1.1 Sensors

Sensor is an analytical device that responds and detects to some type of input feed from the physical environment to a recognizable and readable signal. Sensors are classified broadly into two types based on recognition layer as chemical sensors and biosensors and based on transducer as optical, thermal, mass and electrochemical sensors etc. Chemical sensors detect the presence of chemical substances and elements along with their precise concentrations. In accordance to IUPAC, "Biosensors are integrated analytical devices that works in tandem with the bio-molecular recognition element and a physical transducer creating an electrical or optical signal that correspond directly to the concentration of a specific chemical or biological agent being taken for a specific chemical or biological agent". Biosensors history traces back to year 1962. Clark and Lyons laid the foundation of first generation biosensor that was also the first oxygen biosensor. It was an electrochemical oxygen biosensor wherein the glucose oxidase was integrated with the dialysis membrane for performing quantitative measurements of glucose in the aqueous media. However, consistent requirement of dissolved oxygen was its limitations. Cass et al. 1984 compensated this problem by making use of mediators. Mediators are specific molecules, which can directly transfer electrons produced during a biological redox reaction directly to the sensor probe (electrode). This gave birth to the second-generation biosensors. First commercial glucose biosensor marketed by Yellow Spring instrument al biosensor in 1975. In this line, MediSens Exac Tech also launched the blood glucose biosensor. Arrival of screen-printed technology was around the year 1991. Further integration of bioreceptors / biorecognition element with the physiochemical transducer led to the development of newer and smarter biosensing devices. Various smart materials based on nanomaterials have been developed and used successfully for various biotechnological and

environmental applications.

Main components of biosensor are analyte, the biorecognition element and the transducer. Biorecognition element helps in easy and selective recognition of analyte and its interaction is displayed in form of physical and chemical properties, which varies in direct proportion with the variation in concentration of analyte via the transducer. The word transducer is derived from Latin word "transducere" which means to transfer or translate. A device that translates information from one kind (for example chemical) of system to another (for example current) is called a transducer. Recognition layer comprising of various biomaterials to synthetic biomimetic materials (similar in fashion to that of biological origin) has been developed and used for sensitive and selective biosensors as given in figure 1.1.

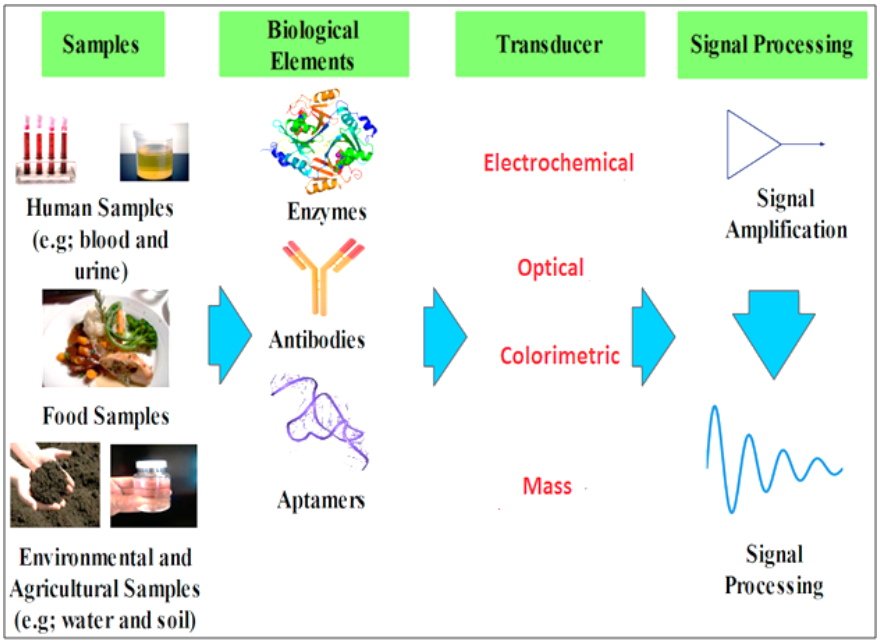

Figure 1.1 Components of Biosensor.

Above schematic is showing three elements of biosensors based on electrical transducer. First is sample, which is having target species present in bio-fluids in form of whole

cell, antigens, enzymes and proteins or in food, or environmental samples as some chemicals or bacteria. The target species is recognized based on the selective material immobilized over the transducers as enzyme, antibody, aptamer or DNA [Luka et al., 2015] and the corresponding signal is obtained.

1.2 Features of Sensors

Some of the essential features of a typical biosensor are:

1.2.1 Sensitivity

Sensitivity is defined as the ability of a device to generate as large a response as possible, on interacting even with small amount of analyte. A sensor is said to be sensitive, if a small change in the analyte concentration causes a large change in the response. Within the linear range of response, the sensitivity is a well-defined value. Sensitivity of a biosensor depends upon (a) sensitivity of the detection system and (b) distribution of the functional biological molecules near the sensor surface.

1.2.2 Selectivity

Selectivity is defined as the ability of a sensor to detect one specific species even in the presence of a number of other chemical species or interferents [Aoi et al., 2013]. High selectivity enables a biosensor to inhibit the interfering species from contributing and thereby imparting the accuracy in the measurements of the analyte.

1.2.3 Response Time

Response time is the time required by a biosensor in order to generate the response as a result of biorecognition event. It is the collective time taken by the interaction event and

transduction of the generated response through the transducer. Response time of an ideal biosensor should be low in order to have a real time quick diagnosis.

1.2.4 Limit of Detection

Limit of detection is the minimum analyte concentration, which must be present to produce an analytical signal that can be distinguished from analytical noise" (the signal produced in the absence of analyte) within a confidence limit with reasonable certainty for a given analytical procedure. International Union of Pure and Applied Chemistry (IUPAC) has defined limit of Detection (LOD) as in given equation [Nien et al., 2006].

$$LOD = kSD/S$$

Here, SD is the standard deviation of the background signal of the system (before injection of analyte), S is the sensitivity of the biosensor and k is the numerical factor chosen in accordance with the desired confidence level. The use of k = 3 allows a confidence level of 99.86% for a normal distribution.

1.2.5 Shelf Life

Shelf life of a biosensor is defined as the length of time until a biosensor (under the specified storage conditions) remains suitable for analyte assay. Also known as storage stability, it is the extent to which a device retains (within specified limits) throughout its period of storage and use, the same properties and characteristics that it possessed at the time of manufacture [Teixeira et al., 2006]. This feature takes into account the time dependent degradation in the performance of a device. Long shelf life is one of the essential features for a commercial reliable biosensor.

1.3 Generations of Electrochemical Biosensors

Based on transfer of electrons from the interface of recognition layer and solution containing analyte to the transducers, the advancement in electrochemical sensors/biosensors is named as first, second and third generations of sensors as described below:

1.3.1 First Generation Biosensor

This generation includes such biosensor that has a recognition layer and transducer that are well separated e.g. Estimation of glucose using GOX. Here the rate of reaction is calculated based on consumption of O_2 or production of H_2O_2.

1.3.2 Second Generation Biosensor

Here the recognition layer and transducer are made in contact with each other by making use of suitable mediator. This mediator competes with the produced electrons during a redox process e.g. Glucometer for home monitoring of blood glucose. It makes use of some artificial mediators such as FAD. Artificial mediators are useful in shuttling the electrons between the FAD center and the electrode surface by the following scheme:

$$C_6H_{12}O_6 + (FAD) + H_2O \rightarrow C_6H_{12}O_7 + GO_x(FADH_2) \quad \textbf{1.1}$$

$$GO_x(FADH_2) + 2M_{ox} \rightarrow GO_x(FAD) + 2M_{red} + 2H^+ \quad \textbf{1.2}$$

$$2M_{red} \rightarrow 2M_{ox} + 2e^- \quad \textbf{1.3}$$

Where Mox and Mred are the oxidized and reduced forms of the mediator. A reduced mediator is formed instead of hydrogen peroxide and then reoxidized at the electrode, providing an amperometric signal and regenerating the oxidized form of the mediator. Such mediation cycle produces a current dependent on the glucose concentration. Because of using these electron

mediators, measurements become independent of oxygen partial pressure and can be carried out at lower potentials that do not provoke interfering reactions from coexisting electro active species. Electron mediators such as ferrocene derivatives, ferricyanide, conducting organic salts (particularly tetrathiafulvane-tetracyanoquinodimethane, TTF-TCNQ) or quinone compounds have been widely used to electrically contact GOx [Frew et al., 1987: Song et al., 2006]. A major drawback of mediator based sensors is the competition between the mediator and oxygen for oxidation of the reduced GOx, which results in the accumulation of hydrogen peroxide near the electrode surface leading to reduced bioactivity of enzyme and so the biosensor response.

