STUDIES OF GLUCOSE OXIDASE IMMOBILIZED CARBON NANOTUBE - POLYANILINE COMPOSITES

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS
FOR THE DEGREE OF

MASTER OF TECHNOLOGY

IN

MATERIALS SCIENCE AND ENGINEERING

By

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July, 2009

Dedicated to

My

Parents

And

Teachers

NEETESH KUMAR

CERTIFICATE

This is to certify that the thesis entitled **“Studies of Glucose Oxidase Immobilized Carbon Nanotube Polyaniline Composites”** submitted by **Mr. NEETESH KUMAR** Roll No. 60702009 in partial fulfillment of the requirement for the award of the degree of **MASTER OF TECHNOLOGY** in Materials Science and Engineering, Thapar University Patiala, is a record of candidate’s own work carried out by him under our supervision. The matter embodied in this report is of the candidate’s own record and not submitted to any other university in any part or full form for the award of such kind of a degree.

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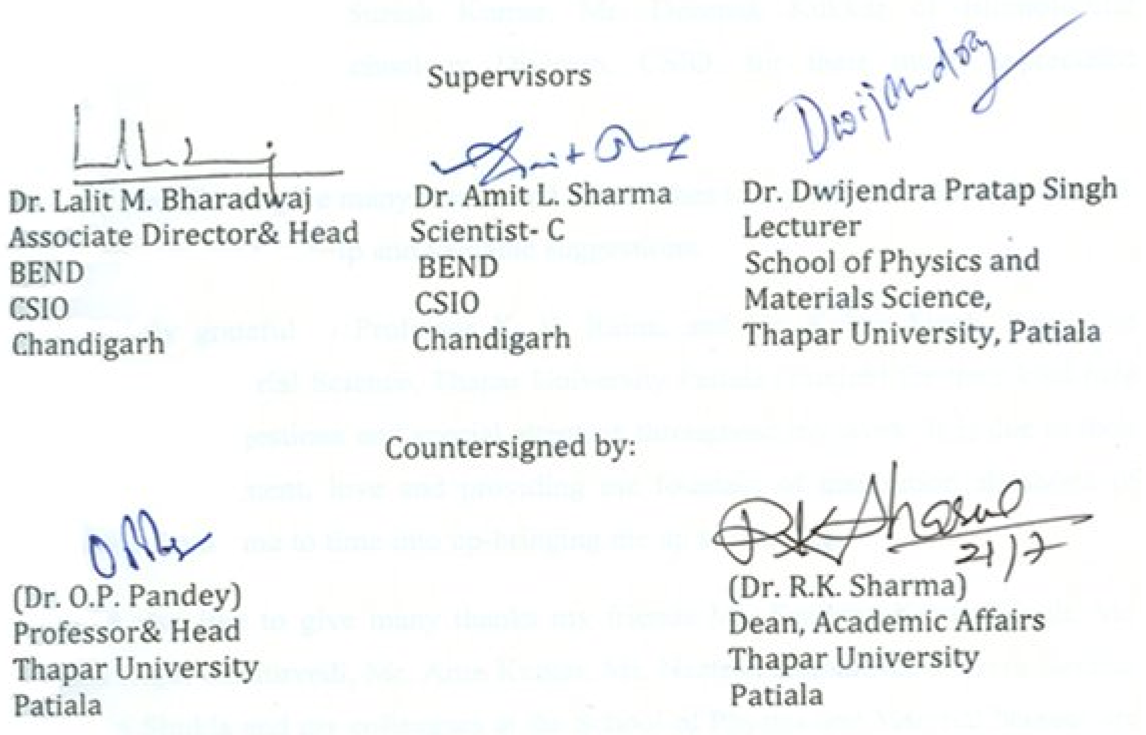

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ACKNOWLEDGEMENT

I would like to express my deepest gratitude to my supervisor Dr. Lalit M. Bharadwaj for the inspiring guidance, support, encouragement and time commitment during this thesis work. I would like to thank Dr. Amit L. Sharma for his help and support. I could not finish my study without their help and encouragement. I believe what I have learnt from them would greatly benefit my future career. I would also like to acknowledge the contribution of my original supervisor Dr. Dwijendra Pratap Singh.

I wish to express my sincere thanks to Dr. Pawan Kapoor (Director CSIO), and Dr. O.P. Panday, Professor and head, School of Physics and Materials Science for permitting and providing the facilities necessary for carrying out thesis work at CSIO.

I am thankful to Dr. Akash Deep, Dr. Indrapreet Kaur, Mr. Ashwani Kumar, Ms. Hersimran Kaur, Mr. Suresh Kumar, Mr. Deepak Kukkar of Biomolecular Electronics and Nanotechnology Division (BEND), CSIO, for their much appreciated guidance.

I would also like to give many thanks and best wishes to my fellow trainees in BEND, CSIO, for any kind of help and valuable suggestions.

I am highly grateful to Professor K. K. Raina, and Dr. Kulvir Singh, School of Physics And Material Science, Thapar University Patiala (Punjab) for their kind help and valuable suggestions and special attention throughout my work. It is due to their moral encouragement, love and providing me fountain of inspiration all shorts of assistance from time to time into up-bringing me upto this stage.

I would also like to give many thanks my friends Ms. Indu Bajpai, Ms. Nidhi Rana, Mr. Rahul Kumar, Mr. Sandeep Kumar Singh, Mr. Karuna Sagar Chaturvedi, Mr. Arun

Kumar, Mr. Akash Katoch, Mr. Rajeev Sehravat and my colleagues at the School of Physics and Material Science are acknowledged for providing me a friendly atmosphere and encouraging me throughout this work.

I am deeply thankful to my Family, their moral support and patience has borne fruit through completion of this Thesis which will result in award of the prestigious degree of M.Tech in Materials science & Engineering.

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ABSTRACT

List of abbreviations used

CNTs	Carbon nanotubes
MWNTs	Multiwall Carbon nanotubes
p-MWNTs	Pristine Multiwall Carbon nanotubes
c-MWNTs	Carboxylated Multiwall Carbon nanotubes
a-MWNTs	Acylated Multiwall Carbon nanotubes
f-MWNTs	Functionalized Multiwall Carbon nanotubes
GOD	Glucose oxidase
GOD-MWNTs	Glucose oxidase attached on Multiwall Carbon nanotube
PBS	Phosphate buffer
PANI	Polyaniline
APS	Ammonium persulfate
MWMT-PANI	Multiwall Carbon nanotubes- polyaniline Composite
p- MWMT-PANI	Composite of polyaniline and pristine MWNT
c- MWMT-PANI	Composite of polyaniline and carboxylated MWNTs
a- MWMT-PANI	Composite of polyaniline and acylated MWNTs
GOD-a-MWNT-PANI	Glucose oxidase attached a-MWNT-PANI composite
GOD-c-MWMT- PANI	Glucose oxidase attached c-MWMT- PANI composite
CP	Conducting Polymer
FT-IR	Fourier Transform Infra-red (FT-IR) Spectroscopy
FE- SEM	Field Emission scanning electron microscopy
ITO	Indium Tin Oxide
POD	Peroxidase

ABSTRACT

Glucose biosensor **is** an analytical device that responds in a direct reversible, continuous, rapid, and accurate manner to changes in the concentration of chemical or biochemical species in an untreated sample. It consists of a sensing microzone where a chemical or biochemical reaction takes place. This is connected or integrated with a transducer.

The performance and usefulness of these types of biosensors are often dictated by the immobilization methods and the immobilization matrixes employed for the deposition of the enzyme layer. For example, the sensor lifetime, its dynamic range, sensitivity, selectivity, response time, and stability are some of the operational parameters affected by the enzyme immobilization procedure and the type of support materials used for the biosensor fabrication.

The aim of this thesis was to study the I-V Characteristic of Glucose Oxidase Immobilized Carbon Nanotube-Polyaniline Composites. MWNTs were functionalized and enzyme is immobilized on the acylated MWNTs. The f-MWNTs and GOD-MWNTs were characterized by FT-IR and FE-SEM.

Polyaniline, MWNT-PANI, c-MWNT-PANI, a-MWNT-PANI composites were synthesized by in situ chemical polymerization process and enzyme was immobilized on the c-MWNT-PANI and a-MWNT-PANI composites. These composites were characterized by FT-IR and FE-SEM.

The films of these synthesized materials were prepared by solution casting method on the glass substrate and I-V characteristic were studied.

The amperometric response of GOD-MWNTs film was studied at constant voltage (Real Time measurement) and variable voltage sweep. The current was found to be increased when glucose solution was dropped on the sensing zone of the film. The result shows the successful synthesis of glucose sensing material.

CHAPTER-1
INTRODUCTION AND LITERATURE REVIEW

The history of biosensors started in the year 1962 with the development of enzyme electrodes by the scientist Leland C. Clark. Since then, research communities from various fields such as VLSI, Physics, Chemistry, and Material Science have come together to develop more sophisticated, reliable and mature biosensing devices for applications in the fields of medicine, agriculture, biotechnology, as well as the military and bioterrorism detection and prevention.

Nano-materials such as, nanoparticles, carbon nanotubes, nano-structured conducting polymers and nanotube-polymer composites have recently attracted much interest owing to their applications in nano-scaled devices, sensors and detectors. Now a day many glucose bio-sensors are available in the market for diabetic patients.

The CNT based biosensor exhibits enhanced stability and approximately eight-fold sensitivity compared metal and carbon electrode based glucose sensors. Today fabrication of CNTs and CNT-conducting polymers based biosensors is an emerging field of research.

1.1 CARBON NANOTUBES

Since their discovery in 1991by Iijima and coworkers at the NEC Fundamental Research Laboratory in Tsukuba, Japan used high resolution electron microscopy to observe carbon nanotubes [1]. He observed tubular features in TEM images (Fig- 1.1) of fullerene soot produced by arc discharge. In 1993, Iijima’s group, as well as Donald Bethune and his colleagues at IBM’s Almaden Research Center in San Jose, California, found that the use of transition – metal catalysts leads to nano-tubes with only a single shell [2].

The quasi one-dimensional carbon nanotubes (CNTs) have captured the attention of researchers worldwide due to their unusual mechanical, electronic, and adsorptive properties as well as their good chemical stability. These features of CNTs make them attractive candidates in nanoscale device applications.

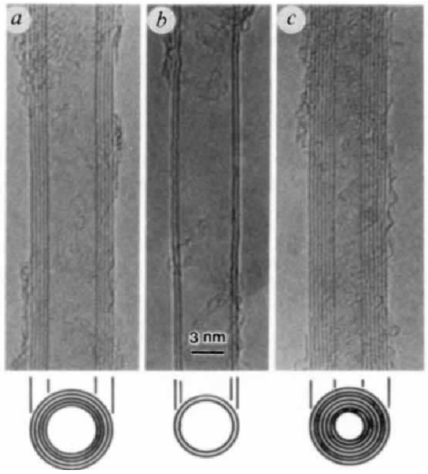

Figure- 1.1: Electron micrograph of microtubules of graphitic carbon [1]

The CNTs are tube like structures of nanometer diameter (10^{-9}) and many microns in length, which easily form bundles. They entangle together in the solid state giving rise to a highly complex network. However, due to strong inter-tube van der Waals interactions, CNTs lack solubility and are difficult to manipulate in any solvent. Hence applications using the materials have been limited. Therefore, strategic approaches toward the solubilization/suspension of CNTs for their integration into inorganic, organic, and biological systems are important.

1.1.1 Structure of Carbon Nanotubes

The few key studies have explored the structure of CNTs using high-resolution microscopy techniques. These experiments have confirmed that these nano-tubes are cylindrical structures based on the hexagonal lattice of carbon atoms that forms crystalline graphite. Three types of CNTs are possible, called armchair, zigzag, and chiral nanotubes depending on how the two- dimensional grapheme sheet is “rolled up”[2].

CNTs are formed from pure carbon bonds have two covalent bonds: sp^2 and sp^3. The former constitutes graphite and the latter constitutes diamond. Figure 1.2 shows the structure of graphite and diamond.

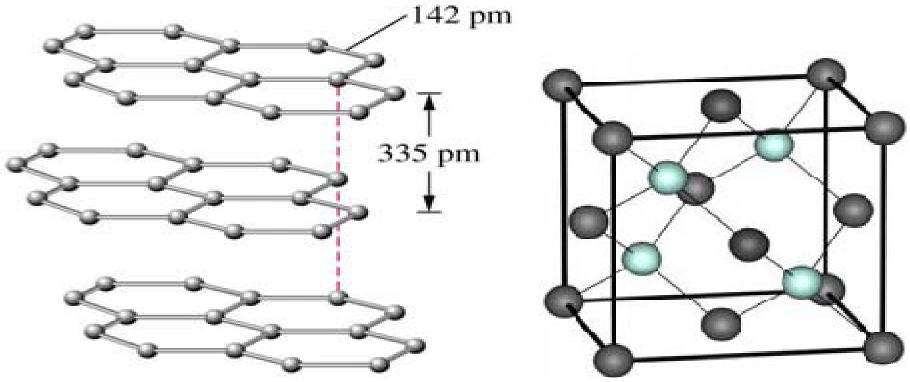

Figure 1.2: Structures of graphite and diamond

The sp^2 hybridization, composed of one s orbital and two p orbitals, is a strong bond within a plane but weak between planes. When more bonds come together, they form six-fold structures, like honeycomb pattern, which is a plane structure, the same structure as graphite. Graphite is stacked layer by layer so it is only stable for one single sheet. Wrapping these layers into cylinders and joining the edges, a tube of graphite is formed, called nanotube.

Atomic structure of nanotubes can be described in terms of tube chirality, or helicity, which is defined by the chiral vector, C_h, and the chiral angle, θ. Figure 1.3 shows, visualized cutting a graphite sheet along the dotted lines and rolling the tube so that the tip of the chiral vector touches its tail. The chiral vector, often known as the roll-up vector, can be described by the following equation: $C_h = na_1 + ma_2$, where the integers (n, m) are the number of steps along the ziz-zag carbon bonds of the hexagonal lattice, and a_1, a_2 are unit vectors in the two-dimensional hexagonal lattice, as shown in **Figure 1.3**.

Chiral angle determines the amount of “twist” in the tube. Two limiting cases exist where the chiral angle is at 0° and 30°. These limiting cases are referred to as ziz-zag (0°) and armchair (30°), based on the geometry of the carbon bonds around the circumference of the nanotube. The difference in armchair and zig-zag nanotube structures is shown in **Figure**

1.4. In terms of the roll-up vector, the ziz-zag nanotube is (n, 0) and the armchair nanotube is (n, n). The roll-up vector of the nanotube also defines the nanotube diameter since the inter-atomic spacing of the carbon atoms is known.

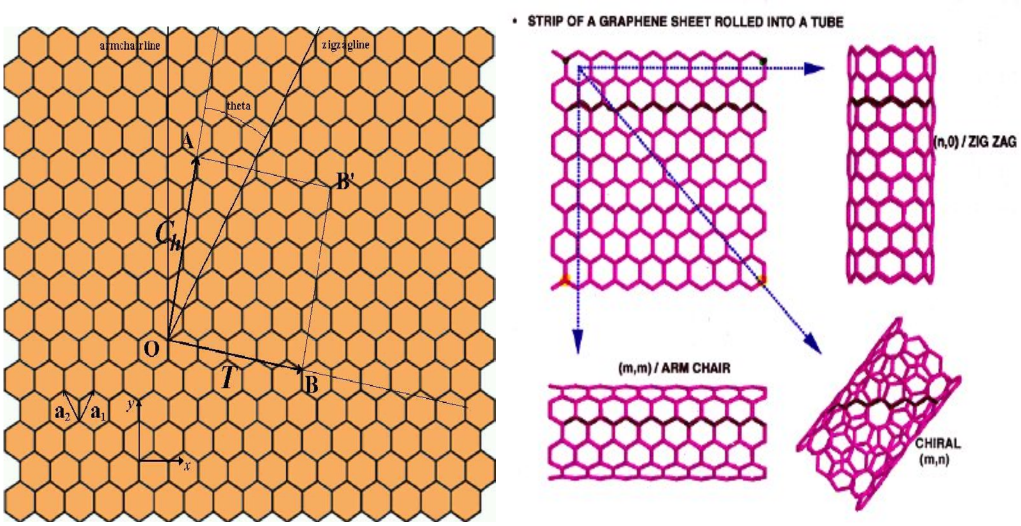

Figure 1.3: Schematic diagrams showing how graphite sheet is 'rolled' to form CNT [3]

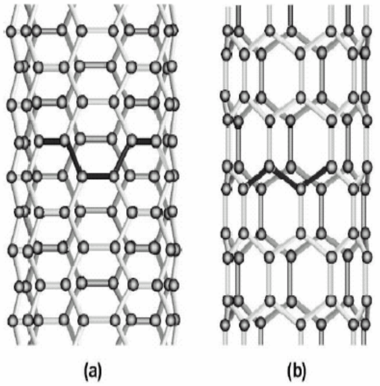

Figure 1.4: Illustrations of the atomic structure (a) an armchair and (b) a ziz-zag nanotube

1.1.2 Properties of Carbon Nanotubes

CNTs have gained in interest as nanoscale materials due to their exceptional, outstanding properties such as: extremely high Young's modulus and ultimate strength, high electric and thermal conductivity. Moreover, CNTs provide a remarkable model of a 1D system. More details on the properties of carbon nanotubes are presented below.

1.1.2(a) Electronic Properties

Due to its special structure, CNTs shows exceptional properties. One of the important properties of CNTs is that it can exhibit characteristics of a metal or a semiconductor. It is determined by chirality and diameter that carbon nanotube acts as metal or semiconductor. If n-m =3q, [2] where q is an integer nanotube acts as a metal otherwise it acts as a semiconductor. All armchair nanotubes are metallic, as are one-third of all possible zig-zag nanotubes. The diameter, d_t, is simply the length of the chiral vector divided by π, and we find that [3]

$$D_t = (\sqrt{3}/\pi)a_{c\text{-}c}\,(m^2+mn+n^2)^{1/2},$$

Where $a_{c\text{-}c}$ is the distance between neighboring carbon atoms in the flat sheet. The chiral angle is given by:

$$\tan^{-1}(\sqrt{3}n / (2m+n))$$

The geometry of the nanotubes determines band structures and thus the energy band gap. The energy band gap of semiconducting CNTs highly depends on the nanotube diameter and is given by:

$$E_{\text{gap}} = 2\gamma_0 a_{c\text{-}c}/d$$

Where, $\boldsymbol{\gamma_0}$ denotes the C-C tight binding overlap energy (2.45 eV), $a_{C\text{-}C}$ the nearest neighbor C-C distance (~1.42 Å), and d is the diameter of a nanotubes.

Based on their structure, CNTs can be divided in singlewalled CNTs (SWNTs) and multiwalled CNTs (MWNTS). In general, MWNTs are quite often found to be one-dimensional conductors with a high electrical conductivity (even >103 S/cm). The metallic properties of the MWNTs are due to their multiple-shell structure consisting of tubes with various electrical properties, where additional electronic coupling between shells takes place. Moreover, MWNTs are predicted to have ballistic electron transport at room temperature (it refers to conduction where Ohm’s law does not apply; the resistance is not dependent on the CNT’s length). The electrical current that could be passed through a multiwall nanotube corresponds to a current density in excess of 107 A/cm^2.

1.1 .2 (b) Chemical Properties

Chemical or physical modification of the surface of CNTs, e.g. by the attachment of certain molecules or functional groups is a very important issue in order to overcome their poor solubility in solvents. This process is known as functionalization. Functionalized CNTs are very attractive for chemical and biological applications because of their strong sensitivity to chemical or environmental interactions. This leads to a broad range of applications, e.g. as sensors. Covalent and non-covalent functionalization, doping, decoration with organic as well as inorganic species of the surface of CNTs lead to direct changes of the properties of CNTs such as optical, electrical, and mechanical [4].

1.1.2 (c) Mechanical Properties

The structural properties of CNTs with strong σ bonds between the carbon atoms give nanotubes a very high Young's modulus and tensile strength. The strength of the carbon-carbon bonds in-plane, along the cylinder axis, retains the structure exceptionally strong resistance to any failure. CNTs also have very good elasto-mechanical properties. Both experimental and theoretical investigations show extraordinary mechanical properties of individual MWNTs with Young's modulus being over 1 TPa and a tensile strength of 10 - 200 GPa, which is several hundred times more than that of steel, while they are only one-sixth as heavy.

1.1 .2 (d) Other Properties

Besides the outstanding mechanical and electrical properties, CNTs exhibit interesting thermal and optical properties. Defect-free nanotubes, especially SWNTs, offer a direct band gap and a well defined band and sub-band structure, which is ideal for optical and optoelectronic applications. The experimental measurements of the optical absorption of a bundle of single-walled CNTs show that there are several groups of absorption peaks and each group is closely related to the nanotube geometry.

CNTs are thermally stable up to 2800 °C in vacuum; their thermal conductivity in the axial direction is about twice as high as of present commercial synthetic diamond (6000 W/mK) but has very small values in the radial direction. CNTs with high aspect ratio and small tip radius of curvature are found to be excellent field emitters (electron emission) [5].

1.1.3 Synthesis of Carbon Nanotubes

The properties of CNTs are closely related to their method of production. A recent processing technique, flame synthesis, is also currently being developed. In early work the arc discharge and laser vaporizations processes were the most common forms of nanotube production, typically resulting in nanotubes with low structural defects and thus excellent physical properties. In these techniques SWNTs are typically formed in the presence of a metal catalyst, which seems to preclude the formation of MWNTs.

In the interest of developing a process that can be scaled-up for industrial production, a great deal of work has been devoted to techniques that may be classified as chemical vapor deposition (CVD). While CVD processes have been used to produce a wide variety of carbon structures, the major drawback of these methods is the reduced structural integrity of the nanotubes.

1.1.4 Potential Application of CNTs

The unique structure of a CNT, different from graphite and conventional carbon has attracted many researchers from chemistry, physics and materials science. The CNTs are used in various applications like, energy storage, hydrogen storage, lithium intercalation, electrochemical supercapacitors, Field emitting devices, bio- sensors etc.

1.2 CONDUCTING POLYMERS

Conducting polymers are increasingly replacing natural and inorganic materials in application requiring excellent mechanical properties and lightweight. The mechanical properties of polymers can be tailored to provide strong materials with high toughness and low resistance. Until recently, the low electrical conductivity of polymers had limited their use in electronics.

In early 1970s, a key discovery in the evolution of conductive polymers was the high conductivity of polysulfur nitride [$(SN)_x$], an inorganic explosive polymer [6], which becomes superconducting at about 0.3K. This was the first polymeric material that was shown to have metallic properties. In the late 1970s many researchers directed their interests to organic conductive polymers because they believed that these materials probably could be processed using conventional plastic technology.

Polymers are insulators because the atoms in the polymer chain are covalently bonded. In the covalent bonded molecules of saturated carbon compounds, there is no scope of delocalization of the valence electrons and consequently, neither charge carriers nor path for their movements are available. Since in the conjugated molecule of a carbon compound, delocalization of electron may occur through the interaction of π-bonded electrons, such molecule may be conducting. Thus it was thought that a long chain conjugated molecule, such as the polymer of acetylene, may prove to be conducting. In fact, it was proposed, purely from theoretical consideration, that properly substituted polyacetylene [PA] molecule would exhibit even superconducting behaviour at room temperature [7].

In 1977, the high electrical conductivity of an organic polymer, doped polyacetylene was reported by H. Shirakawa in Japan, Alan G. MacDiarmid of the University of Pennsylvania and A.G. Heeger, University of California, Santa Barbara. They discovered that partial oxidation with iodine or other reagents made polyacetylene films 10^9 times more conductive than original films [8]. The conjugated polymer polyacetylene could be modified by the action of iodine and other molecular acceptors to produce a material with a metallic conductivity. They proved that plastic could indeed, under certain circumstances, be made to behave very much like a metal, for which H. Shirakawa, Alan G. MacDiarmid and A.G. Heeger were awarded the **Noble Prize** in chemistry for the year 2000.

Amongst the various conducting polymers polyaniline [PANI] has rapidly become the subject of considerable interest for physicists, chemists and material scientists. Polyaniline exists in several oxidation states with electrical conductivity varying progressively from 1-10 S/cm to more than 10 S/cm. However, only one form, called the emeraldine salt, is electrically conducting. It can be synthesized easily by electrochemical or chemical oxidation of aniline in aqueous acidic media, using common oxidants, such as ammonium peroxydisulfate.

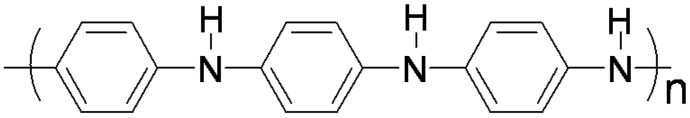

Fig.1.5: Structure of Polyaniline

Mechanically flexible, dark-blue films of conductive polyaniline also have been achieved by protonic doping of emeraldine films cast from N-methylpyrrolidone (NMP) solutions. Protonic doping accomplished either by dipping the emeraldine films in acid or passing a gaseous acid over them, protonates the imine nitrogen atoms in the backbone of the polymer. The conductive emeraldine salt becomes the insulating emeraldine base when treated with aqueous alkali.

The conductivity of polymers depends on the method of polymer synthesis and purification techniques, physical treatment of the polymer etc. Presence of moisture and oxygen in the electrolytic medium affect the conductivity of the doped polymer. Electrical conductivity increases with increasing degree of crystallinity. Generally the degree of crystallinity may be induced in polymers by mechanical stretching or in a better way by simultaneous mechanical stretching and heat treatment.

The mechanism of conduction and behaviour of charge carriers in the conducting polymers have been explained using the concept of solitons, polarons and bipolarons. A radical cation that is partially delocalized over some polymer segment is called a ***polaron***. It stabilizes itself by polarizing the medium around it. It is really a radical cation and has a spin of 1/2.

When an electron is removed from the top of the valence band of a conjugated polymer, a vacancy (hole or radical cation) is created that does not delocalize completely, as would be expected from classical band theory. Only partial delocalization occurs, extending over several monomeric units and causing them to deform structurally. The energy level associated with this radical cation represents a destabilized bonding orbital and thus has a higher energy than the energies in valence band. This rise in energy is similar to the rise in energy that takes place after an electron is removed from a filled bonding molecular orbital.

If another electron now is removed from the already oxidized polymer containing the polaron, two things can happen: This electron could come from either a different segment of the polymer chain, thus creating another independent polaron, or from the first polaron level (remove the unpaired electron) to create a special dication, which is called a ***bipolaron***. Low doping levels give rise to polarons, whereas higher doping levels produce bipolarons. Compared to polaron, bipolaron is doubly charged but spinless. The bipolaron also has structural deformation associated with it. The two positive charges of the bipolaron are not independent, but act as a pair, much like the cooper pair in the Bardeen-Cooper-Schrieffer (BCS) theory of superconductivity. Both polarons and bipolarons are mobile and can move along the polymer chain by the rearrangement of double and single bonds in the conjugated system that occurs in an electric field. If many bipolarons are formed, say as a result of high doping, their energies can start overlapping at the edges, which creates narrow bipolaron bands in the band gap.

In polyacetylene, which has a degenerate ground state (two equivalent resonance forms), the bipolaron dissociates into two independent cations, which are spinless and are called ***solitons***. Solitons do not form in polymers with nondegenerate ground state, such as polypyrrole, polythiophene and polyphenylene. These polymers are called nondegenerate because their resonance forms are not identical if they are superimposed. Doping with a suitable dopant can increase the concentration of charged solitons.

Conducting polymers possess poor processability. Due to extended conjugated chain structure, these polymers are insoluble and infusible and hence are not easily processable. Polyacetylene, for example, shows electrical conductivity in the semiconducting range. But on exposure to atmosphere its conductivity falls rapidly. A number of general techniques have been developed for improving the processability of conducting polymers.

1.3 CNTs-POLYMER COMPOSITES

Nowadays polymers play a very important role in numerous fields of everyday life due to their advantages over conventional materials such as lightness, low-cost production, and ease of processing.

Altering and enhancement of the polymers' properties occur, for example, through doping with various fillers such as metals, semiconductors, organic and inorganic particles and fibers, as well as carbon structures and ceramics; thereby enabling polymers to be used as a structural unit.

Fillers are used in polymers for a variety of reasons: improved processing, density control, optical effects, thermal conductivity, control of thermal expansion, electrical properties, magnetic properties, flame resistance, and improved mechanical properties, such as hardness, elasticity.

The extraordinary properties of carbon nanotubes make them very promising and favorable as fillers for fabrication of a new class of polymeric heterostructures. In CNT-polymer composites, chemical functionalization is used to enhance the nanotube-polymer interface.

Functionalized nanotubes are also typically easier to disperse in organic solvents and water which can improve the dispersion and homogeneity of the CNTs within the polymer. While property improvements based on carboxylated functionalized MWCNTs in a polymer and SWCNT in epoxy have been obtained, results for amino (-NH_2) functionalized CNT composites have yet to be reported. In one study, amino-terminated MWNT/epoxy showed improved dispersion and increased interfacial interaction between nanotubes and polymer but no quantitative thermo-mechanical or chemical results are provided.

There are different methods by which CNT-conducting polymers composites can be synthesized, depending on the desired applications. CNT-Conducting polymers composites can be synthesized in presence of different protonic acid media by different methods. The most widely accepted methods are chemical oxidative polymerization method and electrochemical methods. Chemical oxidative method is preferred over electrochemical polymerization because of its cost effectiveness and bulk quantity of the polymer that can be prepared during the onset of a reaction. By electrochemical technique thin film of the polymer can be deposited on an electrode surface. These have been found to be useful for electrochromic devices, biosensors etc.

Tzong-Ming Wu et al. reported synthesis of doped polyaniline in its emeraldine salt form (PANI-ES) with carboxylic groups containing multi-walled carbon nanotubes (c-MWNTs) via in situ polymerization [9]. S. Hrapovic reported preparation of polymer–C\carbon nanotube composite materials and their applications for enzyme entrapment [10].

1.4 BIOSENSORS

What is a biosensor? Various definitions and terminologies are used depending on the field of application. Biosensors are known as: immunosensors, optrodes, chemical canaries, resonant mirrors, glucometers, biochips, biocomputers, and so on. A commonly cited definition is: "a biosensor is a chemical sensing device in which a biologically derived recognition entity is coupled to a transducer, to allow the quantitative development of some complex biochemical parameter", and also: "a biosensor is an analytical device incorporating a deliberate and intimate combination of a specific biological element (that creates a recognition event) and a physical element (that transduces the recognition event)".

