A Project Report

On

"Single-Walled Carbon nanotube (SWCNT) based chemiresistive/FET biosensor for the detection of Biomarkers"

Submitted in partial fulfillment of the requirement for the award of the degree of

MASTER OF TECHNOLOGY

In

NUCLEAR SCIENCE & ENGINEERING

Submitted by

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(2K14/NSE/17)

Under the Guidance of

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CERTIFICATE

This is to certify that the project entitled "***Single-Walled Carbon nanotube (SWCNT) based chemiresistive/FET biosensor for the detection of Biomarkers***" Completed by Mr. Binay Kumar Prasad, Student of M.Tech, Nuclear Science and Engineering in Applied Physics Department at Delhi Technological University, New Delhi embodies the original work carried out by him under my supervision and guidance. His Work has been found very well for the partial fulfillment of the requirement of the degree of M.Tech.

It is further certified that, the student has developed the project during the period starting from 11th January, 2016 to 30th June, 2016.

This report has not been submitted in part or full in any other University for award of any other degree or diploma.

Mr. Binay Kumar Prasad is student of good moral character. We wish him success in future

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CERTIFICATE

This is to certify that the Project report entitled ***"Single-Walled Carbon nanotube (SWCNT) based chemiresistive/FET biosensor for the detection of Biomarkers"*** *is a bonafide work done by Binay Kumar Prasad bearing Roll No.* **2k14/NSE/17**, *a student of Delhi Technological University, in partial fulfilment of the requirements for the award of Degree in Master of Technology in* "**Nuclear Science & Engineering**". *As per declaration of the student this report has not been submitted in part or full in any other University for award of any other degree or diploma.*

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Candidate Declaration

I hereby declare that the work which is being presented in this thesis entitled ***"Single-Walled Carbon nanotube (SWCNT) based chemiresistive/FET biosensor for the detection of Biomarkers"*** my own work carried out under the guidance of Dr. Nitin K Puri, Assistant Professor, Applied Physics Department, Delhi Technological University, Delhi And Dr. Rajesh, Principal Scientist National Physical Laboratory, New Delhi.

I further declare that the matter embodied in this thesis has not been submitted for the award of any other degree or diploma.

Date: Binay Kumar Prasad

Place: New Delhi 2k14/NSE/17

Dedicated to

My Parents

ACKNOWLEDGEMENT

I sincerely thank my guide Dr. Rajesh, Principal Scientist, Liquid Crystal and SAM Section, National Physical Laboratory, New Delhi for his guidance, constant support and encouragement throughout my project.

It is my great respect and profound gratefulness that I record my indebt and deep felt devotion to my supervisors. Dr. Rajesh, Principal Scientist, Crystal and SAM section, National Physical Laboratory, New Delhi and Dr. Nitin K. Puri, Assistant Professor, Department of Physics, DTU for their precious and encouraging supervision, valuable discussions and inspiring guidance throughout the work. Despite having a busy work schedule, they helped me a lot throughout this project with great interest. Things that I have learnt from them will continue to guide me further in my academic career.

I would like to extend my thanks to Prof. S. C. SHARMA, Head of Department of Physics, DTU for his kind guidance and support during the course for allowing me to carry out my project work in this esteemed research laboratory, NPL, New Delhi.

I would like to thank to Dr. R.K. Kotnala, Chief Scientist, for his constructive suggestions, constant inspiration and timely help. Also I would like to thank to Mr. Vikash sharma, Mr. V.K. Tanwar, Mr. Mangeram for their guidance and help at each and every step of my project. Besides my advisor, I would like to thank the rest of my thesis committee: Shobhita Singal, who rescued me from various red tape crises and reviewed my work on a very short notice and Jitender Kumar for assistance and encouragement. I am grateful for the whole Liquid Crystal and SAM section for providing me all the facilities to complete my work.

I am extremely thankful to Dr. D K Aswal, Director, National Physical Laboratory, New Delhi, for his Kind Permission to carry out the project work at NPL I express my sincere thanks to Dr. Rajeev Chopra, HRD group, NPL, for allowing me to do my M.Tech research Project at NPL.

I heartedly thank to my classmates for their support during my studies.

With deep sense of gratitude, I recorded here my happiness in thanking to my parents with continuous presence and inspirations which guided me all along in developing this project work.

Binay Kumar Prasad
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ABSTRACT

Field Effect Transistor (FET) based biosensor is a very specific and ultrasensitive, label- free chemiresistive biosensor. Here, SiO_2/Si wafer (FET) with gold microelectrode (Source and Drain electrode) was aligned with SWCNT by dielectrophoresis process. It was then modified with 1-Pyrenebutaric acid N-hydroxysuccinimide Ester (PyBt-NHS-Ester) which is a cross- bilinker. It was further passivated with 6-methylcapto-1-hexanol (MCH) to block the non-specific sites. Antibody C - reactive protein (CRP) with the concentration of 100 ng/mL was then drop-cast onto the modified SWNT. There is a covalent immobilization between PyBt-NHS ester and antibody, anti-CRP. Bovine serum albumin (BSA) was then used to block the non-specific sites at the SWCNT channel. The sensing measurement was performed by monitoring the changes in the current versus voltage (I-V) characteristics of the device on antibody-antigen interaction over SWCNTs channel. The sensing performance of the CNT-device was investigated for antigen (CRP) over the concentration range of 10 ng/mL to10000 ng/mL.

CHAPTER-1

INTRODUCTION

1.1. BIOSENSOR

In each and every ingredients and diet we take as food contains microorganisms which are invisible to our eyes. They can be harmless or harmful. Also called as pathogenic and their presence in our body need to be ascertain at a very early stage as per as safety is concerned. And the introduction of biosensors has open a new way in pathogenic or microorganism's detection.

Biosensor can be broadly defined as a device which combines a biological entity with a transducer which acts as a signaling device. The signal generated is synthesize using software and thus characteristics of the entity are interpreted. Thus it provide user with a very handy and useful tool for recognition.

A biosensor consists of a biological recognition entity, often called a bio-receptor and a transducer which generates signal. It so happened that the analyte interact with the bio-receptor to produce an effect measured by the transducer, which converts the information into a measurable effect, such as an electrical signal. Some common recognition elements used in biosensors are: enzymes, nucleic acids, antibodies, whole cells, and receptors.
With the emergence of nanotechnology many nanomaterials-based electrical biosensors have developed and applying them in ultrasensitive bio-sensing has paved a new way of sensing. Examples of such nano based materials include carbon nanotube, nanowires, nanoparticles, nanopores, nanocluster and graphene. Compared with conventional techniques like optical, biochemical and biophysical methods, nanomaterial-based electronic biomarkers offers significant advantages, which include high sensitivity and new sensing mechanisms, high spatial resolution for precise detection, facile integration with standard wafer-scale semiconductor processing and label-free, real-time detection in a nondestructive manner.

Among various electrical biomarkers architectures, devices based on field-effect transistors (FETs) have fascinated researchers a lot because they work as an ideal biosensor which can directly translate the interactions between targeted biomolecules and the FET surface into

readable electrical signals. In a standardized FET, current flows along a semiconductor path that is connected to two electrodes, (the source and the drain). The channel conductance between the source and the drain is flipped on and off by a gate electrode that is capacitively coupled through a thin dielectric layer. In conventional complementary metal oxide semiconductor-fabricated transistors (MOSFET), the conducting channel is buried inside the substrate. While in FET-based biosensors, the channel is in direct contact with the environment, and thus giving better control over the surface charge. This indicates that surface FET-based biomarker might be more sensitive. Biological activities occurring at the channel surface could directly result in the variation in the surface potential of the semiconductor channel which then modulates the channel conductance.

The easiness of on-chip fabrication of device arrays and the cost-effective device production, and the surface sensitivity at its ultra-level places FET-based biosensors as attractive alternatives to existing biosensor technologies. In this project particular attentions have been paid to carbon nanomaterials: single-walled carbon nanotube (SWCNT) and graphene.

1.2. History of Biosensor

The biosensor was firstly conceptualized by Professor Leland C Clark in 1956, who was later recognized as the father of the biosensor concept. In 1956, C Clark published his definitive paper on the oxygen electrode. Clark's idea became popular commercial in 1975 when he successfully launched the glucose analyzer based on amperometric detection of hydrogen peroxide. This was the first biosensor-based laboratory analyzers to be built by companies around the world.

1.2.1. Generation of Biosensors

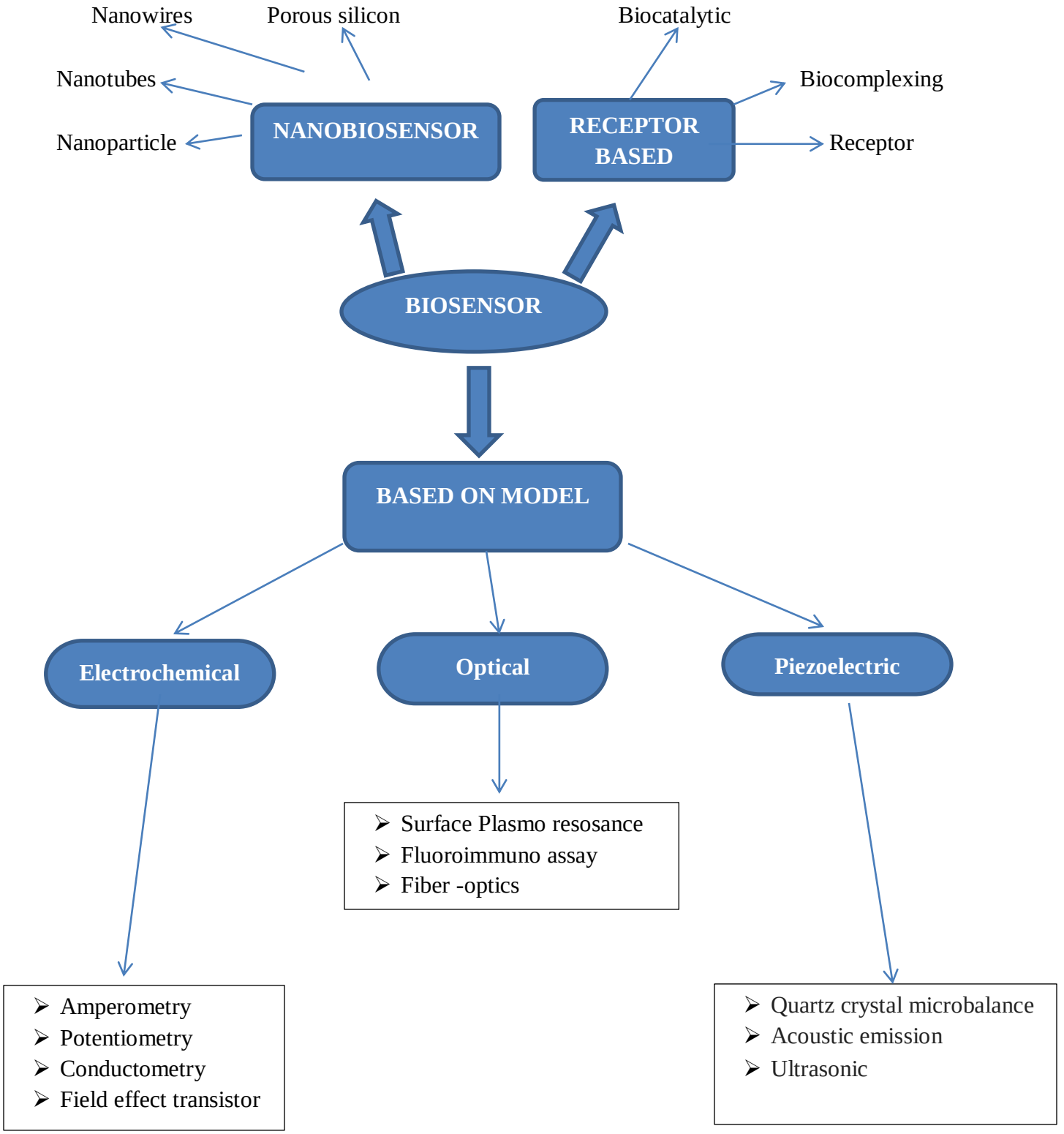
Considering the level of integration, biosensors can be categorized into three generations:

- **First generation biosensor:** during the first generation biosensors the product obtained during the reaction diffuses to the transducer and as a result electrical signal was

generated. It was proposed by Clark and Lyons and implemented by Updike and Hicks who coined term enzyme electrode.

- **Second generation biosensor:** In second generation biosensors, a specific "mediators" was used between the reaction and the transducer so that an improved response can be generated. In ideal case mediator remains inactive. It is highly specific only for the desired electron transfer process between the recognition element and transducer. It usually involved the adsorption or covalent fixation of the biologically active component to the transducer surface and allowed the elimination of semi-permeable membrane. In this generation biosensor's auxiliary enzymes and /or co-reactants were co-immobilized with the analyte, so that there could be improvement in the analytical quality and to simplify the performance.
- **Third generation biosensor:** There is vast improvement during the third generation biosensors. Here, reaction itself generates the response and no product or mediator diffusion is directly involved as was in previous generations. Conducting polymer-based biosensors come under this category. The binding of the biocatalyst to an electronic device directly to that of transducer which is then is amplified to generate the required signals is the basis for a further miniaturization of biosensors. In this generation, biosensors have the mediator integrated along with the enzyme and the electrode to have direct electron transfer. And this direct electron transfer has been highly realized with the use of carbon nanotubes.

1.3. Classification of Biosensors



1.4. Single walled carbon nanotube (SWCNT) and Graphene

Graphene is a two-dimensional (2D) crystalline mono layer made up of sp^2-hybridized carbon atoms and structured them in a honeycomb/hexagonal lattice. In other sense graphene act as the basic unit or building block for graphitic materials of all other dimensions. For example, by folding up a graphene sheet into a cylindrical form in a certain lattice vector, a well-defined, hollow graphitic nanomaterial, classified as a single walled nanotube (SWNT), is formed. Both of these two allotropes of carbon have the simplest chemical composition along with atomic bonding configuration in a two dimensional manner that maximizes its surface-to-volume ratio. Each and every carbon atom on the surface of the nanotube is exposed to the environment and any minute changes in the environment can result in drastic/enormous changes in the electrical properties/characteristics of the carbon nano tube device, thus it forms the basis for ultrasensitive biosensing. The second significant feature of SWCNTs and graphene is that they are 2D conducting nanomaterials that are inherently the same size as the molecules. This specific size of SWCNT offers the opportunity of sensing single-molecule events. Thirdly, SWCNTs and graphenes are molecular chemicals entirely composed of carbon atoms, and this suggests a compatible to natural biomolecules. This also allows controlled functionalization to specifically immobilize bio sensitive agents on their surfaces.

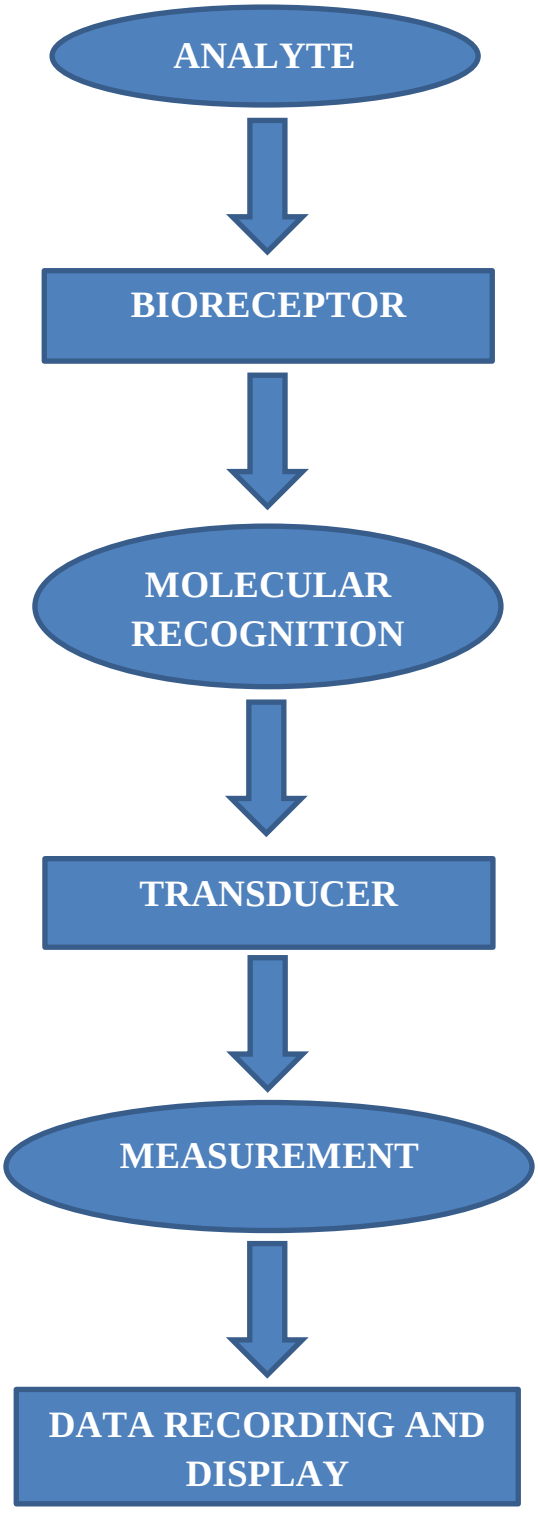

ANALYTE
BIORECEPTOR
MOLECULAR RECOGNITION
TRANSDUCER
MEASUREMENT
DATA RECORDING AND DISPLAY

Fig 1.1: Basic Layout of a biosensor

1.5. Bioreceptor

Bioreceptor can be defined as the entity used to recognize the molecules to be detected. The significance of a biosensor comes from the specialty of the bioreceptor molecule used. A bioreceptor should be very stable one, specific and must be properly immobilized on the transducer surface.

Following are a few types of bioreceptors generally used:

- Antibodies as bioreceptor
- Aptamers as bioreceptor
- Nucleic acid as bioreceptor
- Protein as bioreceptor
- Enzymes as bioreceptor

1.5.1. Antibodies as a bioreceptor

This biosensor utilizes a very unique binding between antibody and its corresponding antigen. The antibody has a very high affinity towards its antigen. This is the reason that this unique property has been generally used in biosensor. The binding of antibody and antigen also shows physical and chemical properties which is then used to generate measurable signal. These signal generated by transducer is further interpreted using graph and plots generated by the corresponding software.

1.5.2. Aptamers as bioreceptor

Aptamers are mono string nucleic acids with well-defined three dimensional (3D) shapes, which enable them to bind to the target biomolecules in a similar fashion as that of antibodies. Aptamers are biomolecules that by their small size and low immunogenicity can replace antibodies in some application.

Aptamers exchanges the following advantages:

- The optimal properties of small molecules such as low immunogenicity, high diffusion, etc.
- As per the characteristics of the antibodies it shows high specificity and affinity, and chemical stability as compared to other bioreceptors.
- And another advantage with respect to monoclonal antibodies is that they can be chemically synthesized rather than biologically expressed.

1.5.3. Nucleic acid as bioreceptor

The nucleic acids are able to detect signal base changes in a complementary DNA sequence. They can be in gene sequenceing and gene expression analysis, sensing of DNA mutations and alternations/changes associated with genetic diseases, also the detection of complementary bacterical/viral DNA sequence. The nucleic acids could replace by the use of PNA chains, with the following advantages:

- High stability against biological degradation of various enzymes.
- Being more resistant to pH it shows high chemical stability or ionic strength changes.
- Reduction of electrostactic interaction due to the absence 2'-desoxy-D-ribose units nor phosphodiester bonds, and ability of establishing stronger bonds.
- Low nonspecific adsorption.

1.5.4. Protein as bioreceptor

Protein based biosensor is generally used for detecting specific small molecules proteins. These are globular proteins that contain two large globular domains with a connecting flexible hinge region. Upon binding with analyte, the protein undergoes large conformational/structural modifications.

A few advantages are as follows:

- High stability
- Ability to work in extreme condition, such as high temperature, high salts concentration
- Can be modified in order to bind several molecules together

1.5.5. Enzymes as bioreceptor

The binding capabilities and catalytic activity of enzymes specifically to the corresponding molecules make them one of the highly used bioreceptors. Analyte can be recognized by several possible mechanisms/techniques:

- The analyte gets converted into a product by enzyme, that is sensor-detectable.
- Sensing of enzyme inhibition or activation by the analyte.
- Enzyme properties can be monitored by the results from interaction with the analyte.

The main reasons for the use of enzymes in biomarkers are:

- Ability of enzyme to catalyze a large number of reactions.
- Potential to detect/sense a group of analytes
- Compatibility with several different transduction methods for detecting the analyte.

Enzymes does not consumed in reactions, thus biosensor can easily be used continuously. The catalytic activity of enzymes also increases sensitivity as it allows lower limits of detection compared to other common binding techniques.