1.3.3 Third Generation Biosensor

This generation does not make use of any mediators. Here direct transfer of electron between the recognition layer and transducer occurs. Instead of mediators, the electrode can perform direct electron transfers (DET) using organic conducting materials based on charge-transfer complexes. The direct electron communication between the deeply buried redox active center of the enzyme and the electrode surface alters the conformational change of the enzyme, resulting in the loss of enzymatic activity. The third generation amperometric glucose biosensors still has lots of scope for improvement. Most of the biosensors currently being investigated belong to either first or second generation. The unique properties of nanostructures are being exploited to achieve parameters like high sensitivity, fast response, low detection limits, wide range linearity etc.

1.4 Classification of Sensors based on Detection Mode (Transducers)

1.4.1 Electrochemical Sensor

In the electrochemical sensor, the transduction element used is an electrode and it represents an important subclass of sensors. In accordance with IUPAC recommendation in 1999, an electrochemical biosensor is well defined as a self-contained integrated device, capable of providing specific quantitative or semi-quantitative analytical information in association with a biological recognition element (biochemical receptor) which is kept in direct spatial contact with an transduction element electrochemical in nature [Thevenot et al.,1999, 2001]. The current produced from oxidation and reduction reactions is measured by electrochemical biosensors. This current produced is directly proportional to either the conc. of the electroactive species present or its rate of production of electron/ consumption of electrons. In other words, the resulting electrical signal corresponds to the extent of recognition process occurring between target and analyte, varies directly with the analyte concentration. Basic classification of electrochemical sensors or biosensors is given in Figure 1.2. Some basic characteristics of this type of sensor is mentioned below:

- Specificity
- Selectivity
- Immense dependence on choice of material
- High sensitivity and low detection limits
- Gives results in real time or close to real time
- Can be further employed as miniaturized sensor

In such measurements, we get results based on potential (that relate to the qualitative properties based on thermodynamic and kinetic control and current). Here the species which is be adsorbed onto the electrode and electro analyzed should react directly or indirectly through coupled reaction. Moreover, the environment (medium) should be sufficiently conducting for the measurement purposes.

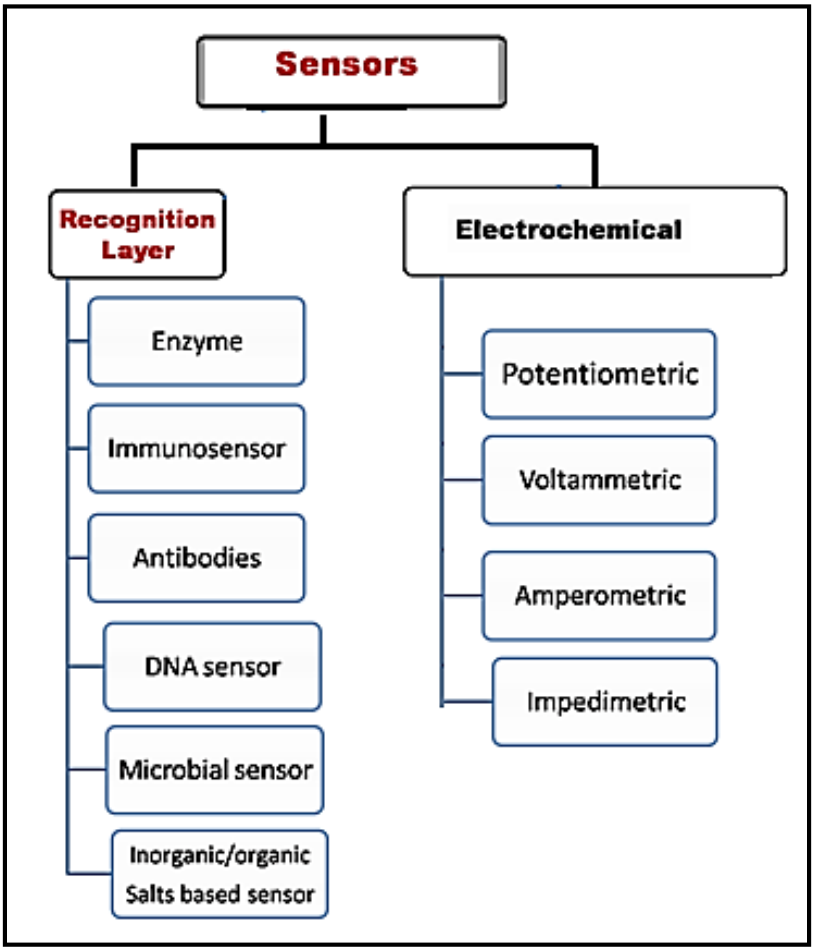

Figure 1.2 Classification of Electrochemical Sensors/Biosensors.

1.4.1.1 Potentiometric Sensors

Potentiometric biosensors depend on the use of an ion-selective electrode or ion-sensitive field effect transistor for getting the analytical information. Such sensors have such biological recognition element, which converts the recognition event into a specific signal. The figure 1.3 shows the one electrode potentiometry measurement of the potential of indicator electrode with respect to a reference (nonpolarizable electrode at open circuit with a small anodic or cathodic current to the indicator electrode. In two electrode potentiometry, the resultant curve (titration) is formed by applying the small constant current between two polarizable electrode which is kept same at anode and cathode.

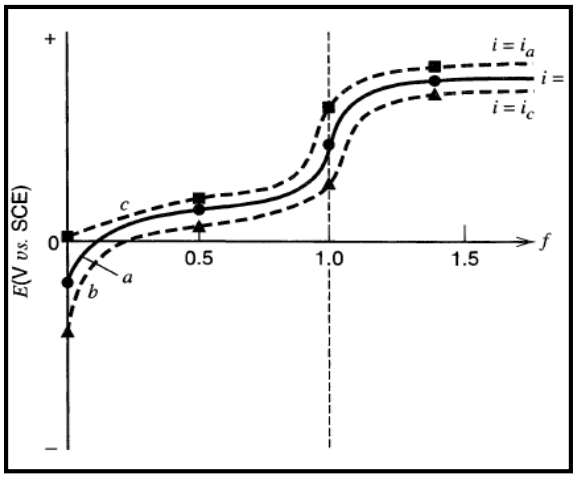

Figure 1.3 Potentiometric titration curves fora platinum indicator electrode vs SCE [Electrochemical methods by Bard et al.].

1.4.1.2 Voltammetric Sensors

It is the technique where the current behaviour at an electrode surface in response to applied voltage is measured. The potential is varied in such a manner that oxidation or reduction of the electrochemical species occurs. Current (Faradic) obtained is in direct

proportion to the concentration of the electrochemical species. Excitation signals in Voltammetry are given in figure 1. 4.

It uses three electrodes which are connected in such a manner to a power source (potentiostat) which precisely control the potential applied to the working electrode. In voltammetric technique when a potential scan is imposed the current that is flowing through the working electrode is measured, which is dipped in solution containing electro active compounds. The voltammetric technique follows Faraday law and Ficks law. Here the current corresponding to the quantity of material transported by diffusion and reacted part at the electrode is measured. The Faradic current is proportional to the concentration of electroactive substance present in solution. There are various methods for varying the potential such as linear sweep, differential staircase, reverse pulse, normal pulse, etc [Chaubey et al., 2002; Katz et al., 2003]. Among it the cyclic voltammetry is the most used type and gives information about the redox potential and electrochemical reaction rates for the concerned analyte in solution.