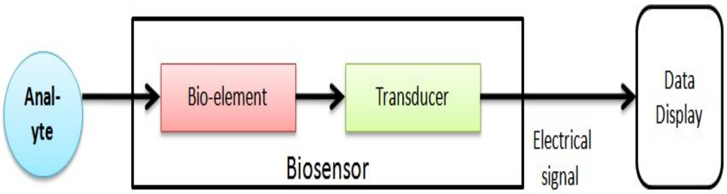

Figure-1.6: Elements of a Biosensor

The name "biosensor" signifies that the device is a combination of two parts: (i) a bio-element, and (ii) a sensor-element. The basic concepts of a biosensor's operation can be illustrated with the help of **Fig. 1.6.** A specific "bio" element (say, enzyme) recognizes a specific analyte and the “sensor” element transduces the change in the biomolecules into an electrical signal [11].

1.5 TYPES OF BIOSENSOR

There are many types of biosensors like resonant biosensors, optical-detection biosensors, thermal-detection biosensors, ion-sensitive biosensors, electrochemical biosensors etc. The important biosensors described as:

1.5 .1 Electrochemical Biosensors

Electrochemical biosensors are mainly used for the detection of hybridized DNA, DNA-binding drugs, glucose concentration, etc. The underlying principle for this class of biosensors is that many chemical reactions produce or consume ions or electrons which in turn cause some change in the electrical properties of the solution which can be sensed out and used as measuring parameter. Electrochemical biosensors can be classified based on the measuring electrical parameters as: (1) Conductimetric, (2) amperometric and (3) potentiometric [12, 13].

1.5.2 Conductimetric

In the conductimetric biosensors the measured parameter is the **electrical conductance / resistance** of the solution. In the analyte (electrolyte solution) when electrochemical reactions produce ions or electrons, the overall conductivity or resistivity of the solution changes, this change is measured and calibrated to a proper scale. The sensitivity of Conductimetric biosensors is relatively low. The electric field is produced using a sinusoidal voltage which helps in minimizing undesirable effects such as Faradaic processes, and concentration polarization.

1.5.3 Amperometric

Amperometric sensors are a type of electrochemical sensor, since they continuously measure current resulting from the oxidation or reduction of an electroactive species in a biochemical reaction. Clark oxygen electrodes perhaps represent the basis for the simplest forms of amperometric biosensors, where a current is produced in proportion to the oxygen concentration. This high sensitivity biosensor can detect electroactive species present in biological test samples. Typically, the current is measured at a constant potential and this is referred to as amperometry.

1.5.4 Potentiometric

In this type of sensor the measured parameter is oxidation or reduction potential of an electrochemical reaction. In other words, potentiometry provides information about the ion activity in an electrochemical reaction. The working principle relies on the fact that when a ramp voltage is applied to an electrode in solution, a current flow occurs because of electrochemical reactions. The voltage at which these reactions occur indicates a particular reaction and particular species.

1.5.5 Voltammetry

Voltammetry is the study of the current-voltage relationships observed when electroactive species in solution are subject to oxidation or reduction at electrodes under carefully controlled conditions. It involves probing a small region of a solution containing, for example, metal ions, by performing small-scale electrolysis between an indicator microelectrode and a reference electrode. A reference electrode, such as the saturated calomel electrode (SCE), is by definition nonpolarizable. That is, its potential remains the same regardless of the potential difference imposed between it and the indicator electrode. The latter is described as polarizable, because it faithfully adopts any potential imposed on it relative to the reference.

If the potential difference between indicator and reference electrode can be controlled accurately and varied uniformly, criteria which modern potentiostatic devices ensure, the corresponding currents that flow reflect the nature and concentration of oxidizable or reducible solutes in solution. Currents flow because of the exchange of electrons between the indicator electrode and electroactive solutes. The latter are frequently metal ions, and the electrode processes monitored are reductions. The indicator electrode then acts as a cathode.

The voltage is measured between the reference electrode and the working electrode, while the current is measured between the working electrode and the counter electrode. The obtained measurements are plotted as current vs. voltage, also known as a voltammogram..

1.6 GLUCOSE BIOSENSORS

Biosensor history started in 1962 when American Scientist Leland C. Clark studied the electrochemistry of oxygen reduction at a platinum metal electrode, pioneering the use of an oxygen sensor. The electrode was eventually coined a “Clark Electrode”. Clark then placed entrapped glucose oxidase next to the platinum electrode and followed the activity of the enzyme by following the changes in oxygen concentration, thereby turning the chemosensor into a glucose biosensor.

Today, mass marketing of home-monitoring glucose systems has become a reality. At present, most of the home-monitoring glucose systems on the market are enzyme-photometric or amperometric methods and are of the in vitro type. However, the commercial market is changing rapidly. Cygnus Gluco-Watch Biographer, a minimally invasive glucose sensing system based on iontophoresis, and the Minimed Continuous Glucose Monitoring (CGM) system, a minimally invasive transcutaneous glucose sensor, have received U.S. Food and Drug Administration (FDA) approval [14].

1.6.1 Physical Principles of Glucose Sensing

Over the years, a wide range of methods for measuring blood glucose concentration has been studied. The first ones to materialize were glucose assays based on glucose oxidase and reagents impregnated in paper strips. When blood is placed on the paper strip, hydrogen peroxide that is produced from the enzymatic reaction oxidizes an oxygen acceptor in the presence of peroxidase to form a color change. The intensity in the color of the paper strips will give the diabetics a qualitative indication of their blood glucose level.

Another method employs the concept of glucose concentration determination using amperometric techniques. The basic underlying chemistry is the catalytic action of glucose oxidase, which oxidizes D-glucose according to the following reaction:

$$\text{D-glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} H_2O_2 + \text{D-gluconic acid}$$

The reaction will become rate limited if either glucose or oxygen concentration is too low. Glucose concentration can be measured by following the decrease in oxygen concentration as the reaction proceeds, the production of hydrogen peroxide or the change in pH with the production of D-gluconic acid electrochemically. To date, the most widely researched methods of detection are the monitoring of oxygen or hydrogen peroxide at an electrode.

For the oxygen-based enzyme glucose sensor, since oxygen is consumed during the enzymatic reaction, oxygen concentration in the glucose oxidase membrane will be a linear function of glucose concentration. The oxygen concentration can be detected by coupling the membrane containing immobilized glucose oxidase to an electrochemical oxygen sensor. Since oxygen is also present in the sample, a similar reference oxygen sensor without the enzyme needs to be incorporated in the system. The signal current is then subtracted from the reference electrode and this result in a glucose-dependent difference current.

The hydrogen peroxide-based enzyme glucose sensor has found wide application in the development of a glucose biosensor, especially an implantable version, due to its simple sensor configuration that facilitates ease of miniaturization. Unlike oxygen, hydrogen peroxide is not present in the sample to be analyzed, so no differential set-up is needed.

- The oxygen and hydrogen peroxide based glucose sensors are the so-called first generation amperometric glucose sensors.

- Second generation glucose sensors make use of mediators to shuttle electrons from the enzyme to the electrode. This type of system is supposed to eliminate the dependency of the enzymatic reaction to oxygen. If the system is oxygen deficient, the glucose sensor will become insensitive to glucose and will only respond to changes in oxygen concentration.
- Third generation amperometric glucose sensors are based on the use of conducting organic salts or polymers. The films are grown electrochemically and glucose oxidase is entrapped in the membranes. Polyaniline and Polypyrrole have been investigated as potential membrane materials for the entrapment of the enzyme. The electrode responds to glucose concentration via peroxide oxidation. The advantage of this system is that manipulation of the electro-polymerization can give a film that extends the linear range for glucose detection and reduces oxygen dependence.

In principle, many transducers could be used in a biosensor for the measurement of glucose, but in practice, electrochemistry has dominated. This is partially historical, but the primary reason for the success of devices of this type, is that they offer suitable sensitivity and reproducibility and, importantly, can be manufactured in great volume at low cost.

The most commonly used enzymes in the design of glucose biosensors contain redox groups that change redox state during the biochemical reaction. Enzymes of this type are glucose oxidase (GOx).

In nature, oxidase enzymes such as GOx act by oxidising their substrates, accepting electrons in the process and thereby changing to an inactivated reduced state. These enzymes are normally returned to their active oxidised state by transferring these electrons to molecular oxygen, resulting in the production of hydrogen peroxide (H_2O_2):

$$\text{D-glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} H_2O_2 + \text{D-gluconic acid}$$

Each of the above enzyme mechanisms can be utilized in glucose biosensors but feature different advantages and drawbacks:

- The oxidase enzyme is inexpensive but requires oxygen as a co-substrate. Consequently, as oxygen is depleted in the sample, performance decreases, whether one is monitoring oxygen depletion, or hydrogen peroxide production.

- The drawback is that the cofactors are relatively expensive and unstable.

Combining these enzyme-based reactions with a suitable transducer enables selective sensor devices to be fabricated.

Table- 1.1 some defining events in the history of commercial Glucose biosensor development [14]

Date	Event
1916	First report on the immobilization of proteins: adsorption of invertase on activated charcoal
1922	First glass pH electrode
1956	Invention of the oxygen electrode by Clark
1962	First description of a biosensor: an amperometric enzyme electrode for glucose by Clark and Lyons
1973–1975	First commercial biosensor: Yellow Springs Instruments glucose biosensor
1976	Miles Bio-stator: first bedside artificial pancreas
1982	First fibre optic-based biosensor for glucose by Schultz
1984	First mediated amperometric glucose biosensor: ferrocene se oxidase for the detection of glucose by Cass et al.
1987	Launch of the MediSense ExacTech blood glucose biosensor
1992	i-STAT launches hand-held blood analyser
1996	Glucocard launched

1998	Launch of LifeScan FastTake blood glucose biosensor
1998	Merger of Roche and Boehringer Mannheim to form Roche Diagnostics

1.7 CNTs *BASED GLUCOSE BIOSENSORS*

Applications of CNTs to biosensors have attracted the maximum attention because of high conductivity, biocompatibility and chemical inertness. CNTs are used as transducers and mediators in biosensors. Recent studies have reported that surface-confined CNTs can dramatically accelerate the electron transfer reactions of important anylates, including hydrogen peroxide, cyctochrome C, NADH and hydrazine compounds. CNTs-modified electrodes have recently been used for studying the direct electrochemistry of GOx-enzymes, since the unique electronic and structure properties of CNTs allow good communication between CNTs and redox active centers of enzyme. Zhang *et al*. [15] has investigated layer-by-layer fabrication and direct electrochemistry of glucose oxidase on SWNTs. His work describes the effective layer-by-layer immobilization of GOx on CNTs based on the electrostatic attraction between positively charged GOx in buffer and negatively charged carboxylic groups in CNTs.

Wang *et al*. [16] studied the bio-electrochemical characteristics of a novel MWNT-based biosensor for glucose detection and compared with those of glassy carbon (GC)-based biosensor. He found that the MWNT-based biosensor exhibits a strong glucose response at applied potentials of 0.65 and 0.45V versus Ag/AgCl, respectively, while GC-based biosensor shows a weak glucose response at 0.65V and no response at 0.45 V and he found that the MWNT-based biosensor shows a high stability of 86.7% of the initial activity to glucose after four-month storage, much higher than 37.2%, the corresponding value for a GC-based biosensor.

Zhang *et al.* [17] have been reported a novel method for immobilization of biomolecules on CNTs based on ionic interaction, which is of universality and widespread use in biological systems. The glucose oxidase (GOD) and SWNT were integrated into a unitary bionanocomposite through an ionic liquid-like unit. The results indicated that the ionic liquid-like unit on SWNTs provided an extra ionic affinity between SWNT and GOD, which facilitated the preparation of bionanocomposite with better redox response of immobilized GOD. Additionally, the bioactivity of immobilized GOD was retained and the electrochemical detection of glucose could be achieved by this novel bionanocomposite.

Chu *et al.* [18] developed a new amperometric biosensor for glucose based on adsorption of glucose oxidase (GOx) at the gold and platinum nanoparticles modified CNT electrode. CNTs were covalently immobilized on gold electrode via carbodiimide chemistry by forming amide linkages between carboxylic acid groups on the CNTs and amine residues of cysteamine self-assembled monolayer.

Li *et al.* [19] have developed a practical and general approach for covalently attaching GOD proteins on the sidewalls of MWCNT under ambient conditions for the preparation of CNTs protein composites. It was found that the GOD–MWNT composites are highly water soluble. Electrochemical characterization of the GOD–MWNT composites that were modified on a glassy carbon electrode shows that the covalently linked GOD retains its bioactivity and can specifically catalyze the oxidation of glucose. The oxidation current shows a linear dependence on the glucose concentration in the solution in the range of 0.5–40 mM with a detection limit of 30 μM and a detection sensitivity of 11.3μA/mMcm^2.

1.8 FABRICATION OF CARBON NANOTUBES BASED BOI-ELECTRODES

For biosensing purpose pristine CNTs electrodes (CNT paste electrode) have been fabricated by mixing CNT powder with deionized water, bromoform or mineral oil and an enzyme is added to the mixture to obtain a CNTPE with incorporated enzymes. The simplest route to CNT-modified electrodes is to cast a solution of CNTs onto a GCE. Since CNTs are insoluble in most solvents, ultrasonication is required during preparation in order to effectively disperse the tubes. Electrodes have been fabricated by dispersing CNTs in a phosphate buffer or concentrated sulfuric acid, and subsequent spin-casting onto a polished GCE [20].

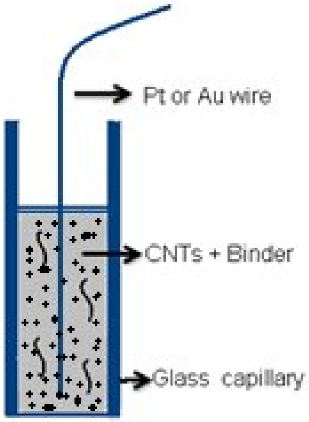

Figure-1.7: Schematic drawing of a CNT paste electrode

The homogeneous and stable CNT film electrode can be obtained through solvent evaporation by the aid of some surfactant like dihexadecyl hydrogen phosphate (DHP) or dicetyl phosphate (DCP) in CNT-paste. Instead of aqueous media, acetone and DMF have also been used as solvents when fabricating electrodes using this technique.

1.8.1 Functionalized Carbon Nanotubes Based Bio-electrodes

Functionalized carbon nanotubes offer enormous potential as components of nanoscale electronics and sensors. CNTs can be functionalized by treating with oxidizing agents like H_2SO_4, HNO_3 or air to produce functional groups like COOH so that its potentiality towards bonding and electron transfer for bio-devices can be improved.

1.8.2 Functionalization of Carbon Nanotubes

In general, the functionalization of CNTs requires chemical modifications of their surface supported by the mechanical agitation methods such as ultrasonication and shear mixing. Several functionalization strategies have been reported recently. They are mainly based on the covalent and non-covalent π - π stacking interaction, adsorption of surfactants coupling of surfactants and functionalities to CNTs, and are described as follows:

1.8.2.1 Covalent Functionalization:

Covalent methods refer to a treatment that involves bond breaking across the surface of the CNTs (e.g. by oxidation) which disrupts the delocalized π -electron systems and fracture of σ-bonds and hence leads to incorporation of other species across the CNTs' surface. Introducing defects to the CNT's shell significantly alters the optical, mechanical and electrical properties of the nanotubes and leads to an inferior performance of the composites. The advantage is that this kind of modification may improve the efficiency of the bonding between nanotubes and the host material (cross-linking). Therefore, the interfacial stress transfer between the matrix and CNTs may be enhanced leading to better mechanical performance.

There are many methods used for covalent functionalization of CNTs the important methods are listed as following: 1.Thermal Oxidation, 2. Acid reflux (AR), 3. Microwave Treatment, 4. Sonochemical, 5. UV - Ozonolysis (UVO)

1.8.2.2 Non-covalent Functionalization

These modifications of the CNTs are of great advantage because no disruption of the sp^2 graphene structure occurs and the CNT properties are preserved. Its disadvantage concerns weak forces between wrapped/coupled molecules that may lower the load transfer in the composite.

The chemical modification of the CNTs' surface improves solubility/separation of the nanotubes in a given solvent. A proper functionalization ensures homogenous and stable dispersion throughout the solvent and in the composite host material. Moreover, functionalities on the surface of CNTs may lead to enhanced interactions between filler and matrix due to the presence of the interfacial bonds between components.

There are some common methods used for non-covalent functionalization of CNTs are:
(1). Ionic interaction and (2) π-π interaction

The enzyme is immobilized on these surfaces by the reaction between carboxylic acid group of CNT and amine residue of enzyme through a linking molecule. The immobilization of biomolecules into CNT modified matrix can be done by two ways:

(1). small biomolecules are entrapped into the inner channel of open carbon nanotubes by physical adsorption and

(2) attachment of the bio-molecule on the outer surface of carbon nanotube either by hydrophobic or electrostatic interactions or through covalent bonding.

Enzyme based electrodes have received great attention because they are very sensitive, highly selective and fast responding. They are effective analytical tools for industrial, environmental and clinical applications. At present, enzyme sensors for determination of glucose, lactate, urease and pesticides based on electrochemical oxidation or reduction of hydrogen peroxide have been widely studied.

S.G. Wang *et al.* [21] have developed glucose biosensor, comprised of electrode of gold/ MWCNTs –glucose oxidase (Au/MWNTs–GOD). The enzyme of GOD was immobilized using MWNTs. The results show that the fabricated biosensor is sensitive and stable in detecting glucose, indicating that MWNTs are a good candidate material for the immobilization of enzyme in glucose biosensor construction.

1.9 BIOSENSORS BASED ON CONDUCTING POLYMERS AND CNTs-POLYMER COMPOSITES

The conducting polymers are being widely used in biosensor applications because it provides stable and porous matrix for the immobilization of biocomponents and it also facilitate the electron transfer process. The widely used conducting polymers for immobilization of enzyme are polyaniline, polypyrrole, polythiophene etc.

Since polyaniline is compatible to most enzymes and can be easily synthesized from aniline monomer in aqueous solution, the polyaniline is more suitable for biosensor applications. Electrochemically polymerized conducting polymers had received considerable attention over the last two decades. The remarkable switching capability of these electroactive materials between the conducting oxidized (doped) and the insulating-reduced (undoped) state is the basis of many applications. The poly-conjugated conducting polymers have recently been proposed for biosensing applications because of a number of useful features such as:

1. Direct and easy deposition on the sensor electrode by electrochemical oxidation of monomer,
2. Control of thickness and
3. Redox conductivity and polyelectrolyte characteristics of the polymer useful for sensor application.

PANI fulfills above requirements together with having the characteristics of easy oxidation, high chemical stability etc. The porosity is an important factor for the facile immobilization of enzyme.

Gaikwad *et al.* [22] have reported polyaniline-polyvinyl sulfonate-glucose oxidase (PANI-PVS-GOD) electrode for determination of glucose. The GOD was immobilized on synthesized PANI-PVS film by cross-linking via glutaraldehyde in phosphate and acetate buffer. The sensitivity of PANI-PVS-GOD electrode in phosphate and acetate buffer has been studied. It was found that the phosphate buffer gives fast response as compared to acetate buffer in potentiometric measurements.

In recent years, the fabrication of PANI/CNT composites has received great interest since the incorporation of CNTs into PANI can result in new composite materials with enhanced properties. Many researchers reported fabrication of CNT-Polymer composites and their application in various types of biological and chemical sensors. For example Ramanathan *et al.* [23] reported Single-walled carbon nanotubes (SWNT) functionalized with amino groups were prepared via chemical modification of carboxyl groups introduced on the carbon nanotube surface. Two different approaches (amide and amine-moieties) were used to produce the amino-functionalized nanotubes. He observed that the amino-termination allows further chemistry of the functionalized SWNTs and makes possible covalent bonding to polymers and biological systems such as DNA and carbohydrates.

Konyushenko *et al.* [24] reported fabrication of MWNT-PANI composite and electrical properties of CNT-PANI composite. MWNTs were coated with protonated polyaniline in situ during the polymerization of aniline. The content of CNT in the samples was 0 - 80 wt%. The conductivity of PANI-coated CNT has been compared with the conductivity of the corresponding mixtures of PANI and CNT. At high CNT contents, it is not important if the PANI coating is protonated or not; the conductivity is similar in both cases, and it is determined by the CNT. It was found that the polyaniline reduces the contact resistance between the individual nanotubes. A maximum conductivity of 25.4 Scm^{-1} has been found with PANI-coated CNT containing 70 wt% CNT. The wettability measurements show that CNT coated with protonated PANI are hydrophilic, the water contact angle being ≈ 40% even at 60 wt % CNT in the composite. The density measurements indicate that the compressed PANI-coated CNT are more compact compared with compressed mixtures of PANI and CNT.

Zengin *et al.* [25] have reported preparation of PANI-MWNT composite films by the in-situ and ex-situ methods. He observed that the composite exhibit an order of magnitude increase in electrical conductivity over neat PANI, the measured increase in electrical conductivity of the PANI-CNT composite may be due to a doping effect of CNTs where the nanotubes composite with chloride ion.

Wu *et al.* [26] reported the synthesis of doped polyaniline in its emeraldine salt form (PANI-ES) with carboxylic acid and acylchloride groups contained multi-walled carbon nanotubes by in situ polymerization. Both Raman spectra and TEM images indicate that carboxylic acid and acylchloride groups formed at both ends and on the sidewalls of the MWNTs. Based on the p–p* electron interaction between aniline monomers and functionalized MWNT and hydrogen bonding interaction between the amino groups of aniline monomers and the carboxylic acid/acylchloride groups of functionalized MWNT, aniline molecules were adsorbed and polymerized on the surface of MWNTs. The conductivities of 0.5 wt% functionalized MWNT containing PANI-ES/c-MWNT and PANI-ES/a-MWNT composites are 60–70% higher than that of PANI without MWNT.

Luo *et al.* [27] reported conducting polymer-based biosensor using carbon nanotube-doped polyaniline. He observed that the application of CNTs in the biosensor system could increase the amount and stability of the immobilized enzyme, and greatly enhanced the biosensor response. Compared with the biosensor without CNTs, the proposed biosensor exhibited enhanced stability and approximately eight-fold sensitivity.

Gaikwad *et al.* [28] reported the polyaniline-sulfuric acid-glucose oxidase (PANI-H_2SO_4-GOD) electrode for glucose detection. PANI-H_2SO_4 film was synthesized from an aqueous solution of distilled 0.2M aniline and 1.0M of sulfuric acid using electrochemical deposition method. The GOD was immobilized by cross-linking via glutaraldehyde (0.1 %) on PANI-H_2SO_4 films.

Hrapovic *et al.* [29] have prepared polymer– CNTs composite materials and their applications for enzyme entrapment. He observed that the entrapment of the enzyme in a polymer matrix preserves the enzyme conformation since it only physically restricts the enzyme movement within the polymer matrix. In the presence of MWCNTs, entrapped enzyme retained 85% of its initial activity. The beads prepared without MWCNTs displayed no enzyme activity after 72 h whereas 50% of the initial enzyme activity was still observed for GOx entrapped in a k-carrageenan-MWNT network.

1.10 *MOTIVATION*

CNT-modified electrodes have been extensively studied for the construction of chemical sensors and biosensors, CNTs can play several different roles, such as promoting the electron-transfer reactions of biomolecules [30]. Functionalization of carbon nanotubes can further improve the performance of an biosensor [31].

The conducting polymer polyaniline (PANI) has attracted much attention due to its interesting electrical, electrochemical and optical properties. Among the most promising applications of PANI is electrochemical biosensing, as it can provide a suitable environment for the immobilization of biomolecules, and the PANI-modified electrodes have several advantages such as impressive signal amplification and elimination of electrode fouling. Based on its excellent conductivity and electroactivity, PANI can act as a mediator for enzyme electrodes, where PANI undergoes redox cycling and can couple electrons directly from the enzyme active site to the electrode surface. Gaikwad *et al.* [32] have reported the development of PANI-PVS-GOD electrode for determination of glucose.

In recent years, the fabrication of PANI/CNT composites has received great interest since the incorporation of CNTs into PANI can result in new composite materials with enhanced properties [24-25]. J. Li *et al.* [33] have reported that the polymer coating on CNTs helps to generate response in the form of conductivity change upon exposure to Cl_2 and HCl vapor at a sensitivity level of 2 ppm and above.

Biosensors are being developed to measure blood glucose levels. GOD is one of the possible enzymes that can be used in biosensor. Biosensors work by keeping track of the number of electrons that pass through an enzyme by connecting it to an electrode and measuring the resultant charge.

Over the years, a wide range of methods for measuring blood glucose concentration has been studied. Various GOD based biosensors are; on line glucose monitoring for fermentations, fiber optic biosensor for analyzing glucose concentrations in soft drinks, disposable strip-type biosensor for blood and serum monitoring, strip type biosensor for blood (GOD-HPR-dye), miniaturized thermal biosensor for whole blood, glucose sensor for whole blood, glucose biosensor for serum from human blood [34-40].

The development of a successful glucose biosensor is one of the most financially attractive areas in medical diagnostics. The various studies have proven that frequent monitoring can reduce the cost of diabetes-related healthcare. The increase in healthcare costs around the world necessitates the introduction of cost-effective, diagnostic glucose-testing kits.

These are among the factors behind the need for continuing research in the area of blood glucose monitoring.

1.11 RESEARCH OBJECTIVES

To realize the full potential of CNTs and polyaniline (PANI) for biosensor applications, functionalization of nanotubes has become an indispensable, viable and effective approach. Through chemical modifications, carbon nanotubes exfoliation and enhanced surface reactivity are expected.

The defects in the nanotubes can be classified into several categories:

- Topological defects, corresponding to the presence of rings other than hexagons, for example pentagon/heptagon pairs, usually at tube cap areas
- Incomplete bonding defects (e.g., vacancies, dislocations, etc.),
- Chemical defects consisting of atoms/groups covalently attached to the carbon lattice of the tubes.

The existence of these defects makes chemical functionalization possible and such defects are considered reactive sites to form covalent interfacial bodings to improve reinforcement/filler effect in the composites.

The main objectives of this work were:

1. Covalently functionalize the MWNTs and study the electrical Properties of these f-MWNTs for glucose sensor application.

2. Covalently immobilization of Glucose Oxidase enzyme (GOD) on f-MWNTs and study the electrical properties of GOD-f-MWNTs composite.

3. To study the activity of immobilized enzyme.

4. To study the electrical properties of PANI, p-MWCNT-PANI composite, c-MWNT-PANI composite, a-MWNT-PANI composites for glucose sensor application.

5. To study the sensor response of GOD-MWNTs and GOD-MWNT-PANI composites.

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CHAPTER-2

EXPERIMENTAL METHODS AND CHARACTERIZATION

2.1 INDRODUCTION

This chapter presents the experimental details of functionalization of MWNTs and GOD immobilization and fabrication of CNT-PANI composites. The functionalization of CNTs involves the generation of chemical groups on their surface. The presence of oxygen-containing groups facilitates the exfoliation of CNT bundles, and increases the solubility in polymers. For covalent immobilization of enzyme the CNTs must be contain chemical groups on their surfaces. The following methods of functionalization, immobilization and fabrication of composites and their characterizations are given in following sections.

2.2 EXPERIMENTAL METHODS

The experimental details of functionalization of CNTs, immobilization of enzyme and synthesis of polymer composites are described in following sections and subsections.

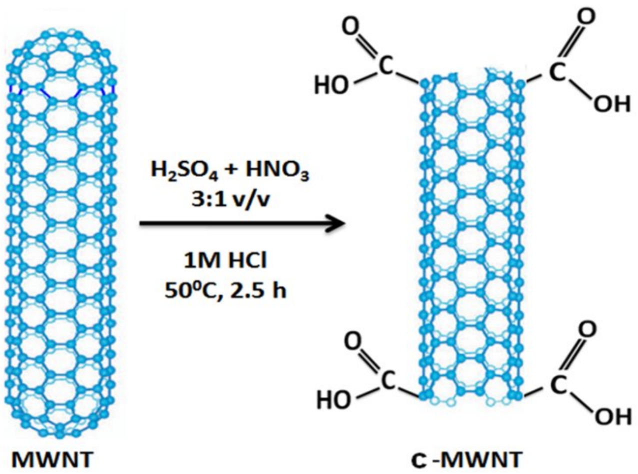
2.2.1 Carboxylation of MWNTs

In a carboxylation process the -COOH and -OH groups are attached on CNTs by a chemical reaction in the presence of HNO_3 and H_2SO_4. The functionalization scheme and procedure are described below.

2.2.1.1 Functionalization Scheme

Functionalization of CNTs involves the generation of chemical moieties on their surface that can improve the solubility and processibility. Y. Wang reported the use of a 3:1 concentrated H_2SO_4/ HNO_3 mixture to cut the highly tangled long ropes of MWNTs into short, open-ended pipes and thus produced many carboxylic groups at the open end [1].

As a result of the chemical oxidation, the ends and walls of the nanotubes are covered with oxygen containing groups such as carboxylate and hydroxyl groups as shown in scheme 2.1. The presence of oxygenated groups on nanotubes is important both to the fundamental research of nanotubes and to their applications in electronic devices.



