record of an original work done by me under the knowledge and guidance of **Dr. Nitin Kr. Puri, Assistant Professor**, Department of Applied Physics, Delhi Technological University. This project work has been submitted in the partial fulfillment of the requirement for the award of the degree of Master of Technology in Nanoscience and Technology. The results reported here are true to the best of my knowledge.

Date:

Place: Yogita Mehta (2K15/NST/11)

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List of Abbreviations

µg/ml: microgram per milliliter

µg: microgram

µl: microliter

µm: micrometer

Abs: antibodies

APTES: 3-Aminopropyltriethoxysilane

Au :Gold

CNT: Carbon Nanotubes

Cu: Copper

CV: Cyclic Voltammetry

CVD: Chemical Vapour Deposition

CuO: Copper Oxide

DC: Direct current

DI: Deionized Water

DOS: Density of States

eV: Electron- Volt

EIS: Electrochemical Impedence Spectroscopy

EFLAL: Electric field in assistance in liquid

FESEM: Field emission scanning electron microscope

FET: Field Effect Transistors

FTIR: Fourier Transform Infrared spectroscopy

hr: hour

HCl: hydrogen chloride

Hz: Hertz

I: Current

i-v: Current- voltage

ITO: Indium Tin Oxide

IR: Infra Red

K_2CO_3 : Potassium Carbonate

KBr: Potassium Bromide

kV: kilo volt

L: liter

LiBs:Lithium Ion Batteries

LSS: Ligand assisted-Solid

M: molarity

mg/ml: milligram per milliliter

min: minute

ml: milliliter

mM: milli molar

mJ/ pulse: milli Joules per pulse

N: Normailty

N_2 Nitrogen Oxide

NaCl: Sodium Chloride

NaOH: Sodium Hydroxide

NiO: Nickel Oxide

nm: Nanometer

NPs: Nanoparticles

ns: nanoseconds

ºC: degree Celsius

O_2 : oxygen

OH^- : hydroxide

PBS: Phosphate buffer saline

PEG: Poly Ethylene Glycol

R:Resistance

SAM: Self assembled monolayers

TEM: Transmission Electron Microscopy

TiO_2:Titanium oxide

V : Volt

VLS: Vapour-Liquid-Solid

VSS:Vapour-Solid-Solid

XRD: X-Ray diffraction

ZnO: Zinc Oxide

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ABSTRACT

In recent years, Nanotechnology has advanced in the field of Science and technology.

Because of small size and unique architecture, all the properties of nano materials (physical, chemical or electronic properties) differ from those of bulk material. These materials can be used for the construction of sensing devices, predominately electrochemical sensing devices. Electrochemical sensing find its application for monitoring glucose levels, cancer cells, cholesterol, cardiac biomarker detection and infectious diseases.

We report the synthesis of CuO (copper oxide) by a simple, low cost, wet chemical route.

Uniform nanowires are grown on Copper foil by oxidising copper. We have reported the growth mechanism for better understanding of optical parameters, that are responsible for controlled growth through a self assembly method. The structural properties, morphology and composition of as synthesized material were explored by X-Ray Diffraction (XRD), Scanning electron microscopy (SEM), and Infrared Spectroscopy (FTIR)respectively. The result obtained, show the formation of highly crystalline metal oxide nanoparticles.

Also the studies related to development of biosensor used for cardiac biomarker detection by APTES modified CuO (APTES/CuO) is done. ITO electrodes (indium tin oxide) were used to deposit APTES/CuO nanoparticles through electrophoretic deposition (EPD) technique and further cardiac biomarker anti-cTnI was used for electrochemical studies.The electrodes of APTES/CuO/ ITO and anti-cTnI/APTES/CuO/ITO were characterised using FTIR, Cyclic voltammetry (CV) analysis, scan rate, and Electrochemical impedance spectroscopy (EIS). The immunosensor showed superior sensing properties, with wide linear range and high sensitivity.

Chapter 1
INTRODUCTION

1.1 Nanotechnology

Nanotechnology is the making of functional system at molecular level. It describes matter manipulation at an atomic, or molecular level with dimension in range 1-100 nm; to develop new appliances with extra properties. Recently nanotechnology is growing in many areas e.g. electronics, optoelectronics, sensing and so on in the twenty-first century. The thought of Nanoscience and Technology was first coined by Richard Feyman (American Physicist) in the lecture in a meeting held at American Physical Society. Kroto *et al.,* 1985 reported the synthesis of nano materials. This was the first report. Rao C N R *et al,* 2006 had written in his article about the growth and applications of nanotechnology and its future endeavours. In recent times, it is growing in all the areas like in Electronics, optoelectronics, and in sensing. Nanostructures refer a connection between molecule and infinite bulk system. These are the structured components with their atleast one dimension less than 100 nm. Nanostructures differ from their bulk both in physical and chemical properties.

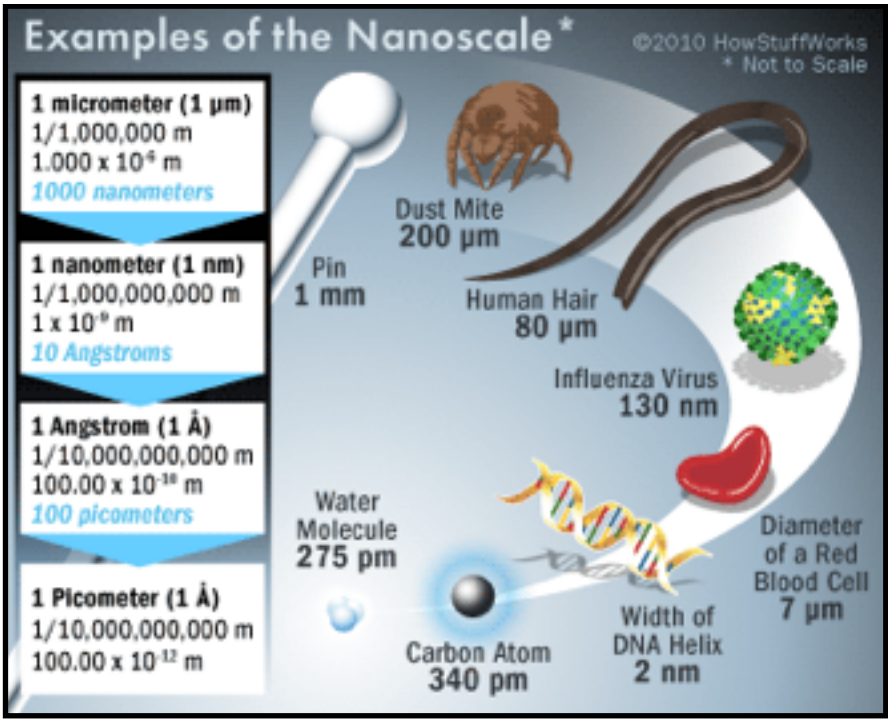

Fig 1.1 Nanotechnology in nature

The major differences between the nanostructure and its counterpart are related to each other with respect to spatial structures, shapes, electronic configuration reactivity, and optical properties.

C N R Rao *et al,* 2001 in this paper explained the size dependent properties of nano materials.

When the dimensions of the bulk are reduced to the range of nanometer, dot formation takes place. There are different categories of structures based on their dimensions, mainly 0 dimensional, 1 dimensional and 2 dimensional.

When a bulk material is cut into few pieces, there will be no change in the properties of that material. If electrical or optical properties are measured , it would show no changes. But if the the size reduction is done at the atomic level , i.e. at electron, proton level, a significant change in property can be observed. This is called quantum confinement. Quantum confinement changes the energy and momentum of the particle, which further alters the properties of the structure. There is a plenty of research going on in field of nanotechnology , and so different classes of nanostructure are studied and explored.(Ajayan,.*et al,* 2006)

The development in the field of Nanomaterials and nanotechnology has shown a high rise ,mainly due to the following two properties-

1. The increase in surface area to volume ratio, greatly increases the reactivity of the surface. This property is used in biosensing and chemical applications.
2. There is an increase in absorption, and emission with the transfer of electrons from one state to other. Mainly this property is used in optoelectronic devices.

1.2 Biosensors

There are many biological components like tissues, cell, cell structures, antibodies, micro-organisms etc.

Analytical analysis combines the sensitive biological component with the physicochemical detector. Biosensors are analytical devices which converts the signal , that arise due to the interaction of analyse with biological event, into a simple and detectable signal. These signals can be light , current, voltage mass etc.

A.Cavalcanti *et al.,* 2008, explained new approach of hardware architect in Biosensors. Biotransducers uses elements such as piezoelectric, optical, electrochemical, gravimetricor pyroelectric materials. Currently Biosensors find their applications in many fields like in human care, food and processing industry, genetic applications, and in environment care.

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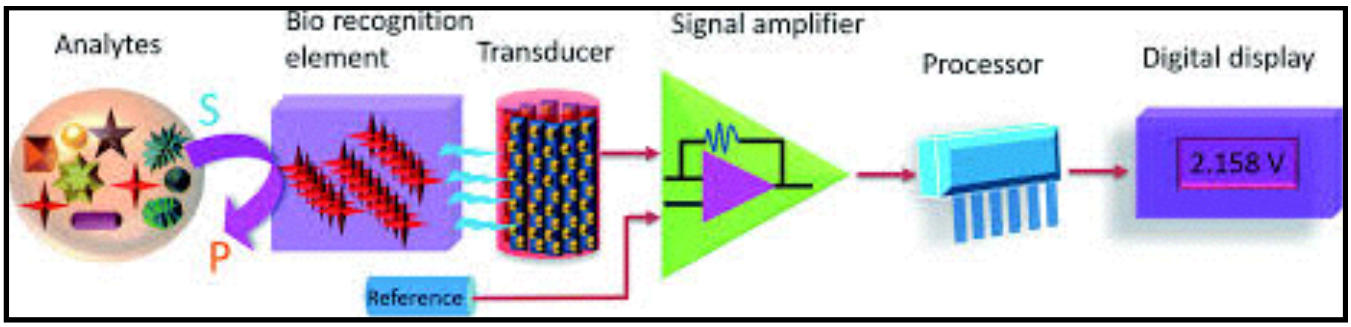


Fig 1.2 Biosensing mechanism

Currently Biosensors find their applications in many fields like in human care, food and processing industry, genetic applications, and in environment care. Because of high sensitivity, high selectivity, easy handling, and fast response, applications of biosensors are increasing day by day. Biosensors composed of nano materials are in trend these days because of its high surface area property. Even a small amount of analyte is detected by it, as nano materials provides fast interaction and nano size surface. (Balasubramanian., *et al*, 2006).

It finds application in pathogenic diagnosis and monitoring applications.Also insertion of nonmaterial inside a biological cell is possible. All these properties make it distinguished from enzyme based biosensors or the bulky material biosensors.

Nanotubes(like CNT), nanowires, magnetic nanoparticles ,gold nano materials and quantum dots are currently used in biosensors. For making a sensitive biosensor, material selection plays a crucial role.

1.3 Metal Oxide Nanostructures

Metal oxide nanostructures are important in field of nano sensors research , because it plays an important role (both theoretical and practical) in analytical chemistry and environmental applications. Recently a lot of work is going on in direction of Semiconductor nano materials. It forms one of the major classes of nano materials. A Semiconductor is a material whose conducting properties lies in between conductors and insulators. There is an intermediate flow of electrons in magnitude with respect to conductors and insulators. The flow of current in semiconductor is due to

both electrons and holes. applications. Recently a lot of work is going on in direction of Semiconductor nano materials. It forms one of the major classes of nano materials. A Semiconductor is a material whose conducting properties lies in between conductors and insulators.There is an intermediate flow of electrons in magnitude with respect to conductors and insulators. The flow of current in semiconductor is due to both electrons and holes.

Table 1.1: Roles of nanoparticles in electrochemical sensor systems

Functions	Properties used	Typical NPs	Sensor advantages	Typical examples	Reference
Biomolecules, immobilization	Biocompatibility; Large surface area	Metal NPs (Au, Ag); Oxide NPs (SiO_2,TiO_2)	Improved stability	Antibody immobilized onto Au NPs	(Zhuo et al., 2006)
Catalysis of reaction enhancement of electron transfer	High surface energy conductivity; tiny dimensions	Metal NPs (Au, Ag); Oxide NPs (ZrO_2, TiO_2)	Improved sensitivity & selectivity; direct electrochemistry of proteins	H_2O_2 sensor based on Prussian blue NPs with a sensitivity of 103.5μA mM^{-1} cm^{-2}	(Fiorito, Gonçales, Ponzio, & de Torresi, 2004)
Labeling biomolecules	Small size; modifiability	Semiconductor NPs (CdS, PbS); metal NPs (Au, Ag)	Improved sensitivity; Indirect detection	DNA sensor labeled with Ag NPs	(Cai, Xu, Zhu, He, & Fang, 2002)
Acting as reactant	Chemical activity	Oxide NPs (MnO_2)	New response mechanism	Lactate biosensor with MnO_2 NPs	(W. Zhao, Xu, Shi, & Chen, 2005)

The significant properties of semiconductor makes it a versatile material in research. Popular research areas of semiconductor nanostructures are sensors and optoelectronics. Chemical stability, low toxicity, high biocompatibility and large surface area attracts research in metal oxide nanostructure. These structures also have superior charge transfer properties, which when used in biometric membrane applications ,improves its performance e.g. it will detect proteins and will retain its activity. In all the metal oxides like ZnO, CuO and NiO that show a variety of morphologies like nanoparticles, nanorods, nanoflowers, attracts the reserchers to fabricate new

nano devices, for electronic(mainly optoelectronic) and biosensing application. Many kinds of nanoparticles including metal nanoparticles, oxide nanoparticles, semiconductor nanoparticles and even composite nanoparticles have been widely used in electrochemical sensors and biosensors. Depends on the role of these nanoparticles play in different electrochemical sensing systems based on their unique properties, the basic functions of nanoparticles can be mainly classified as immobilization of biomolecules, catalysis of electrochemical reactions, enhancement of electron transfer, labelling biomolecules and acting as reactant.