1.6. Biotransducers

A biotransducer is an acknowledgement section of the basic frame of a biosensor. It consists of mainly two parts, a biologically responding part and a physiochemical transducer to respond and convert into recognizable signal part.

Types of Biotransducer

- Electrochemical based biotransducer
- Optical biotransducer
- Field Effect Transistor (FET) electronic biotransducer
- Piezoelectric biotransducer

1.6.1. Electrochemical Biotransducer

Electrochemical biosensors provide an attractive means to analyze the content of a biological sample due to the direct conversion of a biological event to an electronic signal.

Electrochemical Techniques:

- Amperometric
- Potentiometric
- Impedance
- Conductometry

Amperometric: It is a process by which by applying electric current to the solution ions are detected. Here the potential between two electrode is set and the current produced by oxidation

or reduction of electro active species is measured. The amperometric biosensor are reliable, cheaper and highly sensitive for clinical, environmental and industrial purposes.

Potentiometric: In this biosensor the voltage generated during oxidation and reduction of a product, usually at constant current is measured. This technique is very useful for practical application as it is of small size, portable and low cost device. It works in equilibrium condition and the charge potential accumulated at the surface of the electrode is measured as compared to the reference electrode. In other words, potentiometry provides information about the ion activity in an electrochemical reaction.

Impedimetric: in this case Electrochemical Impedance Spectroscopy (EIS) technique is used. It is a very effective technique for label free molecules detection. It provide sensitivity, low cost and selective biosensor systems. Bode plot and Nyquist plot is obtained at a range of frequencies in EIS which is then interpreted to get the changes in the electrochemical processes.

Conductometry: conductimeteric devices can be considered as a subset or part of impedimertric devices. Mostly conductometric devices is strongly related with enzymes, where the ionic strength and thus the conductivity of the solution between two electrodes get changes due to the enzymatic reaction. Thus, conductometric devices can buseful in studying enzymetric reaction that produces changes in the concentration of charged species in a solution.

1.6.2. Optical biotransducer

It offers the largest number of possible subcategories of all the types of the biotransducers. It is because optical biosensor can be used for different types of spectroscopy (e.g. absorption, fluorescence, phosphorescence, raman, refraction, etc.) with different spectrochemical properties. These properties include amplitude, energy, polarization, decay time, phase.

1.6.3. Piezoelectric biotransducer

Piezoelectricity is the potential difference produced across materials due to application of mechanical stress. When a stress is applied mechanically to a piezoelectric material, the change in shape of the material can decrease the separation between cations and anions which produces

an internal potential difference. Piezoelectric Biosensors work by measuring the change in frequency which occurs when the antigen binds to the antibody receptor. Piezoelectric materials are used in a wide variety of detection based applications as biological transducers.

For example: sensing of cancer at early stage by the biomarkers and predicting the effectiveness of drug, DNA hybridization detection, Comparision among DNA strands, Detection of the hepatitis C virus.

1.6.4. Field Effect Transistor (FET) electronic biotransducer

Silicon nanochannel field effect transistor (FET) biosensors are one of the most promising and effective technologies through the development of highly and ultrasensitive and label-free analyte sensing for cancer diagnostics. FET shows exceptional/extraordinary electrical properties and small physical structure and silicon nanochannels which make them ideally suited for extraordinarily high sensitivity. The high surface-to-volume ratios of these systems make mono molecule detection possible. In addition to the above features FET biosensors offer the benefits of high speed, low cost, and high yield manufacturing, without sacrificing the sensitivity typical for traditional optical methods in diagnostics. Top down manufacturing methods leverage advantages in Complementary Metal Oxide Semiconductor (CMOS) technologies, making richly multiplexed sensor arrays a reality.

1.7. FET as a Biosensor

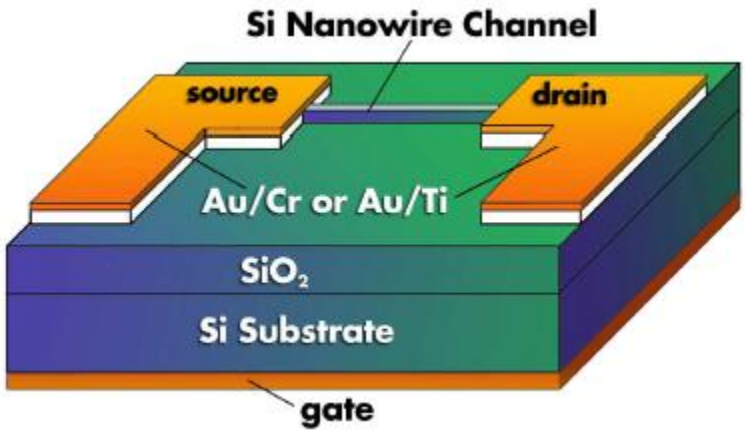


Fig 1.2**:** Schematic diagram of a silicon nanowire FET [Ref: 9]

The field effect occurs as charged particles or molecules bind to the surface of the nanowire and change its conductive properties, analogous to the gate voltage and channel conduction of a conventional FET.

1.7.1. Types of FET biosensor

MOSFET Based Biosensor: Of all types of FETs currently available, the metal oxide semiconductor MOSFET is one of the most highly used FET devices. As the name MOSFET implies, this type has a metal-insulator-semiconductor structure with a metal gate electrode placed on top of an insulating layer of oxide. A MOSFET utilizes an electric field which is controlled by the size and shape of the source-drain channel which is referred to as channel length modulation and channel shape modulation, respectively. In response to a target analyte, a gate electrode controls the flow of the charge carrier (electrons or holes) through the channel formed between the source and the drain, thereby leading to a change in the drain current (I_d). FET measurements depend on the charge density of the biomolecules on the gate surface. FET-type biosensors can detect changes in the surface charge density.

ISFET: The use of an ISFET as a transducer represents a promising tool for biological applications. The ISFET and MOSFET share a good degree of structural similarity. In general, an ISFET device has no metal gate electrode due to the replacement of the metal gate material with an ion-selective electrode, an electrolyte solution and a reference electrode. The current magnitude of an ISFET device depends on the charge density of the analyte molecules on the gate surface. For bio-recognition elements (or receptors), antibodies are one of the most commonly used capture agents for identifying, isolating, and quantifying analytes of interest due to their specificity for binding antigen. When antigen-antibody binding occurs, a substantial change in the gate potential caused by altered the value of the surface charge takes place. The magnitude of the charge density on the surface appears to be an important determinant when measuring the interaction patterns of biomolecules using FET-based biosensors.

Carbon Nanomaterial FET: Carbon nanomaterials are widely used as excellent building blocks for nanoscale devices. It is assumed that one of the most fascinating sensing platforms can be a FET sensor system based on nanostructures, including semiconductor carbon nanotubes and 2D graphene.

- Carbon nanotube (CNT-FET) based biosensor:

CNT FET sensors are considered as sensitive devices because the surface-to-volume (S/V) ratio drastically increases when the diameter of the tube decreases on the nanometer scale, as a high S/V ratio is responsible for the sensitivity of the device.

- Graphene FET (GFET) based biosensor:

A graphene field effect transistor (GFET) consists of a graphene channel between two electrodes (source and drain) with a gate to modulate the electronic response or changes in the channel. The graphene is exposed to enable functionalization of the channel surface and binding of receptor molecules to the channel surface. The surface of the GFET channel is functionalized by binding receptor molecules for the specific target of interest.

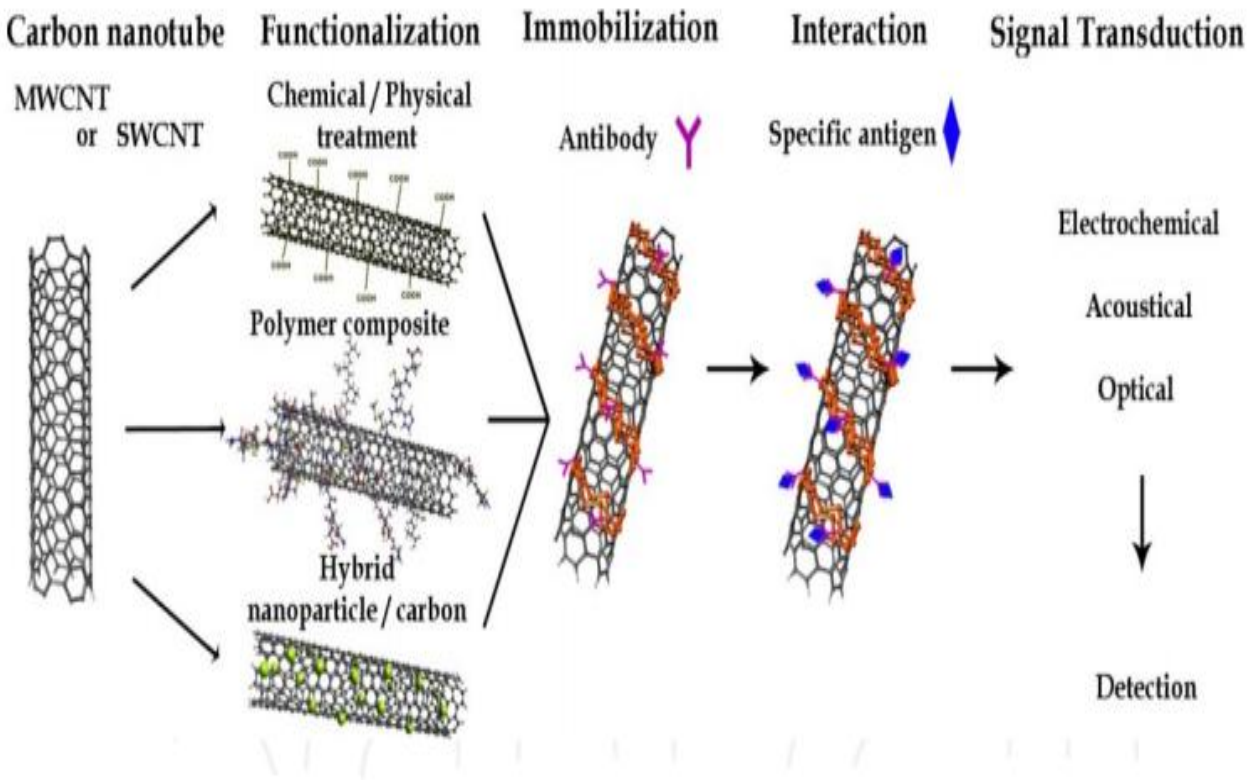
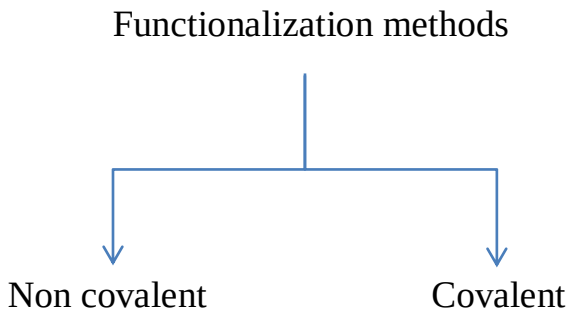


Fig 1.3: The schematic diagram and steps of types of transducer of immunosensors based on carbon nanotubes [Ref: 17]

1.8. Functionalization of Carbon Nanotube

Carbon Nanotube (CNTs) bears unique electrical, chemical and mechanical properties that make them one of the leading materials for variety applications in different fields. But the outer surface of pristine CNTs is, in general, possess chemically inert environment. This condition many a times is not suitable for many applications. One of the most promising ways to overcome this difficulty is to functionalize CNT. Functionalization enhances their properties and consequently their application potential.