Scheme 2.1: Carboxylation of MWNTs

2.2.1.2 Functionalization Procedure

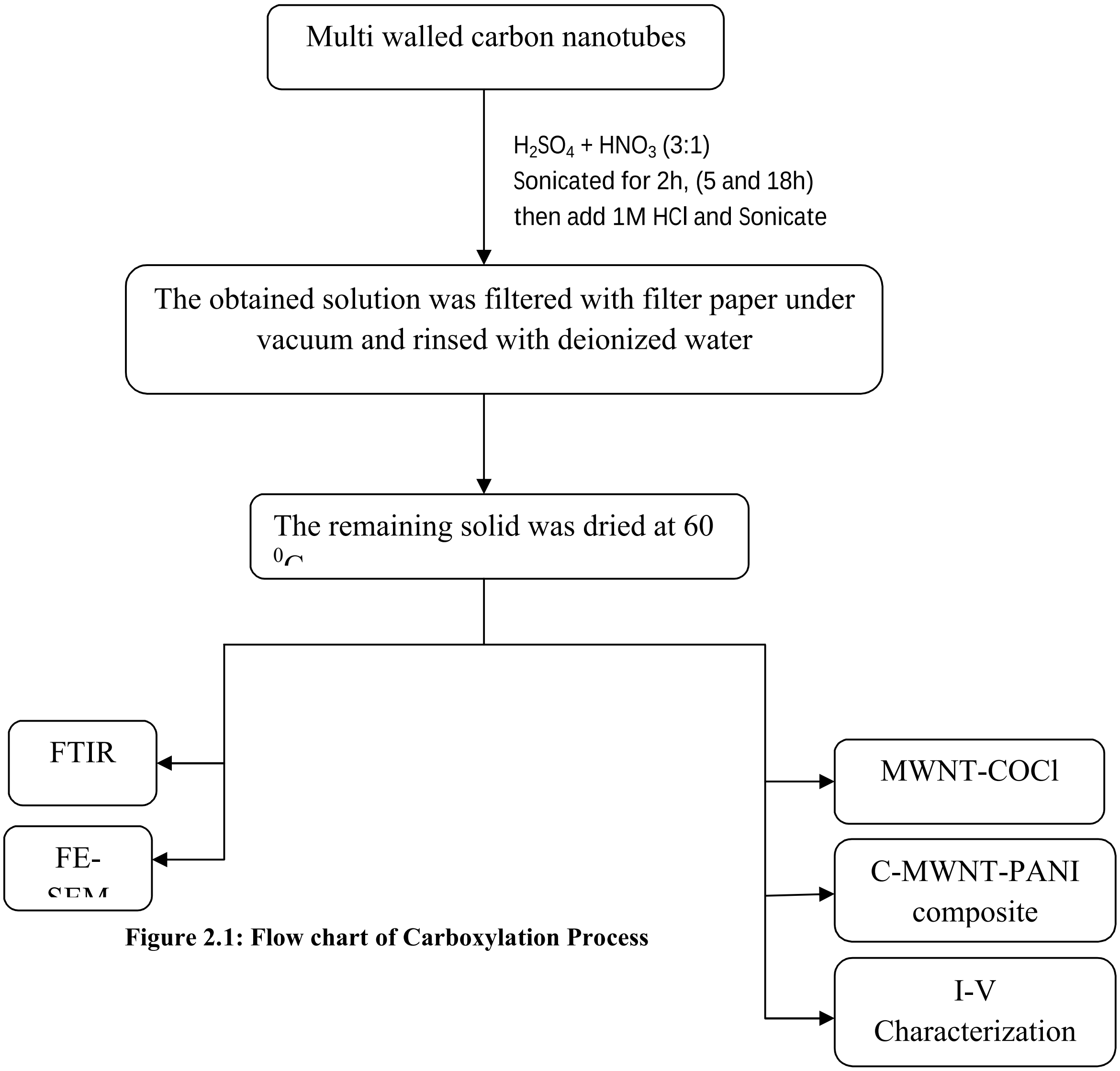


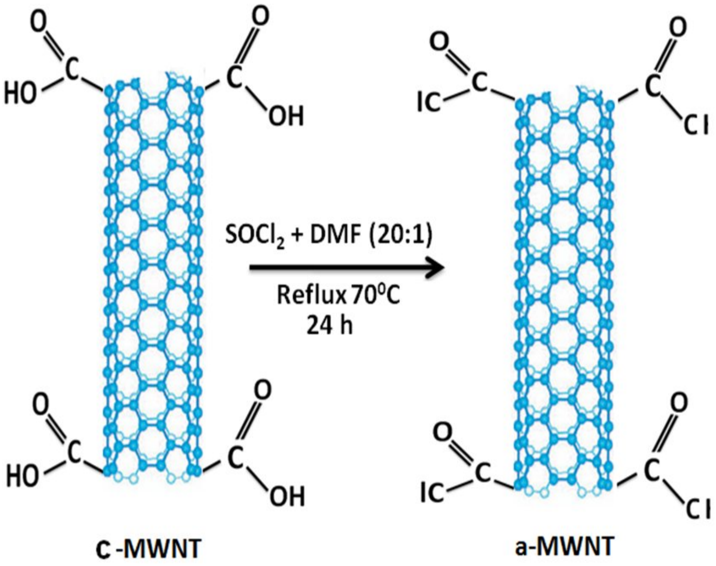
Figure 2.1: Flow chart of Carboxylation Process

In a typical experiment 100 mg of pristine MWNT powder was sonicated in a 3:1 mixture of concentrated H_2SO_4 and HNO_3 at 50 ^{0}C for 2 h, followed by exposure to 1 M HCl and sonication in HCl solution for about 30 min. The carboxylated MWNTs were diluted with deionized water then filtered through PTFE membrane of pore size 0.22 μm, and washed with deionized (DI) water and dried in air at 50 ^{0}C over night. These c-MWNTs were used for acylation, composite fabrication and characterization experiments.

2.2 .2 Acylation of MWNTs

In acylation process the -OH groups are replaced by -Cl groups by a chemical reaction in the presence of $SOCl_2$ and DMF. Y. Wang reported the use of a $SOCl_2$ and DMF mixture in a 20:1 ratio at 70 ^{0}C for 24 hr to produce many -COCl groups at the open end of the c-MWNTs as shown in the Scheme 2.2 below [1].

2.2.2.1 Functionalization Scheme



Scheme 2.2: Acylation of c-MWNTs

2.2.2.2 Functionalization Procedure

The carboxylated nanotubes (50 mg) were stirred in 25 ml of a 20:1 mixture of thionyl chloride and DMF (N, N-dimethyl formamide), at 70 ^{0}C for 24 h. After the acylchlorination, the MWNTs were filtered, washed with anhydrous THF, and dried under vacuum at room temperature for 20 min. These a-MWNTs were used for GOD immobilization and fabrication of a- MWNT- PANI composite and characterization experiments.

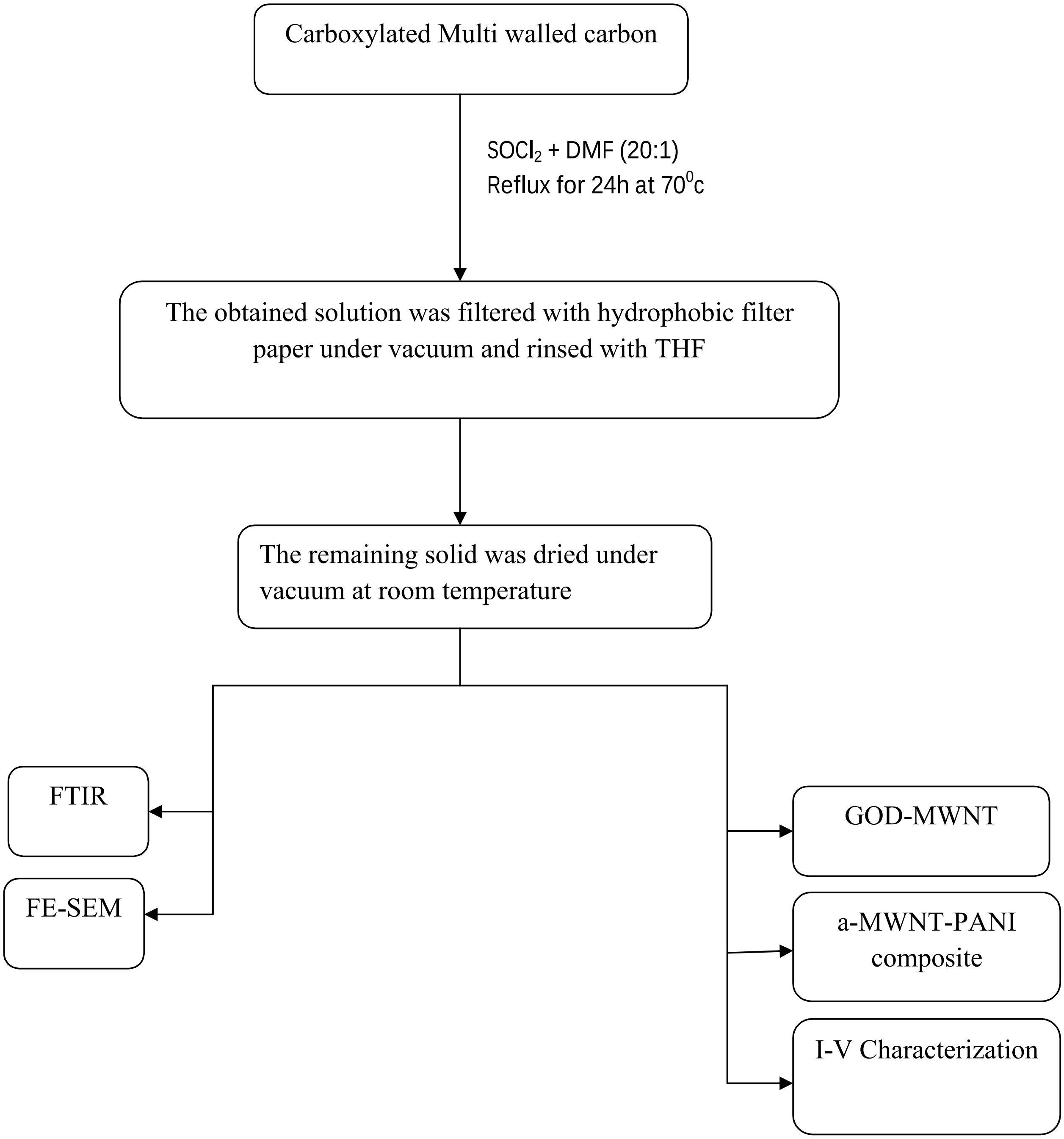


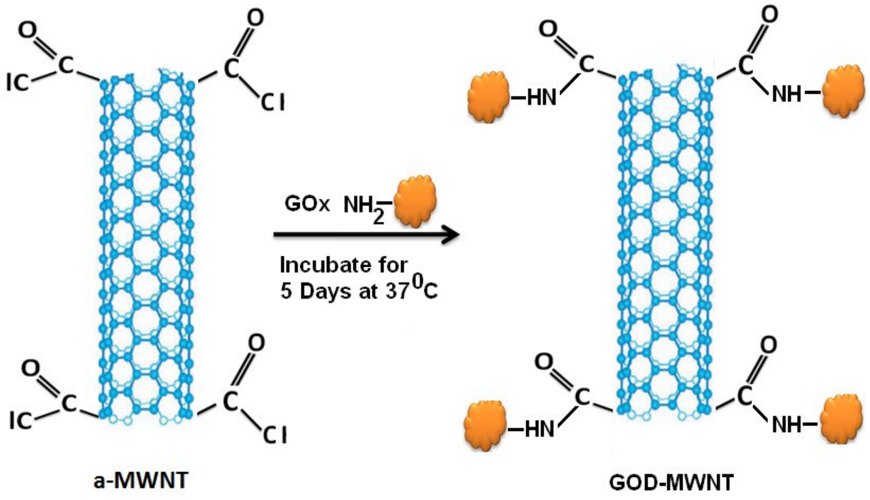
Figure 2.2: Flow chart of Carboxylation Process

2.2.3 Attachment of Glucose Oxidase on a-MWNTs

The meaning of immobilization of enzyme is to restrict the movement of enzyme on the CNTs or any other substrates. The enzyme can be attached covalently or non-covalently. The covalent immobilization is mostly preferred. The immobilization scheme and process is given below.

2.2.3.1 Immobilization Scheme

Y. Wang reported the immobilization of various enzymes on functionalized CNTs using DMF as a solvent [1]. Here PBS buffer (0.1M, pH 6.8) as a solvent and reaction temperature of 37 ^{0}C in case of glucose oxidase. The amide groups of the enzyme react with -COCl groups of the CNTs and forming amide bond - CONH which binds the enzyme to the CNTs covalently.



Scheme 4.3: Immobilization of GOD on a-MWNTs

2.2.3.2 Immobilization Procedure

The acylated MWNTs (15 mg) were dispersed by sonicated in of PBS buffer 10 ml of pH 6.8 for 15 min (solution-1). Glucose oxidase enzyme (10 mg, 25 mg, 35 mg) dissolved in 10 ml of PBS buffer of pH 6.8 (solution-2). The GOD solution mixed in sonicated a-MWNT solution (solution-3). The GOD-a-MWNT solution incubated at 37^{0}C for 5 days. After the attachment GOD-MWNTs composite were filtered through PTFE membrane of pore size 0.22 μm, washed with PBS buffer and deionized water and derided at room temperature overnight.

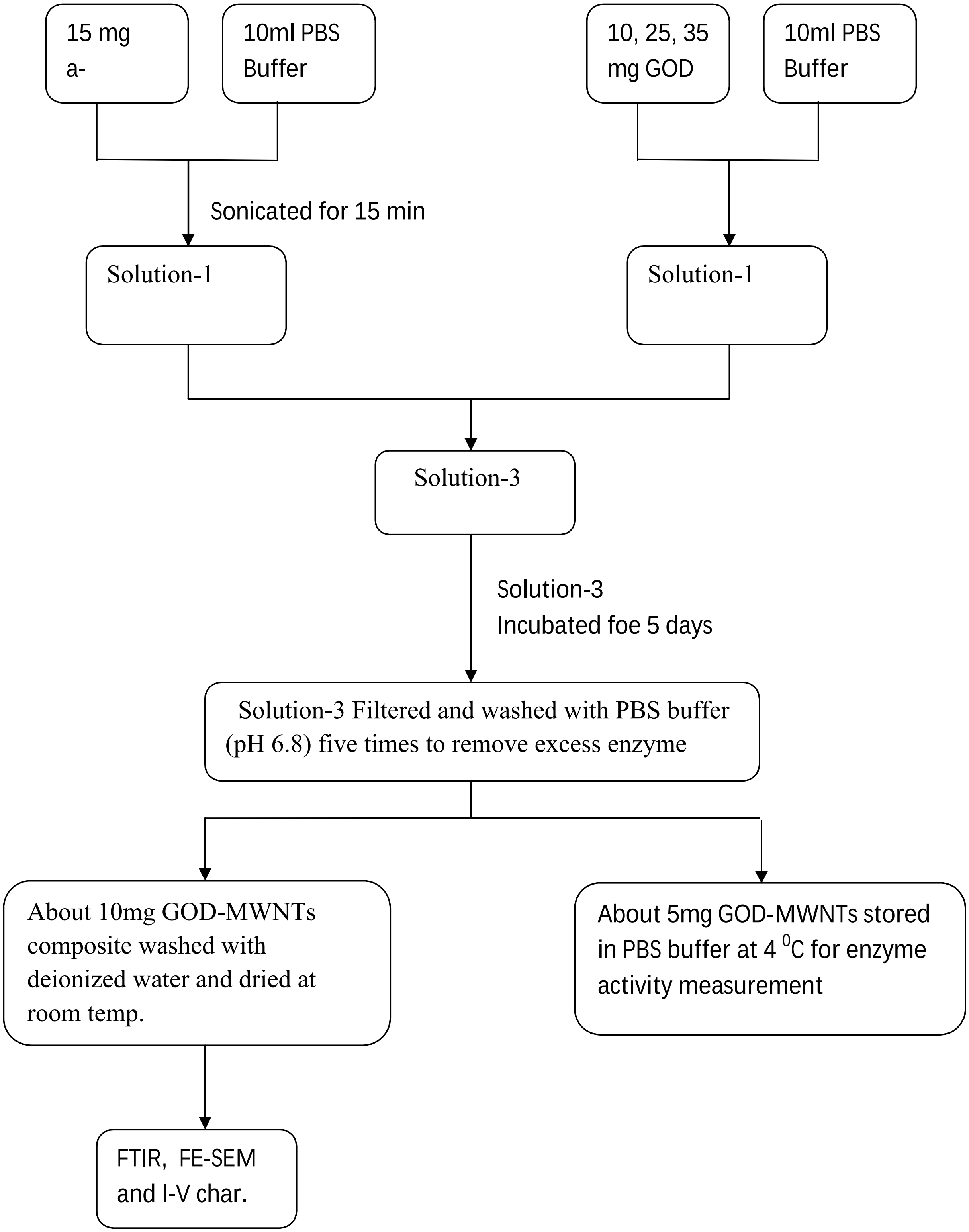


Fig 2.3: Flow chart of enzyme immobilization process

2.2.4 ***Synthesis of Polyaniline (PANI)***

In a typical synthesis experiment of PANI, the Aniline monomer (2 ml, 4 ml) dissolved in 1.0 M HCl solution. A 100 ml 1.0 M HCl solution containing 0.125 M Ammonium persulfate (APS) was slowly added drop wise into the aniline- HCl solution with constant mechanical stirring at a reaction temperature of 0– 5 ^{0}C for 1 h.

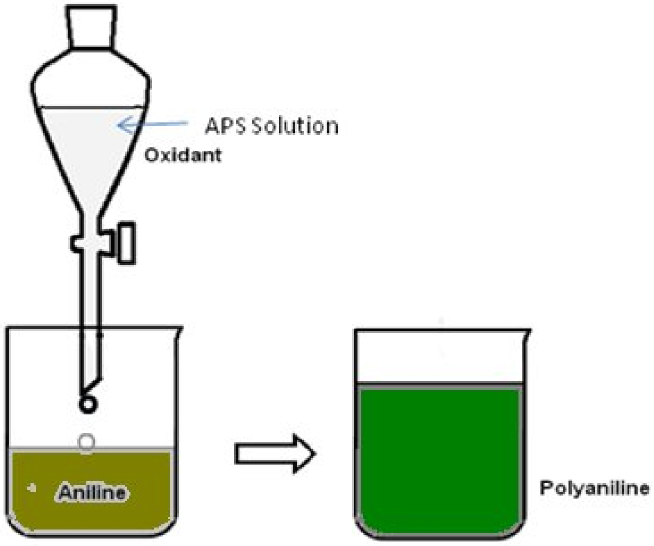


Figure 2.4: The Conventional synthesis of Polyaniline

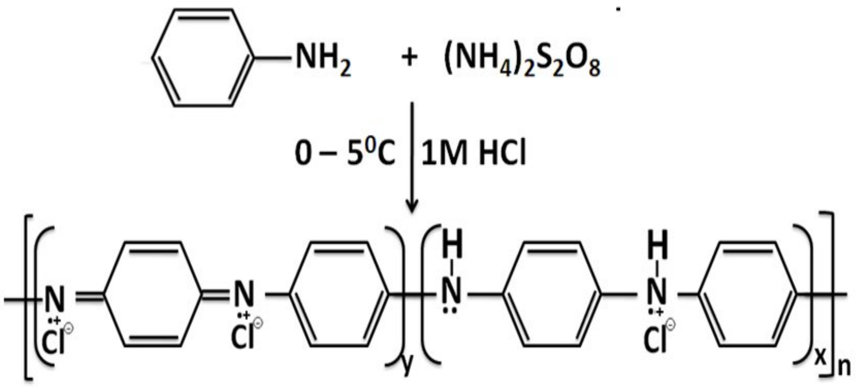


Figure 2.5: Polymerization of aniline

The reaction mixture was stirred for an additional 2 h at 0–5 ^{0}C, and then the resulting green suspension, indicating the formation of insoluble polyaniline in its emeraldine salt (ES) form, was then filtered with whatman filter paper through vacuum and rinsed several times with distilled water. The powder obtained was dried under a vacuum at 50 ^{0}C for 24 h.

2.2.5 Preparation of MWNT-PANI Composites

The composite of protonic acid doped polyaniline (PANI) with MWNT, c-MWNT and a-MWNT was synthesized by in situ chemical oxidation polymerization. Huseyin Zengin [2] reported preparation and characterization of CNT-polyaniline composite.

In a typical synthesis experiment of PANI-MWNT composites, various weight ratio of MWNTs (10 mg, 20 mg) were dissolved in 1.0 M HCl solutions and ultrasonicated over 72 h, then transferred into a 500 ml fourneck flask with an ice-bath. Aniline monomer (2 ml) also dissolved in 1.0 M HCl solution was added to the above MWNTs suspension.

For c-MWNT-PANI composites, various weight ratio of c-MWNTs were dissolved in 1.0 M HCl solutions and ultrasonicated over 3 h, then transferred into a 500 ml fourneck flask with an ice-bath. Aniline monomer also dissolved in 1.0 M HCl solution was added to the above c-MWNTs suspension.

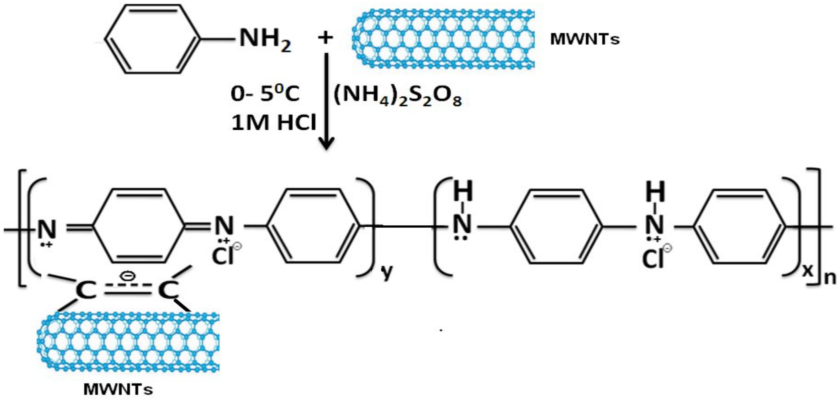


Figure 2.6: Illustration of the interaction between polyaniline and multi-walled carbon nanotube

For a-MWNT- PANI composites, various weight ratio of a-MWNTs were first mixed with aniline monomer and ultrasonicated over 3 h, then transferred into a 500 ml fourneck flask with an ice-bath. A 200 ml 1.0 M HCl solution containing 0.125 M Ammonium persulfate

(APS) was slowly added dropwise into the suspension of all three systems with constant mechanical stirring at a reaction temperature of 0– 5 ^{0}C for 1 h.

The reaction mixture was stirred for an additional 2 h at 0–5 ^{0}C, and then the resulting green suspension, indicating the formation of insoluble CNT-polyaniline composites, was then filtered and rinsed several times with distilled water. The powder obtained was dried under a vacuum at 50 ^{0}C for 24 h. These materials were used for characterization experiments.

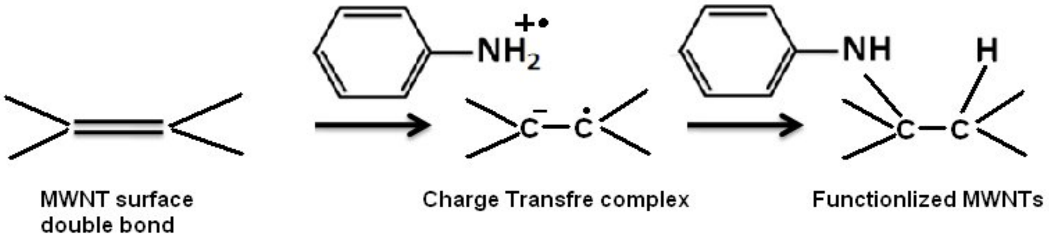


Figure 2.7: Charge transfer complex formation between aniline and the graphitic carbon nanostructure surface, and subsequent functionalized MWNTs

2.2.6 Attachment of Glucose Oxidase (GOD) on MWNT/PANI Composites

The MWNT/PANI composite (15 mg) were sonicated in 10ml of PBS buffer of pH 6.8 for 2h (solution-1). Glucose oxidase enzyme (10 mg, 25 mg, 35 mg) dissolved in 10ml of PBS buffer of pH 6.8 (solution-2). The glucose oxidase solution mixed in sonicated MWNT/PANI solution (solution-3). The GOD-MWNT/PANI solution incubated at 37^{0}C for 5 days. After the attachment GOD-MWNT/PANI composite were filtered, washed with PBS buffer and deionized water and derided at room temperature overnight.

Similarly c-MWNT/PANI and a-MWNT/PANI composites (15 mg) were sonicated in 10ml of PBS buffer of pH 6.8 for 2h. Glucose oxidase enzyme dissolved in 10ml of PBS buffer of pH 6.8. The glucose oxidase solution mixed in sonicated c-MWNT/PANI and a-MWNT/PANI solutions. The GOD-c-MWNT/PANI and GOD-a-MWNT/PANI solutions were incubated at 37 ^{0}C for 5 days. After the attachment GOD-c-MWNT/PANI and GOD-c-MWNT/PANI composites were filtered, washed with PBS buffer and deionized water and derided at room temperature overnight.

The all the materials prepared were characterized by:

- Fourier Transform Infra-red spectroscopy (FTIR).
- FE-SEM – For morphological imaging.
- Electrical characterization.

2.2.7 STUDY OF ENZYME ACTIVITY

Glucose oxidase (GOX) catalyzes the oxidation of β-D-glucose to D-glucono-δ-lactone and hydrogen peroxide. It is highly specific for β-D-glucose and does not act on α-D-glucose.

2.2.7.1 Principle

Glucose oxidase catalyses the oxidation of β-D-glucose to D-glucono- δ-lactone with the concurrent release of hydrogen peroxide.

$$\text{ß-D-Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{D-Glucono-1, 5-Lactone} + H_2O_2$$

$$H_2O_2 + \text{o-Dianisidine (reduced)} \xrightarrow{\text{POD}} \text{o-Dianisidine (oxidized)}$$

Abbreviations used:

GOD = Glucose Oxidase

POD = Peroxidase

Conditions: T = 35^0C, pH = 5.1, A500nm, Light path = 1 cm

Method: Continuous Spectrophotometric Rate Determination

2.2.7.2 Procedure:

The activity measurements for GOx and GOx immobilized on a-MWNTs was carried out by a spectrophotometric method (sigma). The reaction mixture consisted of glucose (2.4mL; 10 % w/v) and o-dianisidine (0.5 ml) in a sodium acetate buffer (0.05 M, pH 5.1) in the presence of horseradish peroxidase (0.1 ml) and GOD-MWNTs solution (0.1 ml) was added in test and sodium acetate buffer (0.1 ml) was added in blank. Both solutions were incubated for 20 min at room temperature and at 500nm, in spectrophotometer and the color was monitored.

The apparent enzyme (GOx) activity immobilized on a- MWNTs was estimated with following formula:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{500nm}/\text{min Test} - \Delta A_{500nm}/\text{min Blank})\ (3.1)\ (df)}{(7.5)\ (0.1)}$$

Where,
3.1 = Volume (in milliliters) of assay
df = Dilution factor
7.5 = Millimolar extinction coefficient of oxidized o-Dianisidine at 500 nm
0.1 = Volume (in milliliters) of enzyme use

2.3 DEPOSITION OF MWNTs FILMS

For film deposition of p-MWNT, c-MWNT and a-MWNT the 1×1 cm^2 glass plates ware taken. The glass plates were cleaned by sonicating in the acetone solution. After cleaning glass plate dried. Now 5mg of p-MWNT, c-MWNT and a-MWNT dispersed in 0.5 ml deionized water by sonicating for 2h. The disperse p-MWNT, c-MWNT and a-MWNT ware solution casted on the glass plate and deride at room temperature. This film was used for I-V measurements.

For film deposition of GOD-MWNTS composite the 1×1 cm^2 glass plates ware taken. The glass plates were cleaned by sonicating in the acetone solution. After cleaning glass plate dried. Now 5 mg of GOD-MWNTs composite dispersed in of deionized water by sonicating for 15 min. The disperse GOD-MWNTs composite was solution casted on the glass plate and deride at room temperature. These films were used for I-V measurements.

REFERENCES

1. Y. Wang, Z. Iqbal, Sanjay V. Malhotra, Chemical Physics Letters **402**, 96 (2005).
2. Huseyin Zengin, Wensheng Zhou, jianyong Jin, R. Czerw, D. W. Smith, L. Echegoyen, D. L. Carrol, S. H. Foulger and J. Ballato, Adv. Mater.**14**, 20 (2002).

CHAPTER-3

RESULTS AND DISCUSSION

The spectroscopic characterization of the MWNTs and MWNT-polymeric samples is usually carried out by either preparing a thin film or making a solution of the polymer transparent to the incident radiation. In case of powders, a few mg is mixed with potassium bromide (KBr) in form of pellets to used for IR studies. The IR spectroscopy identifies and confirms the structure and presence of various groups on MWNTs and various linkage in MWNT-polymer composites. This is a very important characterization technique, specially when substitution, cross-linking and copolymerization are involved.

3.1Fourier Transform Infra-red Studies

Infrared spectroscopy is one of the most powerful analytical techniques, which offers the possibility of chemical identification. One of the most important advantages of infrared spectroscopy over the other usual methods of structural analysis is that it provides useful information about the structure of molecules and bonding quickly, without tire-some evaluation methods.

The technique is based upon the simple fact that a chemical substance shows marked selective absorption in the infrared region giving rise to close-packed absorption bands, called an IR absorption spectrum, which may extend over a wide wavelength range. Various bands present in an IR spectrum correspond to the characteristic functional groups and bonds present in a chemical substance.

- Band intensities in IR spectrum are expressed as transmittance (T).Transmittance is defined as the ratio of the radiant power transmitted by a sample to the radiant power incident on the sample. In most of the spectrum transmittance (T) versus wave number (cm^{-1}) has been plotted.
- In the present work, FT-IR spectra of sample (pellets) has been recorded in the range of 4000 to 400 cm^{-1} at 4 cm^{-1} resolution using FT-IR spectrophotometer of Perkin Elmer spectrum RXI FT-IR system.

3.1.1 FTIR Study of Unfunctionalized MWNTs:

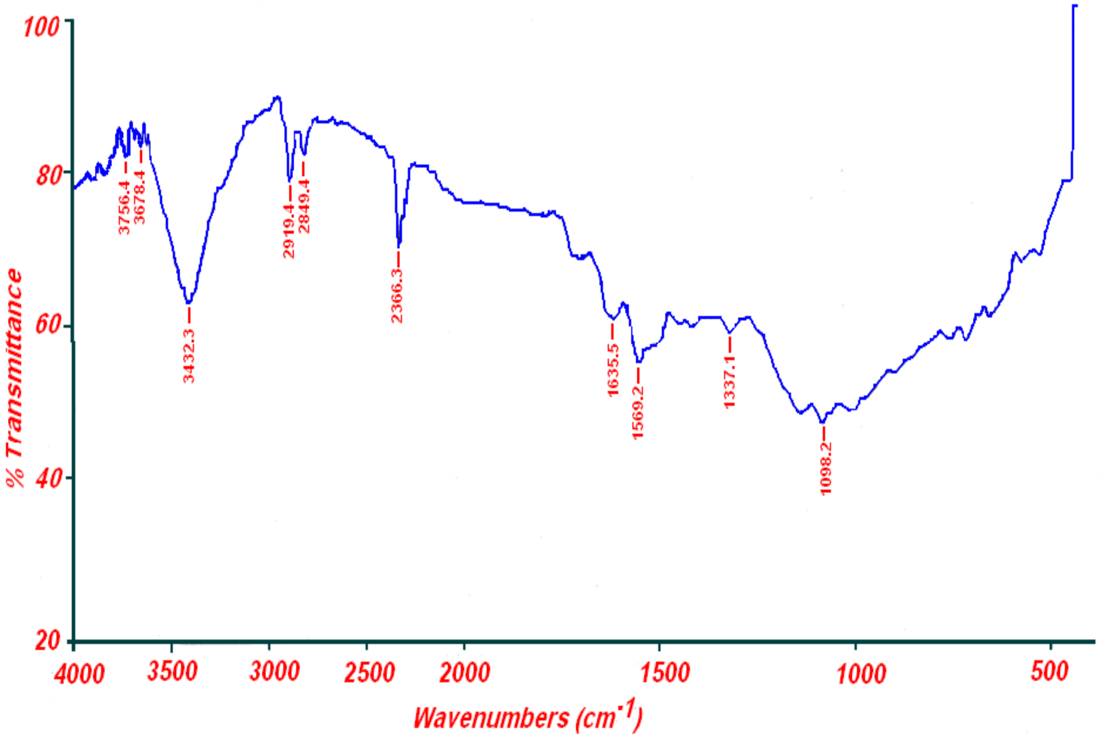


Figure -3.1: FT-IR spectra of pristine MWNTs

In the IR- spectra of p-MWNTs, a very broad peak at 3432.3 cm^{-1}1at correspond OH group present on MWNTs surface this is due to presence of moisture. The peaks at 2919.4, 1635.5 and 1569.2 cm^{-1} due to C≡C bond C=C bond in MWNTs and 1569.2 cm^{-1} due to conjugated C=C bond in MWNTs respectively. The peaks at 1337.1and 1098.2 cm^{-1} were assigned to due to C=C bond stretching and C-C bond in MWNTs respectively.