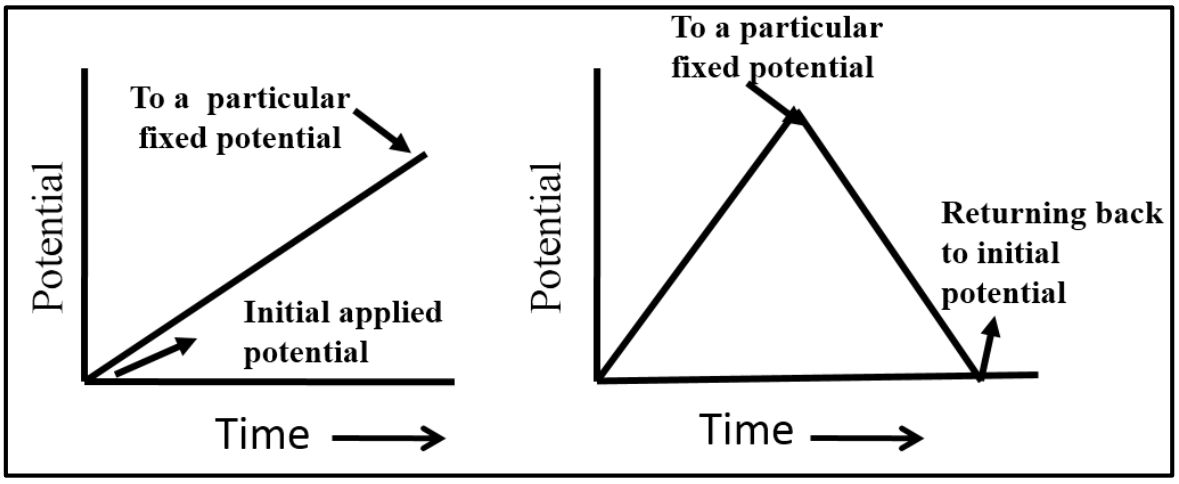

Figure 1.4 Excitation signal for Voltammetry.

1.4.1.3 Amperometric Sensors

It is considered to be a part of voltammetric technique based on the measurement of current at a fixed operating potential. Current estimated after experiment is directly proportional to the potential applied. Amperometric methods can further of two types Similarly for amperometric, when a small cathodic current rent is applied, the potential will be more negative (a) constant applied voltage, one polarizable electrode (one-electrode amperometry) (b) constant applied voltage, two polarizable electrodes (two-electrode amperometry). In the figure 1.4 current potential curves at platinum electrode for Fe^{2+} titration is shown. *One-electrode amperometry* involves maintaining the potential of the indicator electrode at a constant value with respect to a reference electrode and determining the current as a function of the potential of the indicator electrode maintained at a value on the plateau of the *i-E* curve for Fe^{2+} oxidation as given in Figure 1.5.

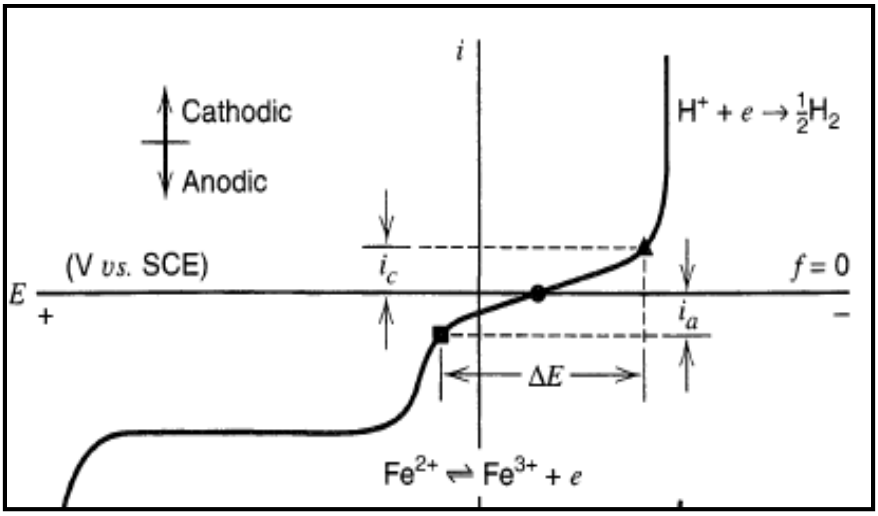

Figure 1.5 Current potential curves at platinum electrodes for Fe^{2+} titration [Electrochemical methods by Bard et al.].

1.4.1.4 Impedimetric Sensors

In this approach, the electrochemical cell is perturbed with an alternating potential in small magnitude until a steady state is reached. Merits of this technique include firstly, the ability to make sensitive measurements and gives averaged value over a long term process for unsteady behavior. Secondly, an ability to give the response theoretically using linearized current-potential characteristics and thirdly measurement is possible for both the wide time scale or frequency range (10^4 to 10^{-6} s or 10^{-4} to 10^6 Hz). Impedimetric experiments are usually performed close to equilibrium, so detailed knowledge about the current-potential curve behavior for over ranges of potential is must and this simplifies the studies of parameters taken under consideration. Impedance spectroscopy share major advantages over other detection methods detection regarding lower concentration of analyte. In a recent study it has been seen EIS based transduction has been employed successfully in tumour growth detection is 100–600 fg/ml shows sensitivity/detection limit of 100 fg/ml [Uygun et al., 2011]. EIS measurement is suitable for real time recording because it provides a label free or reagent less detection [Parkinson et al., 2005]. In this EIS measurement, the sample is positioned on the sensing device such as nanogap, and alternating voltage is applied to the electrode in a controlled manner, and the current flowing through the sample are tracked. Electrical impedance resulting from the sample is estimated as the ratio of voltage over current. Resultant electrical impedance measurement has both a magnitude and a phase and is a complex quantity. For any voltage applied under time-varying conditions, the resulting current can show to be in phase with the applied voltage (resistive behaviour) or out of phase with it (capacitive behaviour, as shown in Figure 1.6).

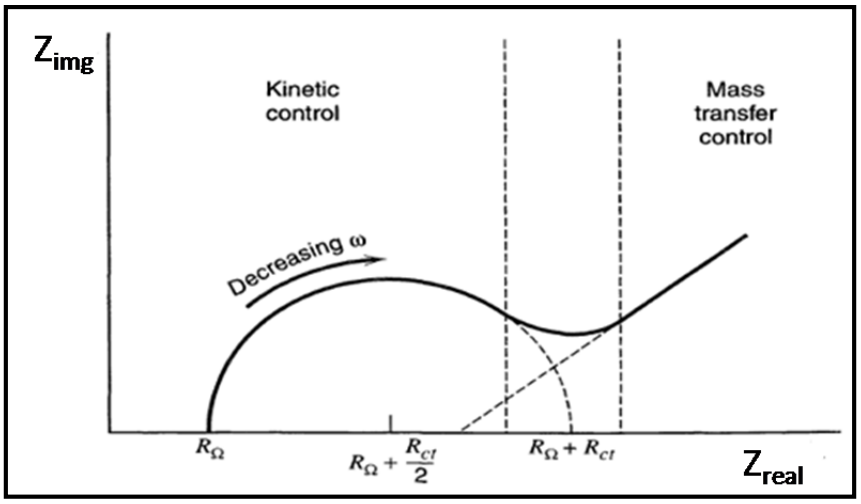

Figure 1.6 Impedance plot for an electrochemical system (showing regions of mass-transfer and kinetic control are found at low and high frequencies).

In a latest review by Yang and Bashir, a detailed discussion on advancement and applications of the impedance biosensor for detection of foodborne pathogenic bacteria has been demonstrated. Impedance spectroscopy finds wide applications in detection of cancer/tumour cells, viruses, bacteria and pathogens [Ohno et al., 2013; Mishra et al., 2012; Wang et al., 2012].