1.8.1. Non covalent functionalization

One of the advantages of non-covalent functionalization is that it does not destroy/modify the lattice of the CNTs sidewalls or outer surface, and it is the reason that it does not affect the final structural and physical properties of the material. The non-covalent functionalization is an

alternative method for tuning the interfacial characteristics of nanotubes. The CNTs are generally functionalized non-covalently by aromatic compounds, surfactants, and polymers, through π-π stacking or hydrophobic interaction. In these cases, the non-covalent modifications of CNTs can do much to preserve their desired properties, while refining their solubilities quite remarkably.
Some of the non-covalent techniques can be summarized as: aromatic small molecule absorption, polymer wrapping, surfactants, biopolymers and endohedral method. Aromatic molecules, such as pyrene, porphyrin, and their derivatives, interact with the sidewalls of CNTs through π-π stacking interactions, thus creating the way for the non-covalent functionalization of CNTs. Polymers, like conjugated polymers, act as excellent wrapping materials for the non-covalent functionalization of CNTs as a result of π-π bonding and van der Waals interactions between the conjugated polymer chains which contain aromatic rings and the surfaces of CNTs.

However surfactants polymers have also been employed to functionalize CNTs. The CNTs surface adsorbed the surfactant which lowered the surface tension of CNTs that effectively prevented the aggregation of CNTs. Also the CNTs treated with surfactant overcame the van der Waals attraction by electrostatic repulsive forces. The effectiveness of this method depends strongly on the properties of surfactants, medium chemistry and polymer matrix.

1.8.2. Covalent functionalization

The caps at the end of the nanotubes are composed of highly curved fullerene-like hemispheres, which are therefore highly reactive, as compared with the side walls. The sidewall itself contains defect sites such as pentagonheptagon pairs called Stone-Walls defects, sp3-hybrideized defects, and vacancies in the nanotube lattice.
Chemical functionalization basically depends on the covalent bond of functional groups onto carbon form of CNTs. It occurs at the end caps of nanotubes or at their sidewalls which contains many defects. Direct covalent sidewall functionalization is related to change of hybridization from sp2 to sp3 and also simultaneously a loss of p-conjugation system on graphene layer. This process can be made by reaction with some molecules of a high chemical reactivity. In the first

approach, fluorination of CNTs has become more popular for initial study of the covalent functionalization because the CNTs sidewalls are expected to be inert. The fluorinated CNTs have C-F bonds that are weaker than those in alkyl fluorides and thus creates substitution sites for additional functionalization. Replacements of the fluorine atoms by amino, alkyl, hydroxyl and carboxyl groups have solved many problems.

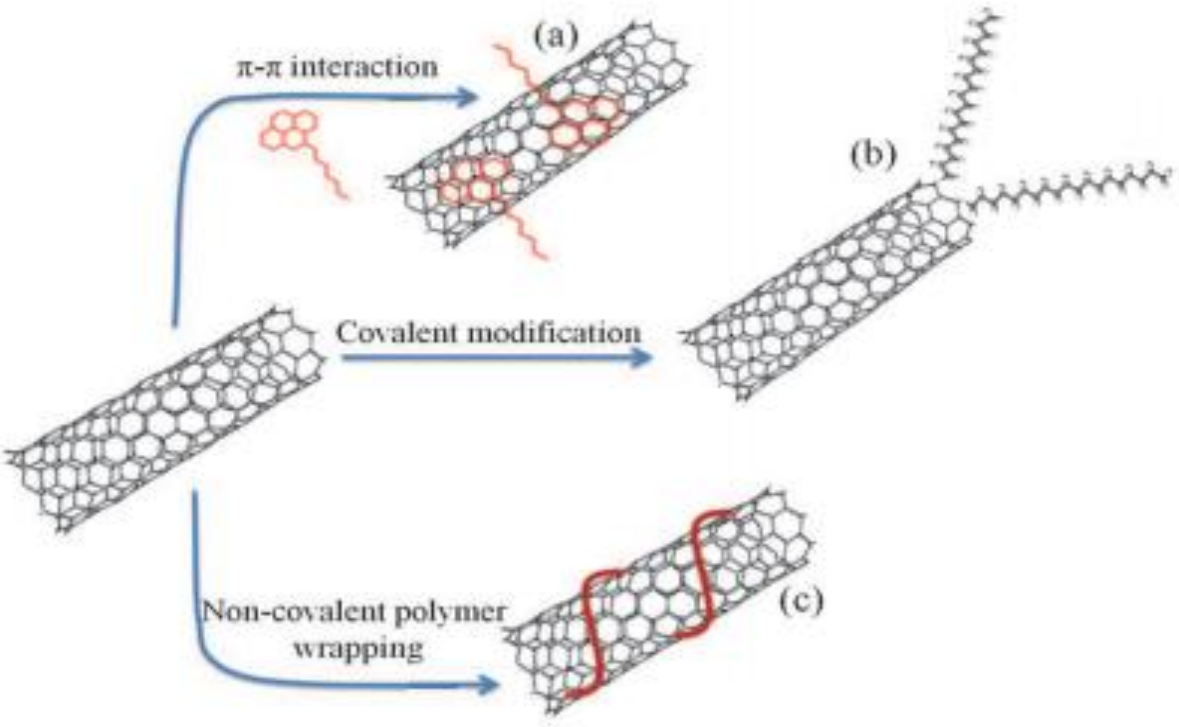


Fig 1.4: Functionalization of SWCNTs with different methods [Ref: 13]

1.9. Immobilization of Biomolecules

Immunosensor is directly related to the immobilization matrix used and orientation and density of antibodies and antigens on the surface of the electrode. There are different strategies used to immobilize the recognition element, which can be directly on the electrode surface or on other solid supports. Conventionally, there are Non-covalent and Covalent techniques generally employed to immobilize antibodies, which are generally based on

- Adsorption
- Entrapment in polymers
- Covalent binding.
- Cross-linking of antibodies aggregates.

Developments in these techniques have great significance and potential application in many areas of biotechnology, including purification of proteins, medicine and drug delivery, regenerative medicine, tissue engineering, and many other applications.

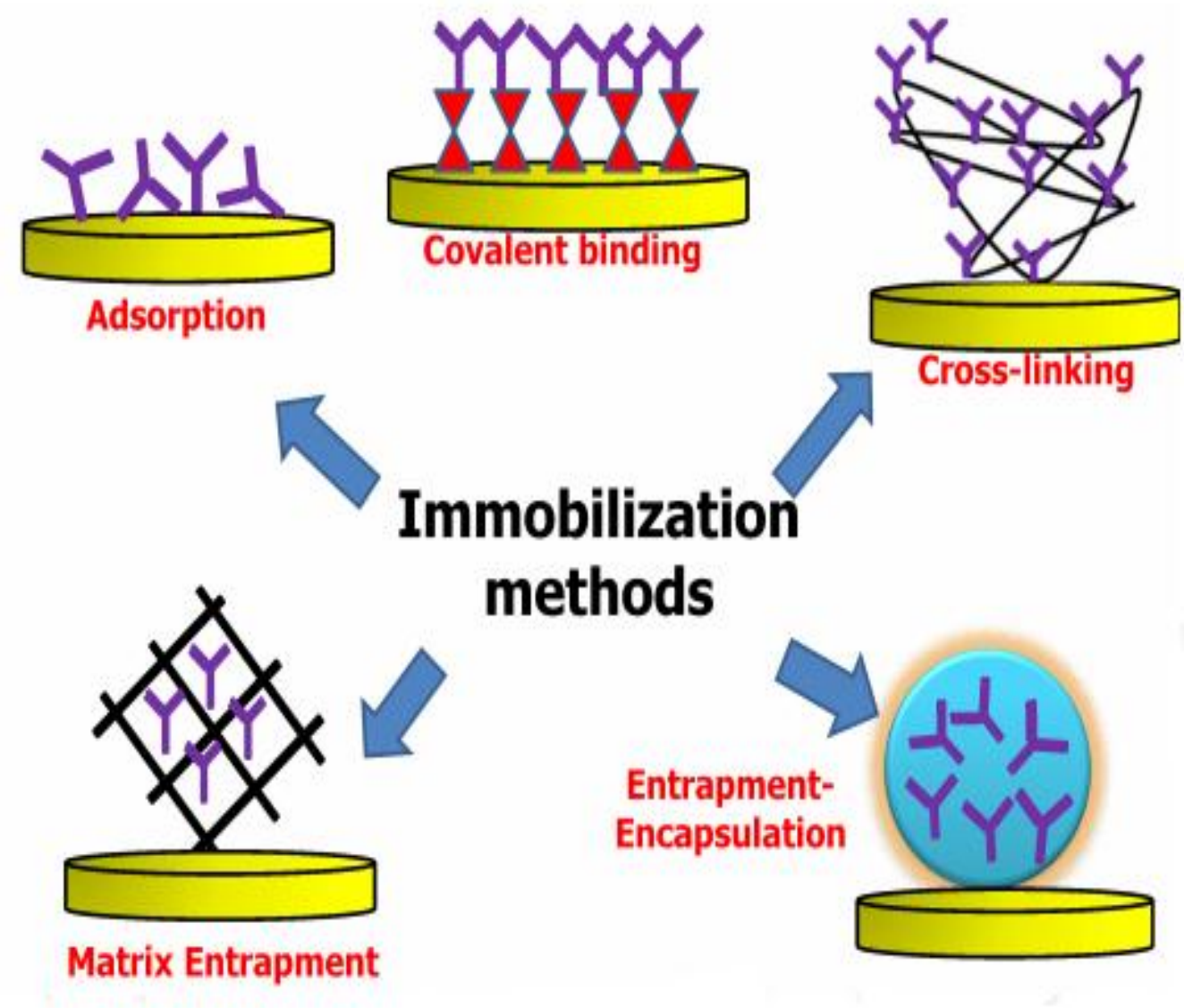


Fig 1.5: Blocks showing different methods of molecular Immobilization [Ref: 29]

1.9.1. Adsorption

This process is generally applied for the immobilization of an enzyme which is based on the adsorption of enzyme protein on the surface of water-insoluble carriers. Thus, this method causes very little or no minimal change of the enzyme or damage of its active center. This method can

be both simple and cheap if employed with suitable carrier. However, it shows the disadvantage that the adsorbed enzyme may leak out from the carrier during use due to a weak binding force between the enzyme and the carrier. One of the major advantages of adsorption as a general method of immobilizing enzymes is that usually no reagents and minimum activation stages are required. Adsorption creates less disruptive to the enzymatic protein as compared to chemical means of attachment because the binding is mainly by hydrogen bonds, multiple salt linkages, and Van der Waal's forces. In this respect, the method bears the greatest similarity to the conditions found in natural biological membranes and has been used to model such systems.