3.1.2 FTIR Study of Glucose Oxidase Enzyme:

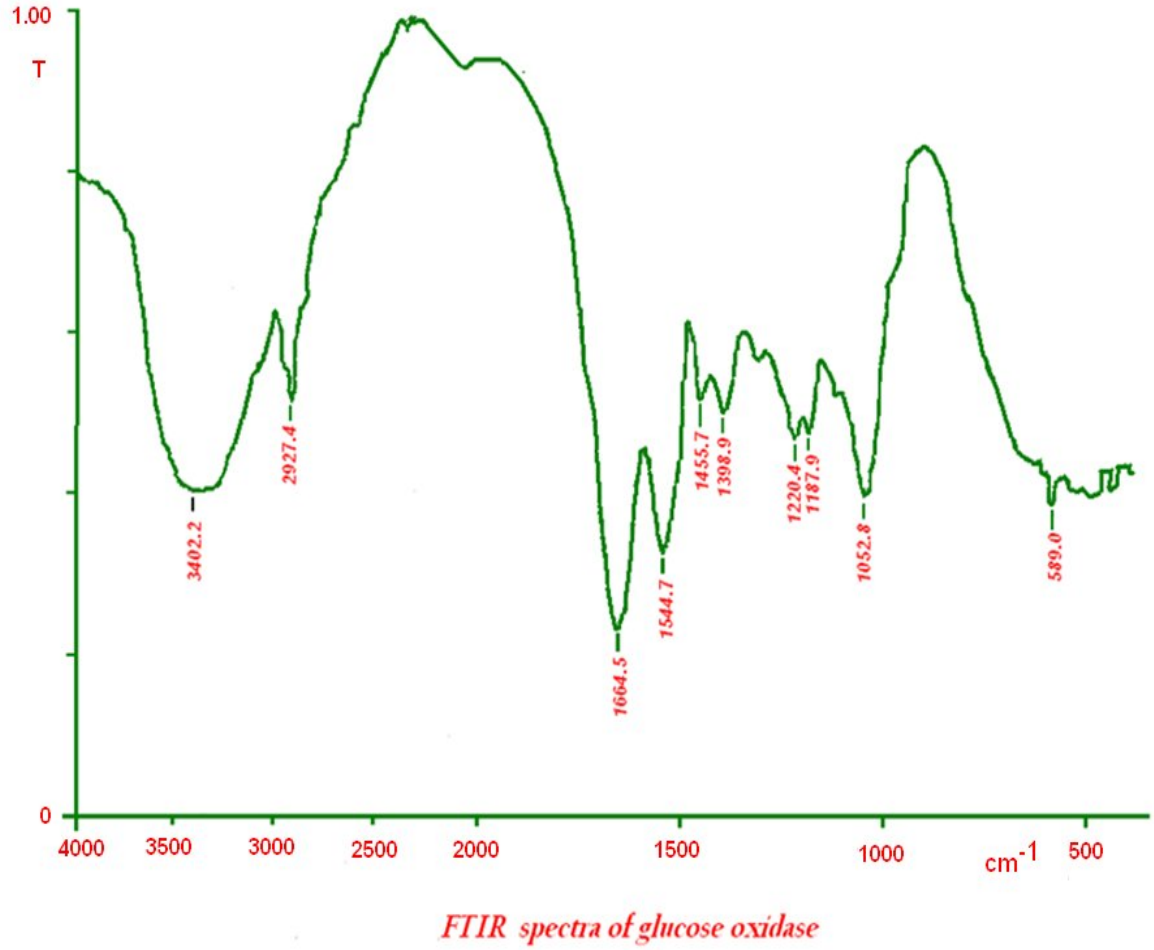


Figure- 3.2: FT-IR spectra of Glucose oxidase

The peaks observed at 3402.2, 2927.4, and 1220.4 cm^{-1} corresponds to N-H, C-H and C-N stretching vibrations respectively. In the case of GOD, the most important features are the amide I band at 1654.5 cm^{-1} and amide II band 1544.7 cm^{-1} of the amide group [1].

The above FTIR of enzyme GOD, the peak are corresponds to the bonds present in the molecules of enzyme.

3.1.3 FTIR Study of Carboxylated MWNTs:

The IR spectra of pristine MWNTs were recorded between the wavenumber rang of 500 to 3500cm^{-1} shown in fig- 3.3.

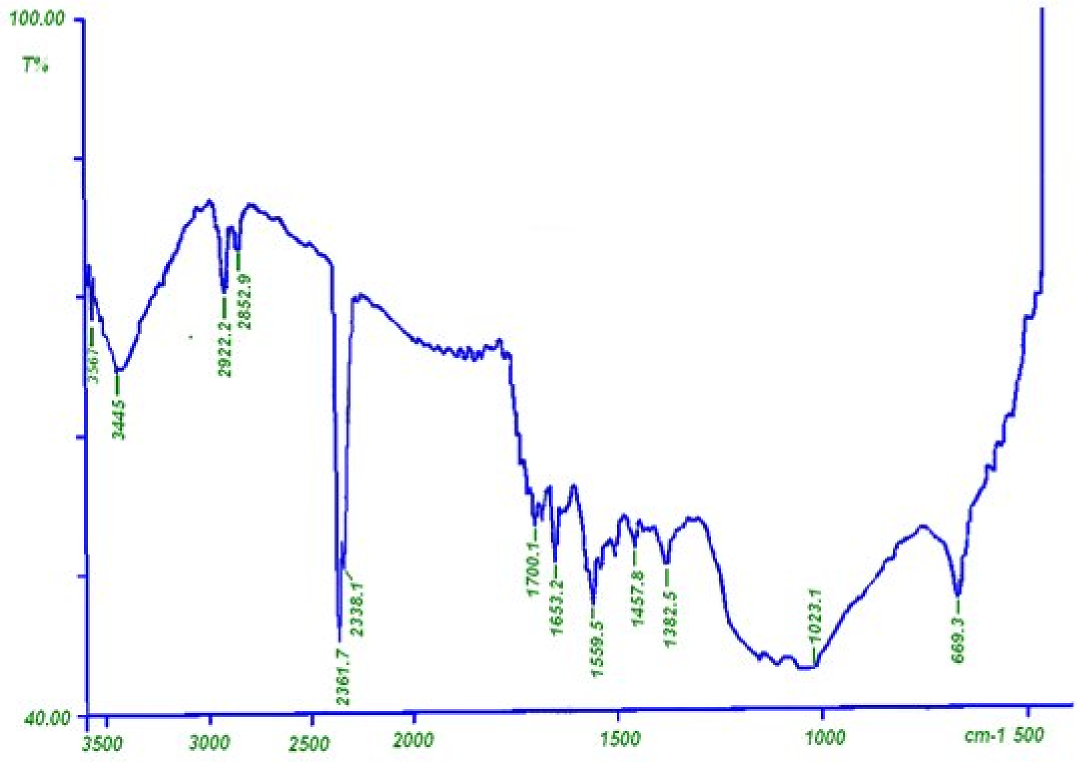


Figure -3.3: FT-IR spectra of c-MWNTs

After acid functionalized MWNTs, **the new peak arises at 1700.1 cm^{-1} which is correspond to C=O of COOH group and** a very broad peak at 3445.0 cm^{-1}at correspond OH stretching vibration in -COOH group**.** The peak observed at 1569.5cm^{-1} due to carboxylate ion COO$^-$.

Therefore, it was considered that carboxylic groups (-COOH) had been attached onto the surface of the MWNTs successfully as a result of acid treatment. The two weak peaks at 2922.2 and 2852.9 cm^{-1} correspond to the –CH stretching mode.

3.1.3.1 Effects of Acid Treatment on MWNTs

The acid treatment variables such as time, temperature, ultrasonication frequency may affect the structure and properties of nanotubes **[2]**. To know the acid treatment effect on the electrical properties of functionalized WMNTs we functionalized MWNTs for different keeping other parameter constant.

From Figure-3.4 (a), it was clear that the FT-IR spectra of raw MWNTs showed peaks with very low intensity at around 3448, 1651.4, and 1168.8 cm^{-1}, corresponding to OH, C=O, and C-C-O stretching present in carboxylic groups (COOH), respectively. In the case of acid treated MWNTs, these characteristic bands appeared with significantly higher intensity. This was attributed to the increased number of carboxylic acid groups which had been successfully attached to the surface of the MWNTs after acid treatment in H_2SO_4/HNO_3 mixture.

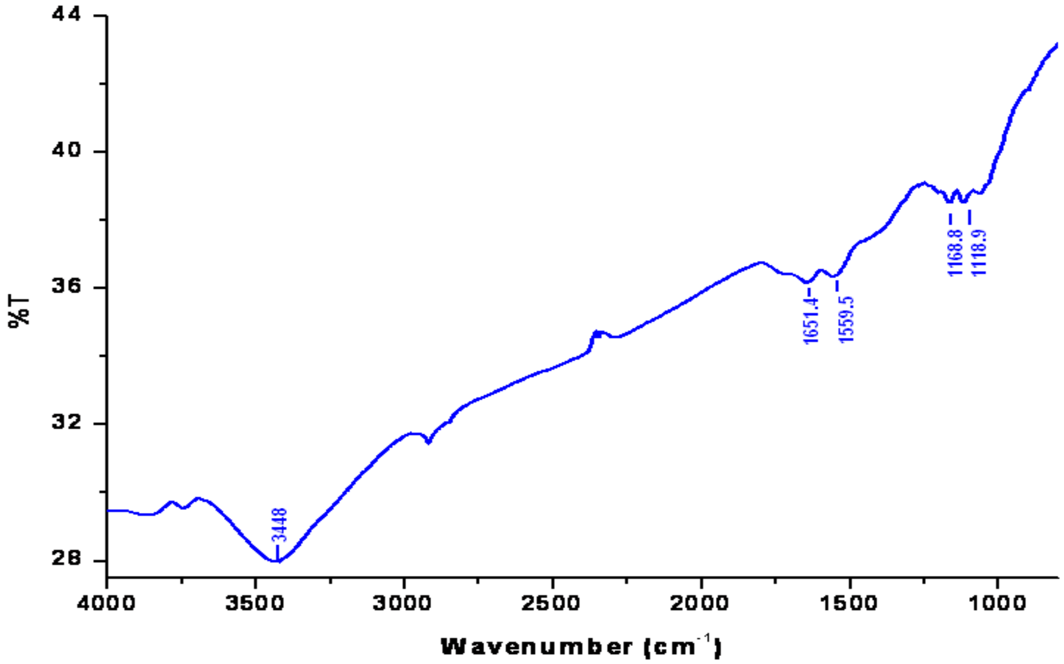


Figure -3.4 (a)

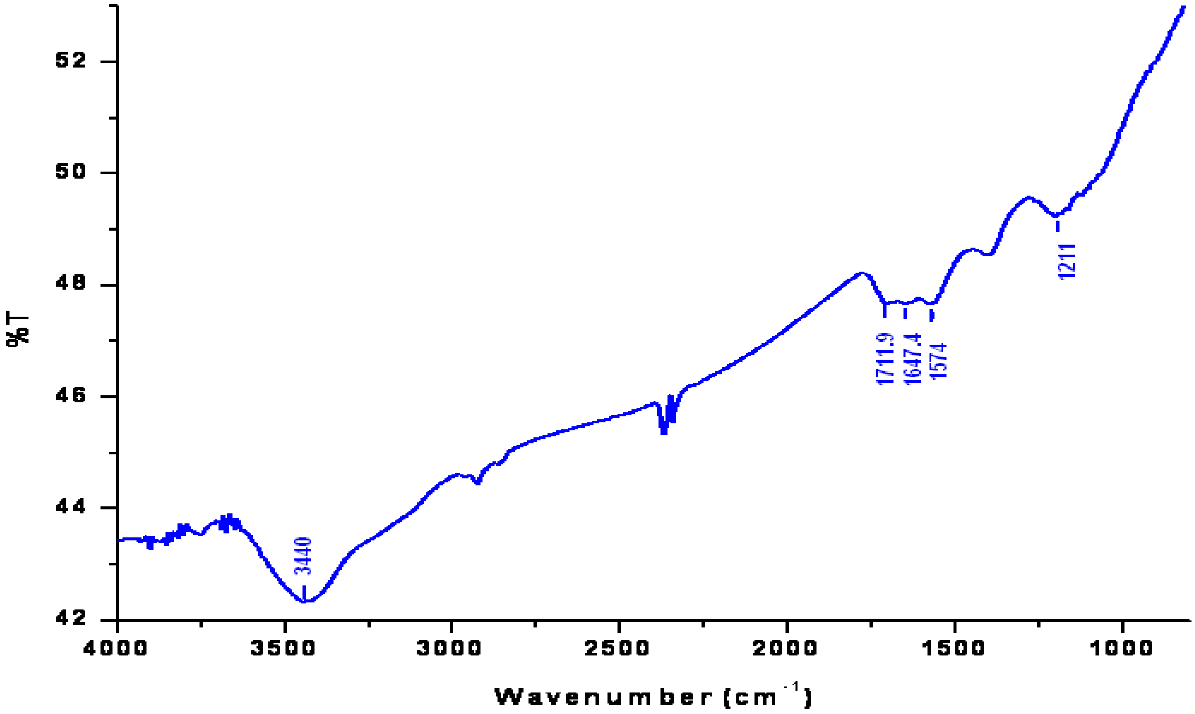


Figure -3.4 (b)

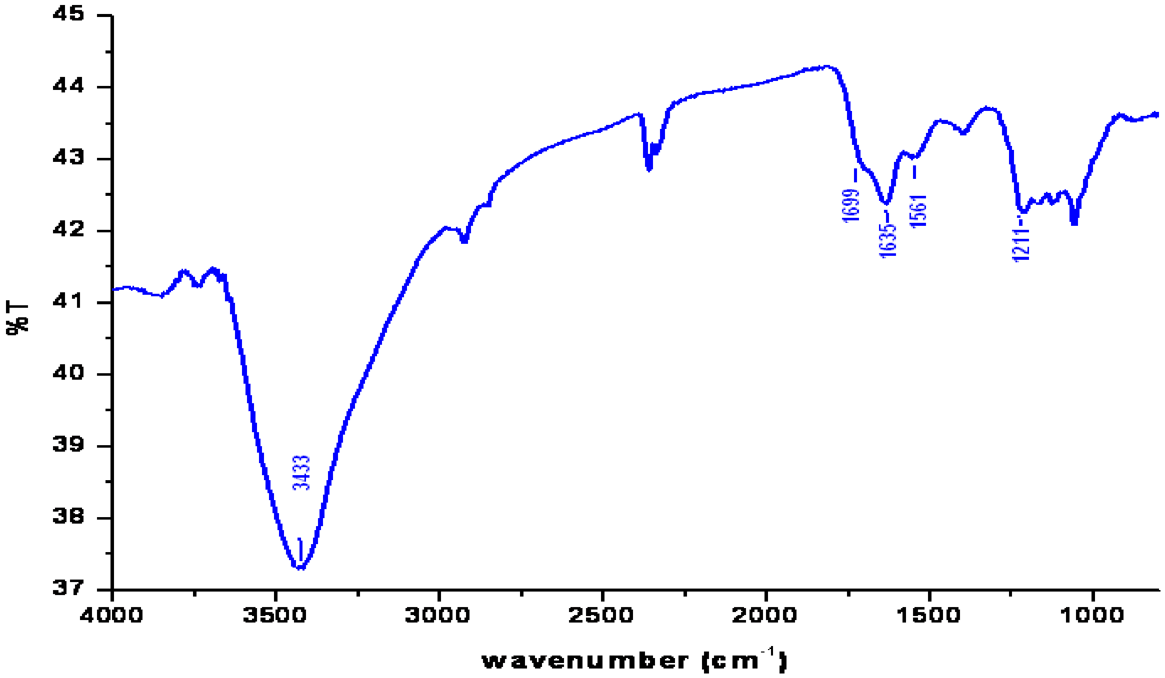


Figure -3.4 (c)

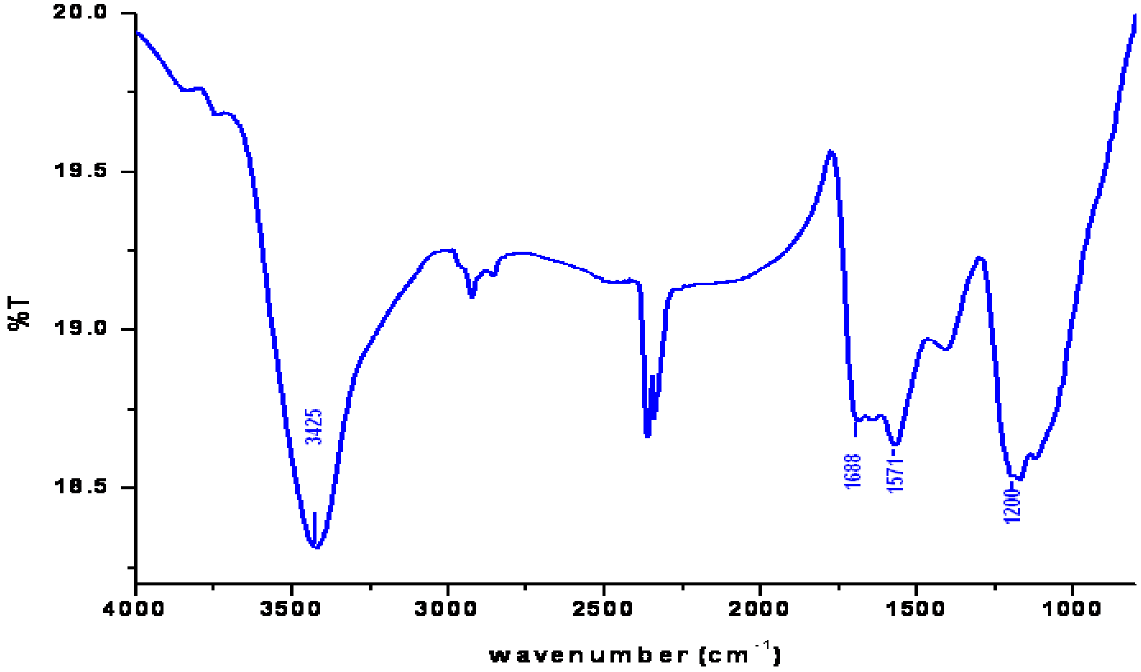


Figure -3.4 (d)

Figure -3.4: FT-IR spectra of MWNTs treated with the acid mixture for: (a) 0h, (b) 3h, (c) 5h, (d) 18h

A typical FTIR spectrum of MWNTs treated with the acid mixture for 3 h is shown in Figure- 5.4 (b), in which a new peak around 1711.9 cm- appears. It is normally assigned to the C=O-strength vibration in the COOH group, which means that the acid-mixture treatment will introduce some C-O groups to the end or the side of the MWNTs. It is interesting that we found that the peak around 1711.9 cm^{-1} shifted to 1688 cm^{-1} as the time of the treatment increased, which suggested that the abundance of -COOH groups increased along with the treatment time.

Meanwhile, the formation of hydrogen bonds between the -COOH groups became more effective. Compared with the as-prepared MWNTs in Figure-3.4 (a), we also found that in the treated MWNTs the peak originally around 1559.5 cm^{-1}, which is assigned to the –C=C- groups, shifted to a higher wavenumber around 1571 cm^{-1}, which may suggest a change in the structure of the MWNTs.

3.1.4 FTIR Study of Acylated MWNTs

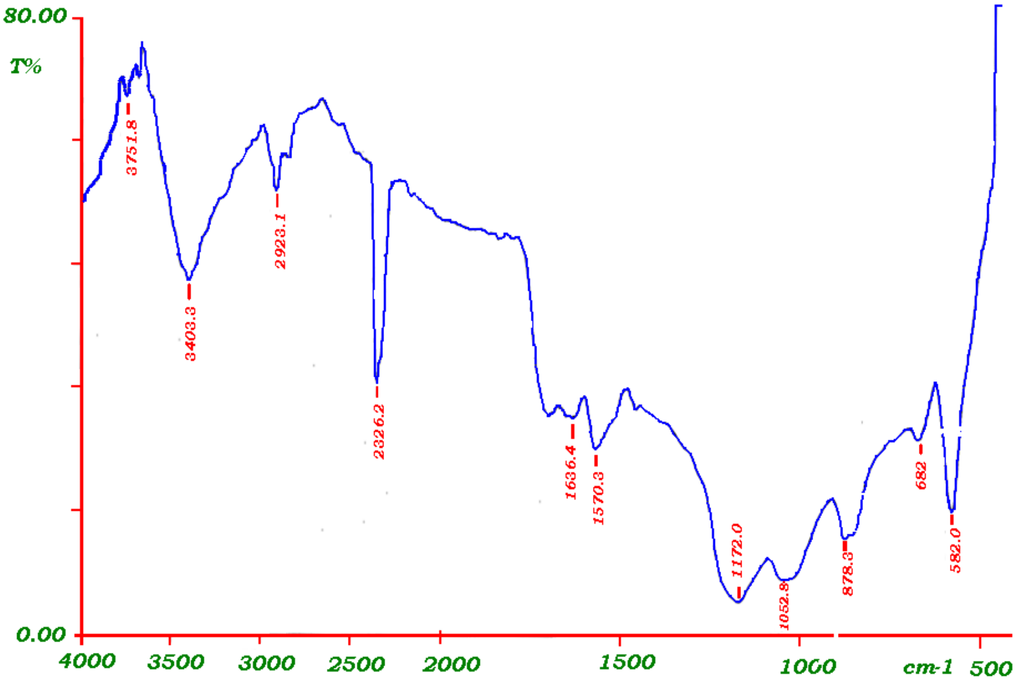


Fig. 3.5: FT-IR spectra of acylated MWNTs (a-MWNTs)

After acylation of carboxylated MWNTs, the peak at 1700.1 cm^{-1} which is correspond to C=O of COOH group is not observed in the IR spectra. The peak at 1636.4 cm^{-1} due to C=O stretching peak of COCl group. The two new peaks appear at 582.0 and 682.0 cm^{-1} corresponds to the C-Cl group which appears generally in the range of 550 to 800 cm^{-1}.

And the peak at 1570 cm^{-1} was attributed to the vibration of carbon skeleton (C-C stretch) of the bulk MWNTs. The other peaks at 3403.3 cm^{-1} due to OH group of carboxylic acid and at 2923.1 cm^{-1} due to C≡C bond in MWNTs

The peak at 2360.2 cm^{-1} corresponds to the CO_2 coating of IR optics which was common in all the spectras. The peak at 1052.8 cm^{-1} corresponds to the C-O stretch in carboxylic acid.

3.1.5 FTIR study of Enzyme (GOD) attached MWNTs

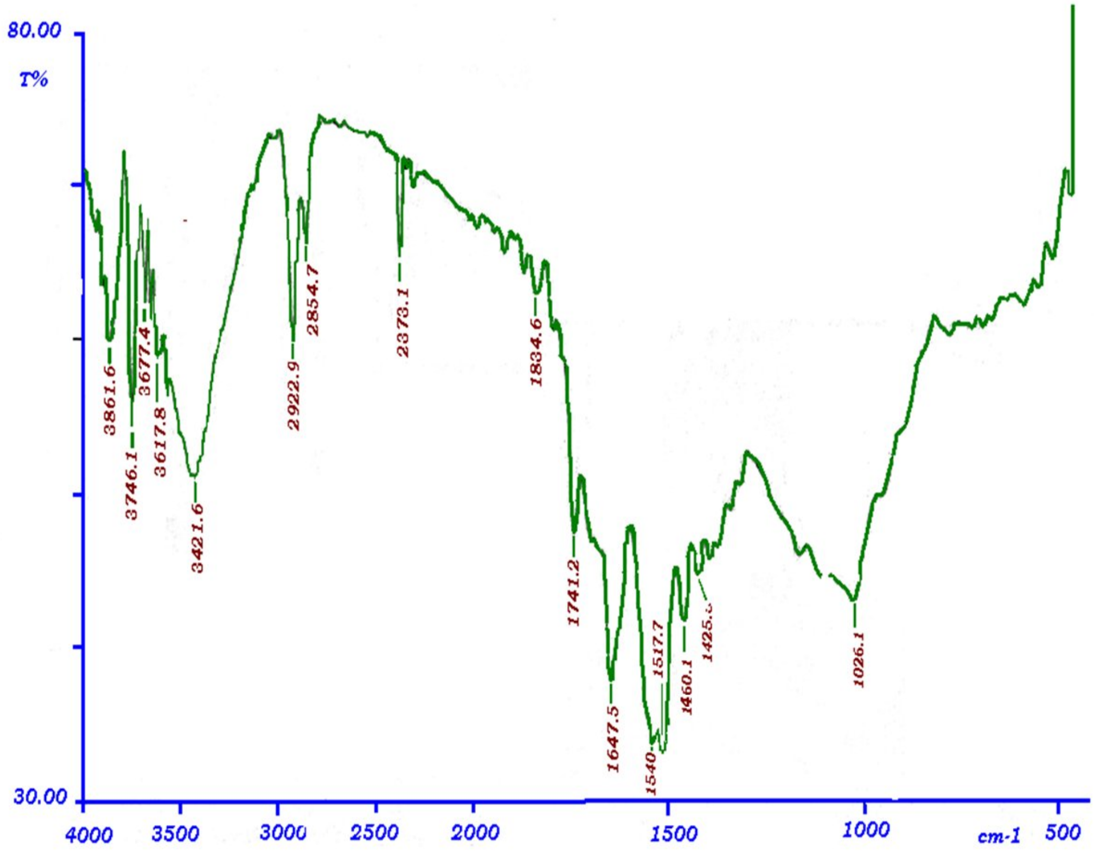


Figure- 3.6: FT-IR spectra of GOD attached MWNTs (GOD-MWNTs)

In the FT-IR spectra of GOD-a-MWNT composite the **peak at 1647.5 cm^{-1} corresponds to the amide-I (C=O) band and peak at 1540 cm^{-1} (N-H bend) corresponds to the amide II band**. Also change in peak position was observed (1636 cm^{-1} to 1647.5 cm^{-1} and 1570 cm^{-1} to 1540 cm^{-1}), this change indicates that the GOD was attached to a-MWNTS.

The other important peaks are at 3421.6 cm^{-1} due to NH stretch of amides, 2922.9 cm^{-1} due to C-H bond in MWNTs, 2373.1 cm^{-1} due to CO_2 coating of IR optics, **1741.2.1 cm^{-1} due to C=O bond in MWNTs**, 1426.0 cm^{-1} due to C-O stretch in carboxylic acid and 1026.1 cm^{-1} due to C-N stretch in enzyme The successful covalent attachment of GOD to MWCNT is indicated by the appearance of the IR absorption of the amide I and amide II [5].

3.1.6 FTIR Study of Polyaniline (PANI)

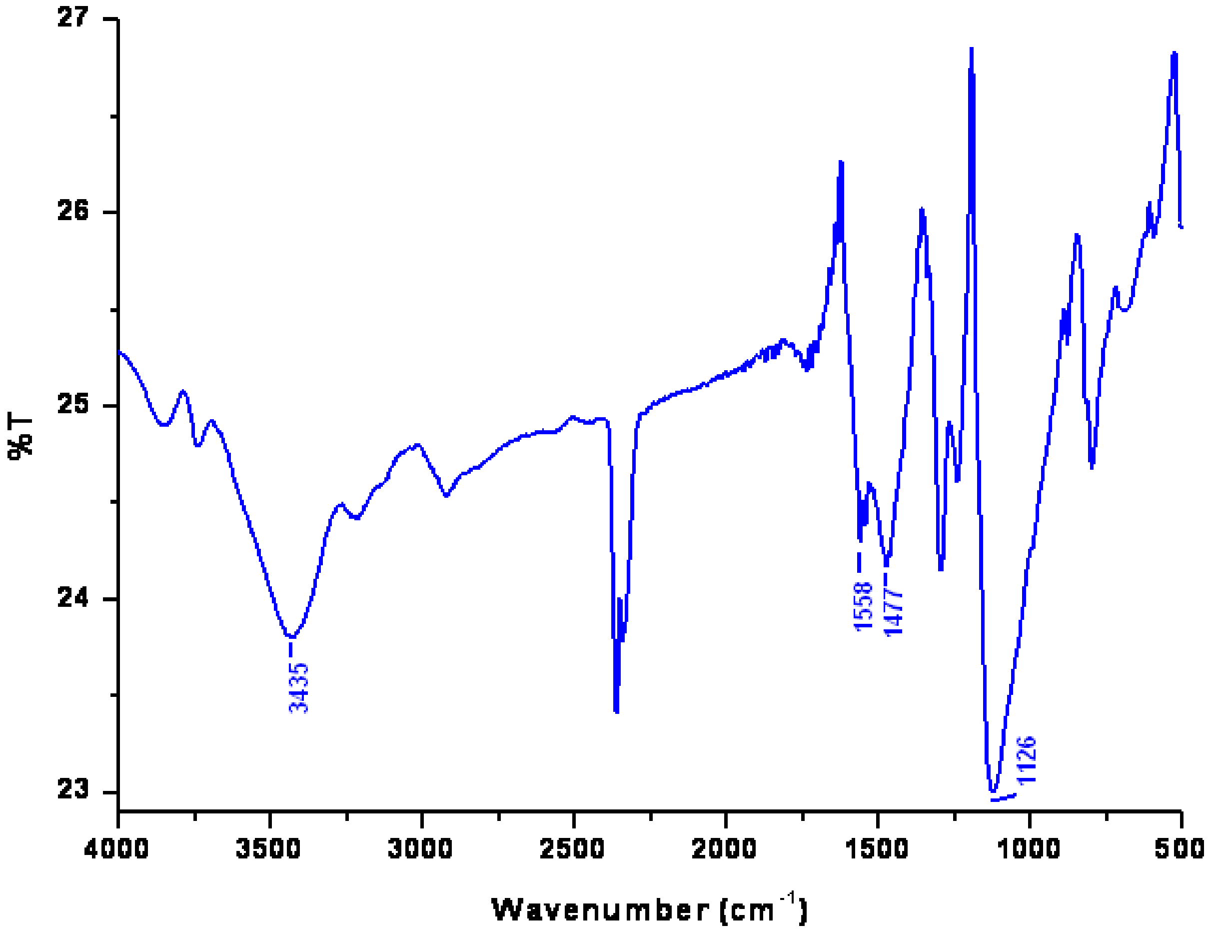


Figure -3.7: FT-IR spectra of chemically synthesize Polyaniline.

