Chapter 1

Introduction & Literature Survey

1.1 General Introduction

A sensor is a device that receives signals due to the interaction of analyte/changes in the environment and converts them into a readable form. The sensors are found everywhere, and the world is full of sensors and their applications. These sensors ease our lives and find profound applications in home appliances, automobiles, fire alarms, robotics, medical diagnostics, etc. In general, the sensors are broadly categorized into three types based on sensing elements: physical, chemical, and biosensor. Physical sensors are used to quantify physical properties like temperature, pressure, mass, distance, etc. Chemical sensors identify and sense chemical species by recording their chemical responses. Biosensors have biological receptor moieties that are used to measure chemical substances and distinguish them from other sensors.

The typical structure of sensors consists of a receptor, transducer, and signal processing unit as shown in Figure 1.1. The receptor is an active or recognition part of the sensor that is able to react with the target analyte selectively among all other molecules. There may be several types of possible interactions between analyte and receptor such as enzyme-substrate reaction, antigen-antibody reaction, chelating interaction through coordination bond, etc. Transducers transform one form of energy into another form. They are able to convert the signal of the receptor to a measurable form such as light, color, current, voltage, resistance, stress, etc., and finally, a readable signal is obtained using a signal processing system that includes a signal amplifier, processing unit, and display [Putzbach et al.; 2013].

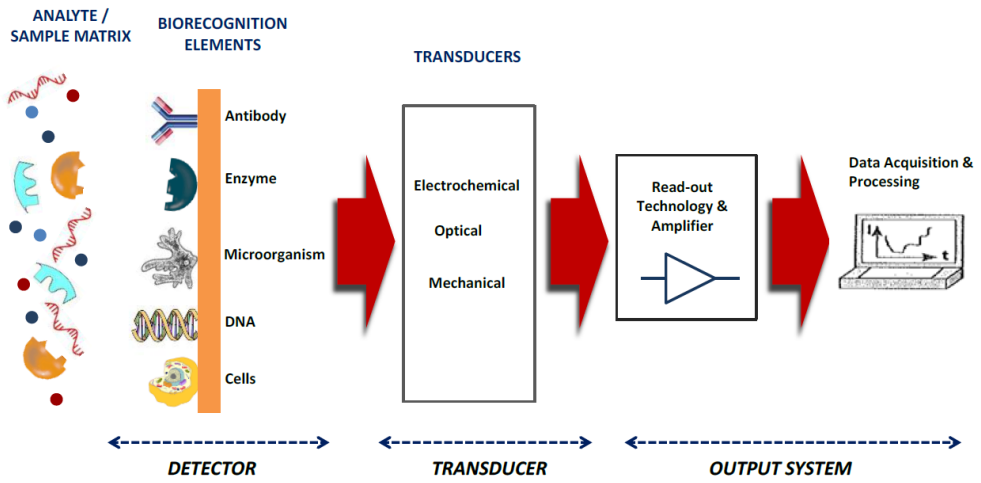


Figure 1.1 Schematic representation of different components of sensors [Mohd Said et al., 2014].

1.2 Brief history of sensors

The history of sensors briefs about the development of a simple idea into innovation in the field of science and technology. Clark and Lyons of Cincinnati Children's Hospital proposed the first-generation sensor in 1962. They performed quantitative analysis of glucose in an aqueous medium using an electrochemical oxygen electrode (Clark oxygen electrode) entrapped with glucose oxidase enzyme (GOx) using a semipermeable membrane [Clark et al., 1962]. GOx entrapped at the electrode oxidizes β-D-glucose to β-D-gluconolactone which concurrently reduces FAD to $FADH_2$. Further, dissolved O_2 gets reduced to H_2O_2 to regenerate FAD from $FADH_2$. Finally, an electrical signal is obtained due to the oxidation of the H_2O_2 on an applied voltage at the electrode surface. Unfortunately, the developed method suffered several limitations. First, the FAD prosthetic group and active site are buried deep inside GOx which restricts the diffusion of reagents. Second, the limited solubility of O_2 and difficulties in controlling its partial pressure ultimately limited the sensor to a narrow range

measurement. The glucose sensor was first time commercialized for the analysis of whole blood samples in the 1970s by Yellow Springs Instrument (USA). The limitation in the first-generation sensor was resolved by Cass et al. by using the redox mediators e.g. ferrocene etc. The electrons generated by the biochemical reaction are now directly mediated to the electrode without any interferences of dissolved oxygen in the analytes. These types of sensors are called second-generation sensors [Cass et al., 1984]. The limited diffusion rates in first-generation sensors were addressed by synthetic mediators which are more accessible than the O_2 in aqueous media. Finally, redox mediators get readily regenerated without applying high potential thus removing the interference and eliminating the background. However, leaching of the synthetic mediators has been a worrying case in some 2nd generation biosensors. Third-generation biosensors are designed by co-immobilizing the enzyme and mediator onto the electrode surface. The mediators immobilized at the electrode have the role of non-diffusion redox relay stations and facilitate the transport of electrons to the electrode surface from the active site of the enzyme. The electron transfer phenomenon is effectively improved due to the close proximity of the redox mediator and enzyme. This has resulted in a faster response time with a higher electron density. The design also allows the prolonged measurement of the analyte without any interference. However, there are cases of the deformation of the active site of the enzyme by close contact with the redox mediator resulting in a decrease in its biocatalytic activity. The schematic diagram for different generations of the sensor is shown in Figure 1.2.