Since weak bonds are involved in it, due to the changes in temperature, pH, ionic strength or even the mere presence of substrate desorption of the protein, is often observed. Another disadvantage is adsorption of other proteins or other substances as the immobilized enzyme which may alter the properties of the immobilized enzyme. Also if the adsorbed substance is a substrate for the enzyme, there would be decrement in the rate depending on the surface mobility of enzyme and substrate.

Adsorption of the enzyme may be necessary to facilitate the covalent reactions. An enzyme when temporarily adsorbed onto a matrix show some stability and has been achieved by cross-linking the protein in a chemical reaction subsequent to its physical adsorption.

1.9.2. Entrapment

This method of immobilization is basically based on the location of an enzyme within the lattice of a polymer matrix or membrane. It is done in such a way as to avoid protein while allowing penetration of substrate.

It can be differentiate into lattice and micro capsule types:

Lattice-Type – In this type of entrapment a water-insoluble cross-linked polymer is involved where an enzymes gets entrapped within its interstitial spaces. Examples of some synthetic polymer such as polyacrylamide, polyvinylalchohol etc. and natural polymer (starch) which have been generally used to immobilize enzymes using this technique.

Also in microcapsule-type entrapping the enzymes gets enclosed within semi permeable polymer membranes. Hollow fiber type of membrane can be used although can be expensive. Lipsome membrane can also be used but for this purpose membrane confinement of enzymes can be achieved by a number of different methods. All of these depend for their utility on the semipermeable nature of the membrane.

1.9.3. Covalent binding

It is the most extensively studied immobilization techniques where the formation of covalent bonds between the enzyme and the support matrix is involved. When considering the selection of the type of reaction by which a given protein is immobilized, there are two characteristics:

- There should not be any loss of enzymatic activity while performing the binding reaction.
- The active site of the enzyme must be unaffected by the reagents used.

The covalent binding technique is based on the binding of enzymes and water-insoluble carriers by covalent bonds. The functional groups that are generally involved in this binding are listed below:

Amino group	Carboxyl group	Sulfhydryl group,
Hydroxyl group	Imidazole group	Phenolic group
Thiol group	Threonine group	Indole group

This method can be further classified into peptide and alkylation methods according to the mode of linkage. The immobilization by covalent binding is much more complex and less mild than in the cases of physical adsorption and ionic binding. Thus, covalent binding may change the conformational structure and active center of the enzyme, which results in major loss of activity and even can bring changes to the substrate. In other case, the force which binds enzyme and carrier is strong enough to prevent leakage of the enzymes, even if there is substrate or solution of high ionic strength.

Covalent binding to a matrix must involve only those functional groups of the enzyme which are not necessary for catalytic action. A number of protective methods have been devised:

- Covalent binding of the enzyme can be done in the presence of a competitive inhibitor or substrate.
- A reversible, covalently linked enzyme-inhibitor complex can be used as protective method.
- A chemically modified soluble enzyme whose covalent linkage to the matrix is achieved by newly incorporated residues.

1.9.4. Cross linking

Immobilization of enzymes using this technique has been achieved by intermolecular cross-linking of the protein, either to different protein molecules or to functional groups on an insoluble support matrix. Cross-linking an enzyme can be both expensive and insufficient, as some of the protein material will inevitably be acting mainly as a support. This can result in relatively low enzymatic activity. In general, cross-linking is more suitable in conjunction with one of the other methods. Cross linking method is used mostly as a means to stabilize the adsorbed enzymes and also to prevent leakage from polyacrylamide gels. The enzyme is covalently linked/bonded to the support matrix, thus very little desorption is expected using this method. The most commonly used reagent for cross-linking is glutaraldehyde. Also it is to be noted that cross-linking reactions are carried out under severe conditions as compared to other methods and these harsh conditions can change the conformation of active center of the enzyme, and thus there may be significant loss of activity.

1.10. C-Reactive Protein (CRP)

C-reactive protein (CRP) is a protein produced by the liver and released into the blood during the acute phase of inflammation. Therefore, CRP is very used as a marker for inflammation process. It is also used in a multi-biomarker system as a predictive biomarker for cardiovascular disease risk. A cut-off level of 2–3 μgmL^{-1} has been reported in the literature for CRP values associated with risk of coronary events. More risk-specific clinical reference ranges for CRP assay are described as ≤ 1 μgmL^{-1} for low risk, 1–3 μgmL^{-1} for medium risk and ≥ 3 μgmL^{-1} for high risk.

CHAPTER- 2

MATERIALS AND METHODS

2.1. Apparatus

The electrical characterization and sensing of SWCNT devices was carried out in air at room temperature by considering the current versus voltage (I-V) characteristics between -0.5 and +0.5V. Source Measurement Unit (Keysight, model no. B2902A), was connected to a Micromanipulator model 450PM-B probe station which provides the electrical contacts between source and drain electrodes. The inverse of the slope of the I-V curve gives the resistance of the device. Micromanipulator is a device which is used to interact with the sample physically under microscope.

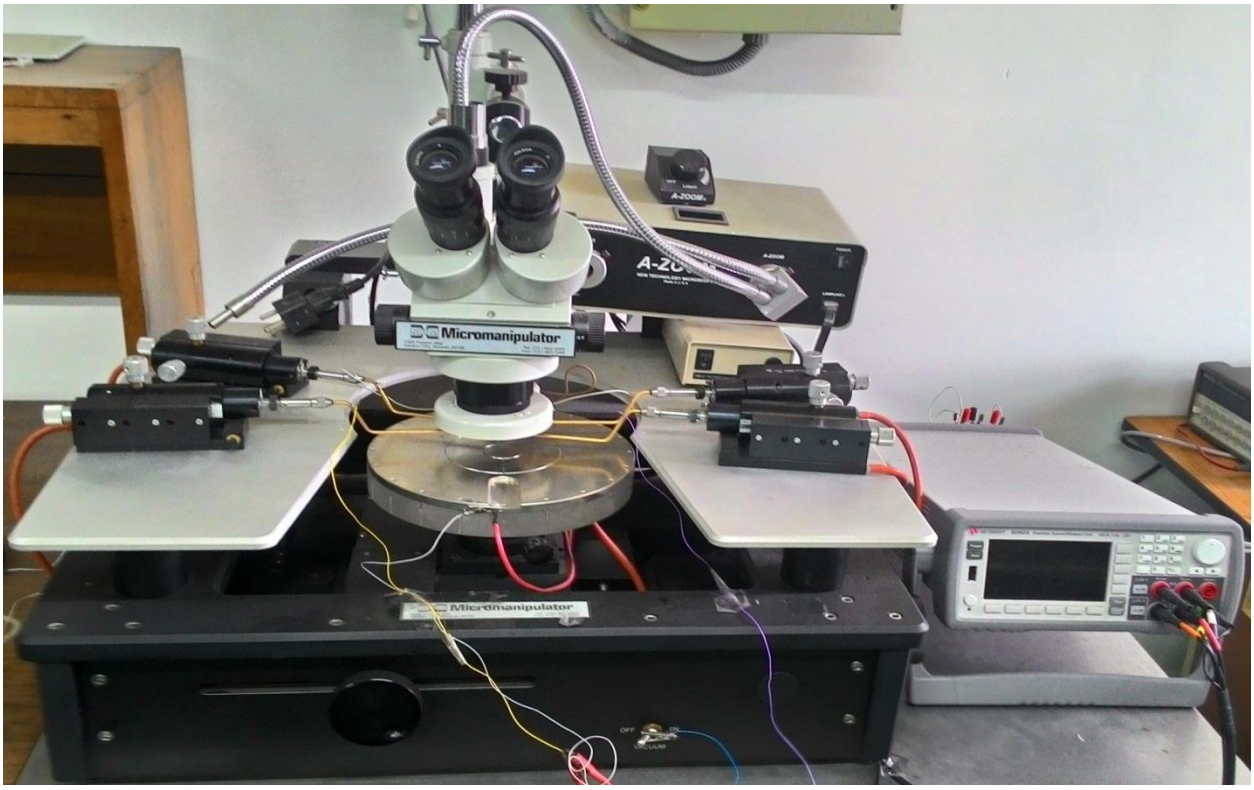
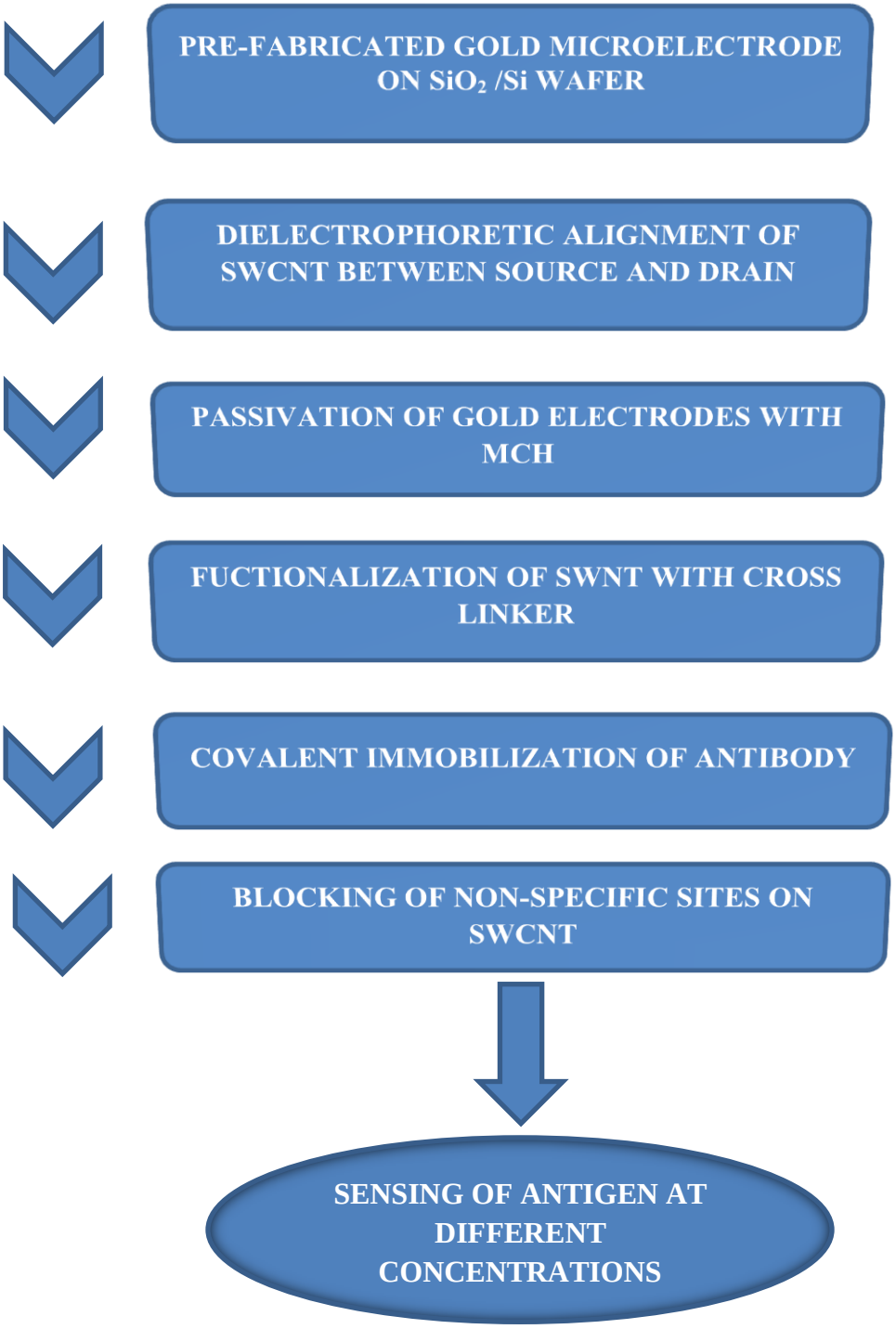