The spectrum exhibits the clear presence of benzoid at 1477 cm^{-1} and the quinoid ring vibration at 1558 cm^{-1}, indicating the oxidation state of emaraldine salt of PANI. The strong band around 1126 cm^{-1} is the characteristic peak of PANI conductivity and is a measure of the degree of the delocalisation of electrons. The very weak and broad band around 3435 cm^{-1} is assigned to the N-H stretching mode.

3.1.7 FTIR Study of MWNT-PANI Composite:

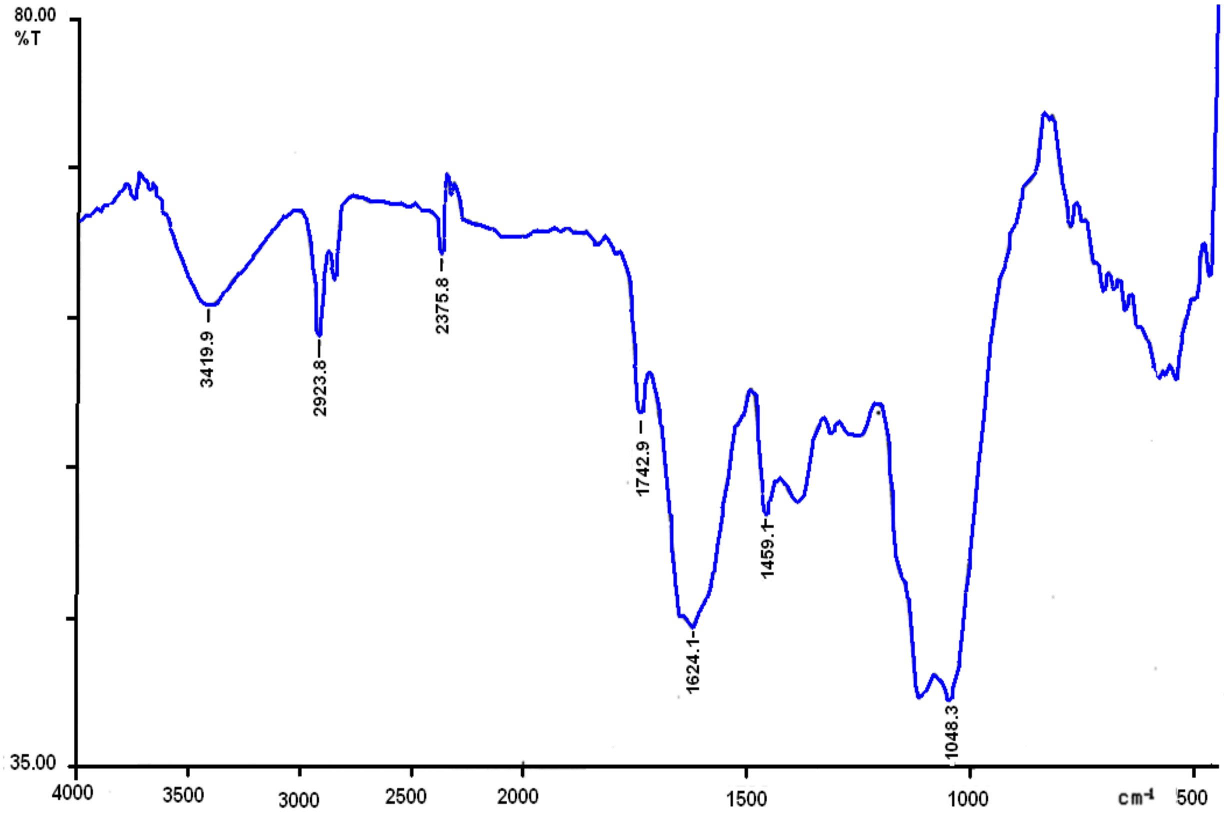


Figure -3.8: FT-IR spectra of chemically synthesize MWNT-Polyaniline composite.

There were clear differences between these and the spectrum of PANI (Fig. 3.7). The N-H stretching region near 3420 cm^{-1} in Figure 3.8 showed strong and broad peaks, but weak and broad peaks were present in the neat PANI spectrum. The interaction between the MWCNTs and PANI may result in “charge transfer”, whereby the sp^2 carbons of the MWCNTs compete with chloride ions [Cl$^-$] and perturb the H-bond, resulting an increase in the N-H stretching intensity. The shift in peak from 3435 cm^{-1} to 3419 cm^{-1} and 1477 cm^{-1} to1459 cm^{-1}. As shown in spectrum of PANI and MWNT-PANI composites, an inverse 1557/1477 cm^{-1} and 1624/1459 cm^{-1} intensity ratios was exhibited [6].

3.1.8 FTIR Study of Enzyme attached MWNT-PANI Composite

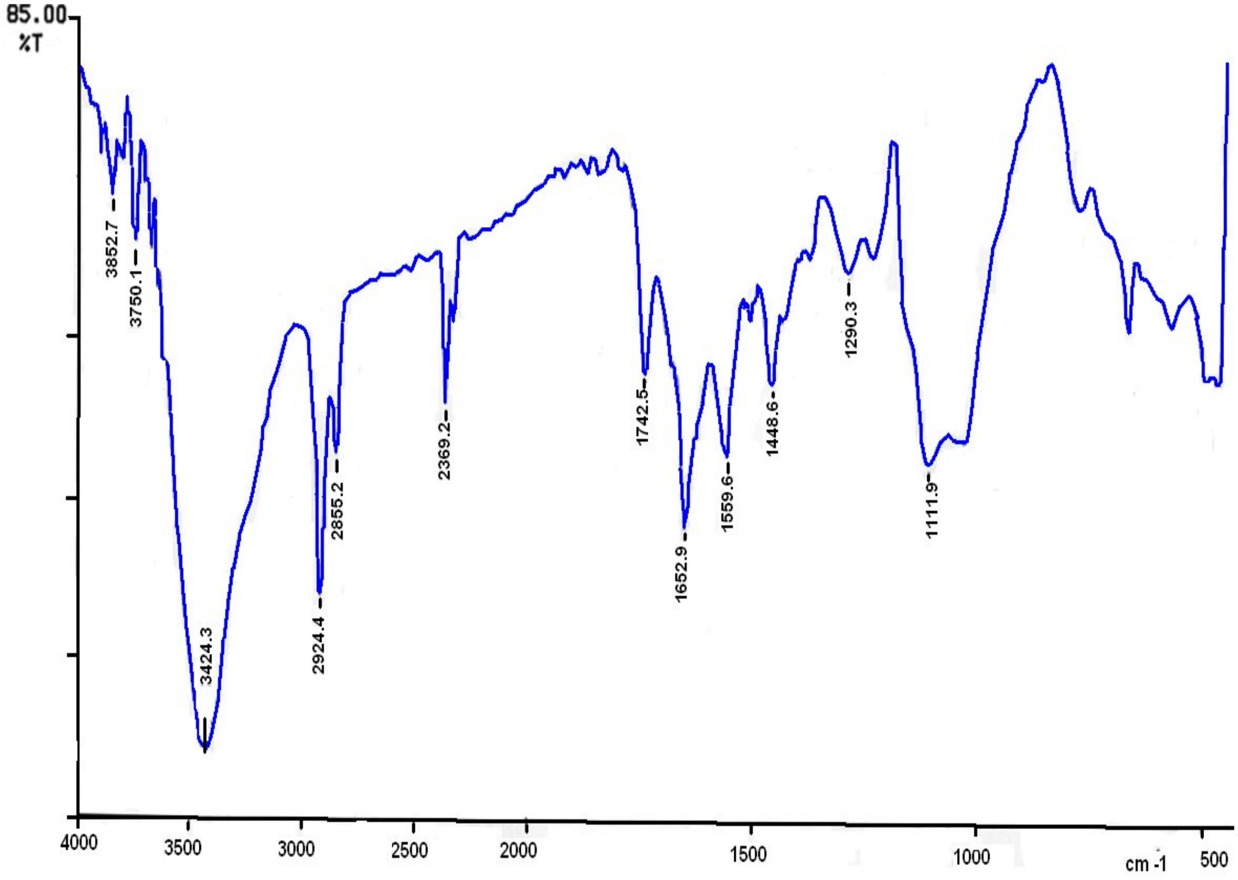


Figure -3.9: FT-IR spectra of GOD attached MWNT-PANI composite (GOD-MWNT-PANI)

In the FT-IR spectra of GOD-MWNT-PANI composite the most important **peak at 1652.9 cm^{-1} corresponds to the amide-I band and the peak at 1559.6 cm^{-1} corresponds to the amide II band [5]**. These peaks confirm that the enzyme is immobilized covalently on the MWNT-PANI composite. For further confirmation, the morphological FE-SEM images of GOD attached MWNT-PANI composite shows the globular structure of immobilized GOD.

3.2 Enzymatic Activity Studies

The measurement of activity of enzymes is an important tool for their study. The graphs shown below are between incubation time and absorbance. These graph results that the activity of enzyme. In the presence of POD, Die and D-glucose chemical reaction takes place and the color of solution changes, this change increases the absorbance. The absorbance increases as GOD concentration increases.

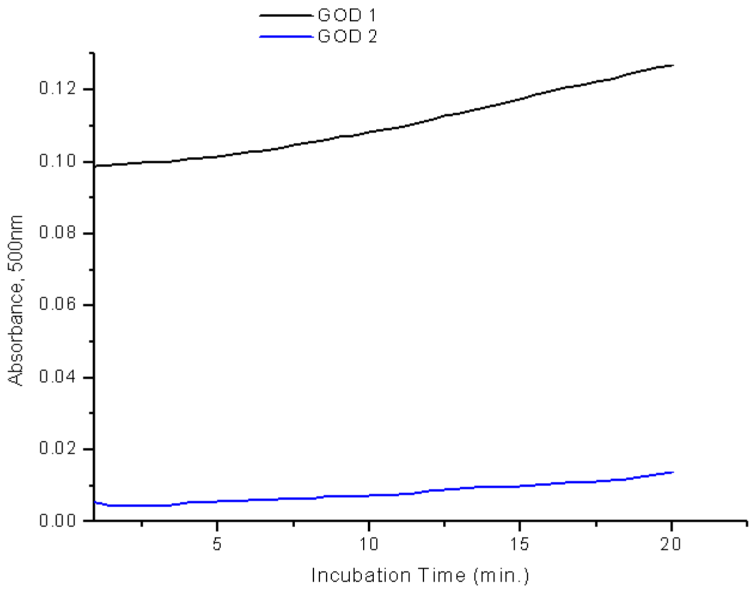


Fig-3.10: (a) Curves for two different concentrations of glucose oxidase in the assay mixture.

The absorbance of GOD-1 is higher than GOD-2 and depends on the concentration of GOD in the reaction mixture. The concentration of GOD-1 and GOD-2 were 0.1mg and 0.05mg.

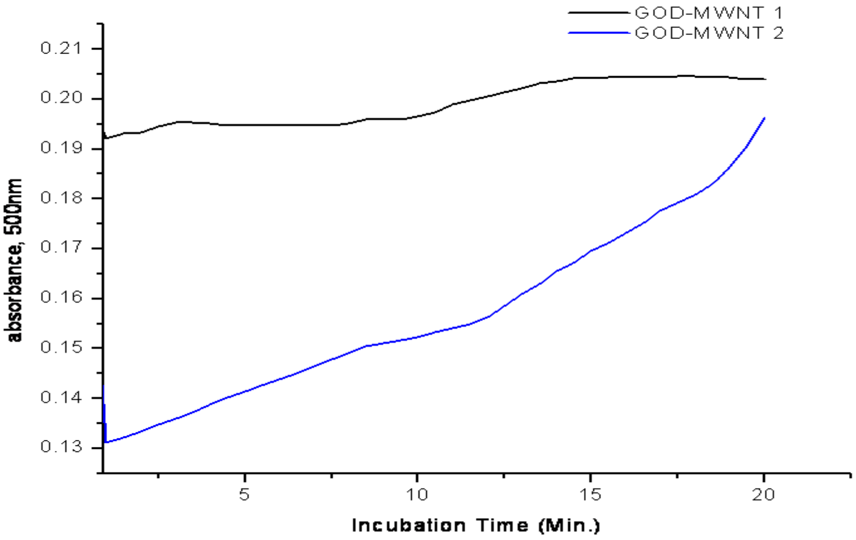


Figure-3.10: (b) Curves for two different concentrations of glucose oxidase immobilized MWNTs in the assay mixture.

3.3 Morphological Studies

Morphological features are the key features that provide the surface information of the materials. A detailed study of the morphological features of all the samples was carried out using FE-SEM is described in following sections.

3.3.1 FE-SEM study of p-MWNTs, f-MWNTs and GOD attached MWNTs

SEM micrographs of raw and acid treatment MWNTs are shown in figure 3.11, 3.12(a), 3.12(b), From the SEM micrographs shown in Figure 3.12(a) and 3.12 (b) it can be seen that the morphology of MWNTs was altered by acid treatment, compared with the raw multi-walled carbon nanotubes. With increasing acid treatment time, the length of the MWNTs decreased and the structure of the tubes became less well defined. SEM images of GOD-MWNTs are shown in the Fig.3.12(c).

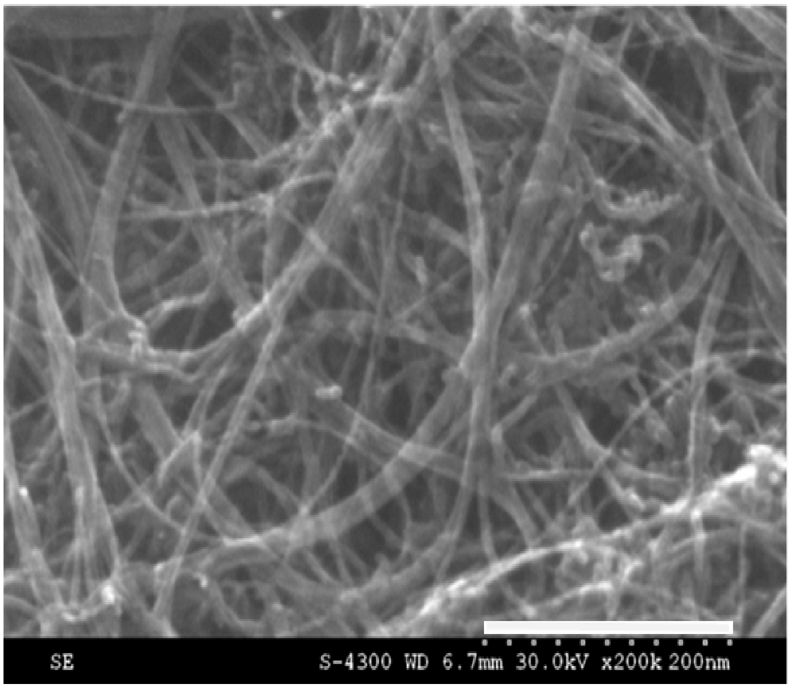


Figure 3.11: FE-SEM images of pristine MWNTs

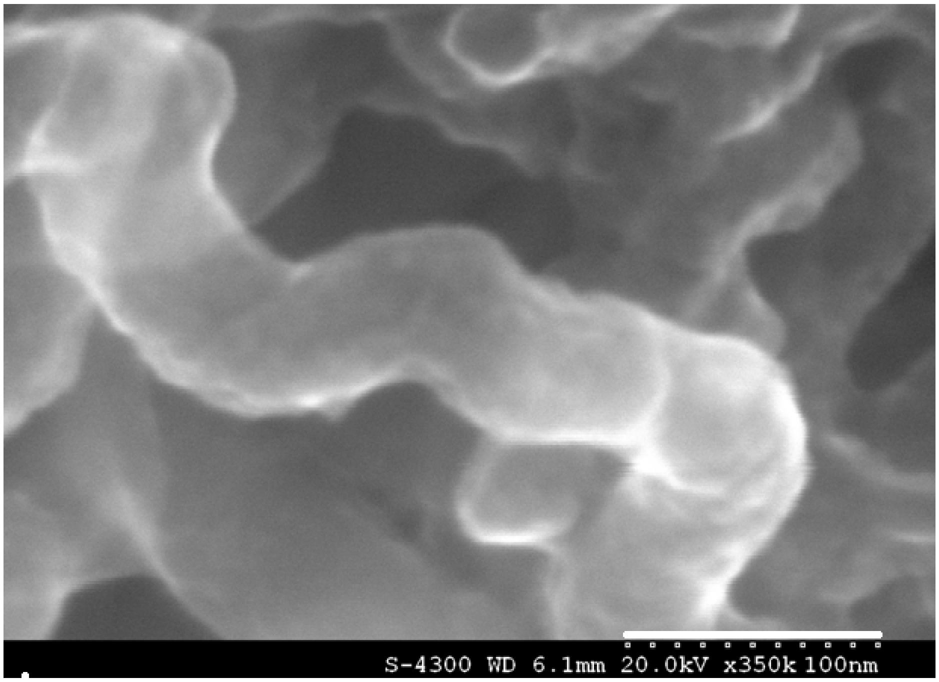


Figure 3.12: FE-SEM images of acylated-MWNTs

The SEM images of functionalized nanotubes at different magnifications are shown above. After functionalization the CNT was well dispersed in solvents. It was also observed that the diameter of functionalized was more than that of pristine.

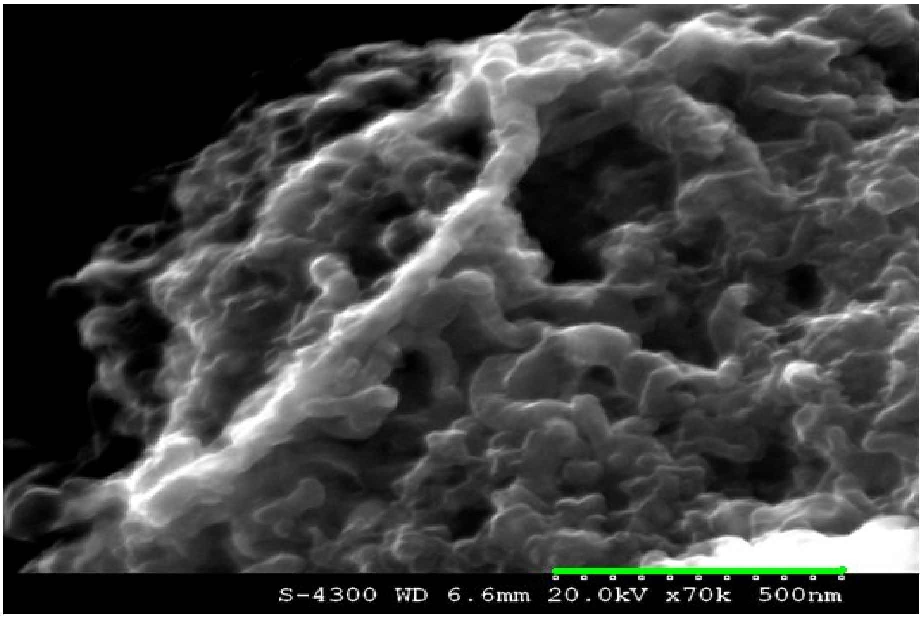


figure 3.12: (a)

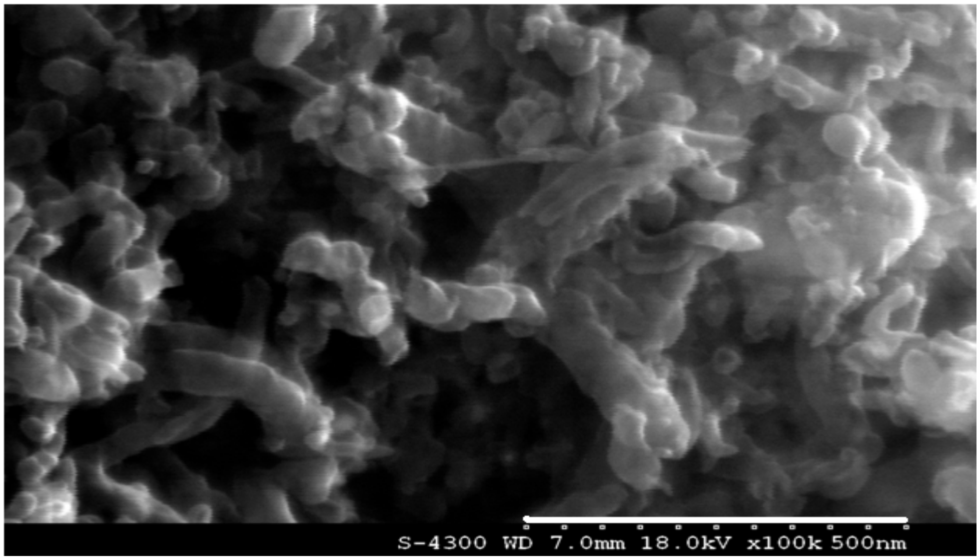


Figure 3.12: (b) FE-SEM images of GOD-MWNT composite

In the figure 3.12 (a), it was observed that the glucose oxidase (GOD) attached at sidewalls as well as at the ends of CNTs.The image (a) was taken at low magnification70 k; the shining reasons are due to enzyme coating. In the image (b) enzyme coating is clearly visible

3.3.2 FE-SEM Study of PANI, MWNT-PANI Composites and GOD-MWNTs-PANI Composites

The SEM images or morphology of PANI, MWNT-PANI composites and GOD-MWNTs-PANI composites are shown in fig. 13 and 14. The PANI and nanotube- PANI composites can be distinguished clearly. The attached GOD visualized as globular in shape.

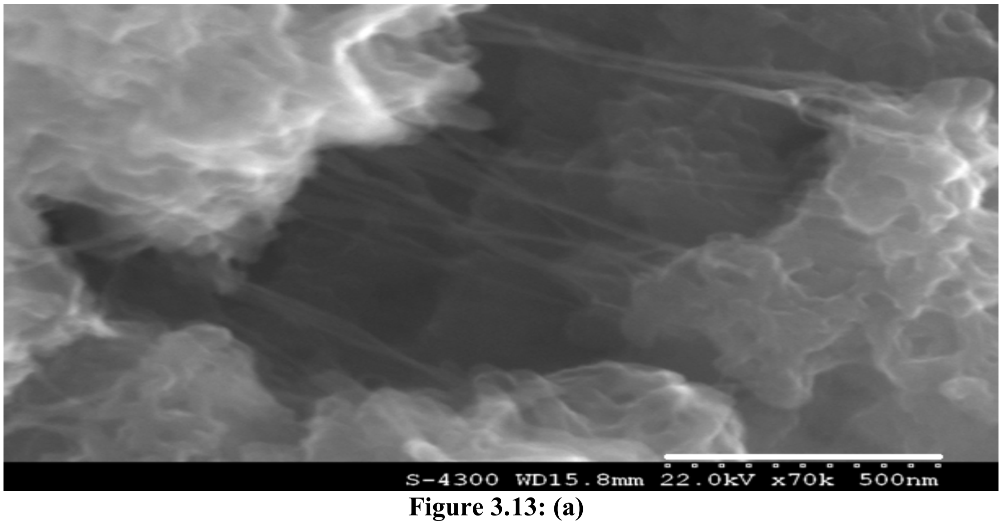


Figure 3.13: (a)

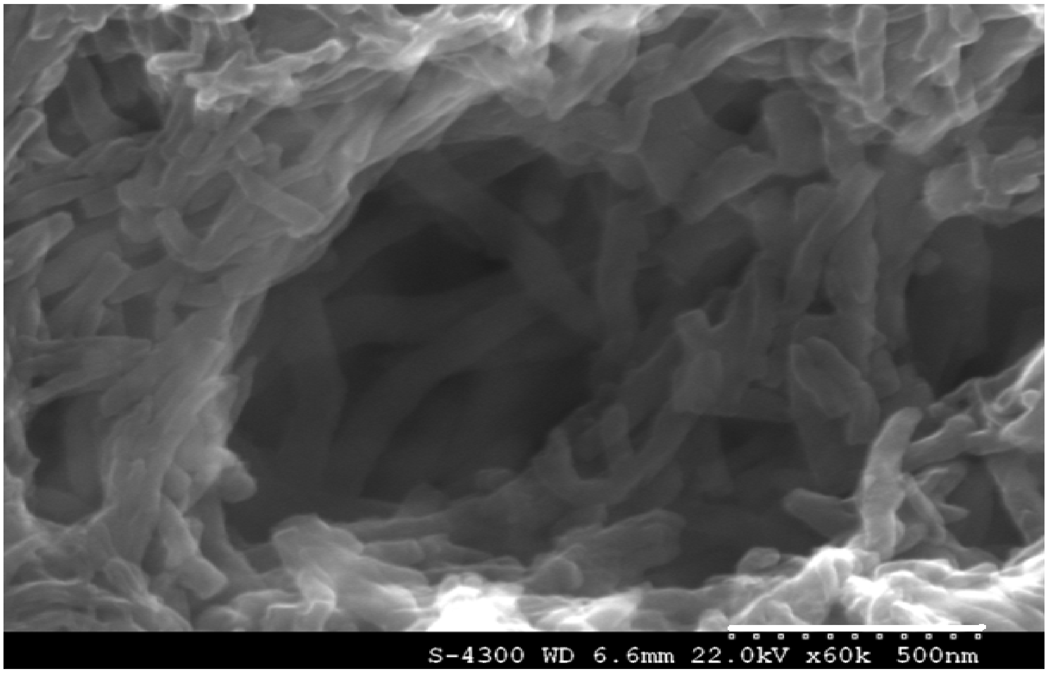


Figure 3.13: (b)

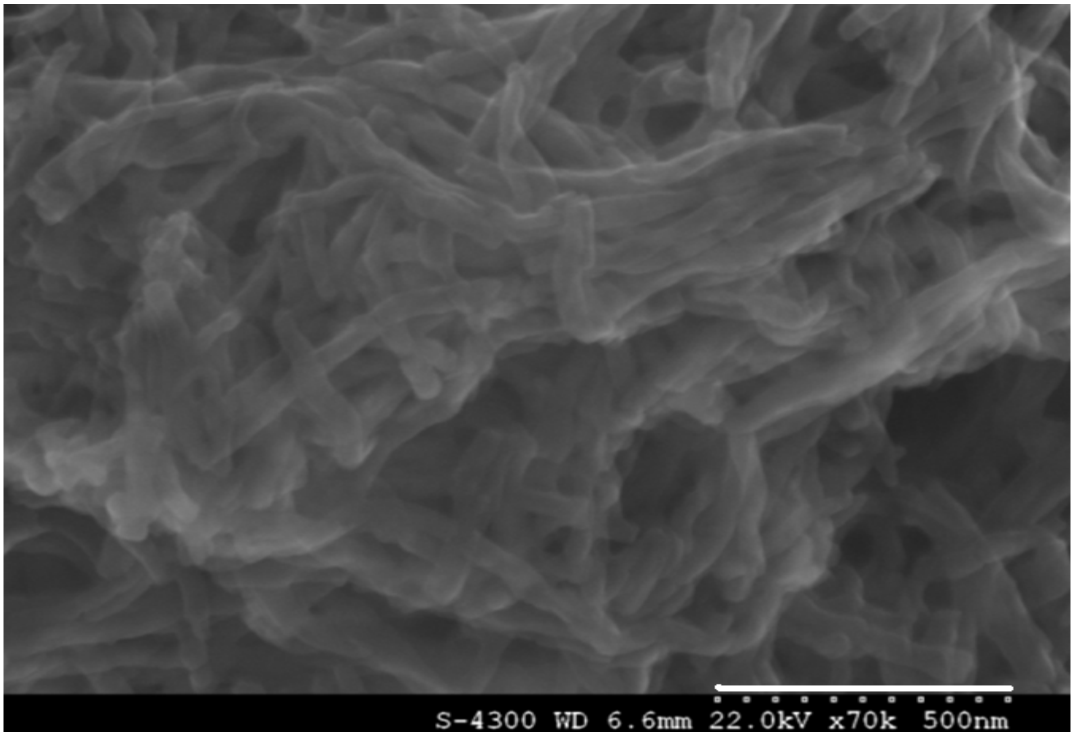


Figure 3.13: (c)

Figure 3.13: (a) FE-SEM images of polyaniline (PANI) and (b &c) p-MWNT-PANI composite

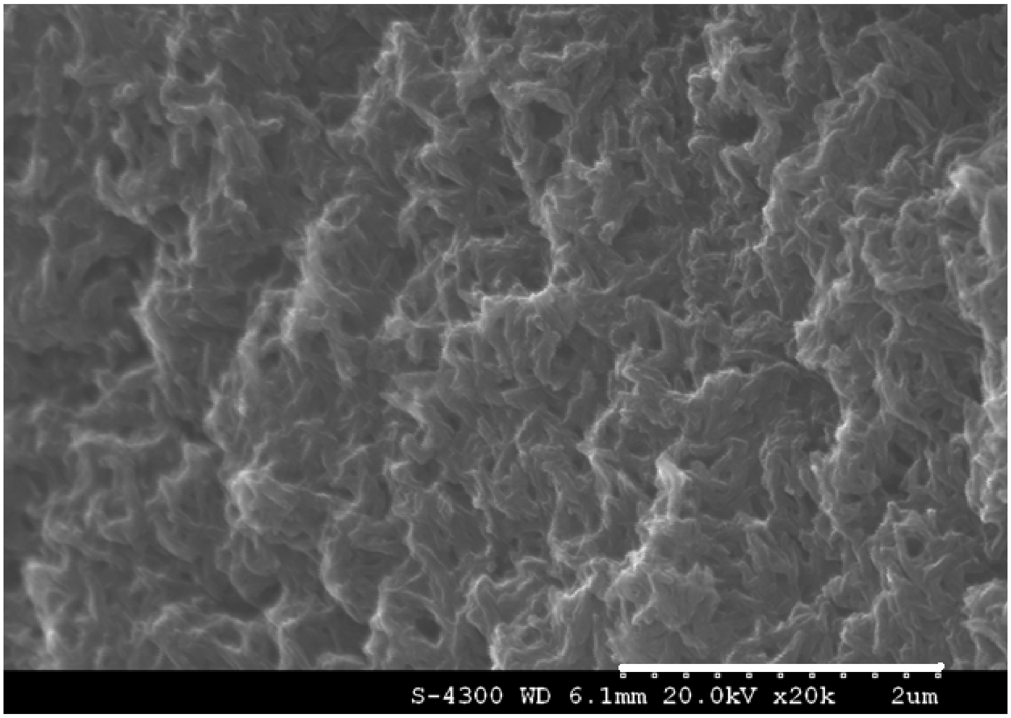


Figure 3.13: (d)