Where, Z_{Real} and Z_{Img} are the real and imaginary parts of the impedance. For example, Z_{Real} and $Z_{Img}=X_c=1/wC$. The magnitude of Z, written Z or Z is given by:

$$|\boldsymbol{Z}|^2 = \boldsymbol{R^2} + \boldsymbol{X_C^2} = (\boldsymbol{Z_{Real}})^2 + (\boldsymbol{Z_{Img}})^2$$

And the phase angle$\emptyset$ is given as

$$\boldsymbol{tan\emptyset} = \frac{\boldsymbol{Z_{Img}}}{\boldsymbol{Z_{Real}}} = \frac{\boldsymbol{X_C}}{\boldsymbol{R}} = \boldsymbol{1/\omega RC}$$

$$\boldsymbol{Z}(\boldsymbol{\omega}) = \boldsymbol{Z_{Real}} - \boldsymbol{jZ_{Img}}$$

1.4.2 Optical Sensors

A fiber optic sensor is a sensor that uses optical fiber either as the sensing element ("intrinsic sensors"), or as a means of relaying signals from a remote sensor to the electronics that process the signals ("extrinsic sensors"). Generally, they make use of optical fibres for measurements and gives spectroscopic results. Depending on the methods of detection, they are sub categorized as given below.

1.4.2.1 Fluorescence Sensors

Fluorescence spectroscopy (also known as fluorometry or spectrofluorometry) is a type of electromagnetic spectroscopy that analyzes fluorescence from a sample. It involves using a beam of light, usually ultraviolet light, that excites the electrons in molecules of certain compounds and causes them to emit light, typically, but not necessarily, visible light. A complementary technique is absorption spectroscopy. In the special case of single molecule fluorescence spectroscopy, intensity fluctuations from the emitted light are measured from either single fluorophores, or pairs of fluorophores. Fluorescence process involves the emission of photons within the nanoseconds as an outcome of absorption event on interaction (the analyte under the excitation beam). Fluorescent sensors e.g. metal detection by fluorometry Fresinius et al., 2000; Fassen et al., 2015]. This sensing makes use of a metal chelating or binding moiety and at least one metal detection fluorophore, which can absorb and reflect light effectively.

1.4.2.2 Absorption Sensors

Absorption spectroscopy is an analytical tool, which can determine the presence of a particular substance in a sample as well as can quantify the amount of the sample. These days

IR and UV-Vis measurements are very common for analytical applications. Absorption spectroscopy finds applications in studies of molecular and atomic physics, astronomical spectroscopy and remote sensing [Jollymore et al., 2012; Bi et al., 2016].

1.4.2.3 Reflection Sensors

Reflectance spectroscopy deals with study of the surface of a material relating to the capacity of reflecting the radiant energy. Mathematically speaking, it is denoted as fraction of incident electromagnetic power that is reflected at an interface. Reflectance spectroscopy is the study of light as a function of wavelength that has been reflected or scattered from a solid, liquid, or gas. As photons enter a mineral, some are reflected from grain surfaces, some pass through the grain, and some are absorbed. Those photons that are reflected from grain surfaces or refracted through a particle are said to be scattered. Scattered photons may encounter another grain or be scattered away from the surface so they may be detected and measured. Remote diffuse reflectance spectroscopy sensor for tissue engineering monitoring based on blind signal separation by Mateos et al., 2014.

1.4.3 Mass Sensitive Sensors

Mass sensitive sensors are based on piezoelectric effect and include devices such as surface acoustic wave sensor. Shift in the resonant frequency of the oscillation occurs which corresponds to change in mass of the surface of the oscillating crystal. The changes of resonators' characteristics had been employed to study the behavior of external perturbations

Currently, the commonly used resonant sensors are quartz crystal microbalances (QCMs), which can correspond the resonant frequency shift to the solid-state mass loading. [Zhang et al, 2003].

1.4.4 Surface Plasmon Resonance Sensors (SPR)

Surface plasmon resonance is the resonant oscillation of conduction electrons at the interface between a negative and positive permittivity material stimulated by incident light. The resonance condition is established when the frequency of incident photons matches the natural frequency of surface electrons oscillating against the restoring force of positive nuclei. Surface plasmon resonance is important technique for monitoring the affinity and selectivity of biomolecular interactions. Biosensors based on the SPR technique can perform real-time detections and on-line controlling of food processing. They also show potential of producing quantitative information, without the use of label (fluorescence) compounds in less than an hour. SPR has proven to be profitable, an easy approach used in food safety industry. This sensing is popularly used in detection of food toxins [Hodnik et al., 2009].

1.4.5 Chemiluminiscence Sensors

Chemiluminiscence is the phenomenon in which light is produced from a chemical reaction. Two chemicals react and form an high-energy intermediate in excited stage, which breaks down to release energy in form of photons. Advance chemiluminiscence detection makes use of CCD camera, which records photons and shows an image in accordance with the amount of light generated during a reaction [Yang et al., 2015].

Both the optical and electrochemical detection methodologies are gaining mutual importance in the development of immunosensor [Bhatta et al., 2010b]. Immunosensor for bacteria and pathogen detection are used in the point of care measurement [Braiek et al., 2012; Holford et al., 2012].

1.4.6 Thermal sensors

Sensors are devices that measure a physical or chemical reaction, such as volume flow, heat flux, through changes in electric resistance, or signal [Kenny et al., 2004]. Some common examples of thermal sensors are thermocouples, themistors, silicon sensors, resistance thermometers.

1.4.7 Pressure sensors

Basically electronic pressure sensors generally use a force collector (such a diaphragm, piston, bourdon tube, or bellows) to measure strain (or deflection) due to applied force over an area (pressure). These types of electronic pressure sensors use other properties (such as density) to infer pressure of a gas, or liquid. It is widely used in sensing of altitude, flow, level, depth.

1.5 Classification of Biosensor based on recognition layer

1.5.1 Enzyme as recognition layer

Enzyme immobilization is the process in which enzyme (biological component) is appropriately attached to the transducer for sensing purposes. Major focus during the preparation of biosensors is on loading capacity of enzyme for sufficient biocatalytic activity and also conducive environmental conditions for the enzymatic activity. Immediate environment of the biosensor is quite important for the enzyme stability. While choosing the immobilization method, various factors are kept in mind such as the recognition (biological) element nature, transducer mode, analyte, chemical and physical properties and conditions in which the bio sensor is operated.[Singh et al.,2008]. Comprehensive data covering the advantages and limitations of enzyme immobilization methods are present in literature [Nunes

et al., 2006]. A typical example of enzyme based biosensor HRP enzyme was immobilized onto CeO_2/rGO for sensing of H_2O_2 in the figure 1.7.

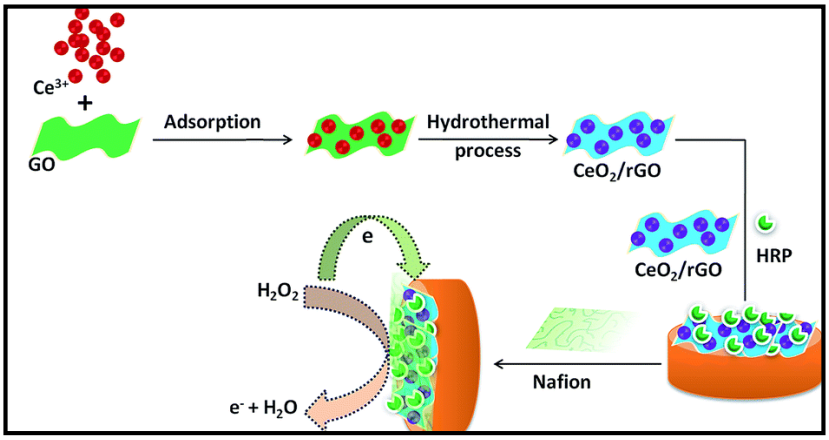

Figure 1.7 H_2O_2 detection scheme using HRP/CeO_2-rGO modified glassy carbon electrode [Radhakrishnan et al., 2015].