Fig 2.1: Photograph of Micromanipulator model 450PM-B probe station interconnected with source- meter.

2.2. Steps


PRE-FABRICATED GOLD MICROELECTRODE ON SiO_2 /Si WAFER
DIELECTROPHORETIC ALIGNMENT OF SWCNT BETWEEN SOURCE AND DRAIN
PASSIVATION OF GOLD ELECTRODES WITH MCH
FUCTIONALIZATION OF SWNT WITH CROSS LINKER
COVALENT IMMOBILIZATION OF ANTIBODY
BLOCKING OF NON-SPECIFIC SITES ON SWCNT
SENSING OF ANTIGEN AT DIFFERENT CONCENTRATIONS

2.3. 1-Pyrenebutaric acid N-Hydroxysuccinimide Ester (PyBt-NHS-Ester)

Chemical formula $C_{24}H_{19}NO_4$ is strongly stacked onto the sidewall of SWNT via π-π stacking of the pyrene group. Its ester group which is active enough to react with the amine groups to form amide bonds. This amide bonds can also be used to attach molecules or biochemically active molecules (e.g. for protein immobilization). Pyrene are generally hydrophobic polycyclic aromatics that makes bond avidly to the hydrophobic CNTs and also donot adversely affect the electrical properties of the CNTs SP2 bonds. The pyrene, a hydrophobic group anchors the reactive NHS ester to the nanotubes and allows it to react with solution phase molecules.

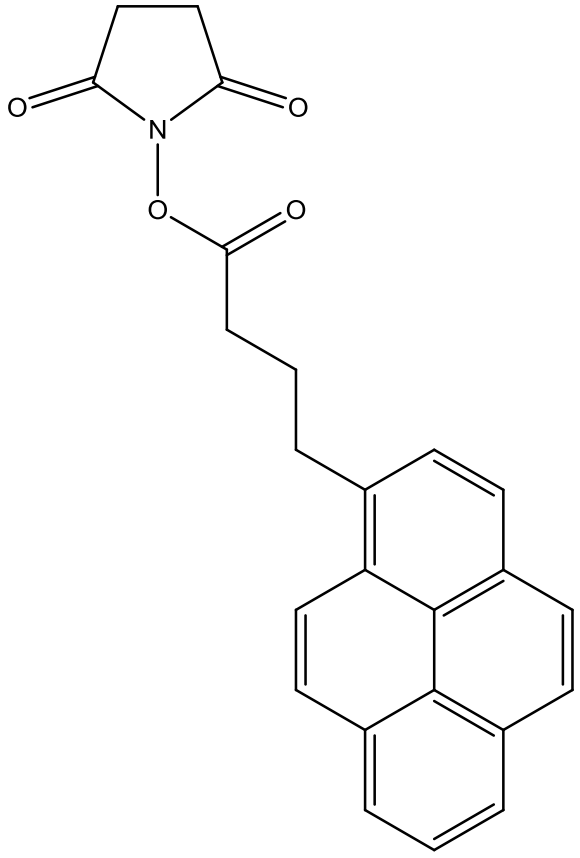


Fig 2.2: PyBt-NHS-Ester

2.4. 6-Methylcapto-1-hexanol (MCH) and BSA

It is generally used to block the nonspecific binding sites. Nonspecific binding refers to the binding other than its specific receptor.

Many a time in addition to binding to its specific receptors, antibody may also bind to other sites. Binding to specific receptor of interest is called specific binding, while binding to the other sites is called nonspecific binding (NSB). Nonspecific binding can be minimized by filling the unoccupied binding sites with a blocking reagent (NSB agent) without taking active part in specific assay reaction.

Some of the Properties of the blocking agent can be rearranged as follows:

- It must inhibit NSB (passive or covalent) of assay components to the surface.
- Must prevent non-specific protein - protein interaction.
- It should exhibit no cross reactivity with subsequent assay components.
- NSB agent should not disrupt the bonds that immobilize the specific protein or biomolecule to the surface.
- Also it exhibit consistent, reproducible performance with every lot.

Protein Blockers – (Bovine serum albumin (BSA), Casein, Fish Gelatin, Whole Sera.) blocks the non-occupied sites on the surface. And also space out and stabilize biomolecules bound to the surface to reduce the steric hindrance.

Bovine serum albumin (BSA) is a serum albumin protein which is extracted from cows and is often used in the lab experiment specially as blocking agent.

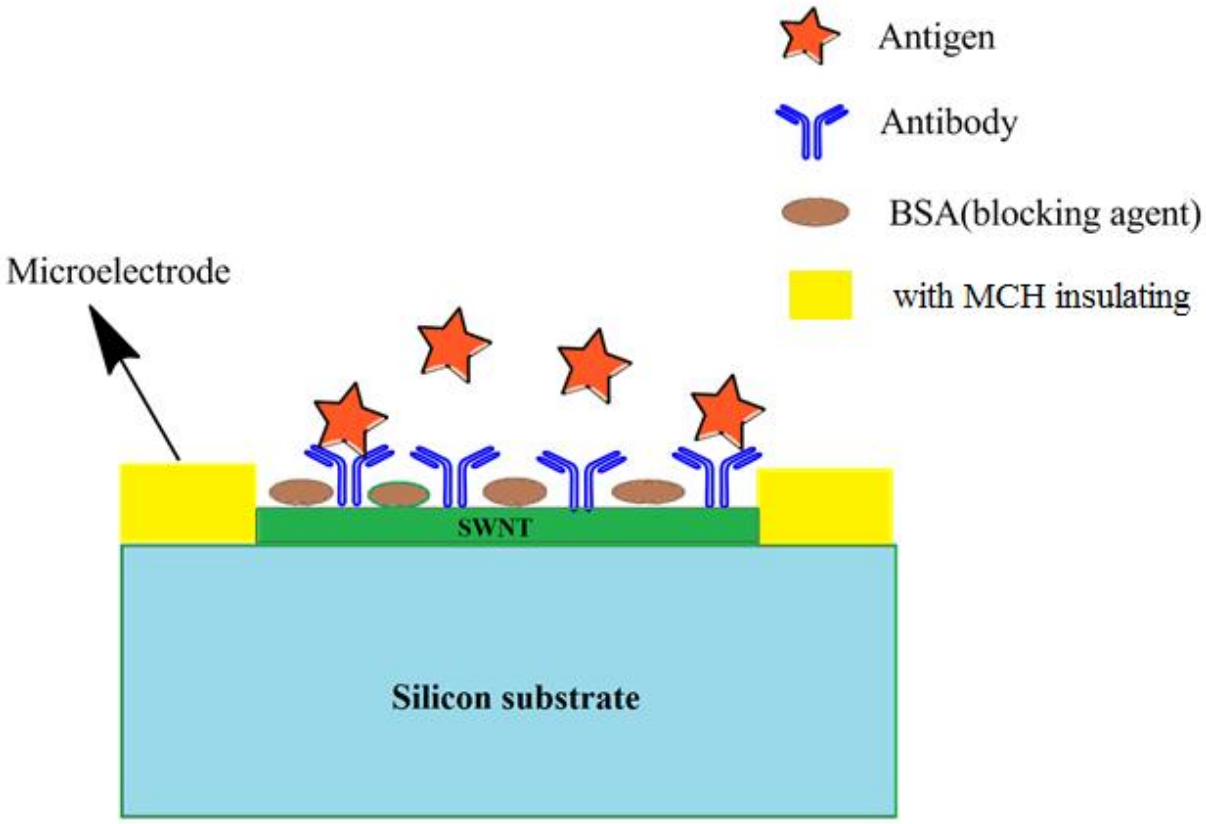


Fig 2.3: schematic showing blocking of Non-specific Sites by BSA and MCH

2.5. Dielectrophoresis

When dielectric particles are exposed to a non-uniform electric field, charges including electrons (-) and protons (+) are moved away from their initial balanced positions and redistributed in these particles. The charge redistribution creates electric dipole moments which force these particles to rotate along the electric field lines. The dielectrophoresis of CNTs is affected by many factors including the dimensions of the nanotubes, the properties of the medium, and the strength of the electric field. The following parameters are adjusted to control the deposition and alignment of the nanotubes: bias voltage, frequency, deposition time, width of the electrodes, and nanotube solution concentration.