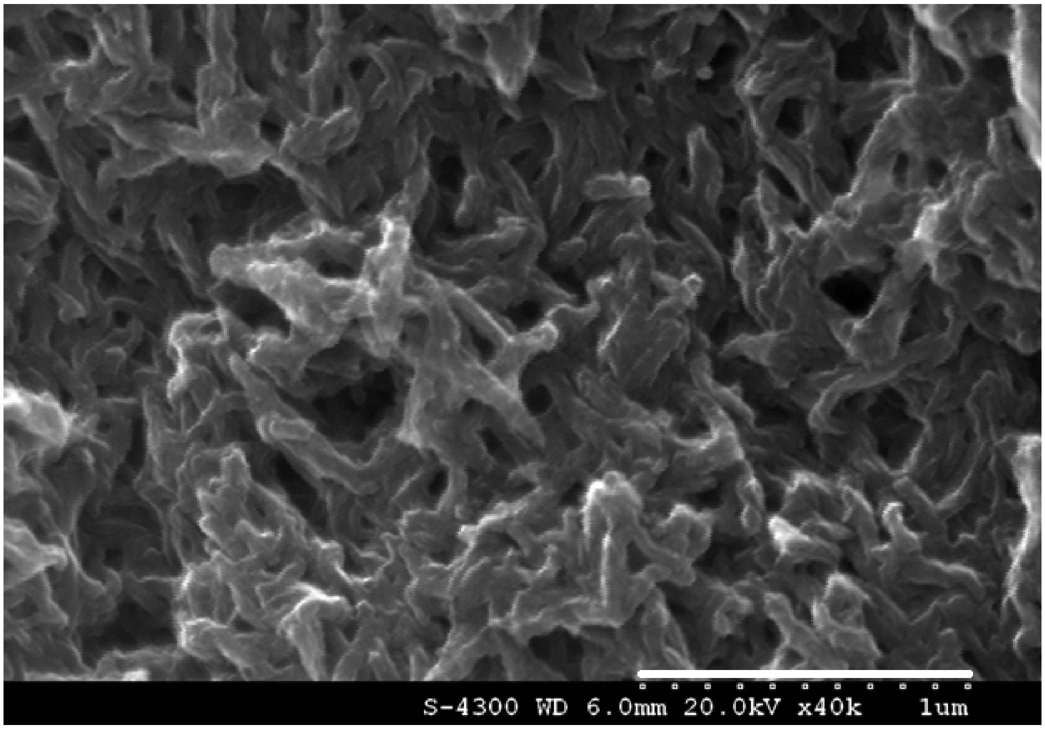


Figure 3.13: (e)

Figure 3.13: (d &e) FE-SEM images of c-MWNT-PANI composite

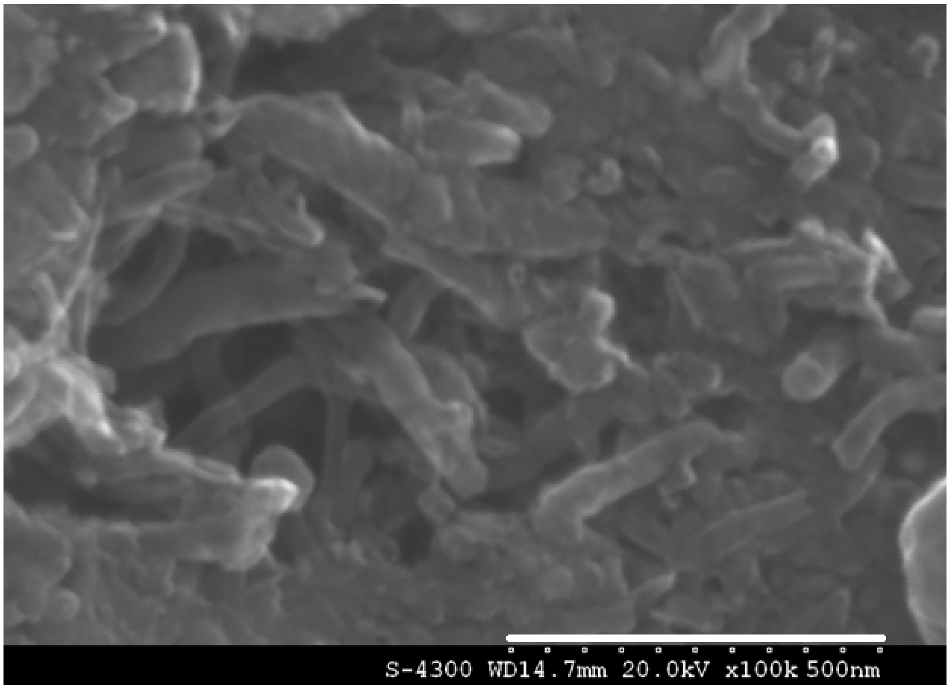


Figure 3.13: (f)

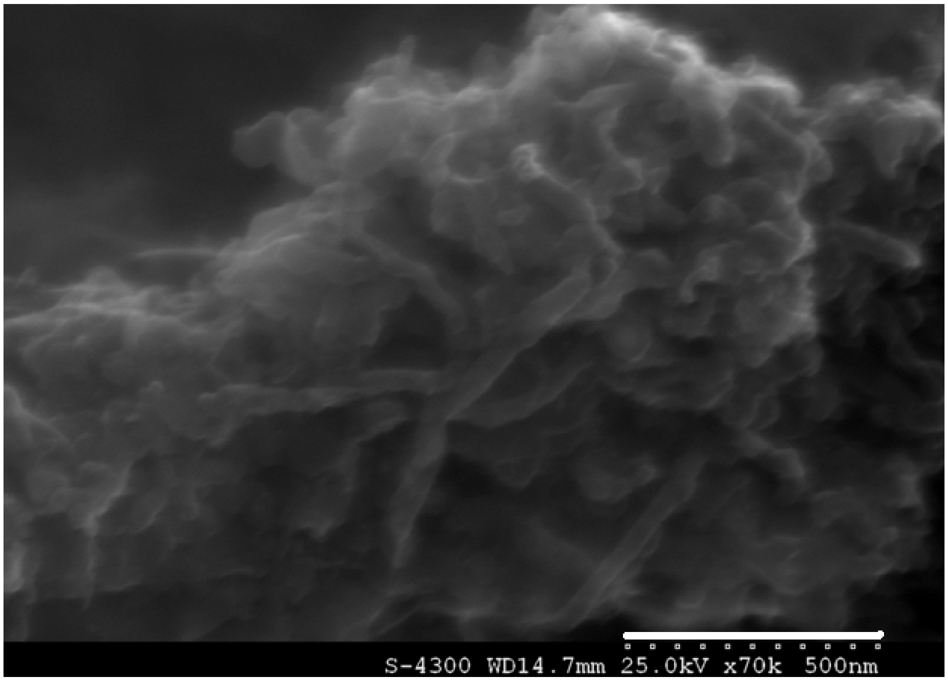


Figure 3.13: (g)

Figure 3.13: (f &g) FE-SEM images of GOD-c-MWNT-PANI composite

In the Figure 3.13 (f &g), it was observed that the glucose oxidase (GOD) attached at the c-MWNT-PANI composite.

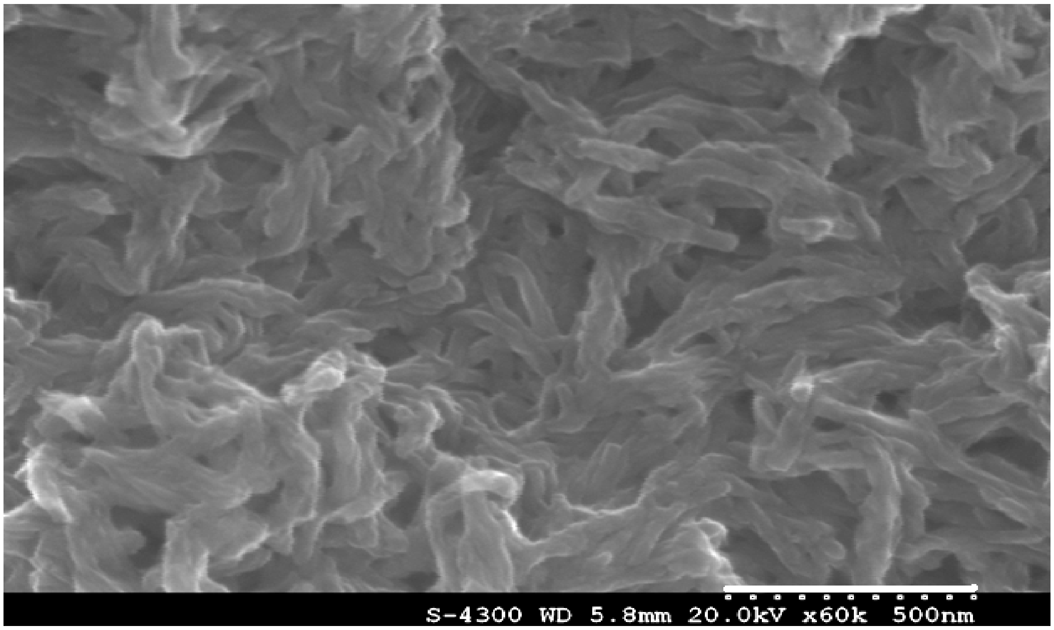


Figure 3.14: (a)

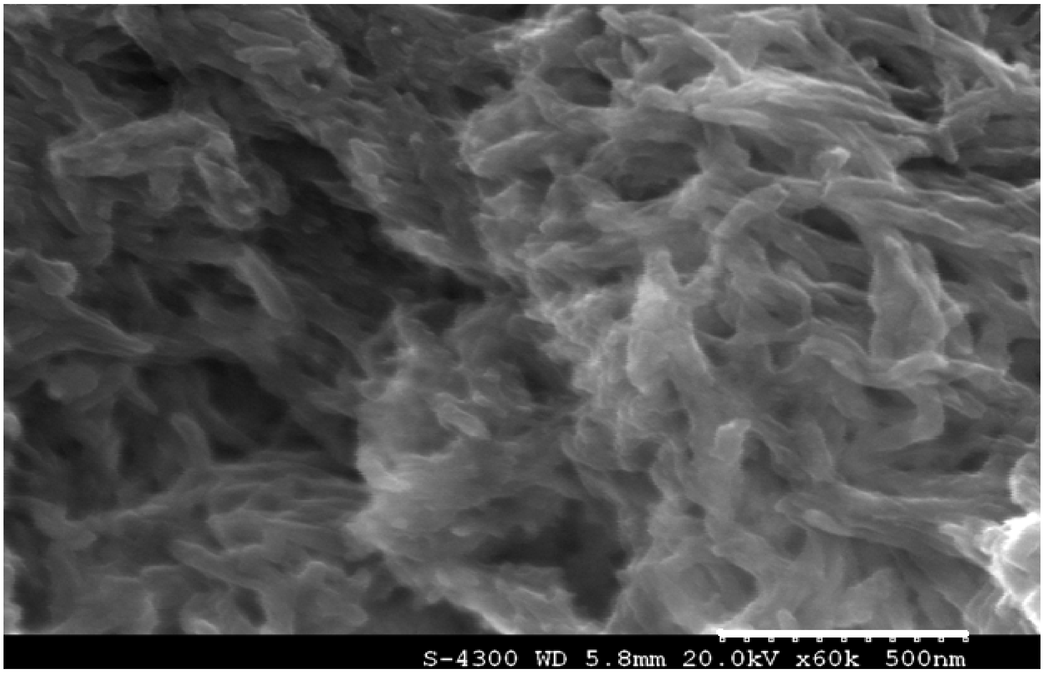


Figure 3.14: (b)

Figure 3.14: (a &b) FE-SEM images of a-MWNT-PANI composites

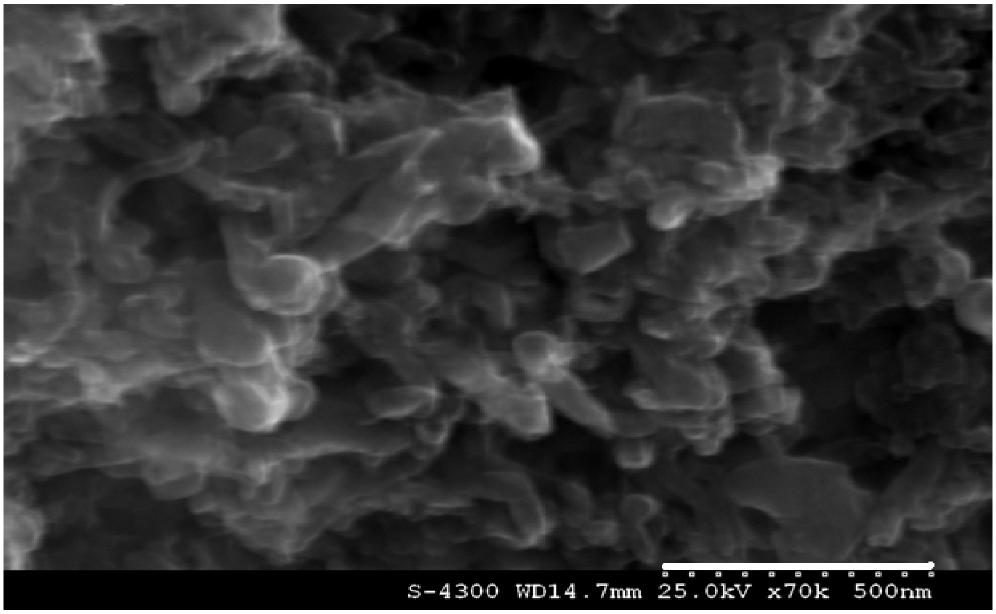


Figure 3.14: (c)

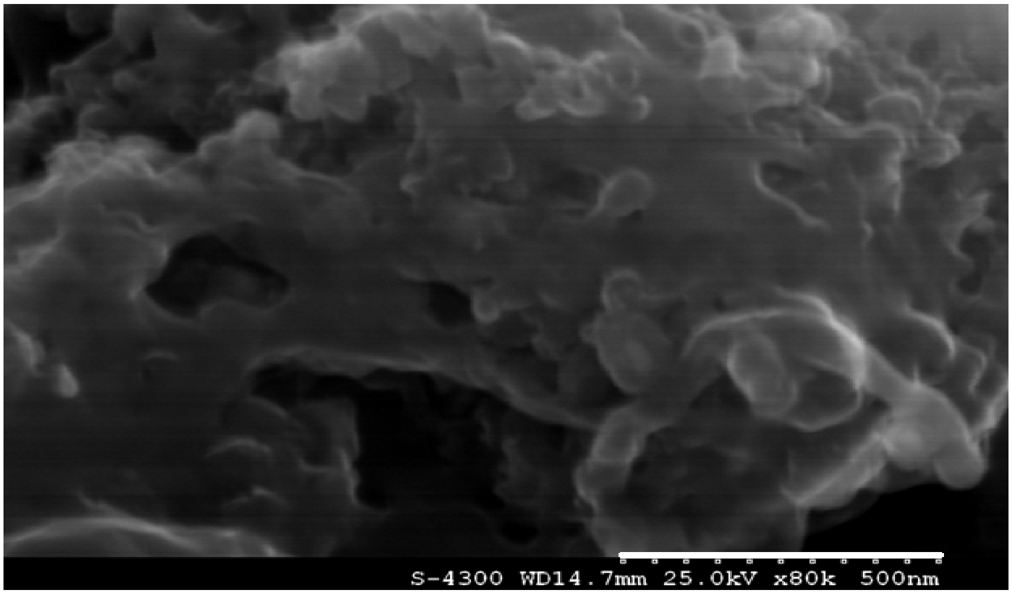


Figure 3.14: (d)

Figure 3.14: (b) FE-SEM images of GOD-a-MWNT-PANI composite

In the Fig 3.14 (C & d), it was clearly observed that the glucose oxidase (GOD) attached at a-MWNT-PANI composite.

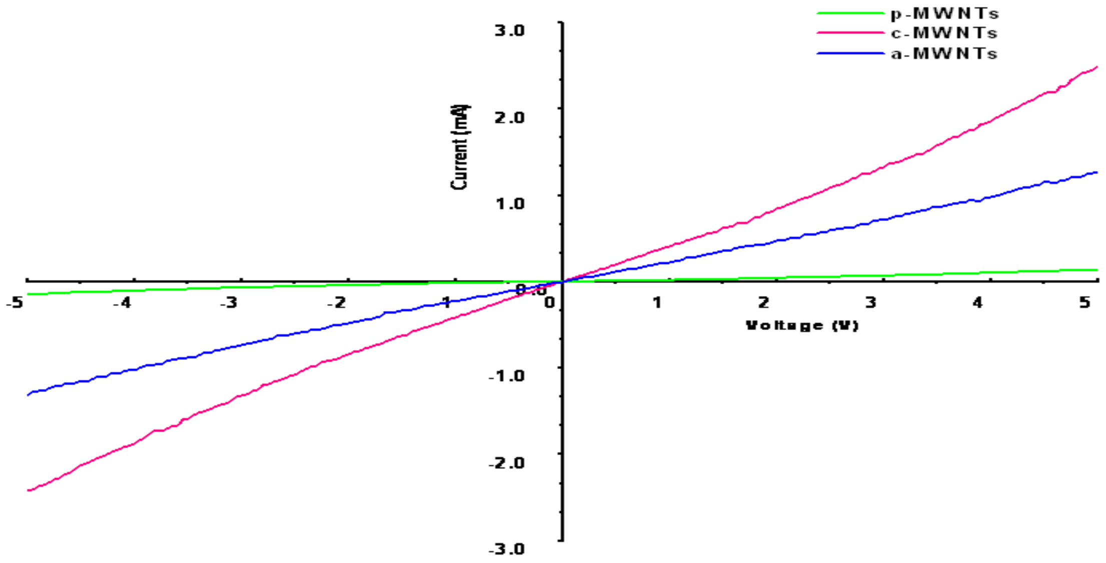
3.4 Current Voltage (I-V) Characteristics Measurements

The effect of functionalization and GOD immobilization on the I-V characteristics of MWNTs, MWNT-PANI composites is a feature of this work. The I-V characteristics of functionalized and GOD immobilized MWNTs and MWNT-PANI composites are shown in figure below.

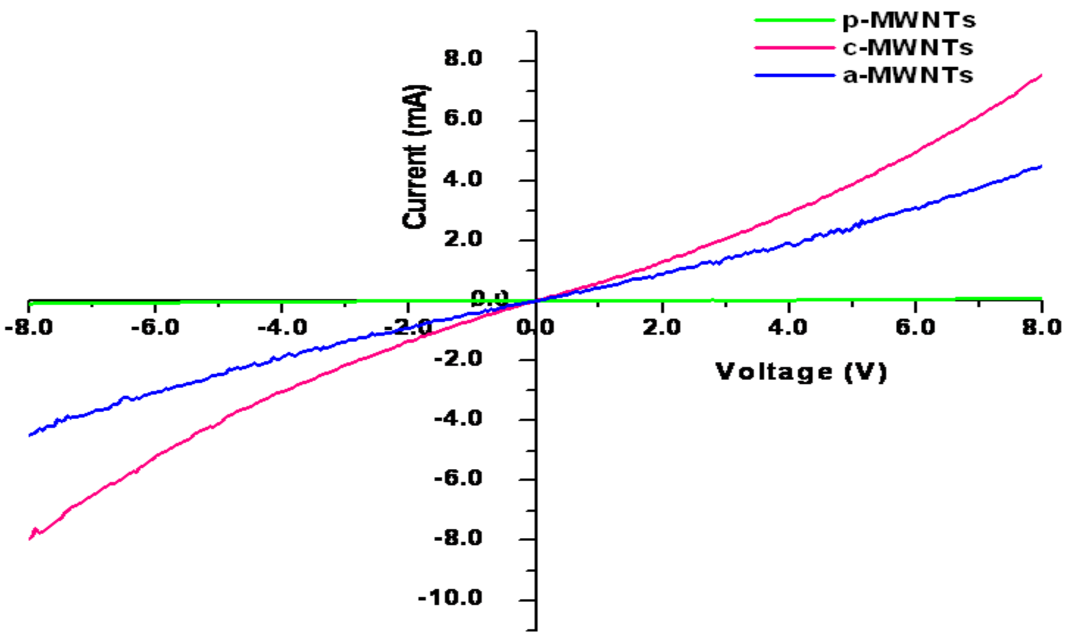
3.4. 1 Study of I-V Characteristic of MWNT Films

I-V characteristics of any material are very important for the construction of any sensor. Here our aim was to know which types of MWNTs are better electrically conducting. The I-V characteristic of the MWNTs films was performed by changing various parameters such as probe spacing, voltage range and voltage step but keeping the film thickness constant. It was found that the change in voltage step size does not contributed significantly, so it was also kept constant.

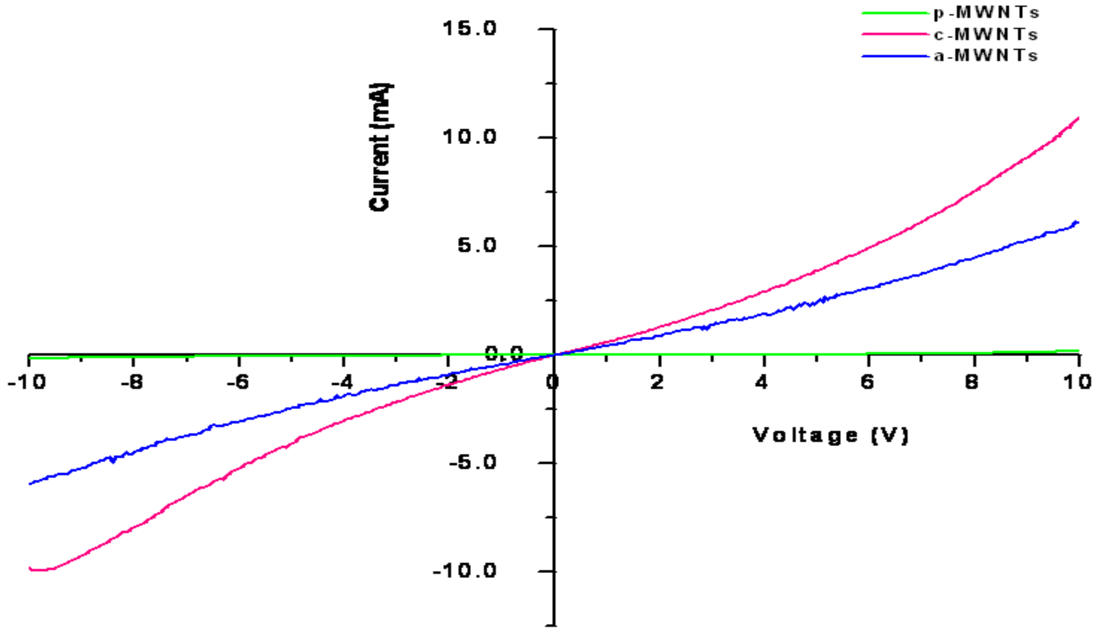
Figure 5.15 shows the I-V characteristic of the MWNTs films was performed by introducing the films between the electrodes followed by a linear sweep of the voltage between -5 to +5, -8 to +8 and -10 to +10 Volts at probe spacing of 200 micrometer and 300 micrometer at voltage step of 0.05V. I-V curves were generated on the dry films at ambient conditions.



3.15 (a)



3.15(b)



3.15 (c)

Fig-5.15: I-V curves of p-MWNTs, c-MWNTs and a-MWNTs at probe spacing of 200 micrometers for voltage range: (a) -5 to +5 V (b) -8 to +8 V (c) -10 to+10 V

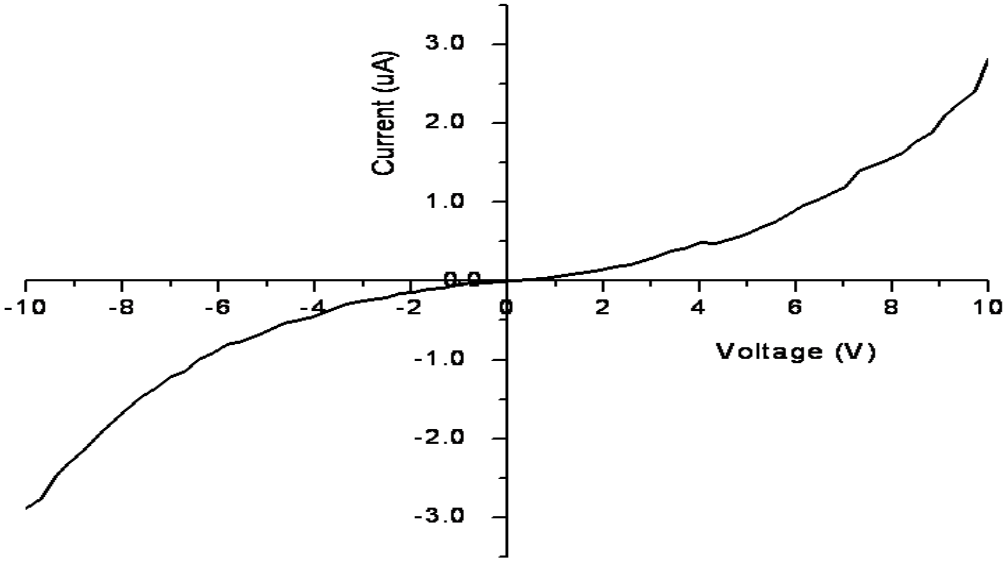
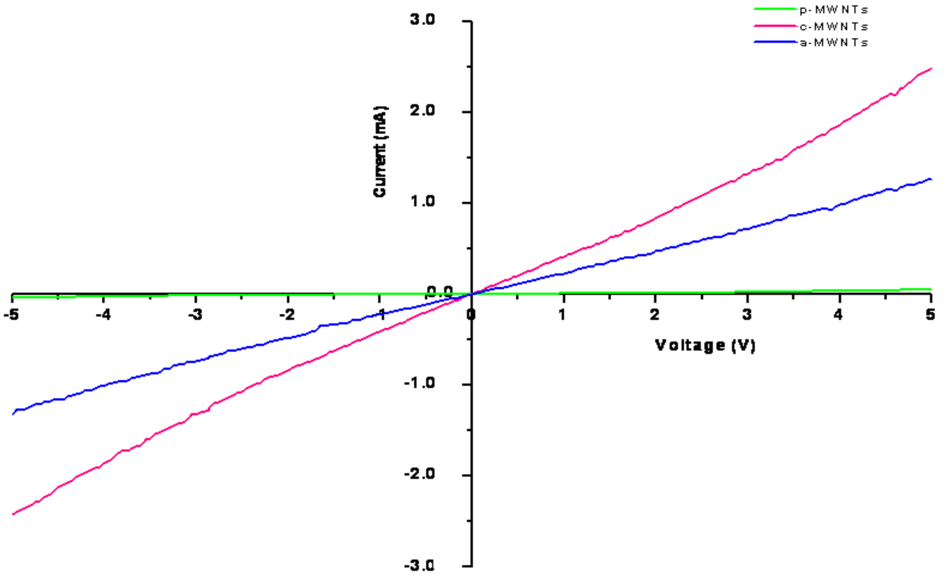
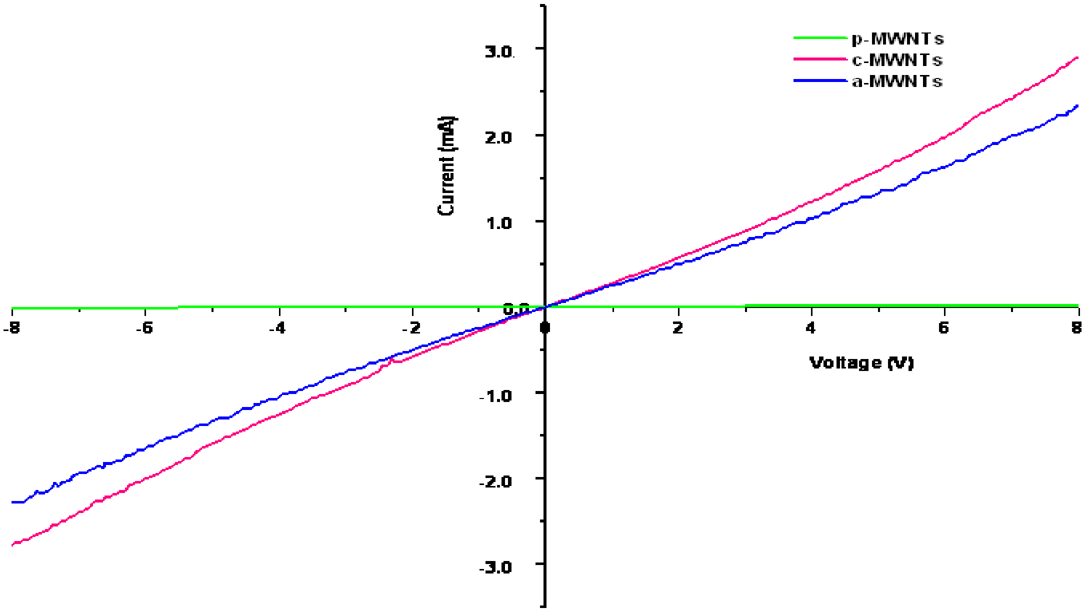


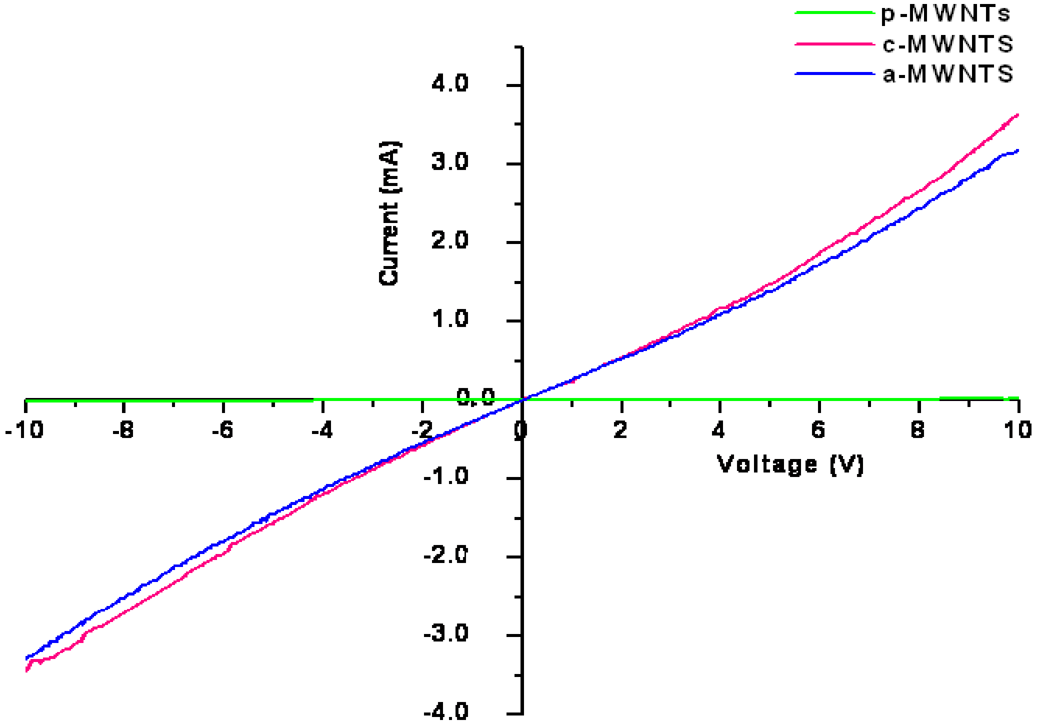
Fig-5.16: I-V curves of c-MWNTs (18 h) at probe spacing of 200 micrometers for voltage range: -10 to+10 V



3.17 (a)



3.17 (b)



3.17 (C)

Fig-3.17: I-V curves of p-MWNTs, c-MWNTs and a-MWNTs at probe spacing of 300 micrometers for voltage range: (a) -5 to +5 V (b) -8 to +8 V (c) -10 to+10 V

From the above graphs it was observed that the enhancement in the I-V characteristics of functionalized MWNTs. The presence of increased concentration of functional groups is one of the main reasons for the increase of conductance value for the functionalized MWCNTs. It has been shown that the new groups increase the number of bands near the Fermi level, promoting electron transfer between the carbon atoms.