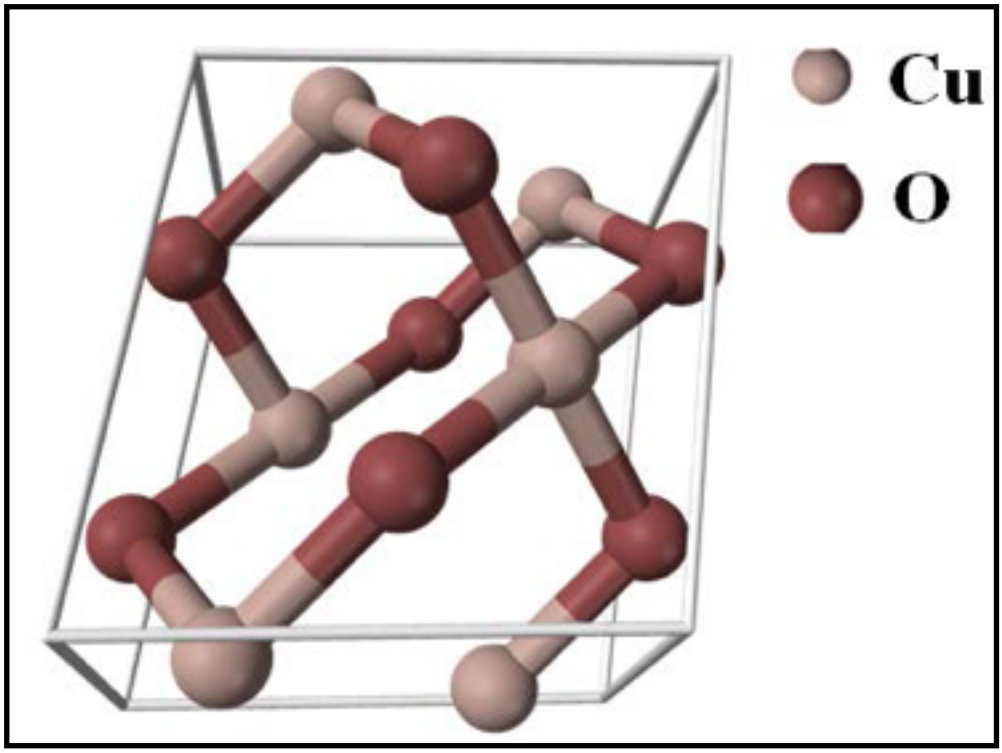


Fig 1.3 The unit cell of CuO structures.

Among all the three metal oxides (ZnO, CuO, NiO) mentioned above, this project focusses synthesis of CuO and its applications in biosensing. The oxides of transition metals are an important class semiconductors. Copper (II) oxide, a p-type material having energy band gap of 1.2eV, has a monoclinic stable phase structure and is used in number of nano devices applications. CuO is so chosen for sensing applications because of its interesting properties like monoclinic structure, nonhazardous source material and it can be prepared by low cost solution methods which are required for gas sensing applications. Sensors ,actuators, High temperature super conductors , catalysts, ion batteries are some of its applications. Many properties of CuO have been witnessed in recent ye

ars like; A variety of substrates can be used for the growth of CuO, CuO are non toxic, can be prepared as different morphologies and low cost..

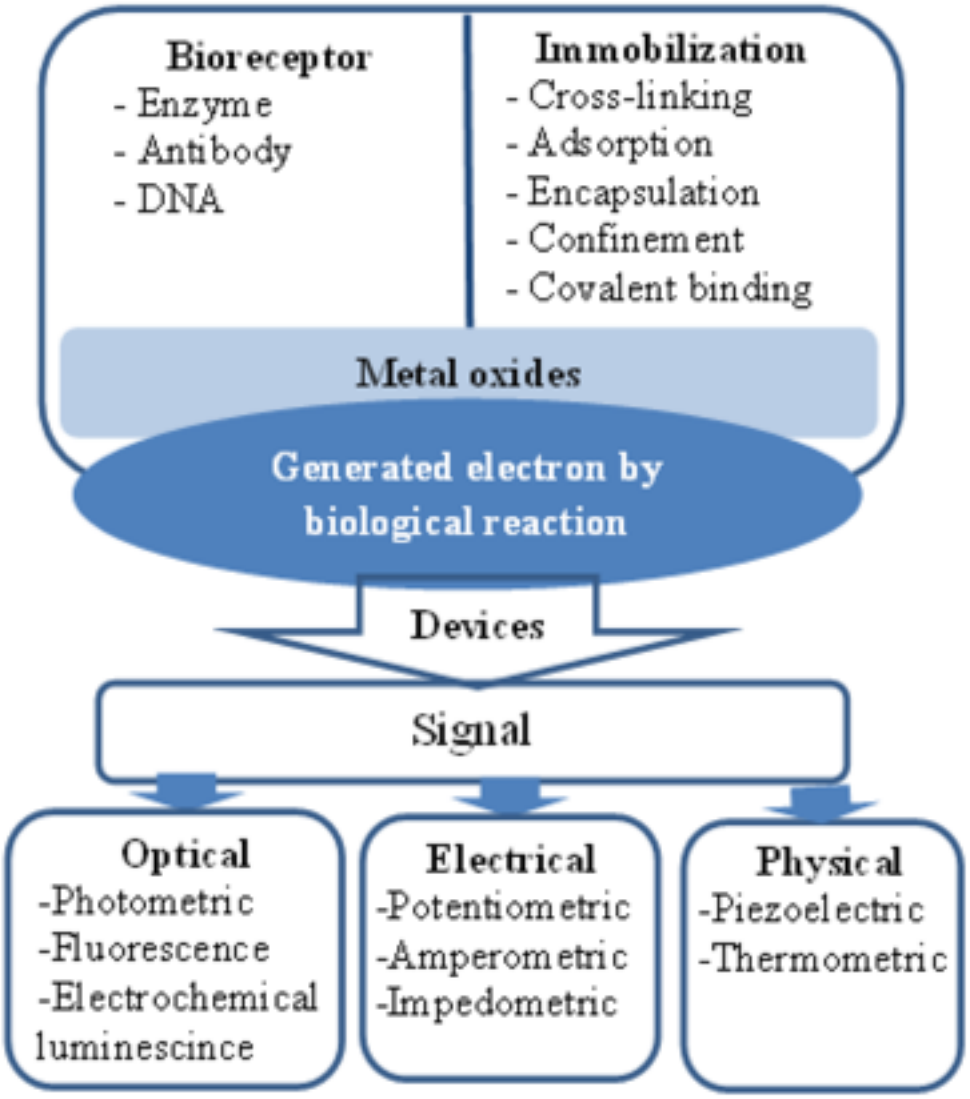


Fig 1.4 Metal oxides in biosensors

1.4. Functionalization of CuO

1.4.1. Indium Tin Oxide Electrodes

For its unique optoelectronic properties, indium tin oxide is used as an electrode material. It is widely used in technical industries for its properties like electrical conductivity and optical transparency. Among all types of thin films the most popular and important are ITO films and are used for electrochemical and optoelectronic applications , because of high transmittance and conductivity.

Over the past year ITO electrode is being widely used as a conducting substrate in developing sensing devices. But one of the major problem of electrodes of this kind is their stability, which also affects its performance. For different fabrication, the surface properties are very different and crucial. Therefore for the development of sensors , the surface chemistry and monolayer modification is very important. Variety of surface treatments for different properties of ITO has been reported. Biomolecule detection requires high specificity for molecular recognition, which is done by modifying ITO electrodes certain molecules.

With distinguished characteristics and properties, use of ITO (as a substrate adhesion) had increased considerably.

In the science related to of electro-optical devices, these electrodes are broadly used as a transparent semiconductor. It being highly degenerated, has many dopants, so the electron concentration of the conduction band can be compared with the DOS in the band. Similarly the concentration of holes (positive ions) in valence band with DOS in p material.

Some superior properties of ITO includes conductivity, electrochemical properties, high optical transparency, Because of these properties , ITO electrodes attraction has increased recently.

1.4.2 Immobilization Techniques

Electrochemical Sensing and Biosensing require molecules that are active (biologically or chemically), therefore the element immobilization over the surface of electrode is important factor in sensor development. A known number of techniques are used , to immobilise an active molecule over the electrode surface. These are covalent bonding, adsorption cross linking ,cross linking, adsorption and entrapment, that are frequently used in immobilisation.(Anusha, J.,*et al,* 2014.)

When the attachment of the recognition element with the molecules of the electrode is done by a diversified chemical reaction accompanied with formation of peptide bond or linkage with any functional group (amino, thiol, epoxy, carboxy, isocynato), it is called covalent bonding. The modified surface provides an advantage of ionic strength ,pH and resistance to temperature changes. But with covalent bonds, the chemical activity of the modified immobilised molecules is lost , because of obstructed dynamics.

When heterogeneous catalysis is there, the entrapment procedure is used. Usuallly it is utilized to trap the element or biomolecule into the polymer matrix , before setting it onto the surface of ITO electrode.

For the procedure, mainly used matrixes are polythiophene, polyvinyl alcohol, polyacrylamide, and polyvinyl chloride. Biocompatibility, easy and mild conditions and freedom of motion are some of its advantages. Whereas weak interaction with surface, and because of variation caused in microstructure there is problem of leakage in active molecules,are disadvantages of this technique. (Md Zaved Hossain Khan *et al.*,2015)

Absorption technique (either physical or chemical) is naive and commonly used method , in the electrochemical sensing. Various methods likes Hydrogen bonding, hydrophobic or hydrophilic interactions , ionic bonding, and weak forces (like van der walls) are used for attaching the active molecule onto the surface of ITO electrode.

The most commonly used technique involves the use of chemical agents like bismaleimidohexane, hexamethylene, disuccinyl suberate and many others. It is called cross linking attachment. These chemicals are used for the deposition of molecules over the surface of ITO. But this method require active molecules in large amount and high complexity .

All the techniques described have share of benefits and drawbacks .The choice of any technique depends on its use in designing the sensor.

1.4.3 APTES

In Biosensing and diagnostic fields, biomolecule immobilisation plays a significant response. For Biomolecules such as enzyme, antibody, aptamers, nucleic acid etc high sensitivity, selectivity and specificity are required for the detection of target analyte.

Immobilisation of biomolecule means to obstruct the mobility of the same by any means either physical or chemical . To achieve immobilisation, the substrate like nanoparticles needs to modified or functionalized, so as to achieve high bonding strength which can bind the biomolecule in proper phase, with long term stability. (Sandeep K. Vashist *et al*., 2014)

The biological recollection event results in a perceptible signal, which is associated with the quantity or mixture of the biomolecule.

Thus, the biomolecule inactivation makes a decisive hold in obtaining absolute sensitivity and the ability to distinguish with extended life period. Functionalization of biomolecule by organic ways make it available several outlooks for the enzymes,antibodies (Abs), nucleic acids immobilisation. The surfaces are then efficiently used to proliferate compelling nano-level architectures. In recent

years, a good amount of work is done in the direction of applications of organosilanes like APTES and other as surface modifiers.

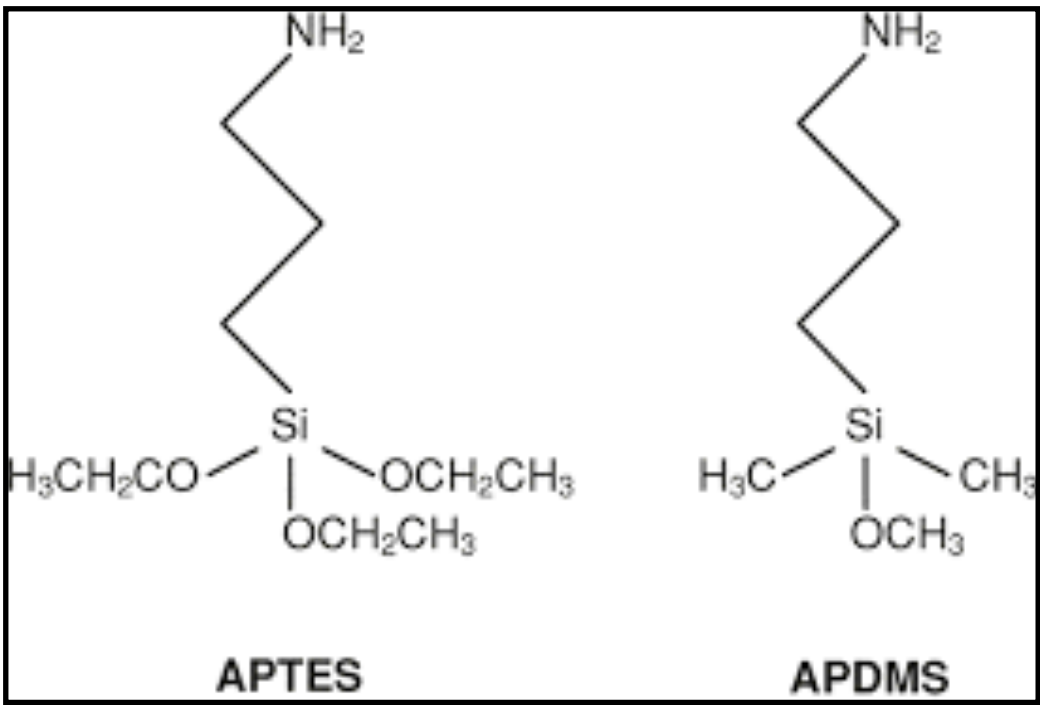


Fig 1.5. Structure of APTES and APDMS

APTES (3-Aminopropyltriethoxysilane) is being used largely for the functionalization of biomolecules for surface alteration. One of the most frequent strategies adopted by the researchers is mimicking nature using biomolecules such as pro- teins and polysaccharides that are selective in their functions and therefore interesting candidates to be associated with dif- ferent classes of materials

APTES is accumulated over the solids or electrode materials with variation in conditions like amount of material, solvent, range of temperature, and duration. With these, abating conditions like air/heat drying must be important with the planned application.The siloxane bonds with surface silanols are formed by aminosilanes, which are strongly interlinked bonds in between biomolecule and an insoluble part. Catalytic activities by amine group provides binding strength which make a bond strong with very less leakage. Layered construction of enzymes into organized systems has attracted considerable attention in recent years due to its potential application in the areas of bio-electronic and biosensors, etc.