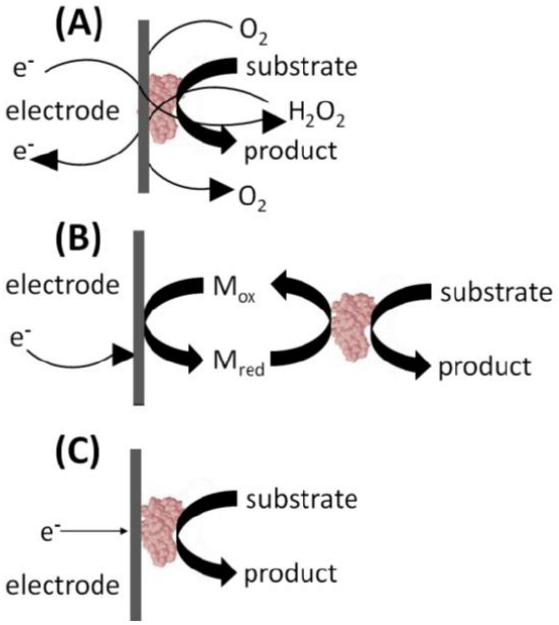


Figure 1.2 Schematic of different generations of the sensor [Putzbach et al.; 2013].

These early studies of sensors moved towards the integration of receptors i.e. recognition elements such as antibodies, enzymes, quantum dots, nanomaterials, etc. onto physicochemical transducers leading to new sensing/biosensing devices. This effort has striking the establishment of major research work towards environmental and biotechnological applications of sensors.

1.3 Transducers

The transducer is an important component of a sensor that converts one form of energy into another. The analyte's interaction with the receptor creates an effect recognized by the transducer and converts it into a measurable signal. The term signalization is designated to the process of conversion of energy into the signal. Transducers commonly give optical signals or electrical signals corresponding to concentrations of the target analyte. Nowadays a new term 'Nanotransducer' is introduced which defines the categories of transducers that perform signal transduction through structures that

satisfy the definition of nanostructures and possess at least one dimension below 100 nm and have another dimension smaller than 1μm. Nano transducers include only such sensors that belong to nanotechnology based on geometrical dimensions [Kohler et al, 2008]. Based on the operating principle, transducers can be classified into optical, electrochemical, electronic, thermal, and gravimetric transducers [Naresh et al.; 2021].

1.3.1 Types of Transducers

1.3.1.1 Optical Transducers

The devices based on optical transducers are based on principles such as internal reflectance and surface plasmon resonance. Light is absorbed when subjected to the optical device through optical fibers or planar waveguides toward a sensing surface. The photodiodes are used as detectors to record the intensity of reflected light. The most common signals recorded through optical transducer include absorbance, fluorescence, chemiluminescence, and surface plasmon resonance.

1.3.1.2 Electrochemical Transducers

Electrochemical transducers measure the electrochemical signal produced due to the interaction of the target analyte with the surface of the electrode [Collings et al., 1997]. The electrical changes can be a voltage difference between the reference electrodes and indicator electrodes (potentiometric), a change in the charge transfer property of the sensing material (conductometric), and a change in current with an analyte concentration measured at an applied voltage (amperometric). Generally, the amperometric transducers are commercially applied to the existing sensors in clinical diagnosis.

1.4 Receptors or Recognition elements

The receptors are an important component of sensors which is intended to recognize and interact with target analytes to give a measurable optical and electrical signal by an integrated transducer. The receptors must be selective in nature for analytes and can identify them in the mixture of other chemical or biological molecules. For example, in the case of glucose sensing, glucose molecules are identified and quantified from the mixture using a specific enzyme glucose oxidase GOx as a receptor. H_2O_2 is generated during the biochemical redox reaction of GOx with glucose, generates two electrons after breakdown, and is used for electrochemical sensing. In another technique, H_2O_2 reduces to $OH^{\cdot}$ radicals in a catalytic reaction and oxidizes chromophores to impart color to the solution in colorimetric sensing of glucose.