The relationship between the amount of oxygenated functional groups and electrical conductivity was reported by **Cher Hon Lau** [3] he observed that electrical conductivity increases as oxygenated group's increases but the acid refluxed MWNTs due to structural damage shows decrease in conductivity.

Here we observed similar that at the higher the sonication time (18h) the conductance of MWNT film decreases as shown in Fig.3.18 (d).

The electron transfer is dependent on the density of states in the electron density near the Fermi energy level (E_F) in the metallic and semiconducting nanotubes. The functionalization of CNTs increases the chemical groups at the CNTs surfaces, these chemical groups adds new energy levels and increasing the density of states and decreasing the bang gap of CNTs. After the functionalization CNTs were more conducting than that of pristine one. According to Ohm's law, the current passing through a conductor is directly proportional to the voltage applied on the two ends of the conductor, and inversely proportional to the resistance of the conductor

$$V=RI$$

Since the voltage applied on the CNT film was changing all the time during I-V measurement, if R is constant, the current will change according to the voltage. If we use ΔI to represent the change of current and ΔV to represent the change of voltage, then the conductance is given by

$$G = \frac{}{} \; \Omega^{-1}$$

This is called differential conductance (G) and the unit is Ω^{-1} or Siemens (S). The conductance in ballistic transport reported by some researchers

G= 4e3/h = 155µs, 1/G= 6.5kΩ

and the mean free path 300 to 700 nm in semiconducting CNTs.

The conductance of different CNTs will be different depending on the electronic structure and diameter, since band gap changes with CNT diameter as E_g = 0.9/d (nm). In the electronic study of CNT-films a large no of CNTs comes together, so in the bulk electronic study the overall conductance of the film comes in the picture. The observed voltage vs. conductance plots of unfunctionalized, functionalized and GOD attached MWNTs are shown below in the Fig. 18 and 20.

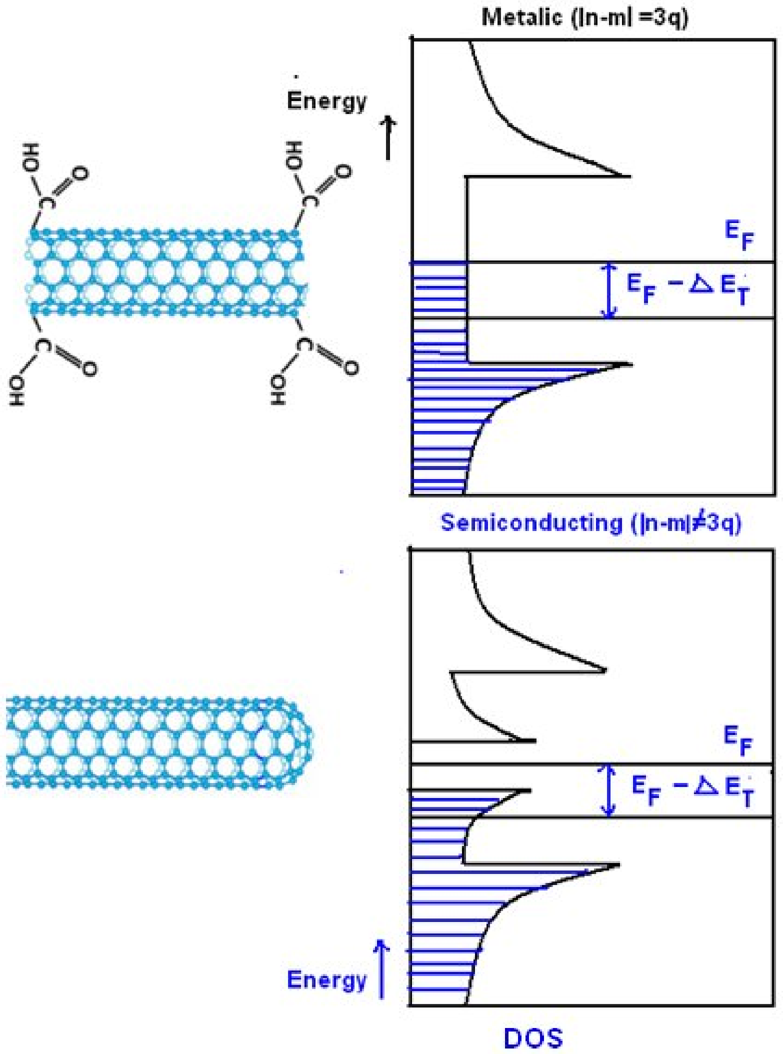
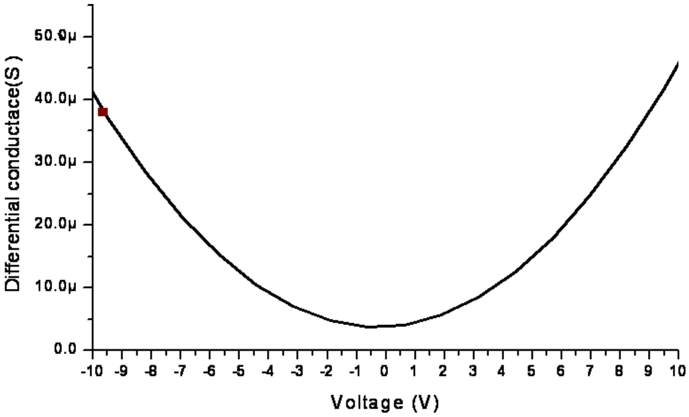
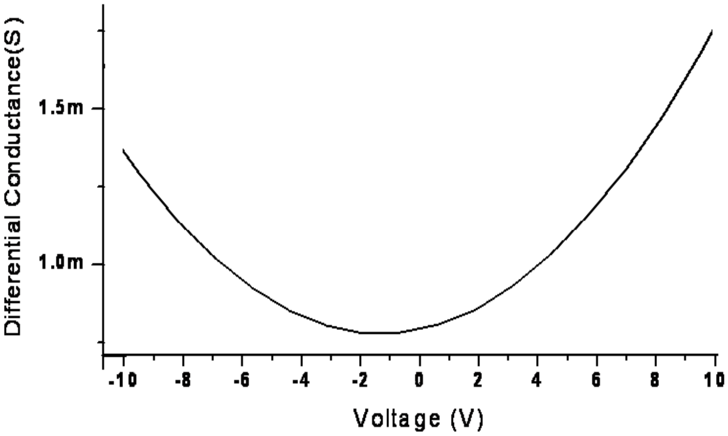


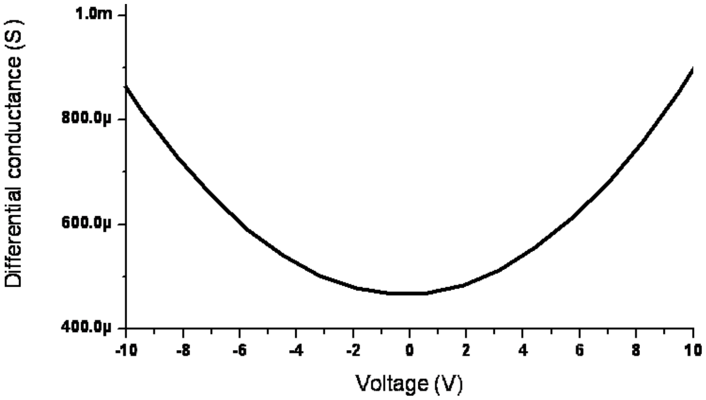
Figure-17: (d) Schematic diagram for unfunctionalized and unfunctionalized CNTs shows the change in electronic structure (DOS) with energy.



3.18(a)



3.18(b)



3.18(c)

Figure **-3.18: Voltage vs. differental coductance plots: (a) p-MWNTs, (b) c-MWNTs, (c) a-MWNTs,**

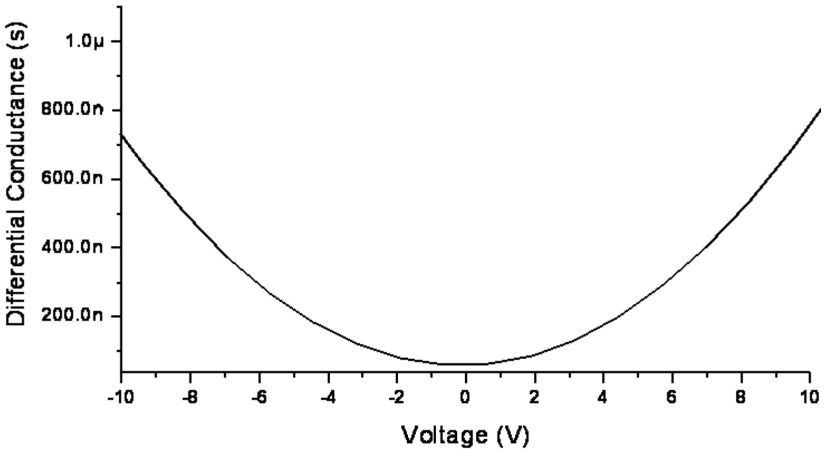
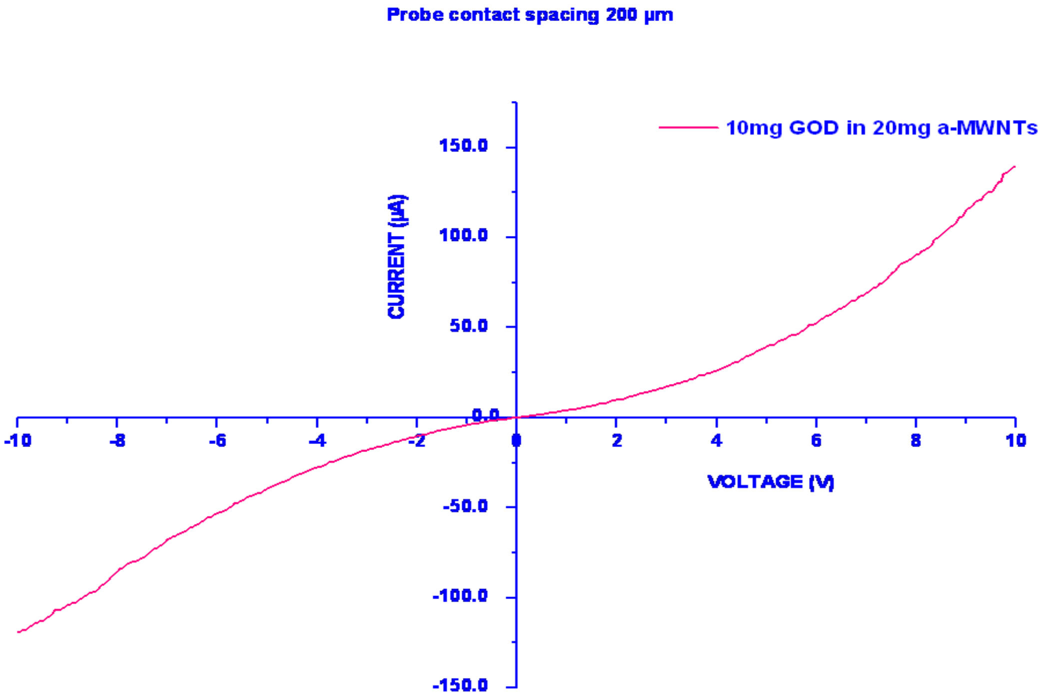


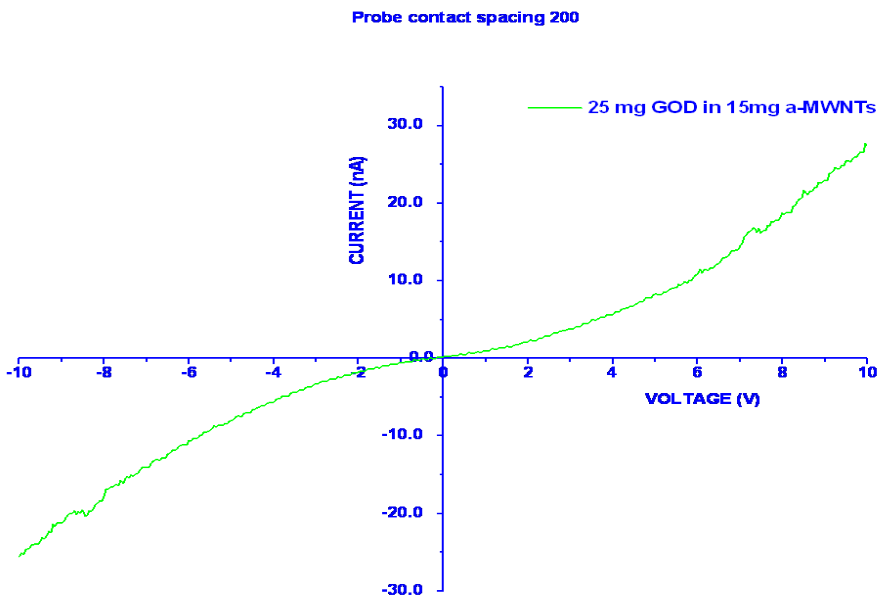
Figure 3.18: (d) Voltage vs. differental coductance plots for c-MWNTs sonicated for 18h

3.4 .2 Study of I-V Characteristic of Glucose oxidase Attached MWNTs Films

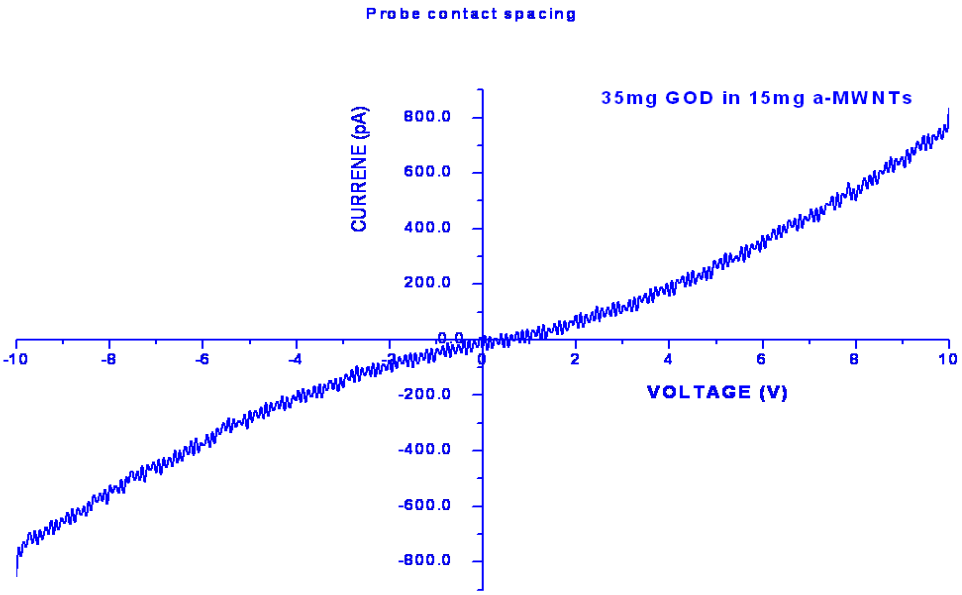
The I-V characteristics of the GOD-MWNTs films was performed by introducing the films between the electrodes followed by a linear sweep of the voltage between -10 to +10 Volts at probe spacing of 200 micrometer at voltage step of 0.05V. I-V curves were generated on the dry films at ambient conditions.



3.19 (a)



3.19 (b)



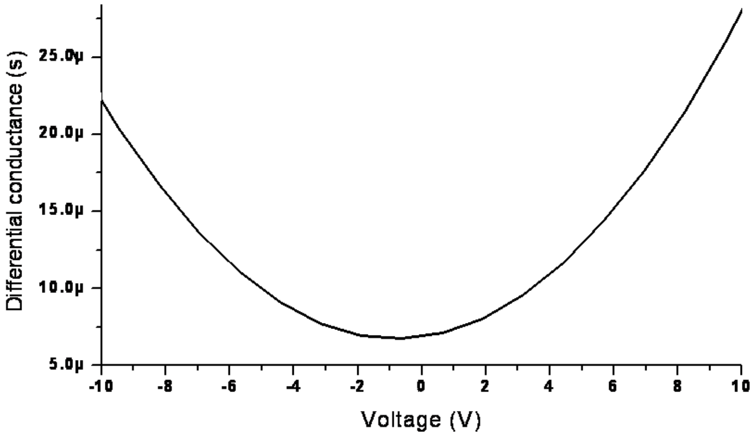
3.19 (c)

Fig-3.19: I-V curves of GOD-a-MWNT composite films at probe spacing of 200 micrometers for different concentration of GOD in the 15mg a-MWNT reaction mixture : (a) 10mg (b) 25mg (c) 35mg

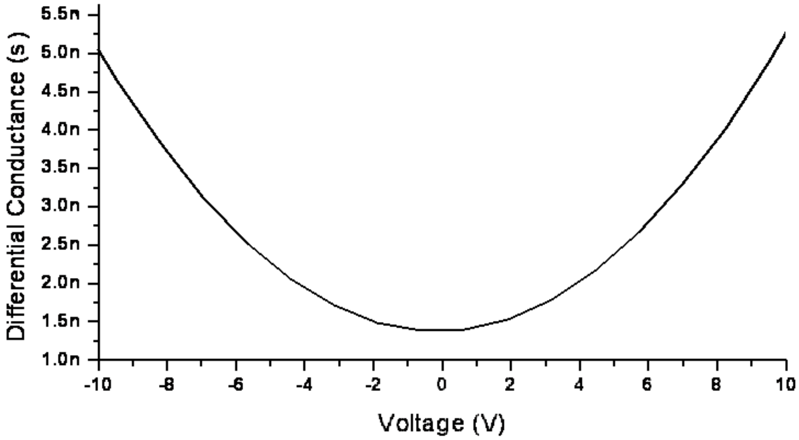
From the above graphs it was observed that the reduction in the I-V characteristics of GOD-a-MWNTs. The presence and increased concentration of GOD is one of the main reasons for the decrease of conductance value of the GOD-a- MWCNTs films. Attachment of the redox enzyme glucose oxidase (GOD) to the nanotube sidewall is achieved through a linking molecule and is found to induce a clear change of the conductance [4].

Immobilization of the enzyme GOD decreases the conductance, which can be attributed to a change in the total capacitance of the tube rather than a simple electrostatic interpretation [4].

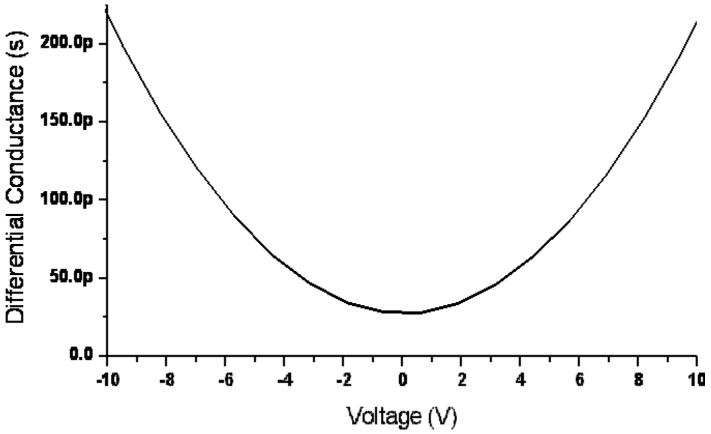
The graph between voltage vs. differential conductance are shown in Fig.3.20 for GOD-a-MWNT composite.



3.20 (a)



3.20 (b)



3.20 (c)

Fig- 3.20: Voltage vs. differential conductance plot of GOD-a-MWNT composite (a) 10mg GOD in 15 mg a-MWNTs, (b) 25mg GOD in 15 mg a-MWNTs, (c) 35mg GOD in 15 mg a-MWNTs,

3.4.3 Study of I-V Characteristic of various MWNT-PANI Composite Films

The I-V characteristic of the PANI and MWNT-PANI composite films was performed by introducing the films between the electrodes followed by a linear sweep of the voltage between -10 to +10 Volts at probe spacing of 300 micrometer at voltage step of 0.05V. The I-V curves were generated on the dry films at ambient conditions.

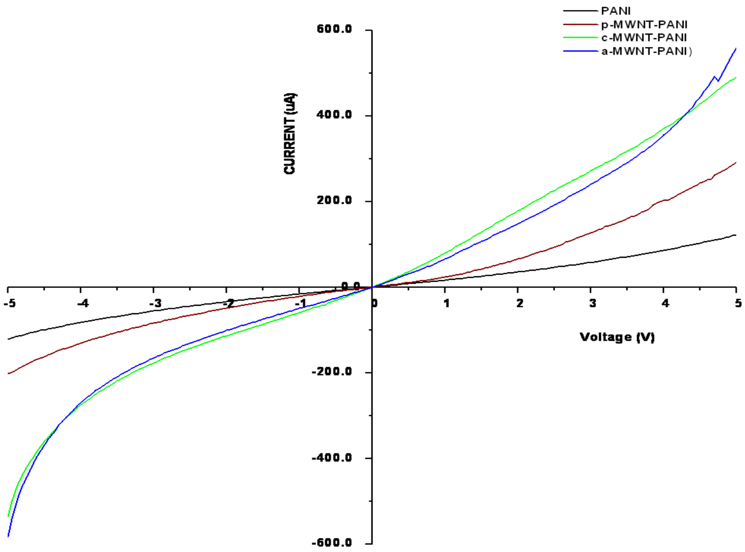


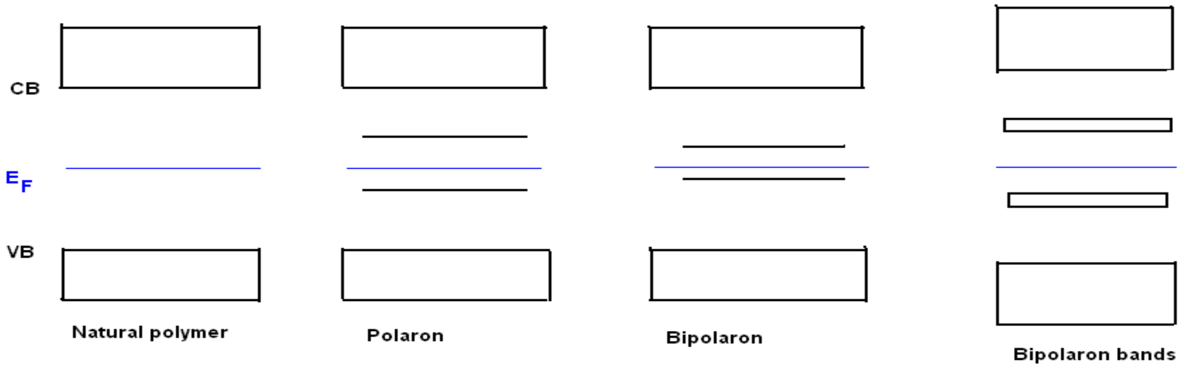
Fig-3.21: I-V curves of PANI, MWNT-PANI c-MWNT-PANI and a-MWNT-PANI at probe spacing of 200 micrometers for voltage range: -5.0 to +5.0 V

From the above graph it was observed that the enhanced electronic properties of MWNT-PANI composites. It was reported that in composites effective site-selective interactions between the quinoid ring of the PANI and the MWNTs facilitated charge transfer process between the two components. In such composites, CNT improves the polymer properties by:

- Inducing additional structural ordering of the polymer
- Improve the compactness and conjugation or chain length
- Higher delocalization of charges in the composites
- Thermal stability
- Charge carrier mobility

Due to all these properties the composites shows a better electrical properties than PANI. Among the composites the electrical properties of c-MWNT-PANI and a-MWNT-PANI composites was higher than MWNT-PANI composite. This is due to covalently linkage between the CNT and polymer serves as a ‘conducting bridge’ between PANI and CNTs.

One early explanation of conducting polymers used band theory as a method of conduction. This said that a half filled valence band would be formed from a continuous delocalized p - system. This would be an ideal condition for conduction of electricity. However, it turns out that the polymer can more efficiently lower its energy by bond alteration (alternating short and long bonds), which, introduces a band width of 1.5 ev making it a high energy gap semiconductor. The polymer is transformed into a conductor by doping it with either an electron donator or an electron acceptor.



Although solitons and bipolarons are known to be the main source of charge carriers, the precise mechanism is not yet fully understood. The problem lies in attempting to trace the path of the charge carriers through the polymer. All of these polymers are highly disordered, containing a mixture of crystalline and amorphous regions.The main mechanism used is by movement of charge carriers between localized sites or between soliton, polaron or bipolaron states. Alternatively, where inhomogeneous doping produces metallic island dispersed in an insulating matrix, conduction is by movement of charge carriers between highly conducting domains. Charge transfer between these conducting domains also occurs by thermally activated hopping or tunnelling.

3.4.4 I-V Characteristics of GOD-c-MWNT-PANI and GOD-a-MWNT-PANI Films

The I-V characteristics of the GOD-MWNTs films was performed by introducing the films between the electrodes followed by a linear sweep of the voltage between -10 to +10 Volts atprobe spacing of 200 micrometer at voltage step of 0.05V

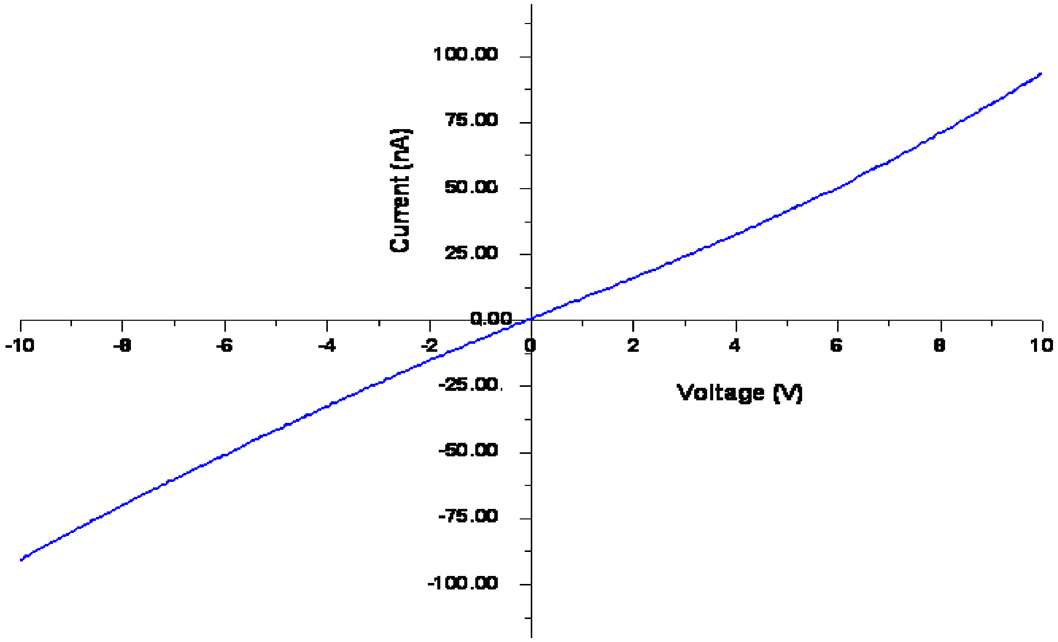


Fig. 3.22(a)

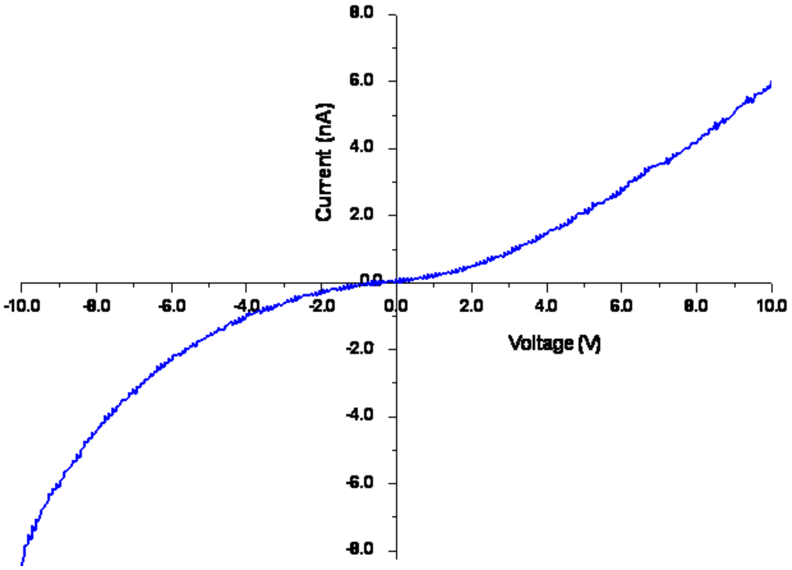


Fig. 3.22(b)

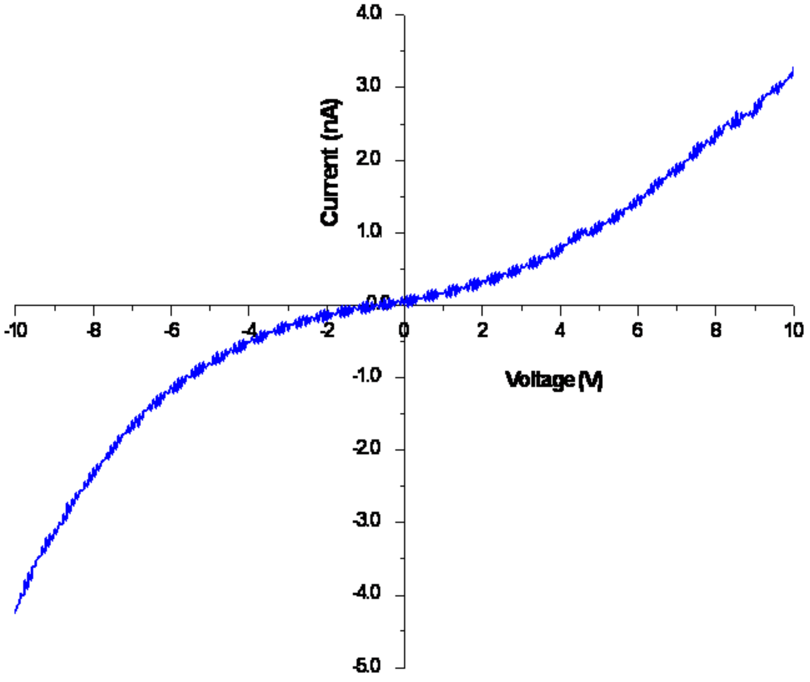
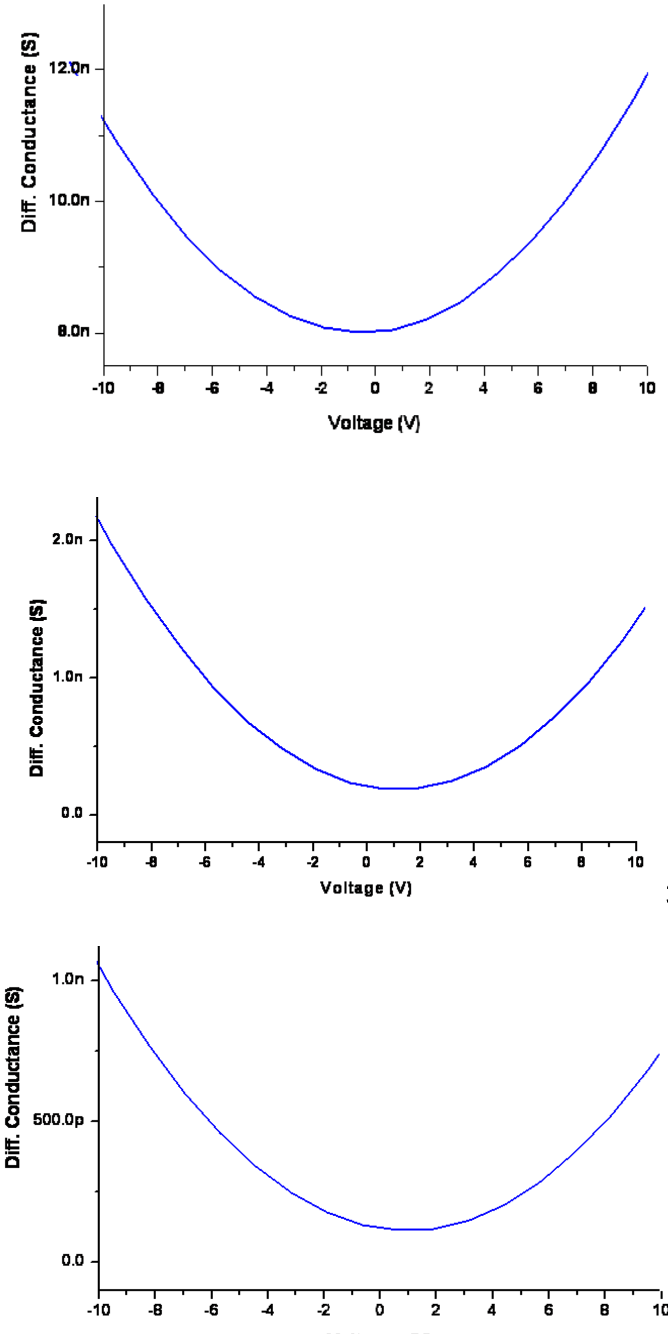


Fig. 3.22(c)

Fig.3.22: **I-V curves of GOD-c-MWNT-PANI composite films at probe spacing of 200 micrometers for different concentration of GOD in the 15mg a-MWNT reaction mixture : (a) 10mg (b) 25mg (c) 35mg**

The graph between voltage vs. differential conductance are shown in Fig.3.23 for GOD-c-MWNT-PANI composite.