APTES combines with the free hydroxyls of an oxidised substrate in three different ways mainly horizontal polymerization, vertical polymerization and mixed polymerization. In horizontal polymerization, APTES attached to the surface forms siloxane with adjacent amine of APTES. It combines with the nearby APTES in vertical polymerization. In the third scheme,the biomolecule possessing surface hydroxyl groups, a hydrogen bond is formed by the APTES with the metal surface or it is protonated by removal of protons from the surface. Alignment of neutral amine with the surface takes place in different conditions and are surface dependent.

Chapter 2
Review of Literature

The most important and crucial part to lay the groundwork is its literature survey.

All the important studies related with the chosen material, the issues and cavity within the work laid previously and new extent raised, forms the legwork of the new discovery. It pinpoints on the variety of scopes of the material, underlines the analysis that is then applied by current scenario, thereby saving a lot of time, funds and the potential of the people. It helps in unique creation of manuscripts around the globe and abstains from the recurrence of the word done previously.

2.1 Synthesis of CuO nanowires

A notable amount of analysis is done to make this project. The chapter outlines the various synthesis techniques for the production of CuO nano rods and architectures, properties highlighting their applications, availability and practical use. It highlights the principal behind using metal oxides in sensing applications. We have used CuO and used the idea of making it a good sensing material.

M. M. Momeni. *et al*. 2010 have put copper (Cu) nanostructures on the substrate of the same material (Cu)for making it available as a super capacitor material. Using entirely different samples Capacitor performance of CuO oxide was illustrated by it. A simple synthesis procedure was given by them for development of CuO nanostructure on the foil at medium temperature.

Genki Saito. *et al*. 2011 had described the synthesis via a solution plasma. The Effect of the copper compound solution and electrolysis product on its morphology was examined. Electrolysis solution containing K_2CO_3 (.001M to .50 M) was used to immerse the copper wire as cathode. The results so obtained stated that by using K_2CO_3 solution , showed the structure of CuO nanofowers in addition to sharp nano rods. Copper nanoparticles showed the occurrence of pores when the voltage was varied from 105 V to 130 V , with the liquefaction of Cu_2O.

Wei-Tang. *et al.* 2005 used the arc discharge method for synthesizing uniform and mono dispersed nano rods , by crystallisation and accumulation of fine CuO nanoparticles. No surfactants were used to generate nanoparticles with solid -liq arc discharge method with less than surrounding

temperature. With Fast oxidation and Ostwald ripening, CuO nano rods are formed with sharp edges.

S. Anandan. *et al.* 2005 showed the synthesis of CuO nano rods, with a facile approach using a chemical compound method at normal room temperature . The method used is easy to perform and cost effective. These CuO structures were then used in the dye syntesized solar cells as a cathode material.

2.2 Metal Oxide Nanostructures and its Application

Amount of work using Metal Oxide nanostructure has been noted for using it in sensing applications. Previously many metal oxides like Nickel oxide (NiO), Zinc oxides (ZnO), have used in the field of sensors. There is also a noted work related to CuO, which is further used in biosensing (mainly glucose Biosensing) .

Espitia *et al.*, 2012 had illustrated in his work, the performance of a metal oxide, mainly ZnO and its application in UV emitters, in device with piezoelectric effect, sensing applications (chemical sensors), etc.

Fujishima *et al.*, 2000 focussed on TiO_2 particles which has remarkable properties and showed its importance in the field of material science and physics. Several applications of TiO_2 particles in Electrochemical sensors, solar cells , photocatalysis , and memory storage devices.

Z Wang *et al.*,2008 had focussed on metal oxide nanostructures due to its superior properties like controlled growth, its composition and size and their use in LiBs (Lithium Ion Batteries). Effect of the structures were studied with main focus on electrochemical behaviour . Oxides such as SnO_2, TiO_2, Co_3O_4 and their complex oxides are studied thoroughly and its progress as a material for future electrodes.

A Kolmakov *et al*., reported that Nano wires made from metal oxide can be used as a sensitive sensors. These nano wires uses their active sensor element either to develop into FET (Field effect transistor), or as resistor. FETs properties are controlled by altering the potential applied to gate of Field Effect Transistors (FET). In case or resistors, with charge transfer process, the conductance is varied. Also functionalization of these nanostructures to improve the sensing efficiency is also reported.

For biosensing activities functionalisation of biomolecule is required. Vey less research has be done in the field where biosensing is done by metal oxide nanostructure. The properties of SAM (Self

assembled monolayers) and its importance in biosensors application has also been studied because ITO film electrode forms the basic of biosensing applications. Some of the major literary articles reported in this field are mentioned below

2.3 Functionalization of CuO

Sandeep K. Vashist *et al*., 2014 pen down that in Biosensing and in the field of diagnosis , the properties like selectivity, and quick response are mainly dependent on the immobilization of bio molecules. APTES (3-Aminopropyltriethoxysilane) has been extensively used in the surface modification. Also application of APTES in assay platforms has been reported. Mainly the article focussed on the preparation of APTES surface ie. functionalisation of metal oxide nanostructure with APTES. The reactions of the surface, and silanization mechanism are explained. Bonding architectures for APTES on substrate surface provides the complete analysis.

Marco E. Marques *et al.,* 2012 presented the new approach to develop biosensors on the basis of enzymatic systems. Two organosilanes MPTES and APTES are used for the functionalization of the substrate surface. MPTES (3-mercaptopropyltriethoxysilane) are used just as APTES for the immobilization. A 3D enzymatic structure was analysed with help of enzymatic structure along with glucose oxidase. A comparison was also presented in this article comparing chemical functionalities with organic functionalities.

Md Zaved Hossain Khan *et al.*,2015 presented a review article , explaining the effects caused by Indium Tin Oxide on the assembled layers and the performance of these layers in biosensors. It clearly demonstrates the properties and characteristics of ITO. It has shown the way ITO electrodes are modified, by attaching silane molecules in different different conditions. Potential stability was analysed and its dependency with surface roughness was analysed. The relative value of work function decreased after the monolayer modification among various samples. This accounted the role of orientation and surface attachment area in the value of work function.

2.4 Electrochemical Sensing

Saurabh Srivastva *et al*.,2014 reported the biosensing study with Au-rGO nano composites. Immunosensor with high sensitivity ,reproducibility, and stability, was prepared with gold salt (Au) with reduced graphene oxide. Electrochemical studies were done this nano composites to confirm its use as a biosensing device. CV for various modified electrodes were performed to obtain the

desired results . Cv analysis and impedance spectroscopy (EIS) were performed on autolab. Three electrode system was used, where increased conductivity and high storage stability was reported. With optical and electron microscopy, the size ,shape and crystal structure of Au@rGO was investigated in this paper.

Suveen Kumar *et al*., 2015 reported the results related to biosensing for the cancer biomaker (CYFRA-21-1). Functionalisation of ZrO_2 with APTES was completed and electrophoretic deposition on ITO electrode was done. Improved hetrogeneous transfer of electrons was reported with faster kinetics . Also investigations regarding morphology and structure was carried out by different surface techniques like XRD, FTIR and TEM.

Chapter3

Methods Of Synthesis and Growth Mechanism

3.1 Methods Of Synthesis of CuO

There are various ways to synthesise CuO nanostructures, depending on the morphology, functional group requirements etc. Hydrothermal route, Impregnation, Sol-Combustion, Precipitation, plasma, combustion, CVD, Microwave, laser ablation, solution (liquid-solid) synthesis, vapour (solid-liquid) synthesis, aqueous reaction, oxidation of copper foil, lithography (e^- beam), thermal decomposition of CuO precursor, arc discharge, etc. are some of the methods commonly used for synthesis.

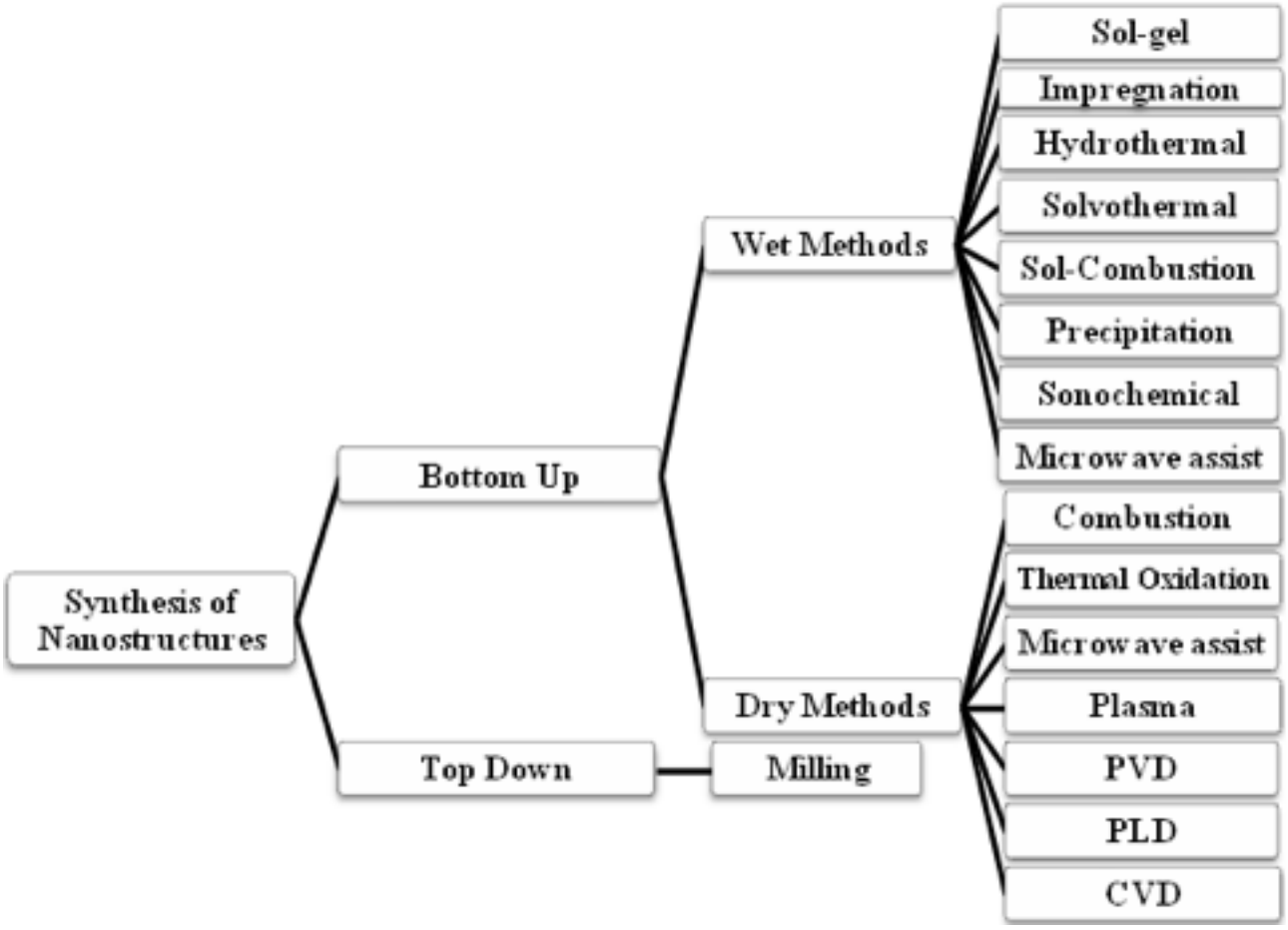


Fig 3.1. Rotello et al., 2004, Synthetic methods of nanoparticles.

Among these methods, many require high temperature , high production cost, precise and accurate instruments which further require attention and utmost care, and perfect anbience. Among all other methods, wet chemical method is predominately used more as this method is easy and cost effective

3.1.1 Copper Oxide Nanoparticles Via Solution Plasma

Genki Saito *et al.,* 2011 presented the synthesis of Cu (copper) and chemical compounds of copper nanoparticles , with solution plasma. Electrolytic solution contianing K_2CO_3 with concentration range 0.001M to 0.500M was used. Copper wire was immersed in it as a cathode material. With the application of K_2CO_3, CuO nano flowers were formed along with sharp nano rods. The dimension of nano rods varied linearly with the solution concentration. Spherical particles of copper with/without pores shaped once the citrate buffer was used. Pores are visible in CuO nanoparticles, when the applied voltage is in the range 105-150V.

3.1.2 Laser Ablation

XZ Lin *et al.,* 2009 have used laser ablation method to synthesise CuO nanostructures for the controlled growth. They described a unique nano manufacturing ,which may bring home the bacon from nanocrystal synthesis to successive purposeful structure manufacturing inside one step. A single step procedure was used for nanostructure formation. The technique uses electric field in assistance in liquid (EFLAL), Sequentially CuO nanostructure were fabricated over the CuO nanocrystal . Both the process , synthesis and assembly, are completed in a single step. With this method, Nano spindles are formed that are many applicable in the field of biology or medicine.