1.5 Classification of sensors

1.5.1 Based on transducers

Based on the operating principle and transducers, sensors are broadly classified into optical, electrochemical, electronic, thermal, and gravimetric sensors

1.5.1.1 Optical sensors

Optical sensors are analytical devices that measure optical signals generated due to catalytic and affinity reactions between receptors (integrated with transducer) and target analytes. The working principle of these sensors is to produce optical signals corresponding to the analyte concentration and facilitate their real-time label-free detection. In optical sensors, the transducer senses the changes in absorption, reflection, transmission, and refraction, as in consequence of chemical or physical changes generated due to a recognition event in the receptor with the analyte. Based on the

principles, the optical sensor can be classified as a label-free and label-based technique. In case of label-free colorimetric technique, there is direct interaction of the analyte with the receptor. On the contrary, the label-based technique is indirect and the signal is given by fluorescence, colorimetric, or luminescent methods. During the last era, optical sensors were extensively investigated with the advancement of technologies and proved that they have the potential for label-free, real-time, and direct sensing of different biological and chemical entities [Soldatkin et al., 2003]. Optical sensors are superior to conventional analytical techniques, owing to their better sensitivity, cost-effectiveness, small size, and operational simplicity [Sant et al., 2003; Luo et al., 2004]. Optical sensors are used in biomedical research, pharmaceuticals, environmental monitoring, healthcare, homeland security, the battlefield, etc. [Luo et al., 2004].

1.5.1.1.1 Colorimetric sensor

Colorimetric sensors present themselves as a promising potential candidate for the detection of different metallic cations, anions, drugs, pesticides, organic dyes, biologically important molecules, and other toxic pollutants due to simple fabrication, low cost, quick detection, high selectivity, sensitivity, and easy naked-eye sensing [Liu et al.; 2020]. At present, there is an increasing demand for sensor development with easy handling for onsite detection of clinically important molecules crucially in third world countries where advanced diagnostic technology and resources are limited. Therefore, colorimetric sensors may bring drastic changes to the health management of these countries without any aid from sophisticated instrumentations.

During the last decade, noble metal nanoparticles have found considerable application in the field of colorimetric sensors. Especially, gold nanoparticles (GNPs)

have drawn great attention due to their biocompatible nature and color transition. The color changes depending on the aggregation phenomenon that affects inter-particle plasmon coupling (red-to-blue or purple) or re-dispersion (purple-to-red) [Zhao et al., 2008]. The gold nanoparticles offer a high surface area available for easy functionalization. The functionalized gold nanoparticles play important role in selective colorimetric sensing for a variety of analytes, for example, DNA [Chandirasekar et al., 2011], cells [Medley et al., 2008], proteins [Ou et al., 2010], and metal ions [Chai et al., 2010]. The GNPs-based colorimetric sensor was also designed by Jiang et al. for simple and effective detection of glucose in rat brains [Jiang et al., 2009].

Nguyen et al proposed a label-free colorimetric sensor for the detection of glucose based on functionalized silver nanoparticles **(**Figure 1.3). The reaction of glucose with GOx generates H_2O_2 which on further reaction with silver nanoparticles leads to a color transition from yellow to colourless. Based on this principle he developed glucose sensors with good sensitivity and selectivity and a low limit of detection (162 nM) [Nguyen et al., 2018].

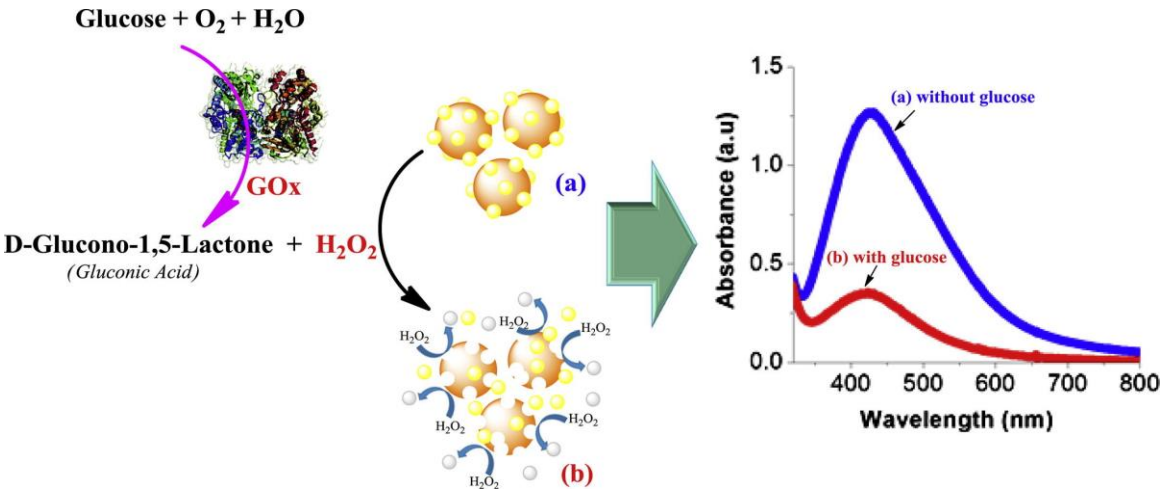


Figure 1.3 Colorimetric sensing of glucose based on graphene quantum dot functionalized silver nanoparticles [Nguyen et al., 2018].