3.23 (a)

3.23 (b)

3.23 (c)

Fig- 3.23: Voltage vs. differential conductance plot of GOD-c-MWNT-PANI composite (a) 10mg GOD in 15 mg a-MWNTs, (b) 25mg GOD in 15 mg a-MWNTs, (c) 35mg GOD in 15 mg a-MWNTs,

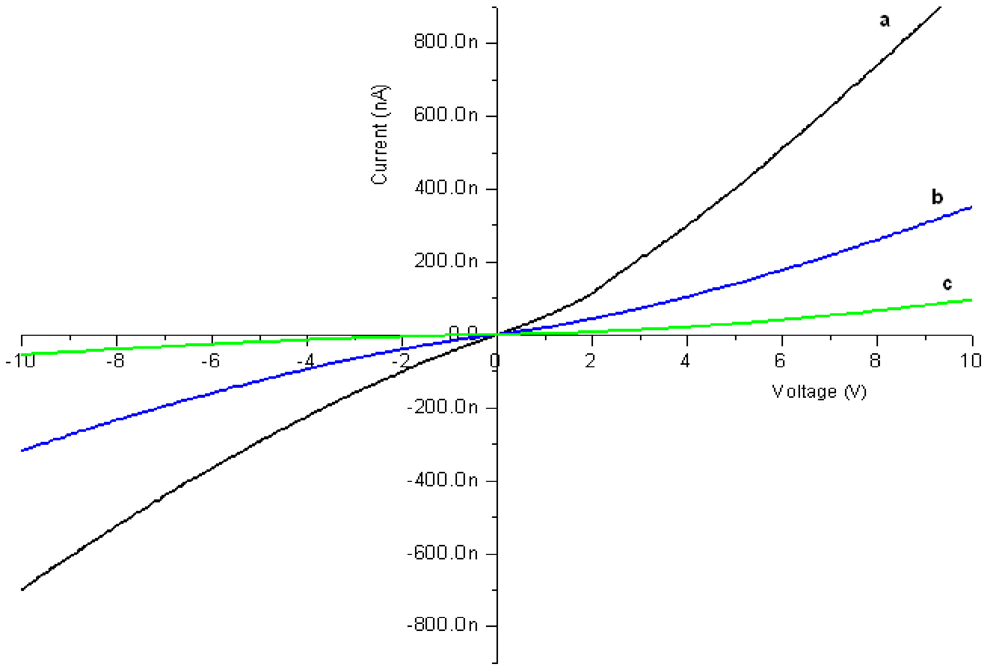


Fig. 3.24

Fig-3.24: **I-V curves of GOD-a-MWNT-PANI composite films at probe spacing of 200 micrometers for different concentration of GOD in the 15mg a-MWNT reaction mixture : (a) 10mg (b) 25mg (c) 35mg**

The graph between voltage vs. differential conductance are shown in Fig.3.25 for GOD-a-MWNT-PANI composite.

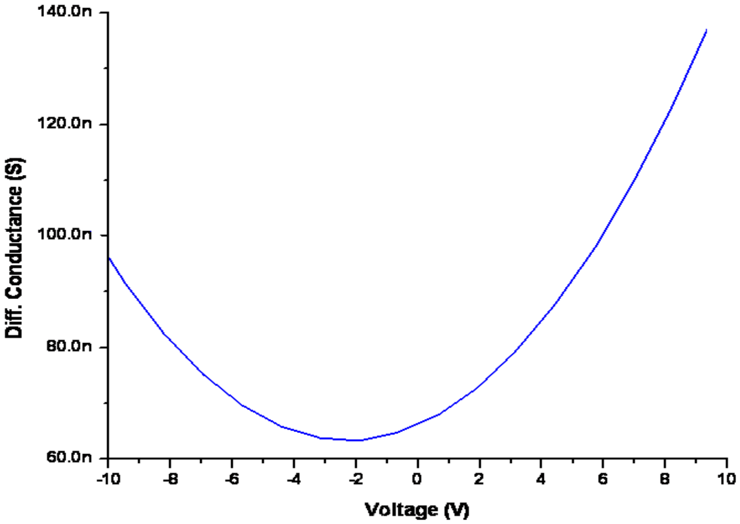


Fig- 3.25(a)

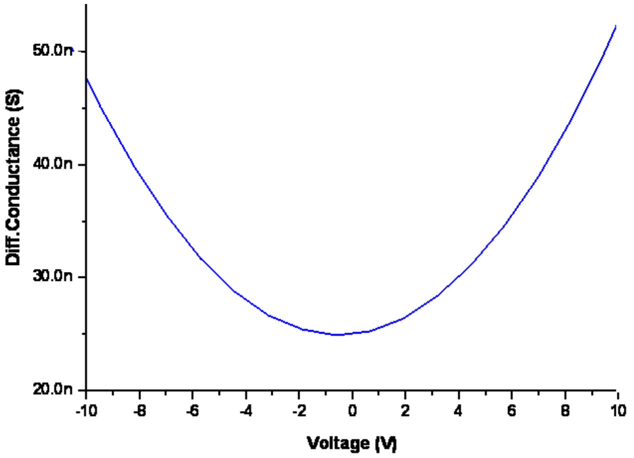


Fig- 3.25(b)

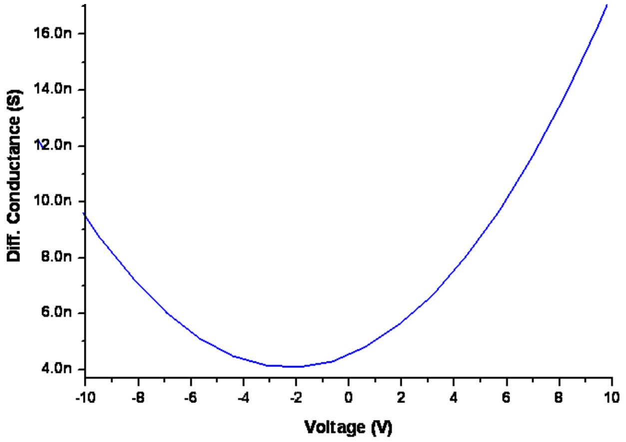


Fig- 3.25(c)

Fig- 3.25: Voltage vs. differential conductance plot of GOD-a-MWNT-PANI composite (a) 10mg GOD in 15 mg a-MWNTs, (b) 25mg GOD in 15 mg a-MWNTs, (c) 35mg GOD in 15 mg a-MWNTs

From these curve it is clear that as GOD concentration increase the conductance of composite film decreases. Electrical conduction in organic materials may occur through movement of either electrons or ions.

The conductivity, σ, is equal to the product of the carrier mobility, μ, its charge, q, and the number of carrier or the concentration, n.

$$\sigma = \mu q n$$

Conducting polymers are usually polyconjugated structures which are insulators in the pure state but when treated with an oxidizing or a reducing agent can be converted into polymer salts with electrical conductivities comparable to metals. *p*- type doping: removal of electrons from the valence band by the oxidizing agent, leaving the polymer with a positive charge. *n*- type doping: donation of an electron to the empty conduction band by a reducing agent.

In the case of CNT-PANI composites, the polyaniline connected to functionalized CNTs covalently by amide bond formation (-CONH). Charge transfer complex formation between aniline and the graphitic carbon nanostructure surface, and subsequent functionalized MWNTs. Due to formation of charge transfer complex between MWCNTs and polyaniline increases the conductance of MWNT-PANI composites. Here CNTs acts as a filler material as concentration or amount of CNTs in the composites increases the conductance also increases that is large no of charge transfer complex in the composite. When GOD is attached to the composite material it was observed that the conductance decreases due to insulating nature of enzyme. As the enzyme loading increases conductance decreases shown in the Fig. 3.23 and 3.25 for GOD-c-MWNT-PANI and GOD-a-MWNT-PANI respectively.

3.5 Sensor Response of GOD-MWNTs Electrodes

The typical configuration is a biosensor consisting of the GOD immobilized MWNT film on the glass plate the electrodes are placed on the film by a silver contact. The glucose solution dropped on the film between the two electrodes as shown in figure 3.26. The biosensor response can be taken by voltage sweep mode and real- time measurement at constant voltage.

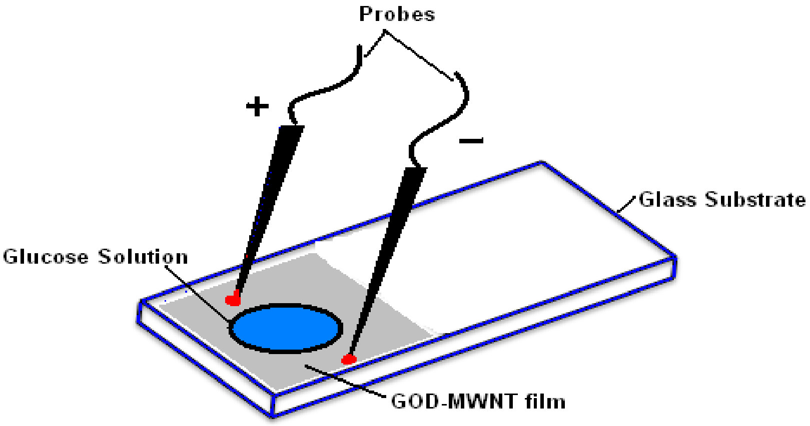


Fig-5.26: Schematic Configuration of Biosensor

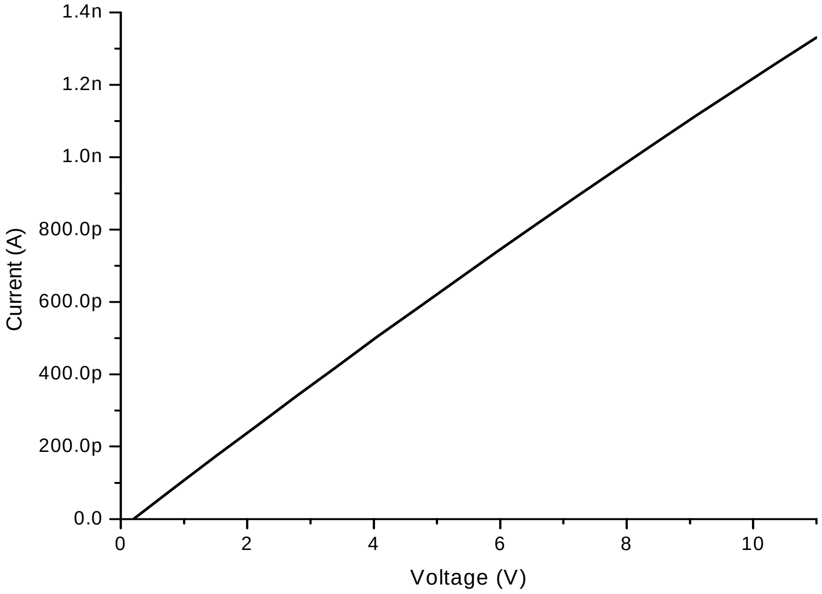


Fig.3.27: I-V curve of GOD-MWNT electrode at 4.5 mm probe spacing

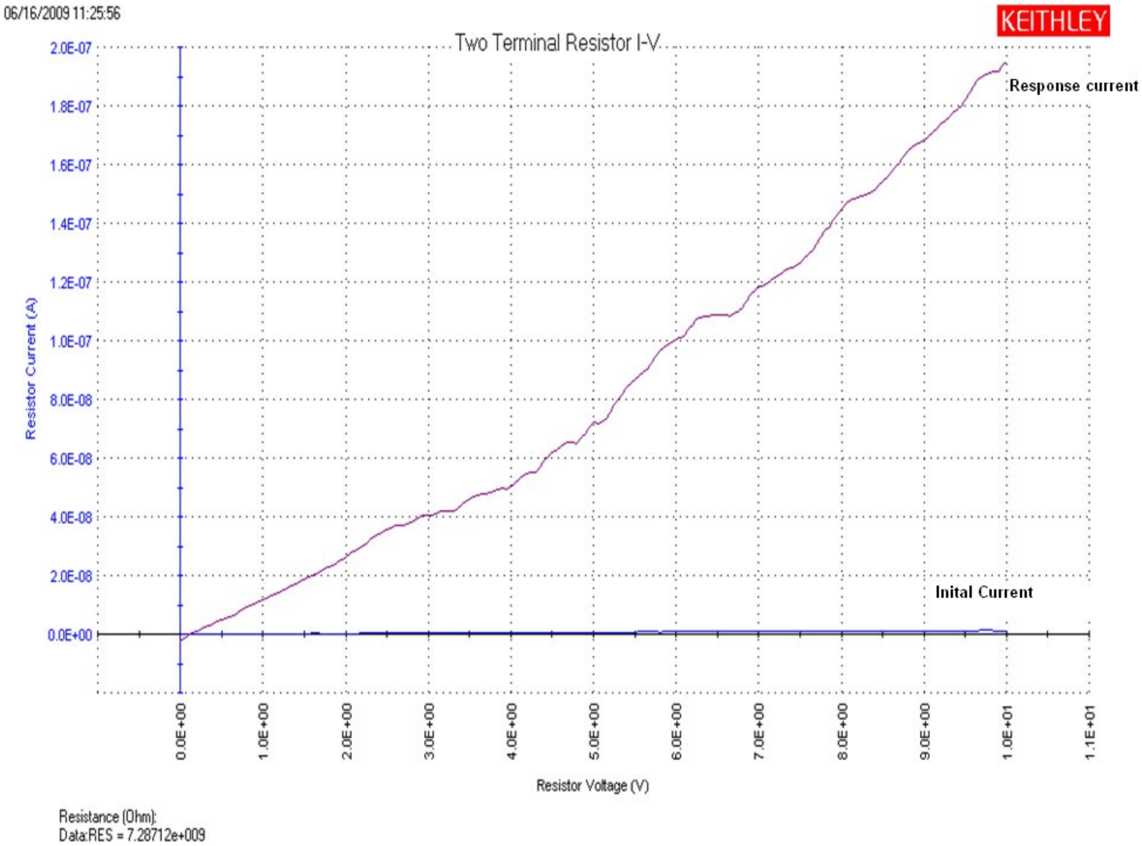


Fig.3.28: – Amperometric response biosensor electrode spacing was 4.5mm.

Amperometric response of biosensor was obtained at electrode spacing of 4.5 mm and 10 µl glucose solution of concentration 5mg/ml was dropped between the electrodes. I-V curve was generated before and after dropping the glucose solution. After dropping the glucose solution the current increases about 140 times from initial value, as shown in the Fig. 3.28.

The result shows that GOD immobilized on the MWNTs is active. Therefore GOD-MWNTs composite can be used for detection of glucose.

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CHAPTER-4

CONCLUSION AND FUTURE SCOPE

4.1 Conclusions

From the study of this thesis work we found the following conclusions are as follows:

1. Multiwalled carbon nanotubes were successfully functionalized with acids. The FT-IR results confirmed the functionalization. The functionalization resulted in uniform dispersion of the tube in solvents. The FE-SEM images confirmed the dispersion of f-MWNTs. The large sonication times reduces the length of CNTs and damage the CNT structure.

2. The glucose oxidase was successfully immobilized on the a-MWNTs and MWNT-PANI composites. The FT-IR and FE-SEM results confirmed the immobilization of GOD.

3. The polyaniline and MWNT-PANI composites were successfully synthesized. The FT-IR and FE-SEM results confirmed the formation of polyaniline and MWNT-PANI composites.

4. The activity of enzyme was successfully measured by Continuous Spectrophotometric method. The increase in absorption (A_{500}) with incubation time indicated that the enzyme is active.

5. The electrical properties of MWNTs, MWNT-PANI composites and its enzyme conjugates were studied. It was found that the functionalized MWNTs was more conducting than pristine CNTs. The increase in the conductance due to the presence of functional groups at the end and side walls of the f-MWNTs. These chemical groups act as a charge carrier mediator between CNT and electrodes.

   The conductance of GOD-MWNT and GOD-MWNT-PANI composites were decreases compared to the without enzyme attachment. The decrease in conductance can be attributed to a change in the total capacitance of the tube because GOD is insulating by nature.

The electrical properties of composites were better than the polyaniline. The electrical properties of f-MWNT-PANI were better than the MWNT-PANI composites because the covalently linkage between the CNT and polymer serves as a 'conducting bridge' between PANI and CNTs.

6. The sensor responses of GOD-MWNT electrode were studied. In the initial study a very little success was found. The amperometric signals generated by sensor chip were not reproducible.

4.2 Future scope

The results of the studies presented in this thesis further suggest some area of scientific and technological interest for the construction of biosensors and electronic devices.

Throughout the experiment, controlling the thickness and size of the films are rather challenging. However, producing films with uniform size and thickness are essential, as it will affect the available enzyme activity and permeability of the membrane.

In this work, the functionalization of MWNT, Immobilization of enzyme on a-MWNTs, MWNT-PANI composites, enzyme activity, and I-V characteristic were studied. But, in order to get a more comprehensive study of the immobilized glucose oxidase, it should include several other tests such as interference test, reproducibility test and optimization of the biosensor.

Reproducibility test should be conducted to determine if the enzymatic membranes are able to respond to the varying glucose concentration in an acceptable time and with reproducible output response.

APPENDIX A

Materials used

- ***Carbon nanotubes (CNTs) -*** During these work multiwall carbon nanotubes (MWNTs) of length 0.5 to 2µm and average outer diameter 8 nm, were used. The purity level was 95%.
- ***Glucose oxidases (GOD) -*** glucose oxidase have been provided by US Biologicals. The biological source of it is fungus *Aspegillus niger* and Its type is X-5.
- ***Filters*** – The filters were used poly tetra floro-ethylene filter (PTFE) hydrophilic and hydrophobic membrane with pore size 0.22µm.

Chemicals used

- ***Sulfuric acid (H_2SO_4) -*** Purchased from Qualigens of product no. 29307, with purity 99%.
- ***Nitric acid (HNO_3) -*** Purchased from LOBA CHEMIE with product no. 7697- 37 -2, in liquid form with purity 69 – 72%.
- ***Hydrochloric acid (HCl) -*** Purchased from LOBA CHEMIE of product no.7647- 01- 0 in liquid form.
- ***Acetone (CH_3COCH_3) –*** Purchased from Merck of product no. A0160 in liquid firm of purity 99%.
- ***Ethanol -*** Purchased from Les Alcols De Commerse Ind in liquid firm of purity 99.9%.
- ***Tetrahydrofuran (C_4H_8O) -*** Purchased from Spectrochem in liquid form of purity 99.5%.
- ***Peroxidase -*** Purchased from Himedia in powder form.
- ***Dimethylformamide ($HCON(CH_3)_2$) -*** Purchased from LOBA CHEMIE purity 99.5%.
- ***O-Dianisidine dihydrochloride dye -*** Purchased from Sigma in powder.
- ***Sodium Chloride (NaCl) -*** Purchased from Himedia in powder form with 99.9% purity.
- ***Sodium phosphate dibase heptahydrate ($Na_2HPO_4.7H_2O$) -*** Purchased from LOBA CHEMIE in powder form of purity 99.5%.
- ***Potassium Chloride (KCl) -*** Purchased from LOBA CHEMIE in powder form with purity 98%.
- ***Potassium dihydrogen orthophosphate -*** Purchased from Himedia in powder form with 99.9% purity.

- ***Sodium hydroxide pellets-*** Purchased from LOBA CHEMIE
- ***Thionyl Chloride ($SOCl_2$) -*** Sd fine chem. In liquid form.
- ***Sodium Acetate (CH_3COONa) -*** Purchased from LOBA CHEMIE.
- ***Demonized water***
- ***PBS Buffer solution (0.1M, pH 6.8)-*** 400 mg NaCl, 10 mg KCl, 72 mg disodium hydrogen phosphate and 12 mg potassium dihydrogen phosphate was added in 500 water. It was stirred vigorously on a magnetic stirrer. Its pH was adjusted to 6.8 with few drops of 1M HCl or 1M NaOH solution. It was sterilized by autoclaving aat 15 lbs/in^2 at 120^{0}C for 15 minutes on laboratory autoclave apparatus (METREX). The buffer was stored at 4^{0}C.
- (A). 50 mM Sodium Acetate Buffer, pH 5.1 at 35°C (Prepared 200 ml in deionized water using Sodium Acetate. Adjusted to pH 5.1 at 35°C with 1 M HCl.)
- (B) 0.21 mM o-Dianisidine Solution (Dissolved the contents of one 50 mg vial of o-Dianisidine Dihydrochloride, in 7.6 ml of deionized water. Diluted 1.0 ml to 100 ml with Reagent A.)
- **(C)** 10% (w/v) ß-D(+)Glucose Substrate Solution (Prepare 10 ml in deionized water using ß-D(+)Glucose,
- (D) 0.17 mM o-Dianisidine and 1.72% (w/v) Glucose Solution(Reaction Cocktail) (Immediately before use, prepared 29 ml by combining 24.0 ml of Reagent B with 5.0 ml of Reagent C. Equilibrated to 35°C and adjusted to pH 5.1 if necessary with 1 M HCl or 1 M NaOH. **PREPARE FRESH.**)
- (E) Peroxidase Enzyme Solution (POD)(Immediately before use, prepare a solution containing 60 Purpurogallin units/ml of Peroxidase, in cold deionized water.)
- (F) Glucose Oxidase Enzyme Solution prepared an initial solution of 20 - 40 units/ml in cold Reagent A. Then immediately prior to use, further dilute to 0.4 - 0.8 unit in cold Reagent A.

Apparatus Used

- ***H***igh strength sonicator
- Hot air oven
- Microbalance
- Magnetic stirrer with hot plate
- Vacuum pump

- Autoclave
- Filtration setup
- Vacuum desiccator
- Eppendroph
- Beaker (100 ml, 50 ml, 25 ml)
- Conical Flask (100 ml, 50 ml, 25 ml)
- Refluxing setup
- pH meter – deluxe (model 101E)
- ITO plates (1×0.5 cm^2), glass plates (1×1cm^2)
- Petri diss
- Measuring cylinder (100 ml, 50 ml, 10 ml)
- Test tubes

APPENDIX B

MATERIAL CHARACTERIZATION INSTRUMENTS

In material characterization basically we have tried to characterize the synthesized material by variety of techniques to assure that the appropriate materials with suitable properties are synthesized. Some of the characterization techniques used for the characterization of materials is described below.

Spectroscopic Characterization

The spectroscopic characterization involves IR and UV-VIS characterization for the study of chemical groups and structure of molecules. The FTIR spectroscopy is described below.

Fourier Transform Infrared (FTIR) spectroscopy

Infrared (IR) spectroscopy is a useful technique for characterizing materials and providing information on the molecular structure, dynamics, and environment of a compound. When irradiated with infrared light (photons), a sample can transmit, scatter, or absorb the incident radiation. Absorbed infrared radiation usually excites molecules into higher energy vibrational states. This can occur when the energy (frequency) of the light matches the energy difference between two vibrational states (or the frequency of the corresponding molecular vibration).

Infrared spectroscopy is particularly useful for determining functional groups present in a molecule. Many functional groups vibrate at nearly the same frequencies independent of their molecular environment. This makes infrared spectroscopy useful in materials characterization. Further, many subtle structural details can be gleaned from frequency shifts and intensity changes arising from the coupling of vibrations of different chemical bonds and functional groups.

Recent advances in computerized IR spectroscopy, particularly Fourier transform infrared (FT-IR) spectroscopy; have made it possible to obtain infrared spectra using various sampling techniques. Infrared spectra have traditionally been produced by transmission that is, transmitting light through the sample, measuring the light intensity at the detector, and comparing it to the intensity obtained with no sample in the beam, all as a function of the infrared wavelength. Techniques such as attenuated total reflectance, diffuse reflectance, specular reflectance, reflection-absorption, and photoacoustic spectroscopy have recently become more common. This article will discuss the sampling techniques, applications, and the molecular structure information the resulting infrared spectra can provide.

The sample is dispersed in a KBr matrix and pressed into a transparent pellet. Approximately 0.4 to 1.0 mg of sample is usually ground in 200 to 400 mg of KBr or other infrared-transparent pressing powder. The sample and KBr must be ground so that the particle size is less than the wavelength of light, minimizing band distortion due to scattering effects.

The IR spectra were recorded between the wavenumber rang of 500 to 4000cm^{-1} with using a Perkin Elmer spectrum RXI FTIR system.

1.2 UV/Visible Spectroscopy

UV/Visible Spectroscopy is used for fundamental studies of the electronic structure of atomic and molecular species. Ultraviolet/visible absorption spectroscopy is almost exclusively the spectroscopy of molecules dissolved in solvents. The spectral region of interest extends from 200 to 800 nm. Ultraviolet/visible spectroscopy is also used for enzyme activity measurement.

For the activity measurement Perkin Elmer Lambda - 35 UV – Visible spectrometer was used. The time scan was taken at 500 nm for 20 min. incubation at a constant temperature of 37 ^{0}C.

1.3Field Emission Scanning Electron Microscopy (FESEM)

The first SEM used to study a solid surface was described by Zworykin et al (1942) working for the RCA laboratories in the United States. As a practice in the early days the gun was located in the bottom so the specimen chamber and was high enough for the operator but the specimen might fall down the column. A resolution of 50 nm was achieved with this microscope.

The first micrographs showing the striking three-dimensional imaging capability were obtained in Cambridge at the Engineering Department in 1952 by Dennis McMullan who was continuing the work by Ken Sander (both under C. W. Oatley supervision). The next important step was also in Cambridge when Oatley improved the secondary electron detector by adding a scintillator to convert electrons to photons, and let the way for improvement in signal to noise ratio.

Nowdays, three-dimensional features can be observed due to the large Depth of Field available in the FESEM. The addition of energy dispersive X-ray detector combined with digital image processing is a powerful tool in the study of materials, allowing good chemical analysis of the material. The FESEM is a major tool in materials science research and development.

A FESEM is used to visualize very small topographic details on the surface or entire or fractioned objects. Researchers in biology, chemistry and physics apply this technique to observe structures that may be as small as 1 nanometer (= billion of a millimeter). The FESEM may be employed for example to study organelles and DNA material in cells, synthetically polymers, and coatings on microchips.

Electrons are liberated from a field emission source and accelerated in a high electrical field gradient. Within the high vacuum column these so-called primary electrons are focused and deflected by electronic lenses to produce a narrow scan beam that bombards the object. As a result secondary electrons are emitted from each spot on the object. The angle and velocity of these secondary electrons relates to the surface structure of the object. A detector catches the secondary electrons and produces an electronic signal. This signal is amplified and transformed to a video scan-image that can be seen on a monitor or to a digital image that can be saved and processed further.

Figure- 1 FE-SEM Model HITACHI S/N 4300

The experiments of all samples were performed on the Al stub. The powder of MWMTs and MWNT-PANI composites was sticked by conducting carbon tape. The images were taken at different magnifications.

I-V Measurement

Conductance of samples was measured with KEITHLEY 4200-SCS system using two probe mechanism.

Figure-2 Two Probe System Keithley Model 4200-SCS Semiconductor Characterization system

A small connect was established between the probe tips & the sample film. Minimal amount of force is applied onto the probes while establishing the contact so that the sharp tips should not damage the film during measurements. The measurements were taken at different probe spacing's and at different voltage ranges.