1.5.2 Immunosensor

Immunosensor employs a wide range of materials for the rapid and sensitive analysis of a range of pathogens and associated toxins are mentioned below.

1.5.3 Antibodies

Various types of antibodies such as polyclonal, monoclonal and recombinant are being used for immunosensor based pathogen detection. Certain characteristics are to be kept in mind, during the selection of any monoclonal, polyclonal or any recombinant antibodies for detection purposes. Figure 1.8 shows a typical example of immunosensor. Primarily, the antibody shows capacity to detect and quantitate even very low cell number (sensitivity). Low cell no issue is major issue for foodborne related bacterial pathogens detection. Another one is

ability to differentiate among related microflora of same species present in the sample (specificity). Potentiometric biosensor based on biotinylated polyclonal antibody for *B. subtilis* has been successfully fabricated [Uithovenet al, 2000]. A potentiometric biosensor based on rabbit polyclonal anti M Vibrio has been made for the *V. cholerae* [Diaz-Gonzalez et al., 2005]. In this line a magnetic sensor for *Y. pestis* has also been developed [Jyong et al., 2006]. Piezoelectric biosensor based on rabbit anti M biotinylated antibody for *M. tuberculosis* is contructed [Meyer et al., 2007].

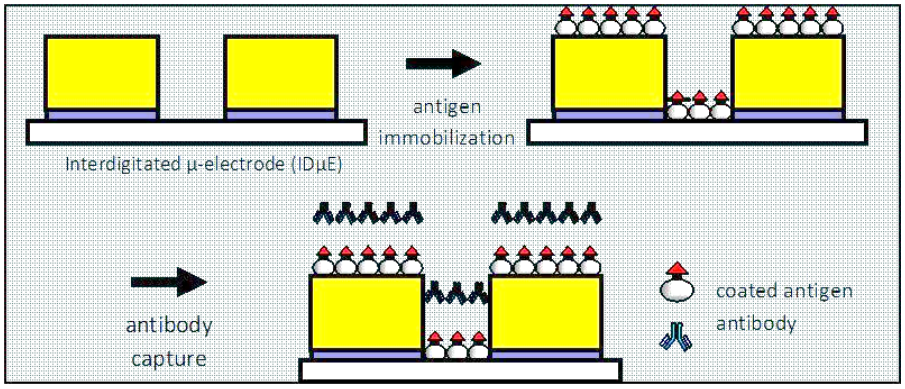

Figure 1.8 An example of immunosensor [Rodriguez et al., 2013]

Wide range of immunosensor-based sensing platforms has also been fabricated also for the detection of other minor bacterial pathogens, including *Yersinia pestis*, *Vibrio cholerae*, *Mycobacterium tuberculosis* and *Brucellaabortus*. Strains such as *Clostridium difficile* and methicillin-resistant *S. aureus* (MRSA) are also been responsible for nosocomial infections and serious health hazardous to humans. A piezoelectric immunosensor for detecting 0.1 µg/mL of SEB (enterotoxin) through the development of a competitio assay [Harteveld et al., 1997]. Figure 1.3 shows interdigitated µ electrode onto which antigen immobilized for

antibody capture.The most popular strategy under it is change in mass detection when antigen interacts with antibody.

1.5.4 DNA Sensor

DNA is a powerful moiety that carries with itself genetic instructions for vital processes of life such as growth, development, functioning and reproduction processes. Three major macromolecules present in nearly all living beings are nucleic acids, proteins and complex carbohydrates (polysaccharides). Mostly DNA molecules is present in native form as two biopolymer strands coiled together form a double helix. A DNA strand chemically composed of polynucleotides since they are composed of simpler units called nucleotides. A typical single nucleotide comprises of a nitrogen-containing nucleobases. A typical single nucleotide comprises of a nitrogen containing nucleotides-either cytosine (C), guanine (G), Adenine (A), Thymine (T)-with a sugar called deoxyribose and a phosphate group [Alberts et al., 2012]. The nucleotides interact with another in a chain via covalent bonds between the sugar of one nucleotide and the phosphate of the next, leading to formation of alternating sugar-phosphate backbone. Base pairing rules (A with T, and C with G) suggests that hydrogen bonds bind with the nitrogenous bases of two individual polynucleotide strands in order to form double-stranded DNA(Figure 1.9).

The basic principle behind the nucleic acid biosensor is affinity based complementary binding between the two single stranded where the nucleic acids was employed as the biological recognition element.

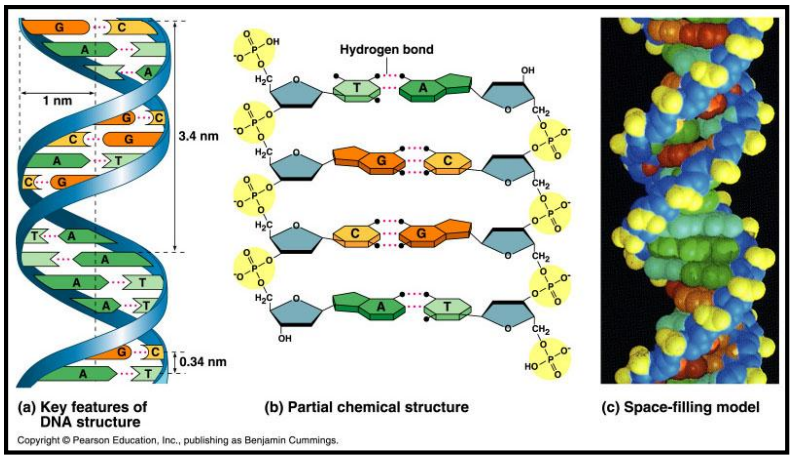

Figure 1.9 Shows DNA structure and interaction between base pairs.

This development in biosensor field led to DNA based sensor platform that was superior from traditional method such as electrophoretic separations which makes use of an radio isotropic agents in terms of cost, hazardous nature, time consumption etc., [Parkinson et al., 2005].

Speciality of nucleic acid based sensor is that it can be easily denatured and show reversibility in binding and further can be regenerated by modifying the buffer ion [Parkinson et al., 2005]. The nucleic acid based biological recognition layer in association with transducer are synthesizable, reusable (when thermal melting of DNA duplex has been done) and highly specific [Teles et al., 2008]. Such biosensors are exclusive in terms of specificity and can distinguish even a single molecule species among complex collection [Brett, 2005]. DNA based biosensor show application in detection of virus and disease in clinical diagnostics [Chua et al., 2011; Thuy et al., 2012]. Manufacturing of an electrochemical DNA biosensor has received a great deal of attention lately and driven by motivation to develop rapid response, high sensitivity, good selectivity and experimental convenience. In the direction of development of hybridization-based sensors, use of linker such as thiol or biotin was used to

immobilize the ssDNA [Lazerges et al., 2012]. A typical hybridization sensor based on nanospheres coated on carbon electrode for immobilization of ssDNA is used for sensing of DNa via cyclic voltammetry (Figure 1.10). More developments in techniques nanocantilevers were used to determine the specific DNA and for monitoring the serum protein marker levels [Rizvi et al., 2010]. Quantum dots, highly fluorescent semiconductor nanocrystals, used to detect specific protein or DNA. *In vivo* screening and treatment also makes use of nanobiosensors for signalling and manufacturing of therapeutic delivery devices for [Kauffer et al., 2014]. Latest advancement in biosensor is a biomimetic biosensor [Yeh et al., 2012]. An artificial or synthetic sensor mimics the natural biosensor in its function e.g. Aptasensor [Hussain et al., 2013: Song et al., 2012: Jarcjewska et al., 2016].