1.5.1.1.2. Fluorescence sensor

Fluorescence occurs due to a three-stage process in some molecules named fluorescent dyes or fluorophores (polyaromatic hydrocarbons). In the process, the light of excitation wavelength is absorbed by fluorophores, and consequence their transition to a higher excited state. The excited state lifetime is very less (1–10 × 10^{-9} seconds) and meanwhile, molecules undergo conformational changes and possible interaction with the environment. These cause dissipations of energy and molecules come to the ground state with the emission of light in the form of fluorescence. In fluorescence measurement, we plot the measured fluorescence intensity obtained at fixed excitation and emission wavelength, and often defined as steady-state measurement. The lifetime is calculated by the decay of emission intensity, which is specific to a particular fluorophore [Shin et al.; 2021]. The fluorophores are designed such that the excitation wavelength is from the UV region while the emission wavelength occurs in the visible region of the electromagnetic spectrum. Thus, when the molecules are excited, they impart color and are visible in a UV lamp.

1.5.1.2 Electrochemical sensor

The electrochemical sensors are typically composed of three electrodes and an electrochemical cell. The electrodes comprise a working electrode where oxidation and reduction of the analyte occurs, a reference electrode, and a counter/auxillary electrode. After the addition of the analyte, the process of electron transfer takes place across a double layer on the surface of the active electrode and gives a current which is measured at a fixed potential. Electrochemical sensors are extensively investigated and applied sensors whose principles of operation depend on the electrochemical behavior

of the transducer and analyte. They show better sensitivity, good selectivity, and bear the ability to sense at a trace level of analytes. Based on the transducer, electrochemical sensors are classified into (a) potentiometric, (b) conductometric, (c) voltammetric, (d) impedimetric, and (e) amperometric. The schematic diagram for different sensors is given in Figure 1.4

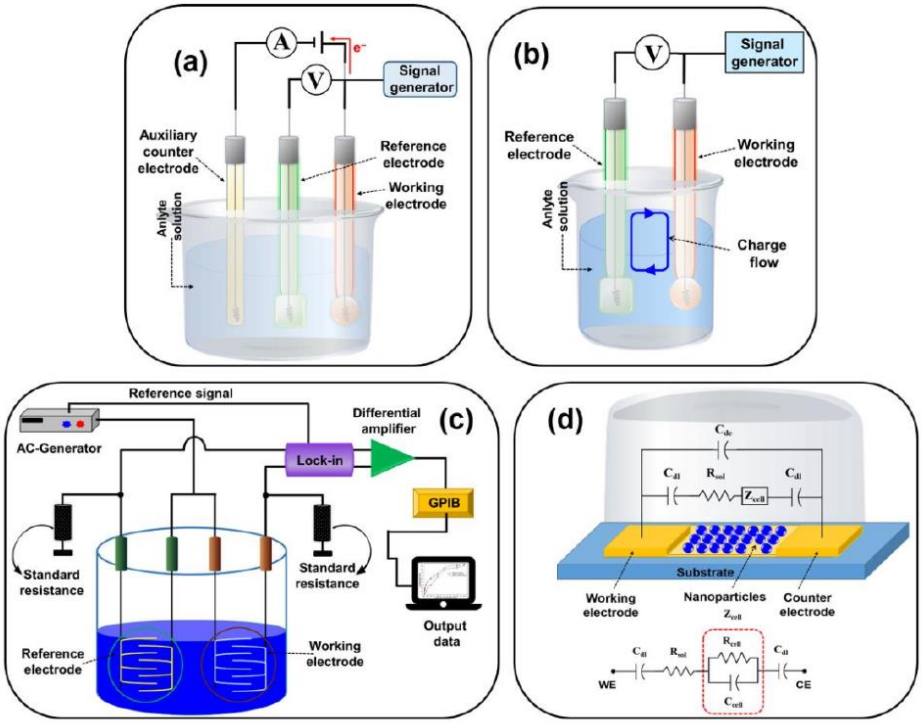


Figure 1.4 Schematic diagram of (**a**) amperometric/voltammetric sensor, (**b**) potentiometric sensor, (**c**) conductometric sensor, and (**d**) impedimetric sensor (equivalent circuit (C_{dl} = electrodes double-layer capacitance, R_{sol} = solution resistance, C_{de} = capacitance of the electrode, Z_{cell} = impedance due to bound nanoparticles, and C_{cell} and R_{cell} are capacitance and the resistance in parallel)) [Naresh et al.; 2021].