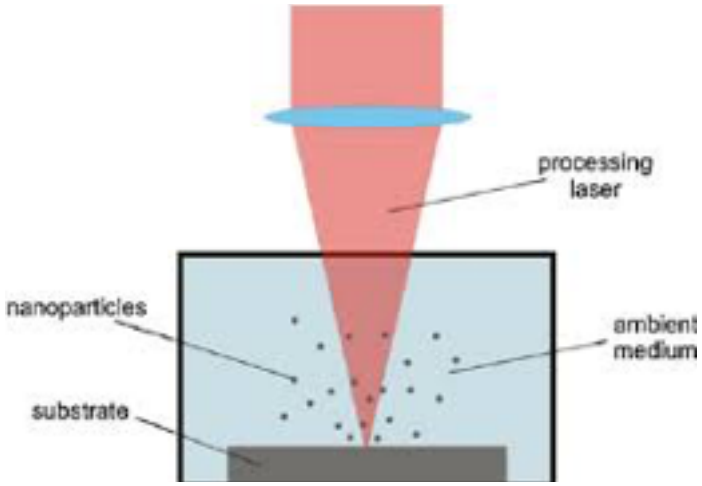


Fig 3.2 Laser Ablation

Laser device (Aluminium garnet) containing Q-switch doped with neodymium, with following specification (pulse breadth 10 ns , frequency value 5 Hz , and pulse energy 100 mJ/ pulse), is used.

Copper target is used with high purity range 99.7% is taken and is kept upon the quartz chamber . Deionising water (DI) is used to fill the chamber upto a height of 10mm above the target.
Copper foil electrodes are used with purity value 99.99% over the chamber walls. Space of around 48 mm is kept in between the two electrodes. Electric field is produced with variable voltage by steady power supply. Optical device was then targeted. In the duration of device ablation, different values of potential are applied mainly 0V, 40V, 80V, and 120 V. With this temperature is maintained and in 60 min duration, a yellow solution is formed . Hence the synthesis and assembly is completed.

3.1.3 Copper Oxide Nanoparticles Synthesis By Thermal Decomposition

Kaili Zhang *et al .,*2007 describes the synthesis of aligned CuO nanowires by the thermal tempering of thin films (CuO films) over the semiconductor substrate. Efect of various physical conditions like film thickness, temperature, gas used for annealing and lithographic patterns were studied. At 400 -500°**C,** long aligned nanowire were obtained. In comparison to thermally deposited film , electroplated copper film is more favourable to nanowire growth. With Hardened copper film, uniform, large area thin films are formed but they are misaligned vertically whereas annealed copper films in N_2/O_2 gas flow, produce vertically aligned nanowire but non uniform . But if thin film formed by lithography is used , aligned nano wires over large area and uniform are formed over the surface of film substrate.

3.1.4 Synthesis Of Cuo Nano Rods By A Solid Liquid Phase Arc Discharge

Mono dispersed CuO nanorods, uniform in nature are made by crystallisation and oriented aggregation of CuO nanoparticle flakes. Wei Tang Yao *et al.,* 2005 reported the procedure for synthesising these nano rods.Solid liquid ar discharge method is used to generate these nanoparticles at temperature below the regular temperature without using any kind of surfactants. Sharp ended nano rods are formed from nanoparticles by super fast oxidation of copper structures and the Ostwald ripening method . The Spontaneous aggregation contributes to formation of these structures with familiar attachment . There is selective synthesis of Cu and Cu_2O NP's by abrupting the oxidisation of Cu with chemical agent.

3.2 Growth Mechanism

For nanostructure of different metal and metal oxides, many growth mechanisms are present. To elaborate growth ways Top to down approach and Bottom up approach are used in nano rods, nanowishkers and nanowires. Some kinds of Growth mechanism are described below-

a)Vapour-Liquid-Solid (VLS) Growth mechanism

b)Ligand assisted-Solid (LSS) Growth mechanism

c)Vapour-Solid-Solid (VSS) Growth mechanism

Here in this work, LSS (Ligand assisted-Solid) mechanism is being used for CuO nano rods and nanowires.

3.2.1 Vapour-Liquid-Solid Growth Mechanism

VLS mechanism was developed by Wagner and Ellis for the growth of whiskers.

Uhland Weissker *et al., 2010* worked on the VLS mechanism. Behind the idea, there was thought that among all the three phases, volatilized phase, liquid phase, and the solid phase , there is an interaction. For the gas, precursor is provided. Over the solid substrate, there exists a liquified molten catalyst. In this growth mechanism , its ascertain that whether decomposition of precursor at specific space of liquid part at T_1 occurs or not. The material is dissolved until solution at concentration c_1 is reached. There is assumption that the particle occupies another locality with concentration c_2 at temperature T_2 . Level and Thermal gradients are assumed at each level. There is diffusion of precursor material and precipitation at T_2 .

3.2.2 Ligand-Assisted-Solid Growth

CuO nanostructures were grown at Cu substrate after a reaction time of 96 hr. Shiny black colour surface was seen to get distributed all over the Cu substrate at the final stage. This further indicated CuO formation and was verified by SEM, XRD.

Satyamoorthy *et al.,* 2013 explains that Simple coordination technique with self assembly that can be used to fabricate CuO in alkaline solution with generation of Cu^{2+} ions from oxidation at the surface.In this work , synthesis of CuO nanostructures were done by decomposing $[Cu(NH_3)_4]^{2+}$

precursor under known reaction time. A solution consisting NaOH and NH_3 were used to oxidize Cu substrate. Following are the reaction equations

$$Cu + NH_3 + O_2 + H_2O = [Cu(NH_3)_4]^{2+}$$

$$[Cu(NH_3)_4]^{2+} + 2OH^- = Cu(OH)_2$$

$$Cu(OH)_2 = CuO + H_2O$$

Dara *et al.,* 2009 explained this process. Ammonia being Alkaline in nature, gives a basic medium and is used to adjust the pH value. $[Cu(NH_3)_4]^{2+}$ complex was generated with Cu^{2+} ions, which further helps in transportation of Cu^{2+} ions to crystals the are growing with OH^- ions , thus producing $Cu(OH)_2$. In alkaline solution , produced $Cu(OH)_2$ is highly unstable and finally forms CuO by reducing itself.

Dayeh *et al.,* 2007 interpreted growth mechanism with VLS (Vapour-liquid-solid) growth and VSS (vapour-solid-solid) growth mechanism and explained that the growth can only be done in the presence of metal catalytic process. If metal catalytic particles are absent , then this growth can only be done by LSS ie. ligands-Aided-Sol. solid . The work presented here is done in absence of catalysts.

Xiaogang Wen *et al.,* 2003 explains the coordination assembly at solid surface for $Cu(OH)_2$ in an aqueous solution. $Cu(OH)_2$, being a layered material, has an orthorhombic structure

which further helps in self assembly of the nanowire. OH^- with Cu^{2+} coordinates and forms extended chains , that make connections with OH^- legends.

Xiaogang Wen *et al.,* 2002 explains that with maximum open surface area, copper foil can be used as a source of controlled delivery with Cu^{2+} ions under alkaline conditions into the aqueous solution. This explains that $Cu(OH)_2$ grows in single dimension in the absence of catalyst with aid of ligands. Also in case of alkaline solution $Cu(OH)_2$ gets reduced to nano rods and nano flowers.

According to the reactions presented here, NH_3 combines with DI water ,and is decomposed to OH^- ions and ammonium ions. For the formation of CuO, it is very important to control the

concentration of hydroxide (OH^- ions) . This concentration shows direct variation with pH value. With increase of pH 11 to 12 , there is considerable increase in concentration of hydroxide ions. Initiation process starts with the generation of building units ($Cu(OH)_2$) at the copper substrate, and is continued till it reaches the super saturation level and until nucleation starts. Fabrication of $Cu(OH)_2$ is done by self assembly , with alkaline solution containing Cu^{2+} ions, obtained from oxidation of Cu at the surface.

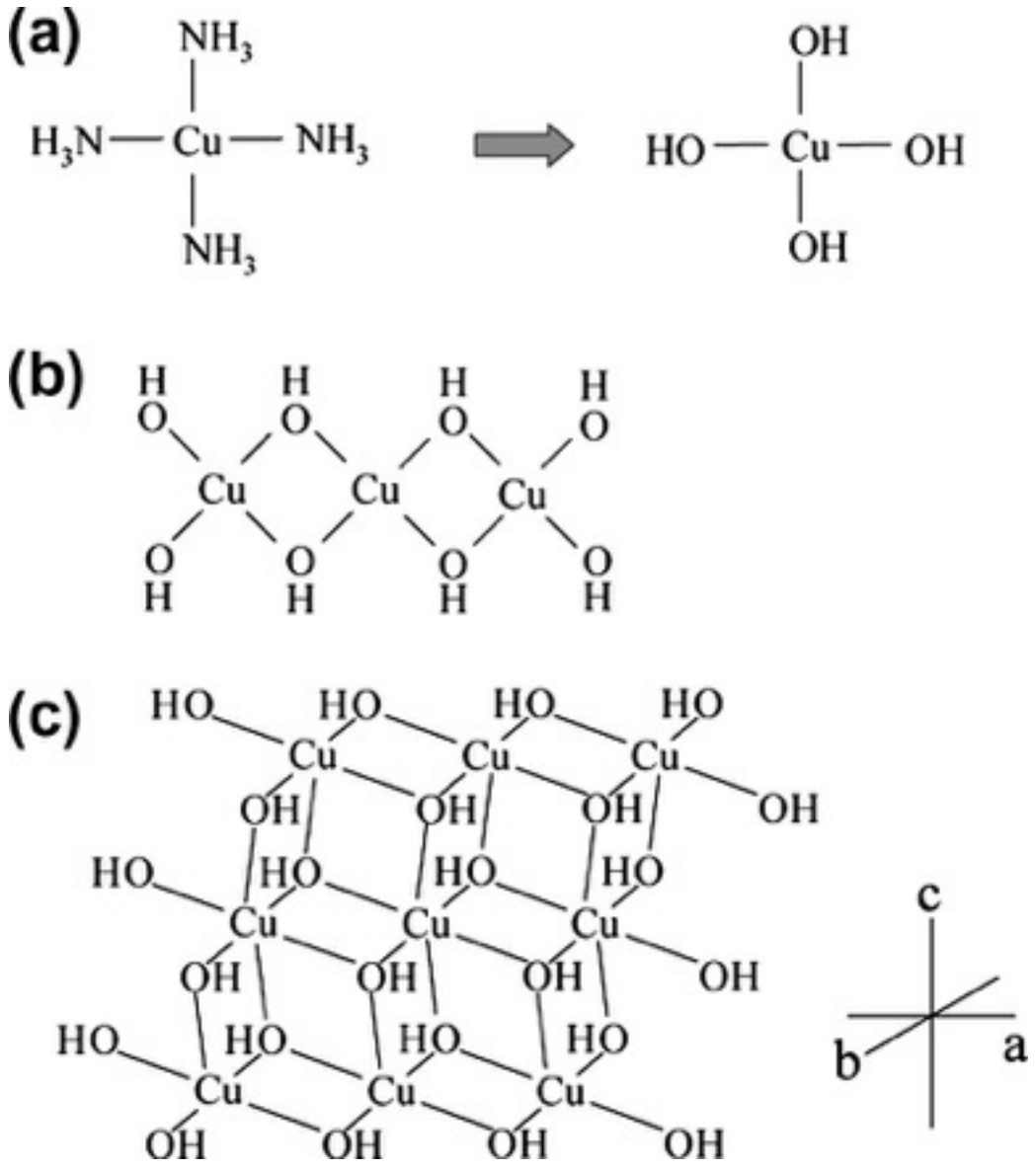


Fig 3.3 The coordination assembly growth of CuO nanostructures

The alkaline nature of ammonia provides a basic media and adjust the pH value of solution.

At pH 11, OH^- ions concentration is relatively low, results to failure of formation of nucleation sites. As the value of pH is increased from 11 to 11.5 , nucleation sites concentration increases. Nucleation sites with High pack density allows the growth of CuO nano rods in single direction, leading to aligned nano rods. With pH 12, there is much increase in nucleation sites which further helps in the formation of highly packed, dense aligned nanorods.

Chapter 4
Characterization Techniques

All the analysing tools that help in analysing the sample with respect to its phase, its surface morphology , its internal structure, and tells about the functional group inside it. are called characterisation techniques. These techniques tell us about all the properties of the sample, its features and its tolerance to the outer environment.