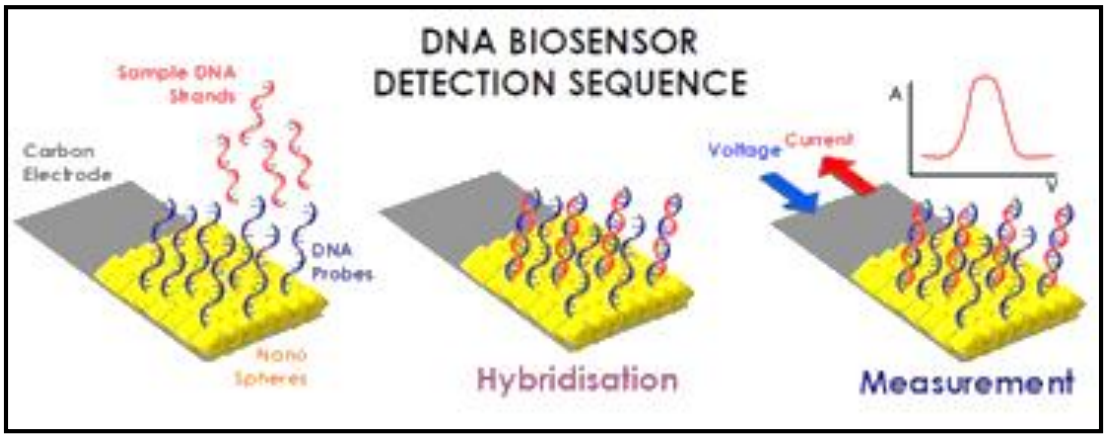

Figure 1.10 A typical DNA biosensor.

1.5.5 *Microbial Sensor*

Turner et al., 1996, first described microbial sensor. Microbial sensor primarily consists of a transducer and microbe acting as a sensing element. Microbial sensors have very different characteristics as that of enzyme sensors or immunosensors especially when specificity of substrates is concerned. RDT (Recombinant DNA Technology) based methodologies has

helped immensely in improvisation of microbial sensor. Microbial sensors show enormous advantages such as tolerance towards physical conditions and being cost effective [Park et al., 2013; Nakumura et al., 2008]. In the figure 1.11 we have shown the sensing mechanism for both mediator and mediator less sensing using microbial cell. Considering the transducer based classification as discussed, we observe that the electrochemical based transduction is superior to other transducer modes and also with association with different recognition layers.

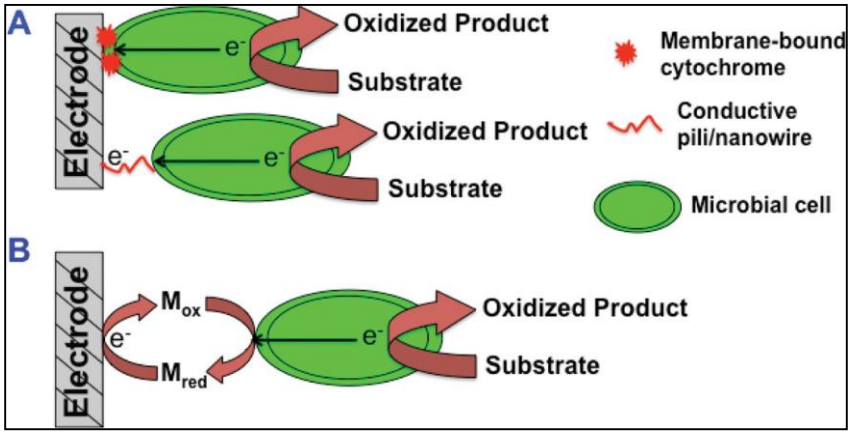

Figure 1.11 Showing a typical example of microbial cell biosensor [Hassan et al., 2012].

1.6 Matrices and immobilization of molecules in biosensors

The formation of recognition layer over transducer is a challenge in development of biosensors. It is most crucial as sensitivity, reproducibility, stability and all types of performance of sensors depends on quality of matrices and immobilization. Representary features of the matrices include physical resistance towards compression, hydrophilicity inertness, biocompatibility, resistance to microbial attack, and availability at low cost [Brena et al., 2006]. Wide range of natural polymer materials as support materials in use are cellulose, alginate, chitin, collagen, etc [Bai et al., 2006] Apart from the natural polymers synthetic

polymeric materials are also employed as support. Characteristic of synthetic polymer are good mechanical stability, ease in modification [Bryjak et al., 2006]. Inorganic supports for the immobilization of enzymes, namely, alumina, silica, zeolites, and mesoporous silicas [Hudson et al., 2008]. Silica-based supports find use in major areas [Humphrey et al., 2005]. These carriers have enormously large surface area which imparts it good immobilization efficiency. Methods of immobilization are of various types as given in figure 1.12.

1.6.1 Adsorption

It is the most simple and quickest way for preparing enzymes for immobilization. Adsorption is either physical adsorption or chemical adsorption.

1.6.1.1 Physical Adsorption

Physical adsorption is both simple and fast method for developing biosensors. It includes methods such as Van der Waals, which are weak interactions We deliberately do reduction of the nanoparticles employing some stabilizing and reducing agents. Stabilizing agents provide the nanoparticles stability (arising due to electrostatic repulsion and also helps in formation of colloidal particles. Physical adsorption methods are simple and fast in application. Its disadvantages lies in orientation issues and tedious functionalization procedures (adding functional groups).

1.6.1.2 Chemical Adsorption

It includes the crosslinking, self assembly monolayer, covalent bonding based methods etc. In comparison, chemical adsorption is much stronger due to the presence of stronger bond such as covalent bonds.

1.6.1.2.1 Covalent bonding

Most common methods of chemical adsorption is covalent binding between the biomolecule and the electrode surface (the colloidal gold surface) occurs here the –SH groups of the cysteine residues interacts with Au on the GNP surface [Brogan et al., 2003]. Covalent bonding does not hamper the catalytic activity since the functional group involved is inert with the corresponding after bonding.

1.6.1.2.2 Crosslinking

Here the biomaterial is connected to the solid supports via the crosslinking agent. Adsorbed biomaterials are immensely stabilized by this method. Glutaraldehyde is the most popular bifunctional agent in use.

1.6.1.2.3 Self-Assembling Monolayers (SAMs)

SAMs considered as a simple and well known tried method of immobilizing nanoparticles and biomolecules onto electrodes (specially the gold nanoparticles). Self assembly provides high degree of control in terms of composition and thickness for the transducer surface [Lin et al., 2009]. Functional groups (–CN, –NH2, or –SH) of SAMs-modified electrode surface are employed for attaching biomolecules e.g. alkanethiols. These are the most profound contains short-chain molecules of 3-mercaptoproprionic acid with

cystamine are self-assembled on the modified electrode for further attachment with nanoparticle [Kumar et al., 2007]. These molecules also furnish the functional groups needed for covalent immobilization of the biomolecules. Zhang *et al.*, utilized SAMs-modified electrodes for the construction of GOx-based biosensor [Zhang et al., 2005]. Another simple immobilization process was demonstrated by Jia *et al.* with horseradish peroxidase (HRP). Here, gold nanoparticles were used to adsorb the horseradish HPR via chemisorption onto the SH group of the SAM layer. Abad *et al.*, gave the strategy for the covalent immobilization of glycosylated enzymes using the binary SAMs for colloidal nanoparticles [Park et al., 2010]. Boronic acids are used to form SAMs for adsorbing the glycoprotein onto the electrode surface by interacting with the sugar groups (of boronic acid). This strategy showed increased immobilization rate and also formation of very stable covalent bonds.