In the case of potentiometric biosensors, the charge accumulation occurs at the working electrode due to the interaction of the analyte with receptors. The accumulated charge under zero current is measured relative to the reference electrode. In the case of biosensors, ion-selective electrodes are generally used to produce a potential signal from a biochemical reaction [Pisoschi et al., 2016]. Amperometric sensors are usually a three or two-electrode system. It measures the current generated at the interface of the

working electrode due to the redox reaction of the analytes or electroactive molecules when a fixed potential is given between the working and reference electrode. The current generated through the redox reaction at the working electrode surface is proportional to the analyte concentration in the solution. Amperometric sensors are sensitive, precise, fast, and have a linear response in comparison to potentiometric sensors but they bear the limitation of poor selectivity and interferences [Alaejos et al., 2004]. Conductometric sensors are used to sense the change in conductance of the solution between a pair of electrodes because conductance depends on the electrochemical reaction and the change in analyte conductivity. Impedimetric and conductometric sensors are generally employed to investigate the metabolic process occurring in biological systems [Grieshaber et al., 2008]. In impedimetric sensors, a small sinusoidal excitation signal is applied to measure changes in the electrical impedance at the interface of the electrode/electrolyte. In this case, a low amplitude of AC voltage is applied at the electrode, and in-phase and out-of-phase current response is measured with respect to frequency [Radhakrishnan et al., 2014]. Voltammetric sensors are used to quantify the analyte concentration by measuring the current obtained with the variation of the applied potential. This method facilitates the simultaneous detection of multiple analytes with high sensitivity [Grieshaber et al., 2008; Borgmann et al., 2011].

The electrochemical methods are widely used in the detection of analytes but they suffer from some limitations of poor stability and sensitivity. But these limitations can be overcome with the introduction of nanoscience and nanotechnology in electrochemical sensing. Nanomaterials have a large potential for improving both sensitivity and stability through signal amplification of electrochemical sensors [Zhu et al., 2015]. Recently, nanomaterials have grabbed prime attention as advantageous tools

for the fabrication and development of electrochemical sensors with better performances [Pandey et al., 2007; Willner et al., 2011]. Shan et al. investigated the potential of graphene as superior electrode material for highly sensitive and selective detection of glucose using glucose oxidase [Shan et al., 2013]. Li et al. proposed a direct electrochemical method for the sensitive detection of cholesterol based on chitosan-graphene oxide composite modified glassy carbon electrode along with cholesterol oxidase [Li et al., 2015]. Nowadays, metal dichalcogenides-based nanomaterials have found great application in the field of electrochemical sensors owing to their good electrocatalytic properties and high surface area. Huang et al. have studied the electrochemical properties of MoS_2–graphene using an Acetaminophen drug. The developed sensor exhibits good analytical performance in acetaminophen sensing over a wide linear range and a good detection limit [Huang et al., 2013]. Solanki et al. have investigated the potential of gold decorated MoS_2 (Au-MoS_2) as electrode material for the impedimetric biosensor for sensitive detection of dengue antigen NS1 [Solanki et al., 2018]. Electrochemical sensors have various advantages over other types of sensors like low cost, high reproducibility, high stability, fast response**,** high sensitivity, and selectivity with a low limit of detection.

1.5.2 Based on Receptors:

Sensors can be classified into different categories based on the type of receptors used and the kind of interactions occurring. Biosensors are categorized as affinity and catalytic biosensors according to the principle of biorecognition. In a catalytic biosensor, receptor moieties are modified with enzymes, tissues, microorganisms, and whole cells where there is the interaction between analyte and bioreceptor which give rise to the new product while in the case of an affinity biosensors, the receptors are

modified with cell receptors, antibodies, and nucleic acids. In these sensors, no new product is formed after the interaction of the analyte with receptors [Nguyen et al., 2019; Shukla et al., 2016]. The receptors used may be grouped as an ion, nucleic acids, cells, enzymes, antibodies, nanomaterials, quantum dots, and biomimetic materials, etc.

1.5.2.1 Enzymes as receptors

Enzymes are biocatalysts, which accelerate the rate of biological reaction. They are made up of proteins and show high catalytic activity and selectivity towards substrates/analytes [Guilbault, 1976]. The working principle of the enzyme-based sensor depends on the binding and catalytic properties of the enzymes toward the target analyte to be detected [Morrison et al., 2007]. There are different probable mechanisms occurring in the recognition event of analyte: (i) monitoring the changes of enzyme characteristics, (ii) inhibition or activation of the enzyme by analyte, (iii) The metabolization of analyte by the enzyme, in this case, the concentration of enzyme is estimated by measuring the analyte transformed by the enzyme [Justino et al., 2015]. Over the decades, various sensors have been proposed and developed based on the specificity of enzymes. Despite that, the structure of enzymes is very sensitive to the external environment which makes them expensive and creates complications in improving the stability, sensitivity, and adaptability of the sensor [liu et al., 2019]. There are limitations like chemical inhibitors, ionic strength, pH, and temperature which affect their catalytic activity. Enzymes lose their catalytic activity at high temperatures (above 60°C) [Pohanka et al., 2013]. The glucose and urea sensors are the most common sensors which are enzyme-based. Ondes et al. have fabricated a potentiometric urea biosensor with good sensitivity and stability using nanoparticles. The limit of detection and response time of the developed biosensor was found to be 0.77 μM and,