In order to analyse the CuO nano structures, few techniques have been studied. The techniques that can characterise it on a microscopic level and at a nanoscale , as the compound grows in nanometer size. All the techniques used further are described here briefly

4.1 X-Ray Diffraction

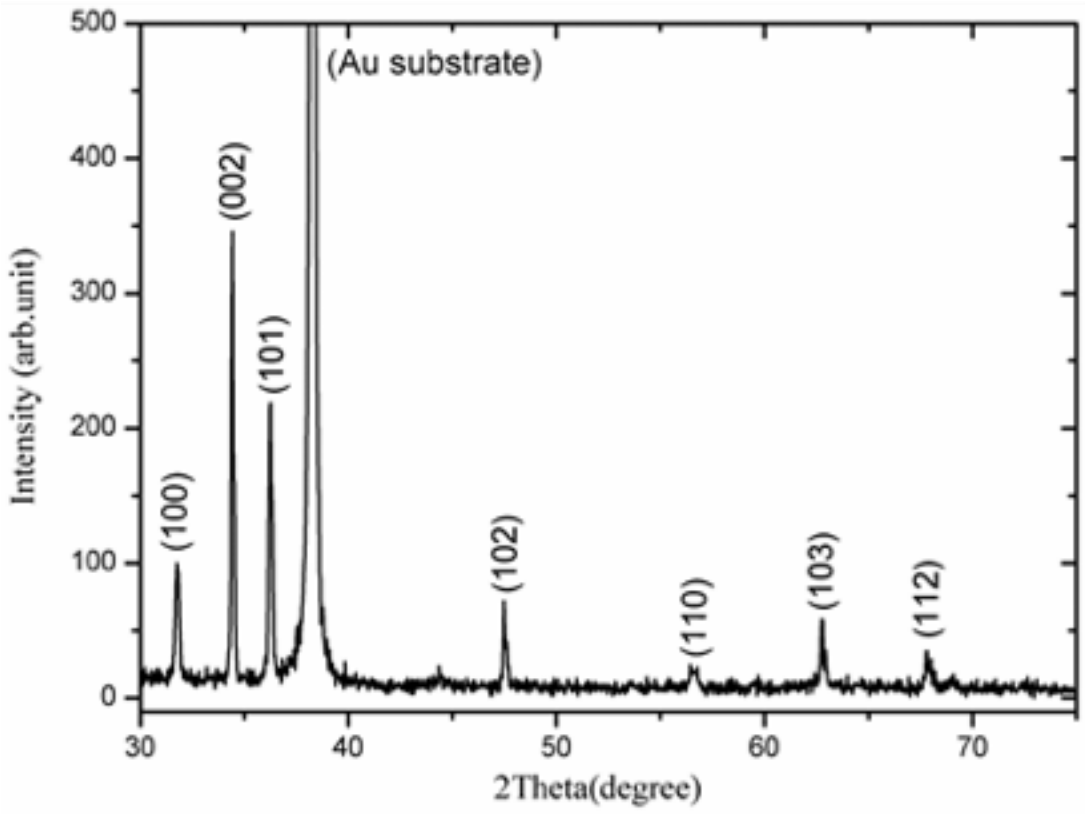


Fig 4.1 X-Ray Diffraction Pattern

To know about the crystal quality, and the composition of synthesised materials , XRD is done. All the information regarding the miller planes, and the crystalline size is obtained by X-Ray diffraction. X-Rays can also distinguish the amorphous from the crystalline material. A variety of

information is obtained from the diffraction performed. Information regarding the lattice parameters, crystal plane and the cell structure is obtained. For a specific material , the formation mechanism used and information regarding all the crystal phases that exist in prepared material is stated. It also states the ratio of amorphous to crystalline in synthesised material. Average size of crystal , can be obtained from the peak width, and the size of particle is inversely proportional to peak width. Lastly it tells about the structural distortion that can be calculated from the inter-planer distance.

Fig 4.2 XRD Setup @ DTU

4.2 Field Emission Scanning Electron Microscope (FESEM)

To Study The Morpholgy Of Any Synthesised Material, Fesem Is Used. It Is A Powerful Technique, That Produces Image By Scanning The Material With Electron Beams , That Are Highly Focused. There Is An Interaction In Between The Atoms Of The Material And The Electrons , Which Produces Signals That Consists Of Composition And Morphology Of The Material.

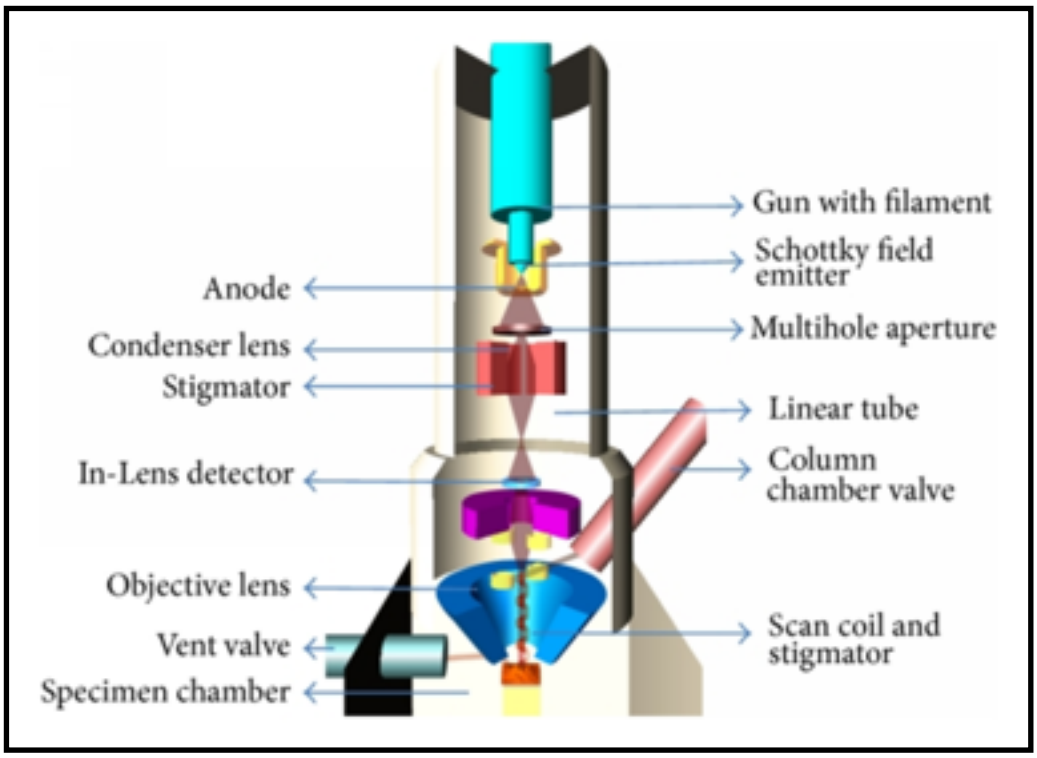


Fig 4.3 The field emission scanning electron microscope

For all types of signals like characristic X-rays , light cathode luminiscent(CL), Secondary electrons, back scattered electrons, and sample current. Standard tool used in all FESEM is SE detector. High-resolution images with size less than a nanometer, can be developed. Since the electron beam used is very narrow, magnification around 500,000 times can be obtained as compared to light microscope.

4.3 Fourier Transform Infrared Spectroscopy (FTIR)

It is analytical technique, which is used to identify the organic groups that are present in the sample. When IR light falls on the material, it absorbs certain wavelengths and rejects other. FTIR measures this absorption by the material of interest. Previously IR instruments uses prism or monochromator. this was dispersive type spectrometer (Ferraro & Basile, 1975).

Firstly interferogram of sample signal is collected with interferometer, which measures a complete range of infrared frequencies. Spectrum is obtained at the output, with several intermediate steps.

The spectrometer digitizes and reads the interferogram , then FT function is performed and then the spectrum is obtained (Smith, 2009). It is a technique that plots infrared intensity with wavelength (wave number).

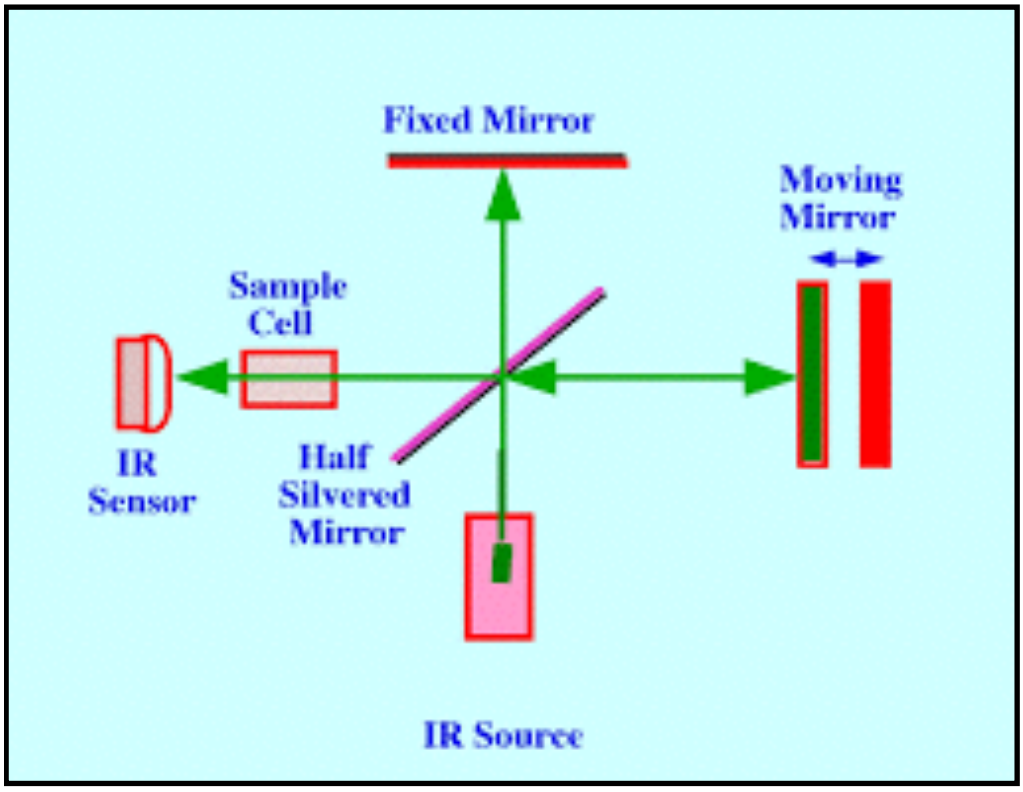


Fig 4.4 Components of FTIR Spectrometers

With reference to wave number, IR can be categorised into far infrared, mid infrared and near infrared with wavenumber in range 4-400cm^{-1} , 400-4000cm^{-1} , and 4000-14000cm^{-1} respectively. This spectroscopy analyses vibration characteristics chemical functional groups in the material(Siesler, Ozaki, Kawata, & Heise, 2008). As the IR interacts with matter, there is a stretch, or a bend in the chemical bonds, that are present in the sample. With this, the chemical functional group , irrespective of the structure of the molecule, tends to absorb a specific wave number IR rays.(N Koji *et al.*,1977)

4.4 Electrochemical Biosensing

Affinity-based biosensors, which use immobilized recognition element for the selctive binding with target molecule, are usually called electrochemical biosensors.(Brett, *et al*, 1993)

A current/voltage change at the surface is generated when the target selectively binds itself to the recognition element.Transducers(potentiometric, amperometric, and impedimetric) can be employed with electrochemical biosensors as it converts all the chemical information to a electrical

signal at the output. Electrochemical sensors finds there application in POC , as cost and size are important parameters for it.(Depika Sandil *et al.,* 2016)

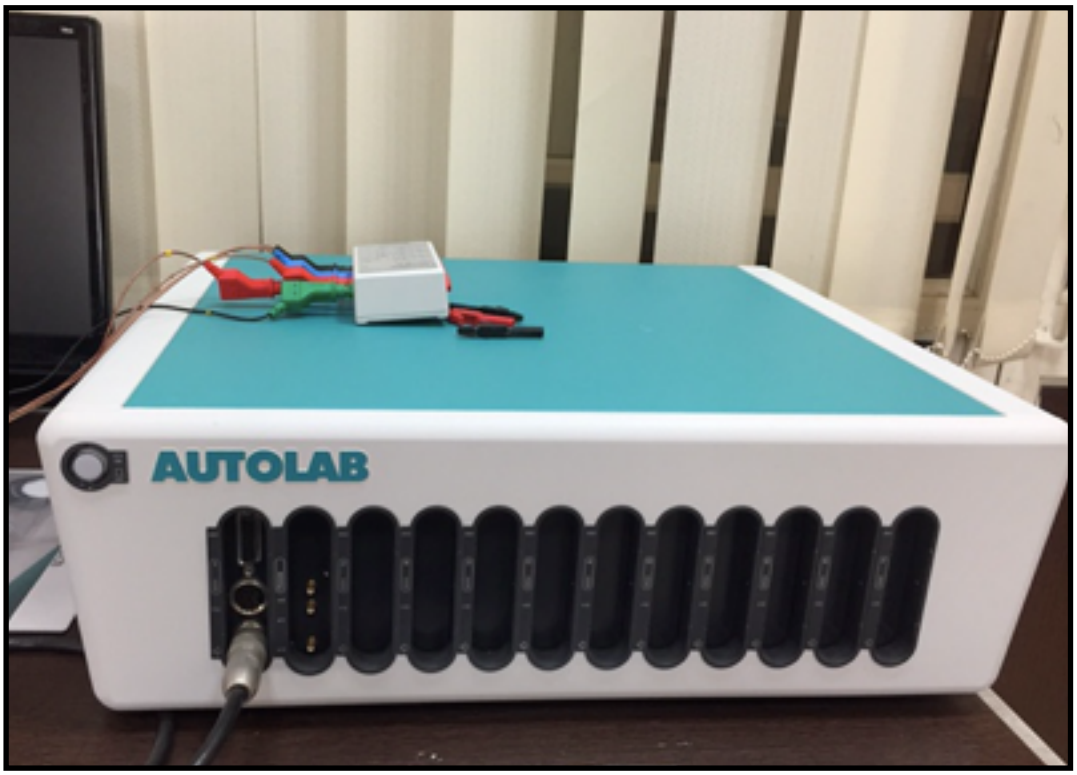


Fig 4.5 Autolab @ Advanced Sensor Lab, DTU

4.4.1 Scan Rate

Mainly Cyclic voltammeter is used for all the information regarding the redox potential and chemical rate constant of analyte solutions. For scan rate, the voltage is varied in between the two points at a certain rate. The voltage is reversed after it reaches V_2 , is swept back to V_1 . $(V_2 - V_1)/ (t_2 - t_1)$, the scan rate, is a crucial factor, as its duration gives sufficient time for the chemical reaction to occur. For different scan rates, different points are

observed.