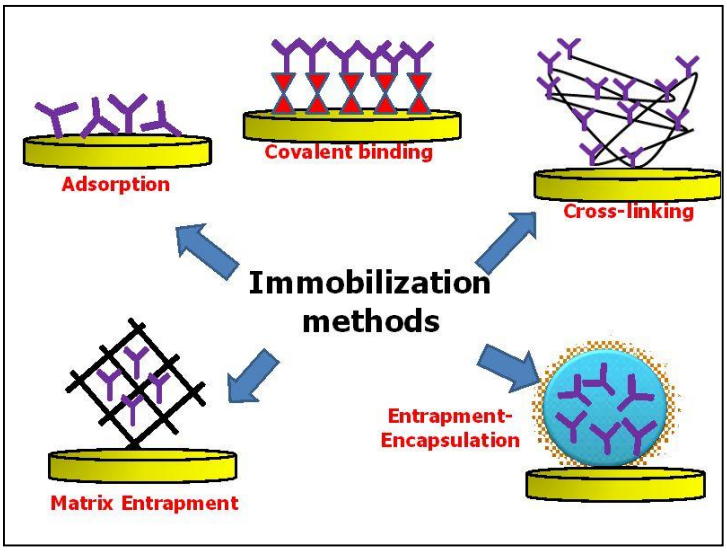

Figure 1.12 Showing Immobilization methods [Rodriguez et al. 2015].

1.6.1.2.4 Co-Modification with Electrode Matrix

This approach involves the co-immobilization of the nanoparticles with the biomolecules species to form a composite material in order to transfer the electric signal from the active site to the electrode, via the polymer-bound colloidal nanoparticles.

Disadvantages of the SAM such as formation of very compact layers leading to overcrowding and steric hindrance on the binding sites, is overcomed by this method [Challa et al., 2010].However, using such composites control the dispersion of the nanoscale species.

1.7 Applications of Biosensors

Biosensors are easy in operation and require no sample pre-enrichment, unlike other methods such as specific nucleic-acid based methods and conventional immunological methods which primarily need the sample pre-enrichment for concentrating the pathogens number before detection [Singh et al., 2013]. It finds major applications in diagnostic market and clinical testing and other markets pertaining to agriculture. In diagnostics field its applications is continually expanding (Figure 1.13). Since diagnostic field is the tool by which the diseases or infection prevention is possible before actual treatment or remedial procedures. The key point is the detection of any illness at the bud stage, before the actual manifestation of disease has occurred. In clinical testing point of care, diagnostics are in use for agriculture and on site environment testing (metal). Need of the biosensor in various field is highlighted as given in given figure 1.15 : increasing trends specially in field of environmental testing(12.6 % to 14.3 %); Biodefense (2.6% to 3.3 %); home diagnostics(19.2% to 20.2%) and decreasing trends in Research lab of care (11.2 % to 10.7 %); Point of care (47.9% to 44.9 %); Process industries(6.8% to 6.6%).

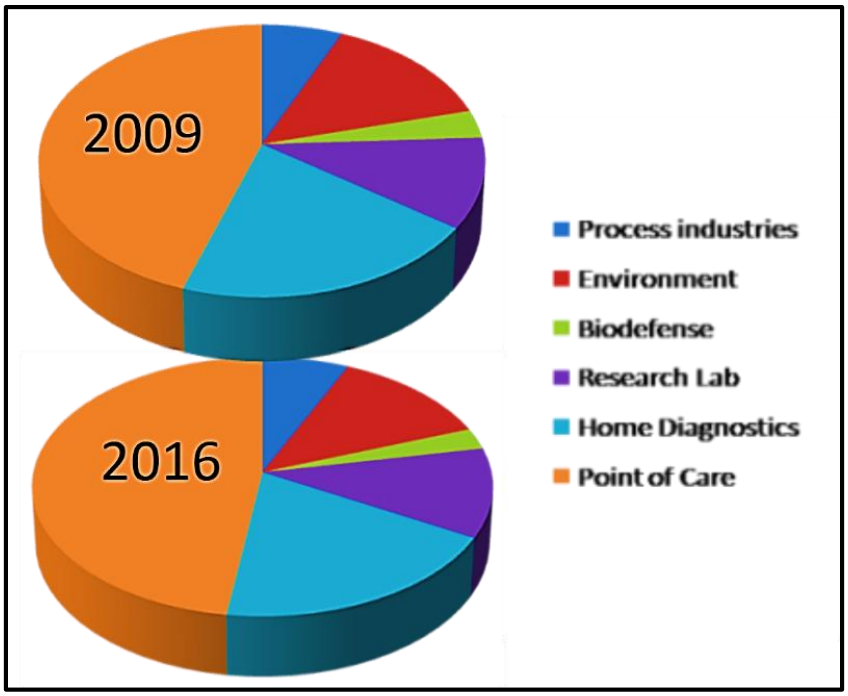

Figure 1.13 Total biosensors market: Percent Revenues (World) [Frost & Sullivan].

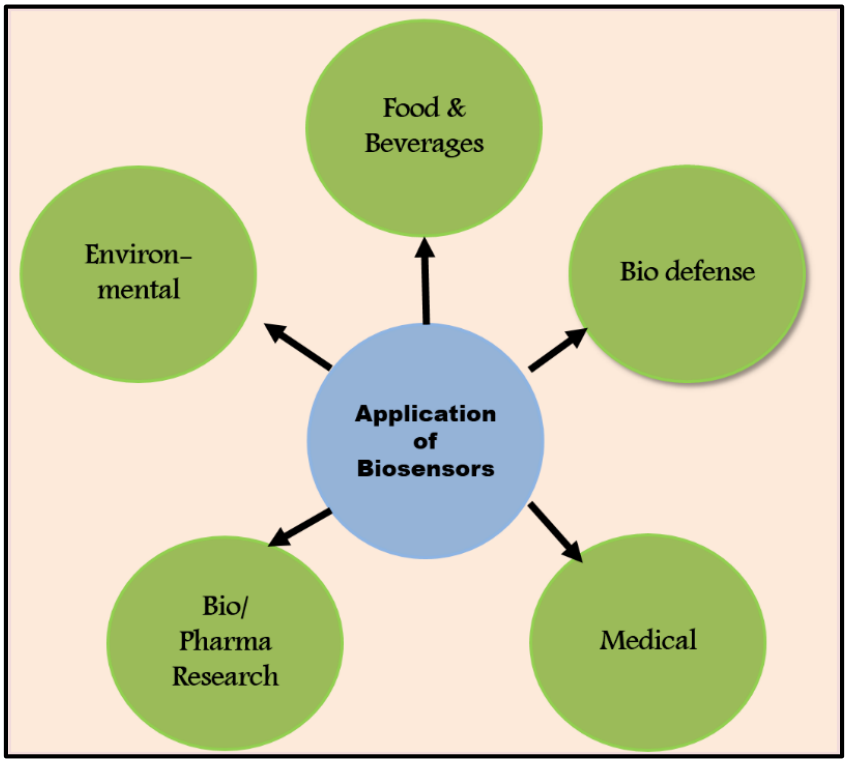

Figure 1.14 Few applications of biosensors.

Its second major applications is in food safety. Also find use in fermentation process for online monitoring of metabolites [Chellaram et al., 2013]. At present biosensors shows enormous potential in medical field. Glucose biosensors are widely used in clinical applications for diagnosis of Diabetes mellitus, in which requires precise control over blood-glucose levels is mandatory [Rhea et al., 2009].

In addition, sensor for multiple pathogen detection has also been manufactured. Pt-Au bimetal nanoparticles based colorimetric sensor has been constructed for detection of *E. coli* O157:H7 [Ziang et al., 2016]. Nanoparticle functionalized multijunction has been based on PEI for the pathogen detection [Yamada et al., 2016]. SPR sensor for food borne pathogen in complex samples has also been developed [Lisalova et al., 2016]. Fluorescent biosensors probe ions, metabolites, and protein biomarkers with great sensitivity. Such sensors can also report the presence, activity or status of the target (serum, cell extracts) in complex solution [Wang et al., 2009; Morris et al., 2010]. In the pharma industry, biosensors are used as effective tools for preclinical evaluation and clinical validation of therapeutic potential, bio distribution and pharmacokinetics of candidate drugs [Caruso et al., 2010; Watson et al., 2011]. Latest are in fields of tissue engineering and regenerative medicine. Both the fields are fast growing fields and can support bioengineering in restoring the damaged tissues and organs and in healing diseases [Rouchi et al., 2014]. Biosensors are gradually playing a cardinal role in microfluidic tissue engineering models.