30 s respectively [Ondes et al., 2021]. Cordeiro et al. proposed W-Au-based glucose biosensors using the amperometric method for monitoring brain glucose in vitro conditions. The introduction of nanoparticles in enzyme-based sensors resulted in a boost in the application of enzymes as receptors in biosensors [Cordeiro et al., 2018]. Enzymes are usually attached to optical and electrochemical transducers. There are several immobilization techniques for enzymes to the surface of the transducer like covalent attachment, adsorption, and entrapment for the fabrication of biosensors [Bartlett et al., 1993; Cosnier, 1999].

1.5.2.2 Antibodies as receptors

Antibodies are the protein molecules with specific binding properties toward specific antigens used for the development of biosensors. They are commonly immobilized on the transducer surface through physical and covalent interaction. The common covalent interactions are the conjugation of amino, carboxyl, or aldehyde groups. Therefore, the transducer should have carboxyl, hydroxyl, amino, or other groups for the immobilization of antibodies. Antibodies are used in the fabrication of affinity biosensors and have found great applications during the last two decades due to their strong complementary antigen-antibody interactions. Antibodies' structures are in the form of a "Y" shape like immunoglobulins (Ig), which comprise two light and two heavy polypeptide chains with connecting disulfide bonds. Based on the differences in their heavy chain, antibodies are classified into five classes: IgA, IgD, IgE, IgG, and IgM [Schroeder Jr et al., 2010]. The biosensors in which antibody is used as recognition element is named immunosensors. Further, immuosensors have been classified as (i) labelled immunosensor, where there is a detectable label is introduced for the assessment of antibody-antigen complex, and (ii) non labelled immunosensor, which are

constructed without using any label and activity is monitored through physical changes occurring due to the formation of an antigen-antibody complex [Lim et al., 2016]. de Castro et al. developed a label-free immunosensor for the detection of ovarium cancer. The immunosensor showed a linear response in the range of 5 to 80 U mL^{-1}, for anti-CA125 of concentration with a detection limit of 1.45 U mL^{-1} [de Castro et al., 2020]. Bhardwaj et al. developed a label-free sensor for Aflatoxin B1 (AFB1) through optical and electrical techniques using gold (Au) nanobipyramids (NBPs) [Bhardwaj et al., 2021].

1.5.2.3 Aptamers as receptor

Aptamers can be defined as short single-stranded nucleic acids (15-80 nucleotides). They are synthesized as sequences of DNA or RNA in the lab and bind selectively to the target molecules. Aptamers have the property to fold in two and three-dimensional structures thus providing better binding sites due to less spatial blocking and greater surface density. Since, aptamers adopt the structure of nucleic acids, they are more stable than antibodies over a long range of temperature and storage conditions [Dhiman et al, 2017]. Moreover, aptamers can also be modified as per the requirement of the sensor. Aptamers can easily be procured from oligonucleotide libraries through an in-vitro selection process, SELEX (Systematic Evolution of Ligands by Exponential enrichment) [Stoltenburg et al., 2007]. Choi et al. developed a label-free detection method for thrombin based on aptamer-capped NIR PbS QDs with a selective charge transfer phenomenon. The developed method showed selective detection even in the presence of interfering negatively or positively charged proteins, with a detection limit of around 1nM [Choi et al., 2006].

1.5.2.4 Nucleic acid as a receptor

Nucleic acids based sensors are called genosensors and their recognition principle is based on complementary base pairing like adenine: thymine and cytosine: guanine. Therefore, in the case, where the target nucleic acid sequence is obtained then the complementary sequences can be designed and labelled. These complementary structures are immobilized at the surface of the transducer during the development of the sensor. The hybridization of complementary probes with target sequences generates an electrochemical or optical signal. The ideal transduction theory is applied during optical detection in this kind of sensor [Leung et al., 2007].

1.5.2.5 Whole cells as the receptor

Whole-cell-based sensors are designed by using microbes such as fungi, bacteria, algae, viruses, and, protozoa because they have the property of biorecognition. These cells are self-replicating to give recognition molecules like antibodies in their pure form [Gui et al., 2017]. whole-cell-based sensors are comparatively easier for us in comparison to plant or animal cells. The cells can easily interact with different analytes and produce measurable electrochemical signals. Whole-cell-based sensors are widely applied in the field of food analysis, environmental monitoring, pharmacology, pesticides, heavy metals, and drug screening due to their high selectivity, and good sensitivity [Berepiki et al., 2020]. Riangrungroj et al. reported a whole-cell Escherichia coli-based sensor to quantify the exposure of pyrethroid insecticide in the linear range of 0.01–2 ng mL^{-1} with a detection limit of 3 ng mL^{-1} [Riangrungroj et al., 2019].