Take one set of redox B/B_n , as the working potial is made more negative , the species B is reduced more to B_{n-}. The solution B concentration when reaches to zero, it yields cathodic (reduction) current peak (i_{pc}). At the moment, when the current reach to its highest value, the concentration gradient is steepest in electrode-electrolyte interface, and the value of current is in proportion to concentration gradient

To study the CV , working bioelectrode potential is scanned in a cyclic way, i.e. potential is applied in one side and it is switched in reverse. Working electrode current is then measured .

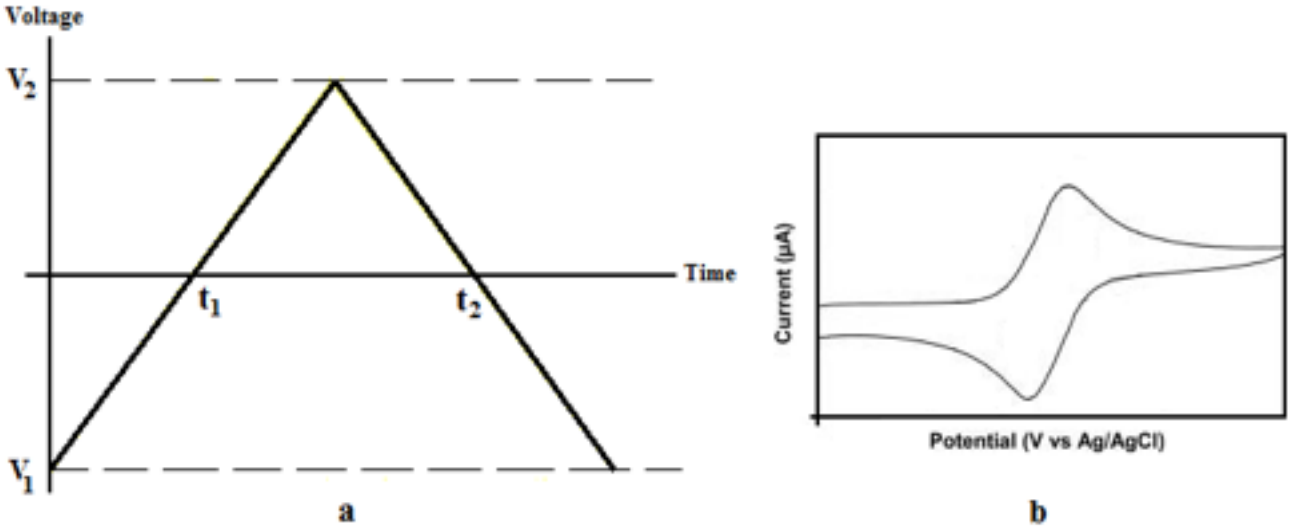


Fig 4.6 C V Study of electrodes

4.4.2 Cyclic Voltammetry

Cyclic Voltammogram is a graph of current vs. potential .

A stationary solution is used , which consists of an analyte and ion excess of background electrode, so as to suppress the migration of analyte transport directly.

When the current finally reach the peak, it starts decreasing. This is because, the concentration gradient decreases as the area composed of depleted concentration spreads further away from surface of the electrode. With the reversal of direction of the potential scanning. the same scenario repeats itself, but the B_{n-} gets oxidised back to B , which results oxidation(anodic) current peak (i_{pa}).

4.4.3 Electrochemical Impedence Spectroscopy (EIS)

EIS gives more wide view of resistance. When DC (Direct Current) is used , there is only resistor, that obstructs the flow of current. For DC current , the resistance is measured by ohms law i.e.

$$R=E/I$$

Resistors provide simplicity to the circuit due to the following properties-:

1. For the complete range of voltage and current, the relationship is linear.
2. It is frequency independent.
3. In-phase relationship between the current and voltage (i-v graph)

The real case comes when Alternating Current is there. In case of Alternating current (AC) , the capacitors also effects the electron flow along with the resistors. Here the simple model of resistor cant be used. The representation of electrochemical cell is done by simple electronic model .AC potential is used in EIS (Electrochemical Impedence Spectroscopy) . Stradiotto.,*et al,(* 2003) Small amplitude , generally 5 to 10 mV is applied, and current through the electrochemical cell (working electrode) is measured. (Bard., *et al*, 1980.). The advantage of EIS is that the electrochemical cell can be modeled by using a purely electronic model.

The potential is kept low to such value so that a pseudo-linear response can be obtained.By pseudo linear response, it can be interpreted that with application of sinusoidal voltage signal, a same sinusoidal frequency current signal is produced but with the phase shift .

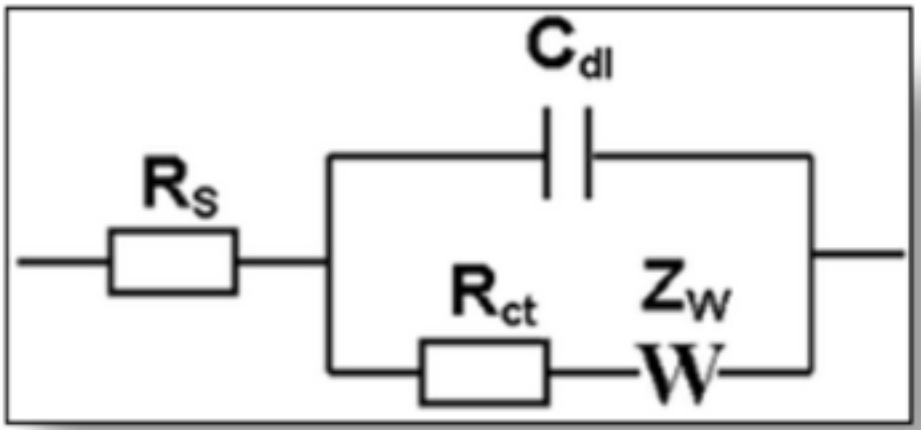


Fig 4.7 Randles circuit

The Electronic circuit consists of both the resistors and capacitors. This is the most frequently used Randles circuit, presented in the fig.4.7 R_S shown is the solution resistance, C_{dl} - double layer capacitance and Feradic reaction impedence which includes R_{ct} -charge transfer resistor and very well known Warburg diffusion element(W). Such plot displays the kinetic control in the region of high frequencies and a mass transfer (Warburg diffusion) control at low frequencies. A parallel combination of faradic impedance and double layer capacitance are connected to solution resistance in series as shown in fig.The parameters of the electronic Randles circuit, are obtained by a least square mechanism , that is present in the modern EIS software used . The plot, through which results of the model are represented is commonly known as Nyquist plot.

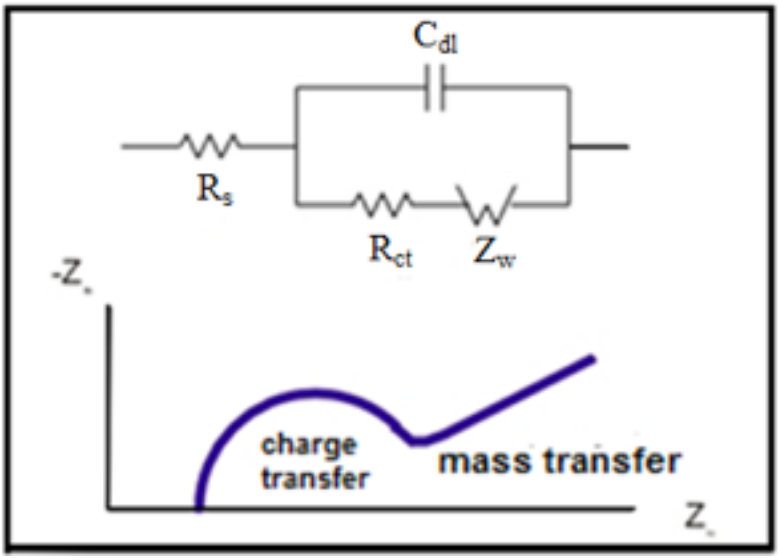


Fig 4.8 Typical fitting Nyquist plot

High frequency region-kinetic control and low frequency region-mass transfer control is shown in this typically fitted plot. Diameter of the semicircle gives the value of R_{ct} (transfer resistance) of the prepared electrode.

Chapter 5
Experimental Procedure

Here in this chapter, all the experimental details are shown. The complete process, synthesis of n-CuO and electrochemical sensing of modified n-CuO was carried out in the department of APPLIED PHYSICS, DTU.

5.1 Materials

In this project, high purity AR grade chemicals were used. Copper acetate [$(CH_3 COO)_2.H_2O$] as precursor and Poly Ethylene Glycol (PEG) as directional agent were brought from Merk. potassium ferrocyanide $K_4[Fe(CN)_6]3H_2O$ 1-(3-(dimethylamino)-propyl)-3-ethylcarbodiimide hydrochloride (EDC) ($C_8H_{17}N_3$), potassium ferricyanide $K_3[Fe(CN)_6]$ sodium diphosphatedihydrate ($Na_2HPO_4.2H_2O$), 3-aminopropyltriethoxy saline (APTES) , sodium monophosphate (NaH_2PO_4), and N-hydroxysulfosuccinimide (NHS), were obtained from Fisher scientific. For the electrolyte, 5mM phosphate buffer saline (PBS) solution of pH 7.2 of [$Fe(CN)_6^{3-/4-}$] was used as a redox species.. The milli-Q water (18.2 MΩ) was used for formation of all buffers, as solutions and also was used to clean all materials and glass wares.

5.2 Apparatus

X-ray diffraction (XRD) obtained on Bruker D-8 Advance with $\lambda = 0.154$ nm (Cu Kα) was used to study The structure and crystalline phase of the synthesized sample and the identification of the phase were made with the help of the JCPDS files. Scanning electron microscope (SEM) from TESCAN, MIRA II LMH CS has been used for studying surface morphology and the size of the synthesized samples. The FT-IR [Perkin Elmer Spectrum BX 11] was used to find all functional groups in the samples. The electrochemical response of the electrode has been studied using cyclic voltammgraph (CV) and impedance measurements (EIS) conducted on an Autolab Potentiostat (Netherlands) using 3-electrode cell. In a three electrode cell, fabricated electrode worked as the working electrode, Ag/AgCl as the reference electrode and platinum (Pt) as the counter electrode in the 50mM, 7.2 pH phosphate buffer saline (PBS) containing $[Fe(CN)_6]^{3-/4-}$.

5.3 Experimental Procedure-

5.3.1 Synthesis of copper oxide:

0.5 M solution of Copper acetate was prepared by taking a known amount of Copper acetate in 20 mL deionized water. The solution was stirred for 10 minutes on magnetic stirrer. It was labeled as Solution A. Solution B of 5M was prepared by taking a known amount of NaOH in 20 mL DI water. It was also stirred for 10 min. Sol. A and Sol. B were then mixed and stirred thoroughly. 1mL PEG was added drop-by -drop to the solution. Along with PEG, water was added and the solution was stirred continuously for some time. The solution was centrifuged then to obtain the precipitate. The precipitate was dried overnight and was collected as powder and was used for further characterization.

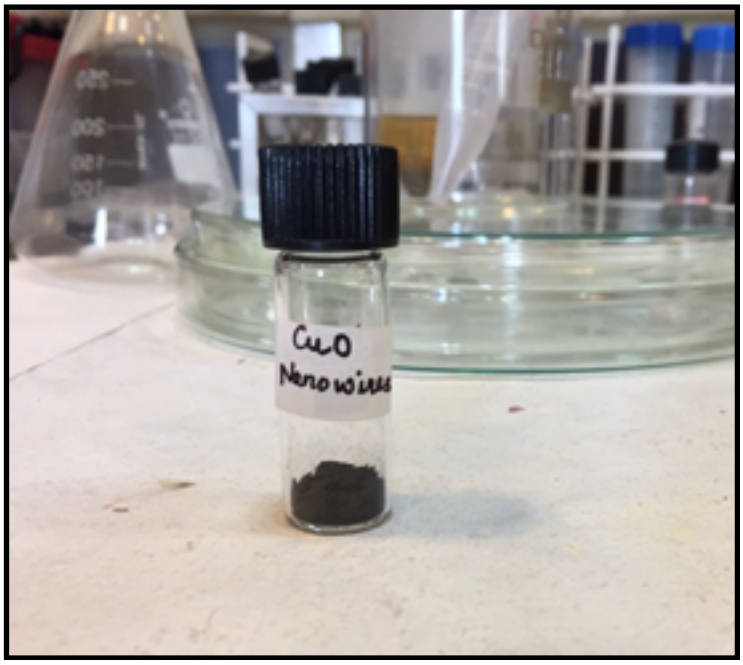


Fig 5.1 As synthesised n-CuO powder

5.3.2 Functionalization of Copper oxide and its electrophoretic deposition on ITO electrode (APTES/n-CuO/ITO):

For the functionalization of n-CuO, 5 mg/mL concentration of n-CuO was prepared in isopropanol and was ultrasonicated for half an hour and kept on stirring at room temperature. After continuous stirring for more than one hour, 100 µL of APTES (98%) was added to it drop wise and stirred at 400 rpm for 45 h at room temperature (27 ˚C). To remove the unbound APTES, the suspension was filtered and washed thoroughly with deionized water. Finally it was centrifuged and the precipitate was collected after washing it properly with DI water and Ethanol.