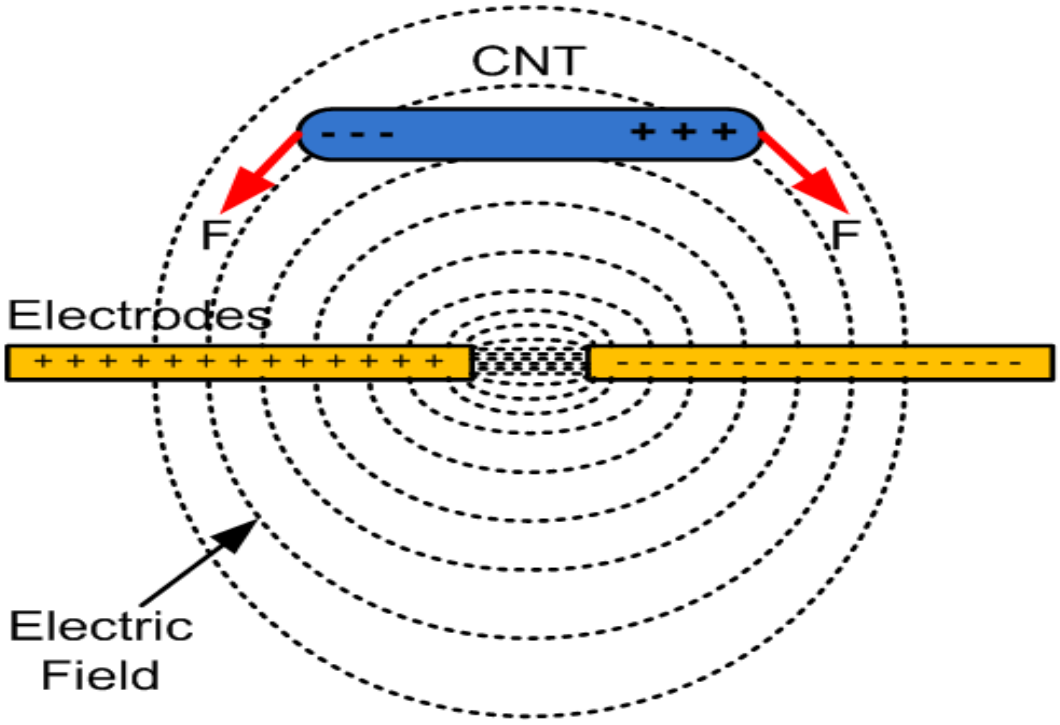


Fig 2.4: Principle of dielectrophoresis deposition and alignment of a carbon nanotube [Ref: 12]

1.0µl drop of SWNTs suspended in N, N-dimethylformamide(DMF) were aligned across a pair of 3µm apart microfabricated gold electrodes by ac dielectrophoresis by applying an sinusoidal voltage of 1.5 v at 4MHz frequency. The aligned SWNTs were then annealed at 300^0c for 1 hour in an inert flow environment (95% N_2 and 5 % H_2).

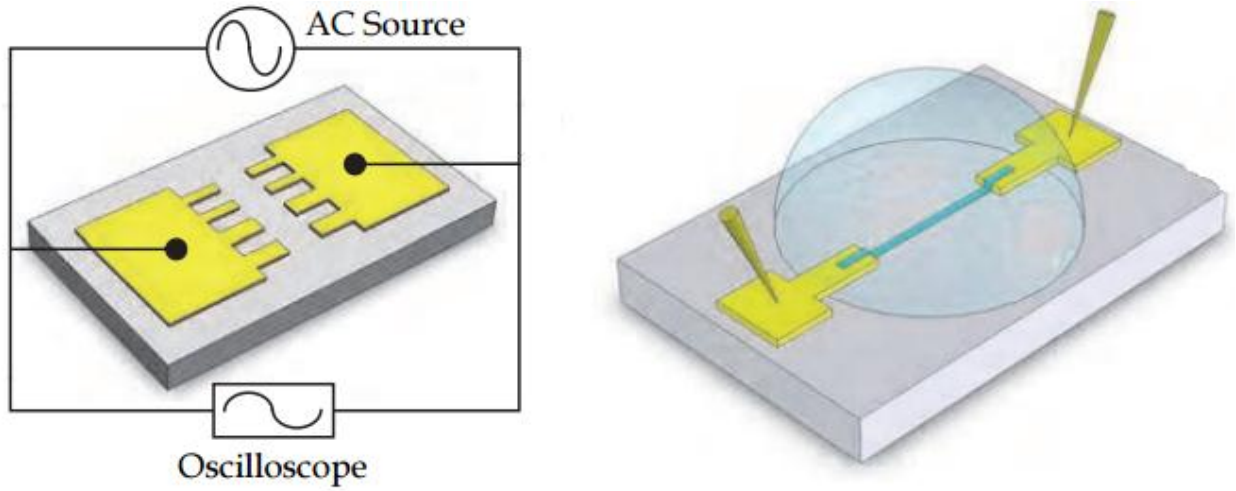


Fig 2.5: Experimental system for the dielectrophoresis and schematic diagram of the CNT alignment [Ref: 12]

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Process Involved

The prefabricated gold microelectrode on SiO_2/Si wafer which is of P-Type and through dielectrophoresis technique the SWNT was aligned between Source and Drain. This SWCNT provide a channel to allow charges to flow through it. After the SWCNT alignment on FET the next step is to modify the SWNT layer with 1-pyrenebutaric acid N-hydroxysuccinimide ester (PyBtNHS). This PyBtNHS get stacked onto the sidewalls of SWCNT as π-π binding leaving NHS ester exposed to the solvent and accessible to proteins. The most relevant features of the PyBtNHS is that it gives π-π stacking which does not damage the physical or chemical properties of CNT. After this, the gold electrodes were passivated with MCH in DMF (6mM for 1hr) to prevent nonspecific binding of the protein. Further, antibody C - reactive protein (CRP) with the concentration of 100µg/ml was drop cast onto the modified SWCNT. There is a covalent immobilization between PyBtNHS and CRP. The non-specific sites of the matrix were further blocked using BSA protein. Often after the transfer of the proteins it is important to block the remaining surface of the membrane to prevent non-specific binding of the detection antibodies during subsequent steps. In general any protein that does not have binding affinity for the target or probe components in the assay can be used for blocking.
To investigate the sensing capability of the device the modified SWCNT device was sensed with antigen CRP at different concentrations ranging from10 ng/mL to 10000 ng/mL.

At each and every step of device fabrication monitoring was done through current voltage (I-V) characteristics obtained using micromanipulator probe station. The source voltage was varied from -0.5V to +0.5V. The FET wafer was placed on the Hot Chuks where a fixed gate voltage was applied. Using needle of the probe station, the current was measured between source and drain at different stages.

3.2. Field effect transfer and I-V characteristics of the SWCNT-FET device

To understand the charge transfer mechanism corresponding field dependence electron transfer characteristics (FET) study was carried out before and after the chemical and biological modification of the SWCNT device in dry condition. Fig 3.1 represents the graph showing the dependence of the source drain current, I_{sd} (Y-axis) on the back gate voltage, V_g(X-axis), of the SWCNTs FET in the range of -40V to +40V, at a bias voltage(V_d) of 0.1V. It shows a P-Type behavior for the SWCNT due the electron withdrawal of adsorbed oxygen molecules from the air

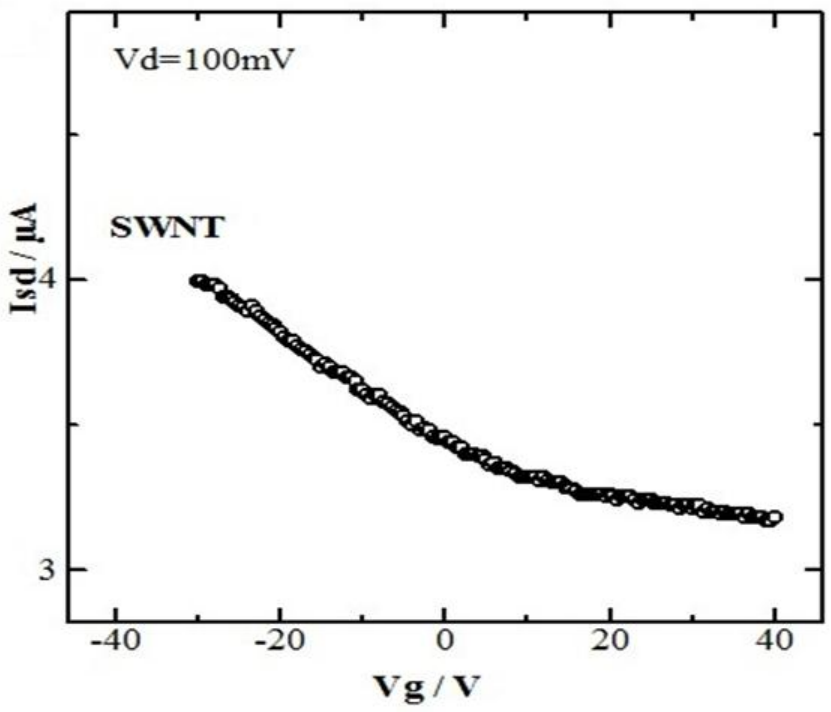


Fig 3.1: Typical gate voltage dependence of the normalized source drain current (I_{sd}) at V_d=0.1V of SWNT