1.5.2.6 Ion as receptor

Ions-based sensors are the most primitive sensors which are designed in large numbers. F. Haber and Z. Klemensiewicz were the first to propose an ion-based sensor for pH using a glass electrode. In this kind of sensor, the electric charge becomes the basis of recognition. Selectivity can be introduced for ion recognition by some other factors like analyte-receptor and bond size of the receptor [Dole et al., 1980].

1.5.2.7 Nanomaterials as receptor

Besides the above receptors, nanomaterials open a new category of receptors that have attracted considerable attention in the field of sensors with the advancement of nanoscience, and nanotechnology. Nanomaterials have a high surface-to-volume ratio which facilitates immobilization of the analytes in a small volume. The immobilization of the analytes onto the surface of nanomaterials takes place through adsorption, covalent interaction, and entrapment method. Among nanomaterials, graphene, gold nanoparticles, carbon nanotubes, silver nanoparticles, graphene oxide, quantum dots, and polymer nanoparticles, are largely studied [Holzinger et al, 2014]. In some cases, nanomaterial-modified sensors show high sensitivity as they are comparable in size to that of analytes of interest (e.g., biomolecules, DNA, pathogens, antibodies, metal ions) and thus facilitate the study which was not possible before. Further, the incorporation of natural receptors with nanomaterials provides high sensitivity and selectivity toward the detection of target molecules. For example, nano bioelectronic noses and tongues for odorants, and tastants respectively, and G-protein-coupled receptors for dopamine, geosmin, hormones, cadaverine, trimethylamine, etc. [Kwon et al, 2019].

1.6 Nanomaterials in sensing applications.

Sensors find great applications in the everyday life of mankind ranging from fitness, lifestyle, healthcare, and manufacturing. Fundamental issues regarding the development of sensing platforms include increasing demand for inexpensive, reliable sensors with real-time measurement for point-of-care tests. Nanotechnology and nanoscience have supported to great extent in resolving the issue. Nanomaterials-based sensors have great potential for introducing a reliable, cost-effective sensor with high sensitivity and selectivity. Further, the selectivity of a sensor depends on the specific interaction of receptors with target molecules. However, the selectivity, stability, sensitivity, response time, and the limit of detection of the sensors very much rely on the physical and chemical characteristics of the transducer. The transducer properties can be ameliorated by the use of nanomaterials as an interface or redox mediator between receptors and transducer. Nowadays, nanomaterials-based sensors have found leading applications in the field of clinical diagnosis, medical research, biosecurity, food safety, and environment monitoring. Despite several merits, nanosensors have several challenges, especially in their application in biological systems. These sensors may affect cell metabolism and induce homeostasis. It also has challenges in separation methods, mass production, quality control, validation, reproducibility, integration of sensors with elements, etc. [Yang et al., 2021].

Nanomaterials can be defined as materials with a minimum of one external dimension lying in the ranges of 1-100 nanometers [Buzea et al., 2007]. There are various nanomaterials have been investigated for sensing applications ranging from graphene and their derivatives (GO, rGO), metallic nanoparticles (silver and gold nanoparticles), transition metal dichalcogenides (MoS_2, WS_2, $MoSe_2$), semiconductor

nanoparticles (quantum dots) to polymeric, dendrimers, liposomal, etc. In the advancement of nanotechnology and nanoscience, nanomaterials play a prime role because they exhibit high catalytic efficiency, reaction activity, high surface area, good electrical and optical, thermal properties, good biocompatibility, magnetic behavior, high degree of specificity and selectivity, ease of functionalization, and strong adsorption ability that makes them a potential candidate for the application in the development of efficient sensors [Fan et al., 2008; Huang et al., 2010].

The insightful use of nanoparticles led to clearly improved performances with enhanced sensitivities and lowered limit of detection of several orders of magnitudes. The choice of nanomaterials relies very much on the sensing strategy adopted and the target analyte chosen for sensing. One common privilege of all nanoscale objects is their high specific surface area enabling the immobilization of a substantial amount of receptor units. Nanomaterials became a crucial part of analytical devices and enhance the efficiency in terms of sensitivity and limits of detection down to single molecules detection. The specific characteristics of nanomaterials also provide alternatives to classic techniques of transduction. Furthermore, the different combinations of nanomaterials, each with its properties, to increase the performance of sensors is a well-accepted strategy. Nanocomposites play a revolutionary role in the development of sensing platforms. Nanocomposites are made of two or more constituents having significantly different physical and chemical properties. The two different materials with their dimensions in the nano range when added produce new materials with different properties than their individual components. Several nanocomposites or hybrid materials are reported in the literature that shows their usefulness with significantly tuned properties and reduced cost [Daniel et al., 1994; Jones, 1998; Stankovich et al., 2006].