For the electrophoretic deposition, a colloidal solution was prepared of APTES-n-CuO and acetonitrile (0.1mg/ml). Two electrode cell was optimised by varying the voltage. Pt foil was used as cathode in cell and clean ITO glass electrode as anode. $Mg(NO_3).6H_2O$ was added to suspension ,as a catalyst. The optimised voltage was applied to the cell for making thin films of APTES-n-CuO. The as prepared APTES/n-CuO/ITO films were kept out of the solution for drying.

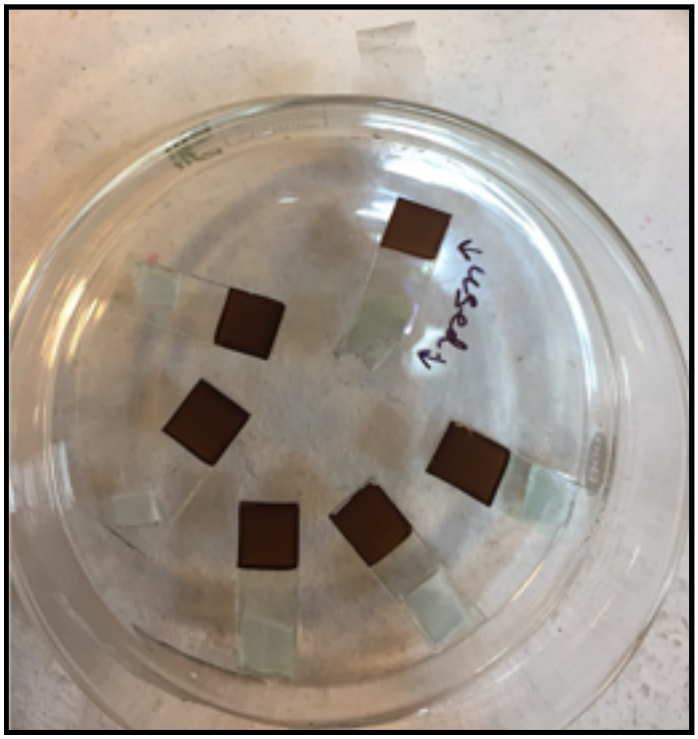


Fig 5.2 APTES modified n-CuO electrodes

5.3.3 Immobilization of cTnI antibodies onto the APTES/n-CuO /ITO electrode

A stock solution of anti-cTnI was prepared in PBS buffer of pH 7.2. Before immobilization, the carboxyl groups present in antibodies were actuated by EDC-NHS chemistry. 0.2 M EDC as a coupling agent was used and 0.05 M NHS as an activator. 7.5 µL of each (0.2 M EDC and 0.05 M NHS) were mixed with 15 µL of anti-cTnI. Subsequently, this solution was uniformly spread onto APTES/n-CuO /ITO electrode. Humid chamber was used to keep The electrode at room temperature and lastly, ethanolamine (EA = 1 mg/mL) was used for blocking the non specific active sites of the electrode. The EA/anti-cTnI/APTES/n-CuO/ITO bioelectrode was refrigerated (4˚C) when ideal.

Chapter 6

Results and Discussion

6.1 Crystallographic study:

The crystallographic study of the as-synthesized n-CuO has been characterized by X-ray diffraction (XRD) performed in the range of 30^0 to 80^0. Predominately five major peaks have been observed in which, the first two peaks represented by dots, are of n-CuO peaks and the rest three peaks represented by arrows are of copper substrate. First peak is observed at 36.86° correspond to planes (111),(002) and next peak of n-CuO is at 38.8133°with miller indices as (111), (200). Both these peaks represents that n-CuO structure is monoclinic.

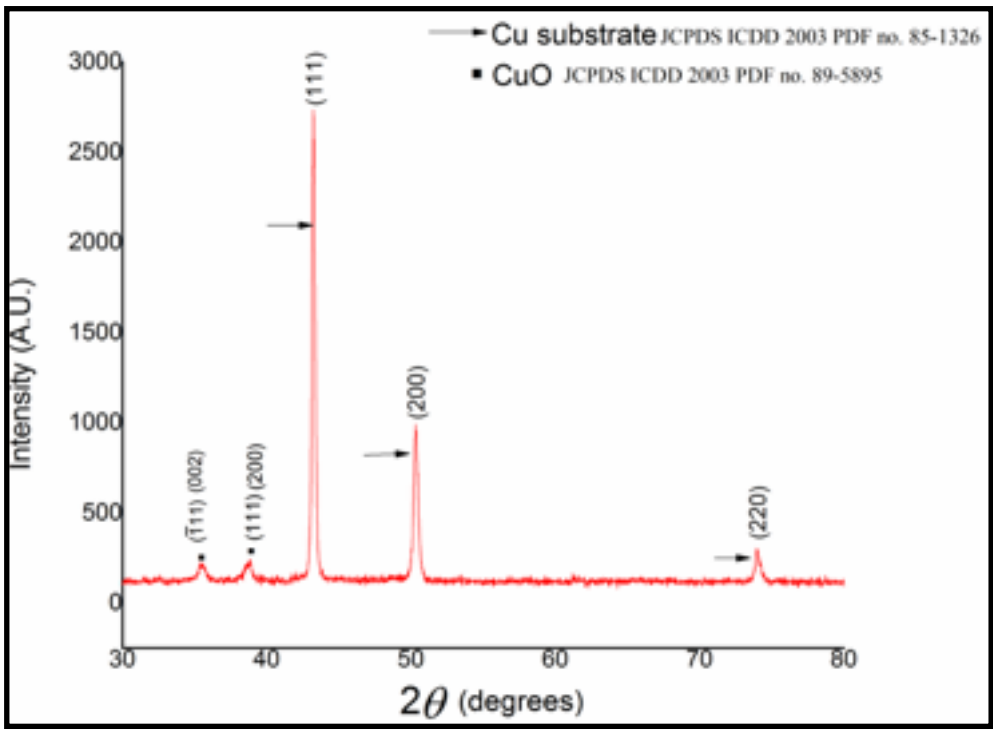


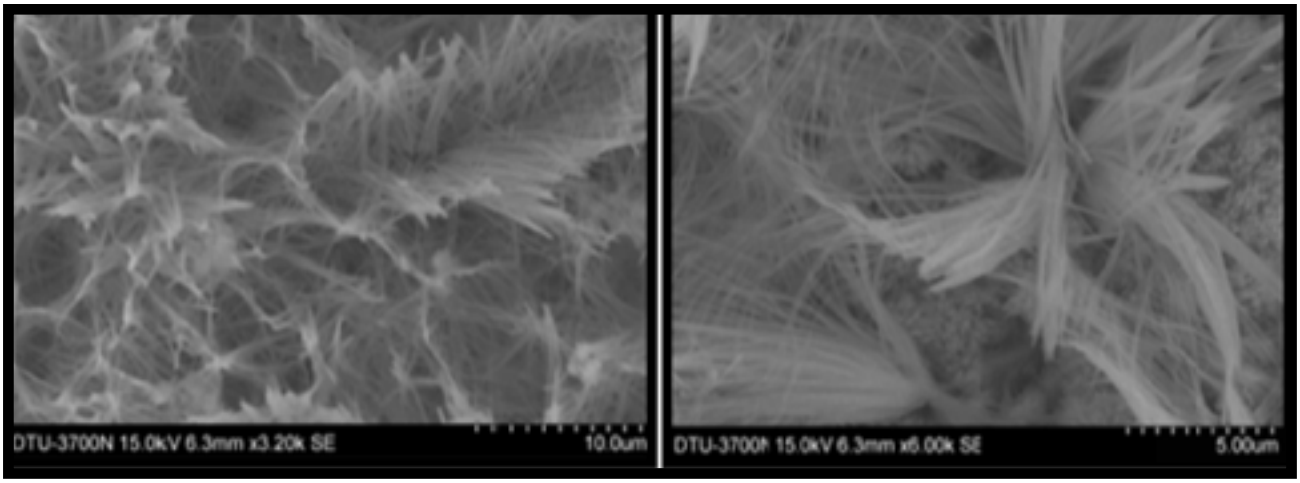
Fig 6.1 X-Ray Diffraction pattern for n-CuO sample

The other three peaks observed at angle 42.5°, 51.6°, and 78.86°. Corresponding miller indices to these peaks are (111), (200) and (220) respectively. From the peaks of Cu substrate, it can be interpreted that the system shows cubic structure with face entered lattice. Lattice parameters obtained are a=b=c=3.461.

The mean size of the crystal is obtained by Scherrer equation . With first peak of n-CuO , mean size was calculated as τ= 14.786 nm and with second peak, mean size was calculated as τ= 15.897 nm. The average size of the n-CuO nanaorods , was calculated as 15.3415nm.

The record Cartesian coordinate range of this instrument is 400-4000cm^{-1}. Every sample was collected with 64 scans co-added at 4cm^{-1}resolution. The traditional operation mode of this spectrometer is temperature stabilised. The transmission spectra give higher distinction between intensities of strong and weak bands as a result of transmission range from zero -100% T whereas absorbance ranges from infinity to zero.

6.2 Scanning Electron Microscopy



(a) (b)

Fig 6.2 SEM image of n-CuO (a) lower resolution (b) higher resolution

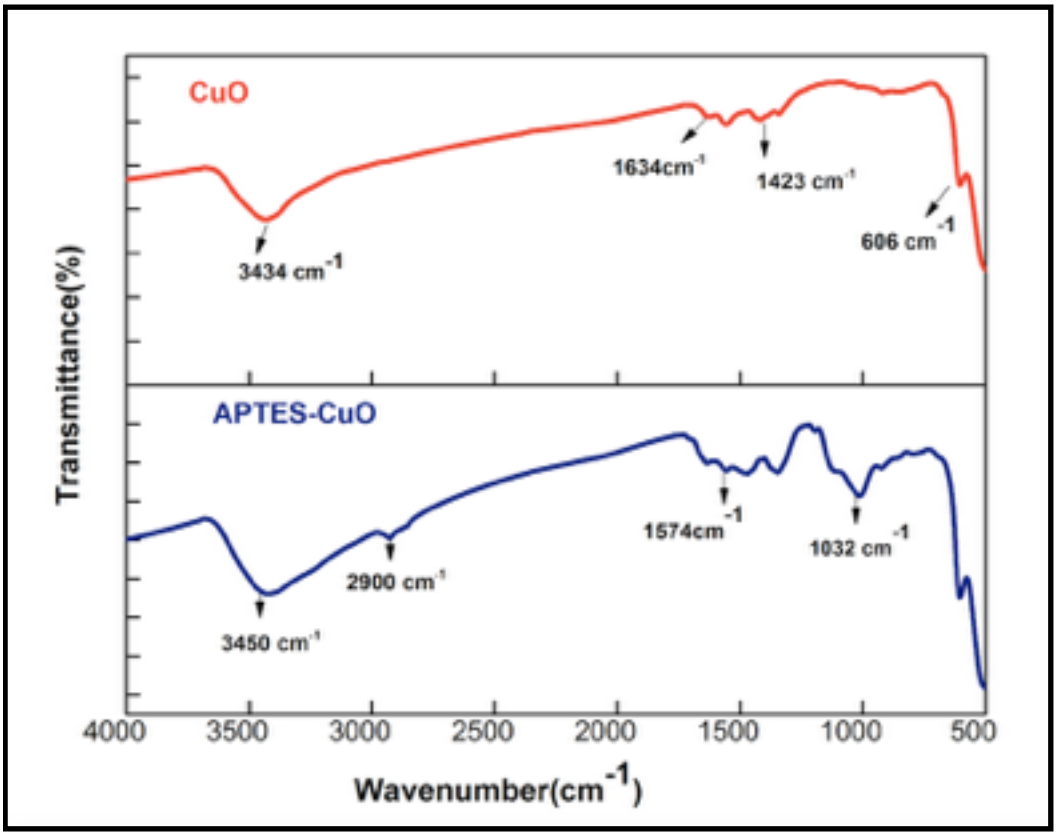
The morphological analysis of the n-CuO has been performed via scanning electron microscopy (SEM).Following fig shows the SEM images of the n-CuO nanowire so formed. It can be seen from SEM images that on increasing pH of solution, density of nanostructures increases. The SEM images clearly shows nanowire which are 10-15nm in diameter. The surface of the Cu substrate has been uniformly covered with these nanowire. These wires are precise and are in range 100nm-200nm in length. Fig (b) has higher resolution. These nanowires are smooth and densely packed, as seen from the top view. (Garcia *et al.,* 2007)

6.3 Fourier Transform Infrared Spectroscopy

The infrared absorbance spectra of n-CuO and APTES functionalized n-CuO (APTES/n-CuO) in transmission mode are shown in Fig. . the FT-IR spectrum of n-CuO(curve…) reveals the characteristic peaks at 606 cm-1,1423 cm-1,1634 cm-1 and 3434 cm-1. The peak 606 cm-1 is of Cu-O stretch (shows that metal oxide is there in the sample). The peaks in the range 1350 cm-1—

1800 cm-1 mainly at 1423 cm-1 and 1623 cm-1 represents the metal oxide(M-O ; Cu-O) inside the plane and outside the plane respectively. At 3434 cm^{-1}, the observed peak is of hydroxide group (O-H) attached. It may be present because with the surface of n-CuO particles, the water molecules get attached. If heated for long time, this byproduct (water) can be removed.

Fig



6.3 FTIR of n-CuO and APTES/n-CuO

In reference with the APTES-n-CuO sample shown in fig, the characteristic band is observed at 1578 cm^{-1} in APTES is present in the fig with a slight shift. The peak at 1574cm^{-1} is earmarked for the N-H stretch and vibration. Other peak at 1032 cm^{-1} denotes the silane group attached. Peak observed at 1032 cm^{-1} represents the stretching (Si-O) of silane group. For the fitting of organic layer, onto the inorganic layer, silanes are important. Surface hydroxide chains attach with Si-O bonds through a reaction to get linked with the proteins or polymers (organic layers). Also the hydroxide (O-H) peak at 3450 cm^{-1} is broadened with APTES-n-CuO as compared to n-CuO sample. This broadening can be explained with N-H stretching.