Regarding the commercial aspects, they show capacity to sense biological molecules using the miniaturized tissue preparations in real-time as well as at very low concentration levels in association with ultrasensitive methods of detection (optical, electrochemical, or acoustic sensing systems). It also shows potential for *in vivo* sensing of diseases specific

biomarkers [Hasan et al., 2006]. The device operating in *in vivo* environment show the capacity of real-time signal generation of biologicals, such as the antibodies production with respect to tissue damage, release of proteins, and in various inflammatory events or infections. Biosensor shows a unique advantage of displaying the progress of disease specially for early stage disease detection and treatment [Speers et al., 2006]. Few popular applications of biosensors are mentioned below (figure 1.14).Commercial biosensors are available in market for glucose, for bacteria *E. coli*, Influenza A and B viruses, for various diseases and food and water analysis and environmental analysis [Chee et al., 2013]. In this regard, miniaturized platform in form of screen printed electrodes are very popular.

1.8 Scope of work

1.8.1 Need of stable sensing platforms

Wide number of materials has been employed such as inorganic and organic materials as matrix for development of stable sensing platforms. The ideal matrix should provide stable and easy immobilization to biomolecules and transfer of signal from biomolecules & analyte interaction to transducer for a readable signal. Recently two matrices got much more attention one organic conjugated polymers (conducting polymers) and second metal nanoparticles impregnated biopolymers. Conducting polymers have emerged as prospective candidates for electrochemical sensors due to having excellent conductivity (i.e. electronics as well as ionic) and compatibility with inorganic and biomaterials both. They show simple preparation methods and peculiar redox properties. Conducting polymers can be electrochemically grown directly on electrodes (transducers) and allowed for immobilization of biomolecules such as DNA, enzyme, antibody, aptamer etc. Various electrochemical detectors have been developed using conducting polymers immobilized with enzymes/antibodies/ssDNA as the recognition

layer for sensors [Shimidzu et al., 1987; Li et al., 2009; Nie et al., 2009; Rehman et al., 2015]. Studies on Polycarbazole-Modified electrode and its applications in the development of solid state potassium and copper (II) ions sensors were done [Pandey et al., 2000]. Polyindole modified potassium ion-sensor using dibenzo-18-crown-6 mediated PVC matrix membrane were synthesized for various applications including sensing [Pandey et al., 1998]. Electropolymerized polyindole were used in the construction of a solid-state, ion-selective electrode by Pandey et al., in year 1998. The second type of matrices as biocompatible metal-polymer nanocompositesfor biosensing has seen a boom in recent decade. Enormous increase in wide variety and categories of nanoscale materials with different shapes, sizes and composition ratio are employed for bio sensing application efficiently. Frequent use of nanoscale materials is used as effective matrixes in fabrication of biosensing and bioelectronic devices. Contributing candidatory features of nanomaterials for biosensing are their high contact surface area, biocompatibility nontoxicity, charge-sensitive conductance and ease in use and handling [Wang et al., 2005]. Both the matrices are having several advantages, however, not much explored for DNA based sensors (genosensors). There is still need to study for stable immobilization of DNAs and oligomers on these matrices. Moreover, compatibility of polymers and nanoparticles is a serious issue in the DNA sensing. Therefore, in this thesis focus was made on these two matrices and a comparative study for DNA immobilization and sensor development. We have fabricated some of the biocompatible conducting polymers like polyindole and nanoparticles using carbohydrate polymers like chitosan etc. These polymers and composites were studied for immobilization of ssDNA (oligomers) and fabrication of genosensors.

1.8.2 Need of ease in immobilization techniques

Initial methods of immobilizations are based on physical methods such as adsorption or matrix entrapments etc. Although they are simple and rapid technique, their drawbacks lie in strength of binding and the orientation of biomolecules during sensing, which can lead to leaching and false results. For effective binding we can opt for covalent binding using EDC-NHS coupling with the immobilization matrices for holding the biomolecules or by providing supports for strong physisorption of biomolecules (viz. nanometals).Therefore, in this thesis we have focused on chemical immobilization using functional groups and nanometals for strong physical adsorption.

1.8.3 Need of detection of food and water borne pathogens

Pathogens that causes foodborne diseases are often referred as foodborne pathogens and they include bacteria, fungi, viruses, and parasites [Zhao et al., 2014]. According to a survey, foodborne pathogens and viruses are the main reasons of illnesses whereas bacteria are the primary causes of hospitalizations and deaths [Scallan et al., 2011]. Most common due to foodborne disease outbreaks are *Listeria monocytogenes, Escherichia coli* O157:H7, [Oliver et al., 2005; Scallan et al., 2011; Zhao et al., 2014]. Increasing number of percentage of people eating street foods and increased usage for processed ready-to-eat products has led to serious health issues among public [Lee et al., 2014]. Diagnostic tools need to be developed for the accurate detection of diseases. Conventional methods of detection were based on culturing and immunological methods and PCR based amplification technique. Limitations of former method lie in false negative results, lesser sensitivity and excess time [Alhalmlan et al., 2015; Ingerson-Mahar et al., 2012]. Scientist [Zhao et al., 2014] have developed novel methods with increased rapidity, sensitivity, specificity and suitability for in situ analysis. Especially with

food industry, fast detection methods are mandatory for quick detection of the presence of pathogens in raw and processed foods. Still a major problem lies in low copy number of pathogen and viable but non culturable pathogens (VBNC such as. *E. coli)*. For that purpose newer methods with time-efficiency, rapid, labor-saving and ability to decrease chances of human errors are required [Mandal et al., 2011].Not only the food borne diseases but water borne pathogens and its related diseases are also a major concern worldwide for mortality and enormous expenditure caused in its prevention and treatment [Castillo et al., 2015]. In water sample, we majorly see ETEC *E. coli* is responsible for major contamination. Initial methods were based on quantitative microbial risk assessment (QMRA) for pathogen contamination and it includes the surveillance, analysis detection methods and decision.

The answer to this problem is the development of Electrochemical DNA sensors, which can efficiently and can directly detect the food pathogen (*L. monocytogenes*) and water pathogen (ETEC *E .coli*.). Therefore, our major focus was on development of water and foodborne pathogens.

1.8.4 Need of low cost miniaturized sensor probes

Miniaturization of analytical systems, allows the handling of even low-volume samples, consumption of reagent is reduced and lesser waste generation. Miniaturization of biosensor platforms got much more attention due to need of less amount of biofluides mainly blood. Latest development is miniaturization of sensor electrodes using screen-printed electrodes in electrochemical sensing. An electrochemical-based device shows unique properties including sensitivity, selectivity and a wide linear range. Moreover they show possibility of miniaturization, supporting portability, little space and power requirement and

inexpensive instrumentation. During the past decade, successful fabrication has been done as microelectrodes, molecular devices, tailored interfaces and smart sensors. These developments gained popularity in field of electro-analysis and tried in new phases and environments [Thiyagaranjan et al., 2014; Honeychurch et al., 2003]. However, it is limited to a few lab scale sensors and hardly for genosensors.

Thus, easy to use sensors have effectively replaced the traditional beaker type electrochemical cells and bulky electrodes. Screen printing technology is a reliable technique for the development of portable, economical and disposable electrode based sensing systems [Hayat et al., 2014]. In view of these major efforts are required on developing screen printed sensors for quick and low cost detection of pathogens.

1.9 Objectives

Taking in consideration of above mentioned scope and need of advancement in genosensors proposed the following objectives for my thesis work.

- Recognition layer improvement based on stable matrix for the immobilization of biomolecules (ssDNA)with both the conducting polymer (5C Pin) and nanometals (Platinum nanomaterial) dispersed biopolymer (Chitosan) using chemical and strong physical binding.
- Development of genosensors specific for water and foodborne pathogens like *E. coli* and *L. monocytogenes*.
- Miniaturization of sensors probes for quick and low cost detection based on screen printed electrodes**.**