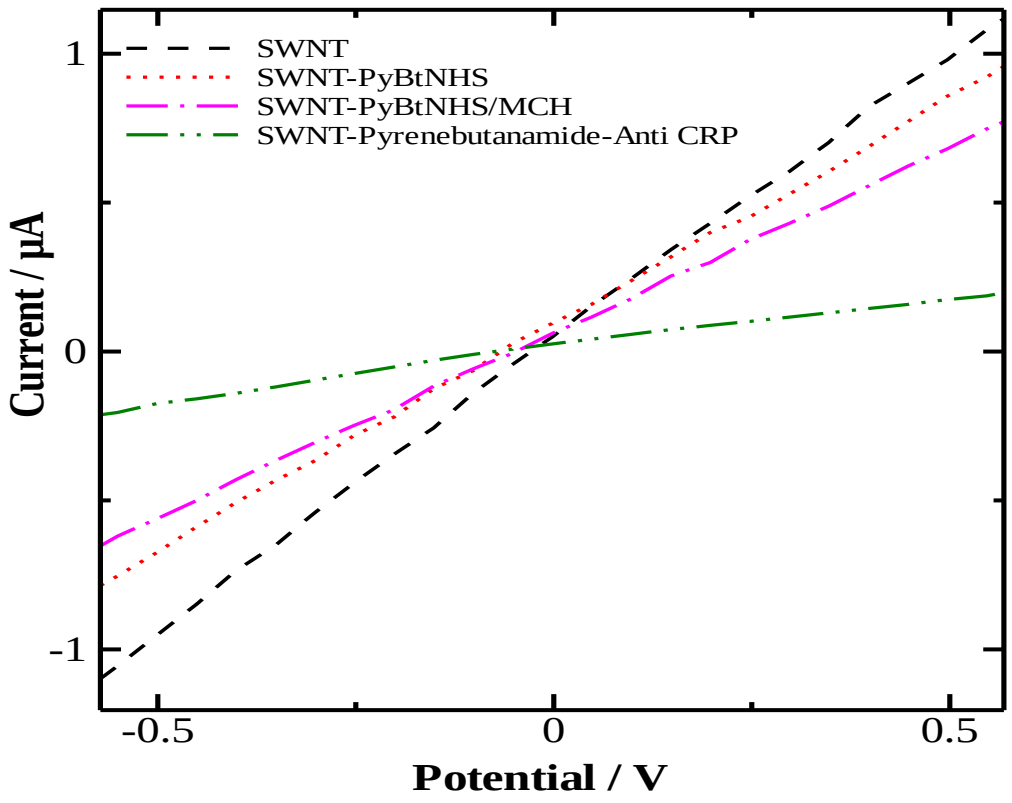


Fig 3.2: Current versus Voltage (I-V) curves at different stage of fabrication

The above fig: 3.2 correspond to I-V characteristics of SWNT obtained at each and every step of modifications. It can be perceived from the graph that there is decrease in the current at a given voltage after functionalization of SWNT with bi-linker PyBt-NHS as compared to the current obtained on SWNT. There is a π-π stacking between PyBt-NHS and the sidewalls of CNT which

might be the reason for increase in resistance and decrease in current due electron scattering on SWCNT channel. Further decrease in current was observed on subsequent application of MCH during passivation process of the gold electrodes to block the non-specific sites. This can be ascribed to the formation of an insulating layer of MCH on the gold microelectrode resulting in a decrease in the channel conductivity. The current was further decreased after the covalent immobilization of with protein antibody, anti-CRP on PyBt-NHS modified SWCNT channel. This might be explained on the basis of the neutralization of positive charge density in P-Type SWNT semiconductor due to accumulation of negative charges of the antibody CRP and scattering potential (reduction in hole density), thereby confirming the formation of the SWCNT-FET biosensor device. The sensing measurement was performed by montitoring the changes in the current versus voltage (I-V) characteristics of the device on antibody-antigen interaction over SWNTs channel. The sensing performance of the CNT-device was investigated for antigen (CRP) over the concentration range of 10 ng/mL to 10,000 ng/mL.

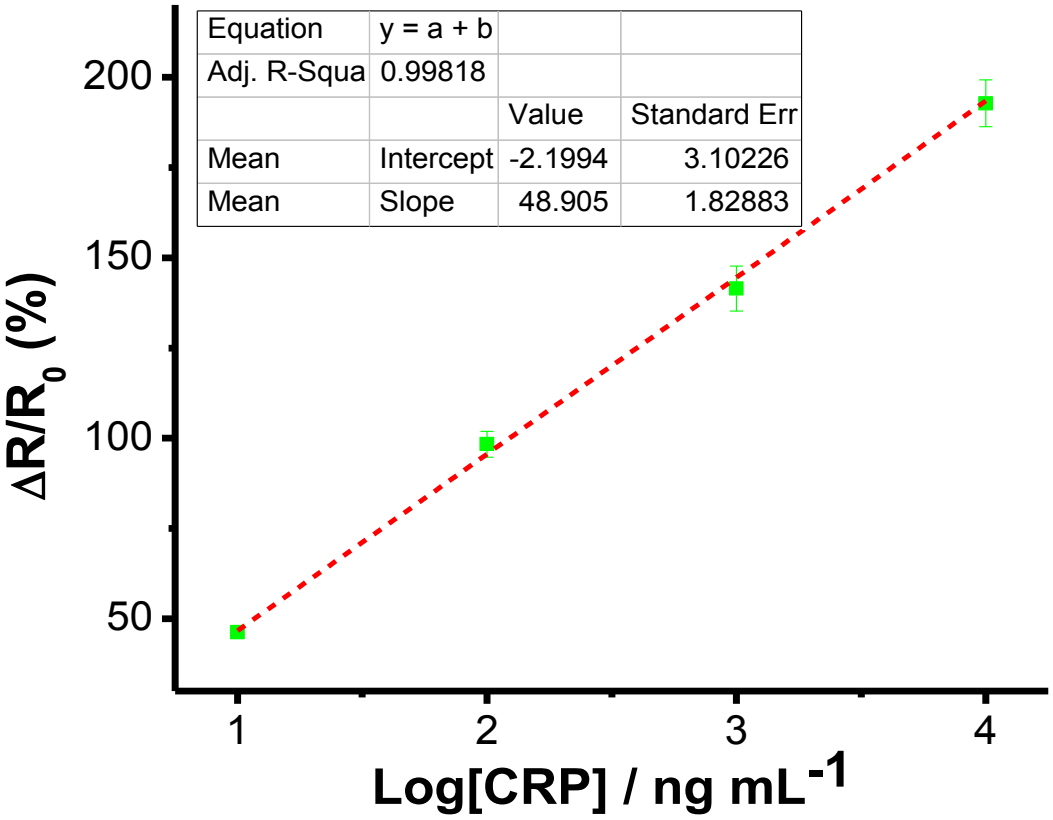


Fig 3.3: Calibration curve of CRP

Fig 3.3 shows the normalized response of the CRP – PyBtNHS/SWNT blend as a function of CRP concentration

$\frac{\Delta R}{R_0} = \frac{R - R_0}{R_0}$, where R_0 and R is the resistance of the device measured before and after exposed to CRP respectively.

The resistance was calculated as the inverse of the slope of the I-V plot between -0.5V to +0.5V in linear range. It was ascertained that conductivity of the SWCNT hybrid device continued to decrease i.e. there was increment in resistances with the increase in the concentrations of CRP.

The device exhibited a linear response i.e. normalized resistance change to target CRP from10 ng/mL to 10,000 ng/mL. The error bars which corresponds to the range of the resistance measured for the three replicates shows that the differences in the resistances was within a range of 6% to 15% at the individual CRP concentration.

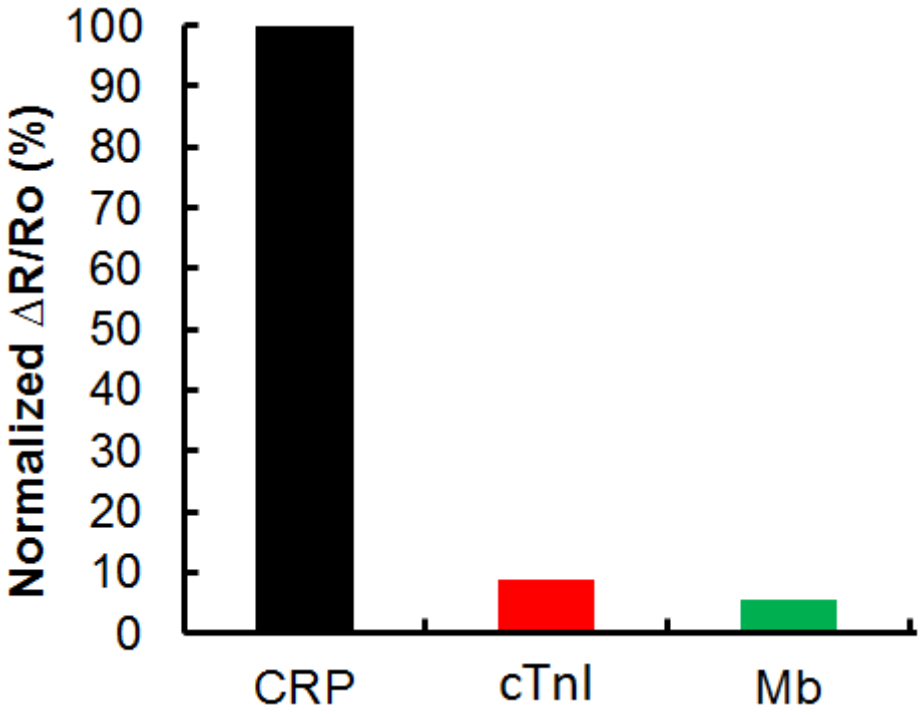


Fig 3.4: shows selectivity of CRP

An antibody (protein) is very much specific towards its corresponding antigen. To confirm the selectivity of the protein, CRP (antibody) was tested with different antigen i.e. antigen not corresponding to CRP antibody.

The specificity of our sensor towards the target CRP was investigated by exposing it with other cardiac specific biomarkers e.g. 100.0 ng mL^{-1} concentration of individual protein antigen of troponin (cTnI), myoglobin (Mb). The change in the normalized response (%) of the device for cTnI and Mb was found to be 8.68 % and 5.62 % and is thus insignificant with respect to target

CRP (Fig 3.4). This means that the specificity of the sensor to CRP only due to the antibody antigen immunoreactions.

This confirms the selectivity of protein (CRP).

CONCLUSION

In this work a SWCNT-FET device has been developed as a biosensor for the quantitative detection of human cardiac antigen CRP. The protein antibody, anti-CRP, was covalently immobilized on SWCNT channel surface by using a bilinker, PyBt-NHS ester. The specificity of the device was determined by exposing it to non-specific biomarkers like cTnI (Cardiac Troponin-I) and Myoglobin (Mb). The device showed linearity in the % change of resistance on reaction to CRP antigen over the concentration range of 10 ng/mL to 10,000 ng/mL with a sensitivity of 48.90 % ($\Delta R/R_0$). Thus, this high sensitivity and specificity together makes this device a better technique in the field of biosensor. Further this FET can also be used for biosensor application by immobilizing different antibody or enzymes for a particular detection of target molecules.

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