6.4 Electrochemical Study

6.4.1 Cyclic Voltammetry Studies:

In electrochemical analysis, CV has been used to scrutinize the electrochemical behaviour of the as modified electrode surface.

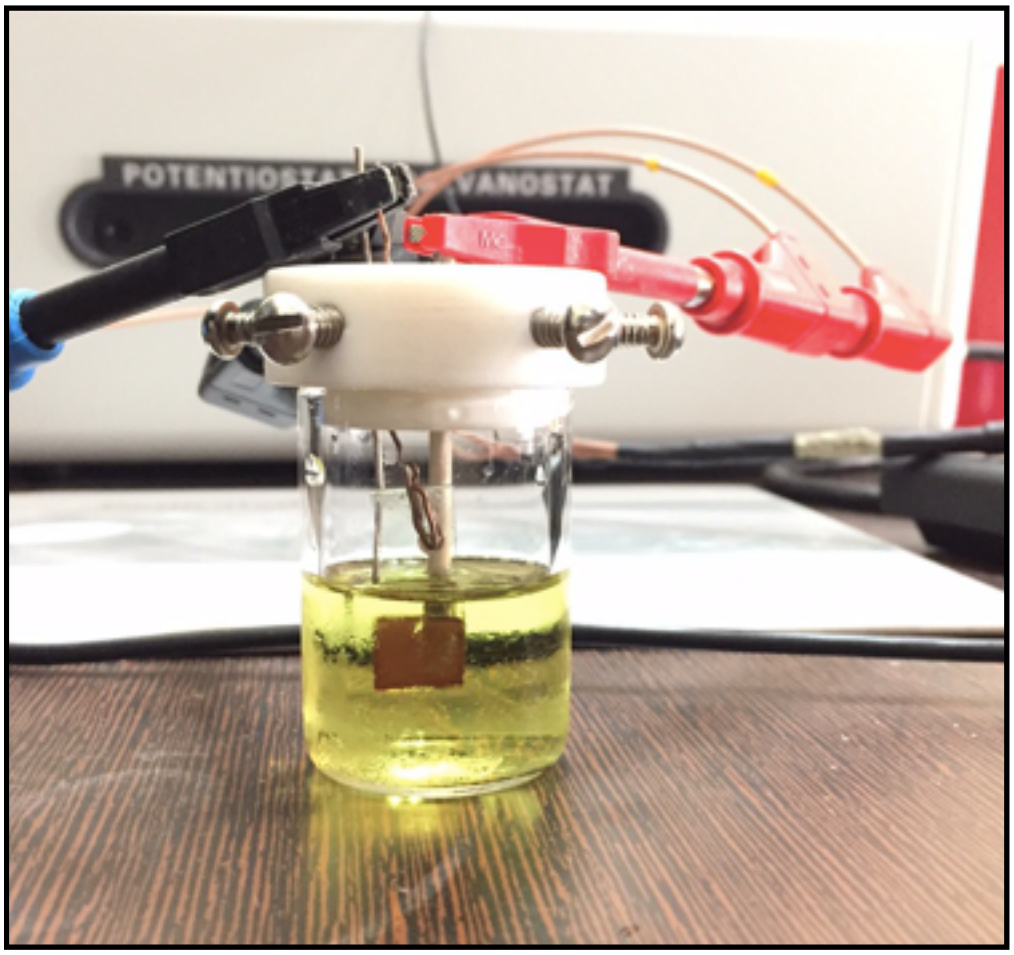


Fig 6.4 Three electrode cell for electrochemical sensing

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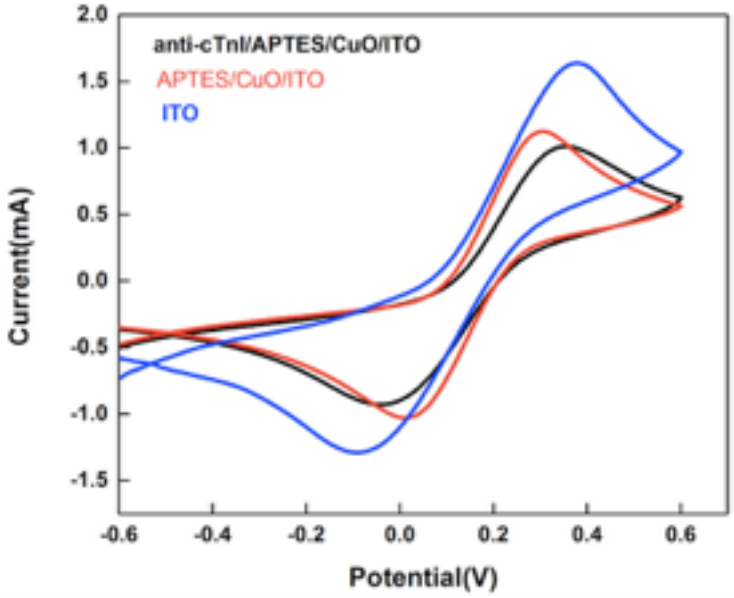
Electron transfer rate constant is accountable to the changes found in peak current and to separation of peak potentials in the CV analysis.

The CV studies have been performed for (i) ITO electrode (ii) APTES/n-CuO/ITO and (iii) anti-cTnI/APTES/n-CuO/ITO electrode at the scan rate of 100mV/s. The peak current for ITO was observed at 0.926mA, for APTES/n-CuO/ITO was 1.186mA and for anti-cTnI/APTES/n-CuO/ITO the peak current was 1.67mA. The variation is attributed with good electrochemical propertie.

Figure… shows the response of the EA/**anti-cTnI/APTES/n-CuO/ITO** bioelectrode in PBS buffer (0.9% NaCl) using cyclic voltammetry (CV). We have chosen pH 7.2 because maximum current is obtained at this value. In general, neutral pH is used as at pH 7, biomolecules remains conserved at their natural form.

(a)



(b)

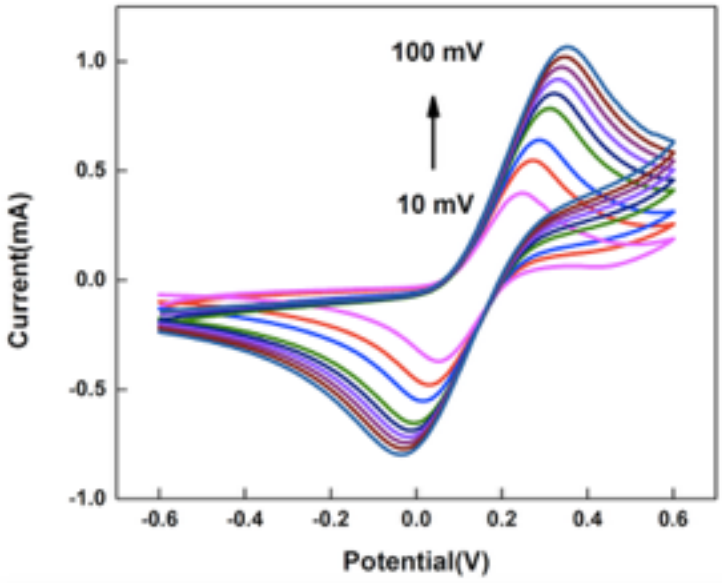


Fig 6.5 (a) CV of bioelectrodes (b) scan rate studies of EA/**anti-cTnI /APTES/n-CuO/ITO** electrode (10-100mV/s)

The CV responses of EA/**anti-cTnI /APTES/n-CuO/ITO** electrode has been taken with scan rate in the voltage range 10-100mV/s. As observed, the peak currents I_{pa} and I_{pc} (anodic peak current and cathodic peak current) , with the scan rate had a direct relationship. This relationship is explained with electrochemical reaction , which uses Diffusion process to control the reaction.

With increase in scan rate, there is a peak shift. The oxidation peak takes a shift towards high potential, and the reduction peak takes a low potential shift indicating a transfer of charge from medium to electrode.

6.4.2 Electrochemical Response analysis

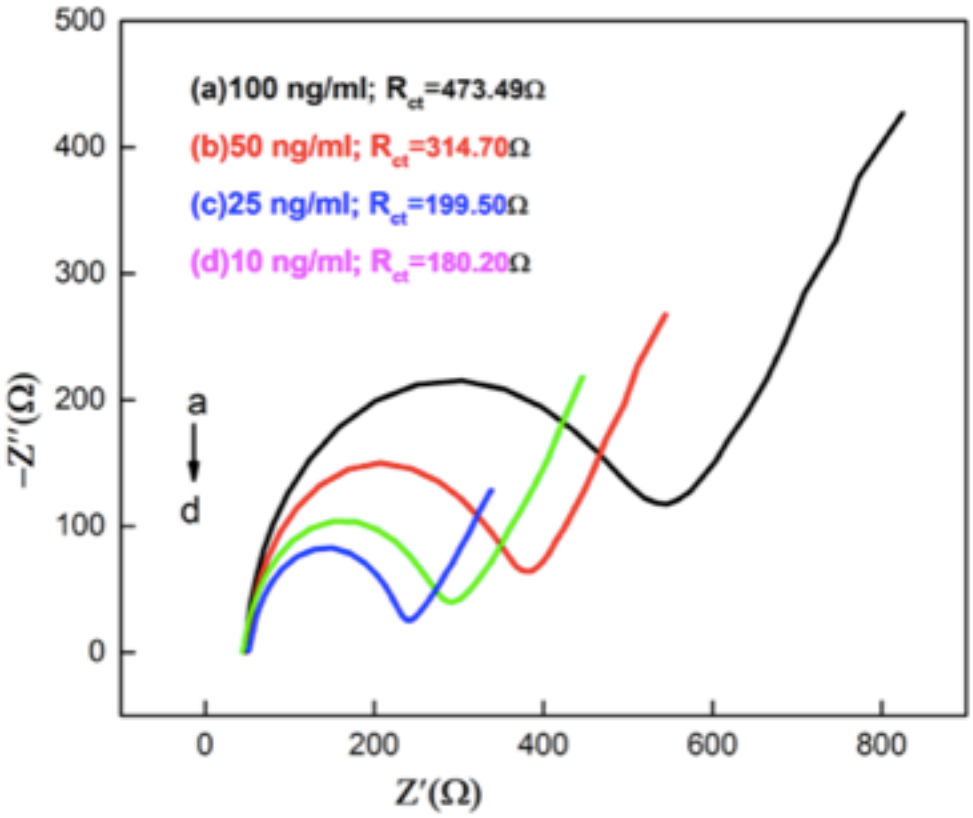


Fig 6.6 EIS of the EA/anti-cTnI/APTES/n-CuO/ITO bioelectrode

Fig. 6.6. represents the electrochemical impedance studies of the EA/anti-cTnI/APTES/n-CuO/ITO bioelectrode as a function of cTnI concentration (10-100 ng/mL). With the use of Randles circuit, value of R_{ct} is evaluated.

It can be seen that the R_{CT} value (diameter of the Nyquist plot) in fig 6.6 , increases cTnI concentration. It shows linear relationship in the entire range of 10-100 ng/mL.

This is explained by the cTnI interaction. The specific interaction between the antibody-antigen may lead to complexes that are electrically insulated, blocking the process of electron transfer from $[Fe(CN)_6]^{3-/4}$. The anti-cTnI/APTES/n-CuO/ITO bioelectrode observes high sensitivity with good stability and linearity.

Chapter-7
Conclusion

In outline, we have successfully synthesized CuO nanowires (25 nm in diameter) using a wet chemical compound technique on the copper foil . The proposed strategy utlize low temperature environment, savvy techniques and provides uniformity and homogenity in nanostructures.

Synthesis of n-CuO depends on the coordination self assembly of square complexes of Cu. The crstraloographic and morphological studies shows the successful synthesis of desired CuO nanowire. This work showed that the obtained CuO NSs are highly dense, uniform and compromise pure CuO crystal phase.

One of the main objectives of my research is to develop the electrocatalytic oxidation in electrochemical sensors by using metal oxide nanoparticles (n-CuO).These nanostructures guarantee numerous potential applications in sensors. We have effectively functionalized nanostructured copper oxide (n-CuO) by APTES. Electrophoretic deposition technique has been used to develop thin films of APTES/n-CuO/ITO and these thin films are covalently immobilised by anti-cTnI for cTnI detection by EIS technique. The infrared spectroscopic (FTIR) studies confirms the effective grafting of APTES on n-CuO. The electrochemical studies conducted via cyclic voltammetery shows oxidation and reduction peaks , thereby showing a good electrochemical behaviour of n-CuO. precision so it exhibited good reproducibility and long-term stability. Also, the fabricated electrode displays a voltammetric response to the ITO, APTES/CuO/ITO, and anti-cTnI/ APTES/CuO/ITO and the results were matched with referenced value obtained by the standard technique.Scan rate studies of anti-cTnI/APTES/CuO/ITO bioelectrode indicated Diffusion controlled process for the electrochemical reaction. Interfacial properties of electrolyte boundaries and electrode were succesfully determined by EIS technique and R_{ct} value was evaluated from the Nyquist plot.

The impedance value increased with increase in anti-cTnI concentration which shows specific interaction between antigen and antibody. Due to the bio-safe properties in many cases and the low cost for fabrication of the metal oxide semiconductors, especially CuO nanomaterial, In correlation with other announced Troponin biomarker discovery techniques including biosensors, the proposed biosensor is simple, facile and label free.

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