Among different metal nanostructures, noble metal nanoparticles like gold, silver, platinum, and palladium nanoparticles have been extensively investigated owing to their unique catalytic, electronic, and optical properties with tunable size and shape [Norouzi et al., 2011; Willner et al., 2011]. Gold nanoparticles possess the ability of electrons to transfer between the electroactive species and the electrode. This principle is primarily applicable in redox enzyme sensing where the receptor moieties catalyse the oxidation/reduction of the analyte. Furthermore, gold nanoparticles have outstanding optical properties and have demonstrated their potential in sensing applications through their surface plasmon resonance transduction. The surface plasmon resonance is firmly dependent on the shape, and size of the nanoparticle and the dielectric constant of the medium. This medium dependency offers a great privilege to analytics as the recognition event changes the oscillation frequency resulting in an observable color change. In this context, a series of efficient colorimetric biosensors were developed for DNA or oligonucleotide detection [Reynolds et al., 2000; Oldenburg et al., 2002]. The immobilization of natural enzyme horseradish peroxidase (HRP) on gold nanoparticles' surfaces has been achieved through biotin-streptavidin linkage. HRP is used as a label in biosensors since they form fluorescent, colored, or redox active molecules with H_2O_2 reduction to water. [Veitch et al., 2004]. Jv et al. have developed a simple colorimetric method for the detection of H_2O_2 and glucose based on positively-charged gold nanoparticles with intrinsic peroxidase-like activity [Jv et al., 2010].

Even though gold nanoparticles have various advantages but it offers some limitations as they are prone to aggregation, long-term storage issues and production cost is a bit higher. The aggregation can be prevented either by using a cross-linker or making composites. Silver nanoparticles bear several advantages over gold nanoparticles in

terms of sharper extinction band, higher molar extinction coefficient, and extremely high field enhancement but the poor chemical stability limits their application. The research is going on to improve the stability of these nanoparticles through surface engineering and functionalization [Pinto et al., 2010]. Handayani et al. reported a colorimetric technique for the detection of Fe^{2+} using silver nanoparticles. The detection principle was based on the color change of the silver colloid on reaction with the ion [Handayani et al., 2019]. Platinum nanoparticles have also gained extensive attention with wide applications due to unique properties such as their volume effect, surface effect, quantum size effect, and quantum tunneling effect [Yu et al., 2021]. Wang et al. have developed an easy and ultrasensitive colorimetric assay for the detection of Ag^{+} using platinum nanoparticles at picomolar levels. The detection principle was based inhibition effect of Ag^{+} on the peroxidase activity of synthesized nanoparticles. Sanzo et al. utilized platinum nanospheres and Pt (II) and platinum nanoflowers, and Pt (IV) to make the bimetallic Au–Pt nanostructure. The composite along with glucose oxidase effectively used for the detection of hydrogen peroxide in the range of 0.1–2.0 mM with a sensitivity of 33.66 μA/mM cm^{-2}.

Although noble metals have been very useful in the development of sensing platforms, their high cost and risk of aggregation limit their storage and use in practical applications. Recently, carbon and inorganic transition metals-based nanomaterials have attracted considerable attention for the development and advancement of stable and cost-effective sensors e.g. graphene oxide (GO), graphitic carbon nitride (g-C_3N_4), reduced graphene oxide (rGO), carbon nanotubes (CNTs), Fe_3O_4 magnetic nanoparticles (MNPs), Prussian blue analogs (PBAs), Vanadium pentoxide nanowire (V_2O_5), Molybdenum disulfide nanosheet (MoS_2), Tungsten disulfide (WS_2), Tungsten diselenide (WSe_2), Molybdenum diselenide ($MoSe_2$), Manganese dioxide (Mn_3O_4), etc.

[Yan et al. 2010; Morais, 2021; Li et al. 2016; Karyakin, 2017]. Two-dimensional (2D) nanomaterials define a new avenue for nanotechnology and have sheet-like nanostructures where transverse dimensions are greater than 100 nm, and the thickness of the sheets is less than 5 nm. Owing to their unique structures and shapes, 2D nanomaterials display huge surface area and anisotropic physiochemical properties [Tan et al., 2017]. 2D nanomaterials are one of the thinnest in dimension and have comparatively greater specific surface areas than other known materials, concluding that they possess a large number of anchoring sites for the receptors and analytes [Qian et al., 2017]. Graphene is structurally one-atom-thick with numerous unforeseen properties like large specific surface area, excellent transparency, good electrical, and thermal conductivities with extraordinary mechanical properties. The success of graphene has ignited great interest which has led to the development of various 2D nanomaterials. For e.g. graphitic carbon nitride (g-C_3N_4), transition metal oxides (TMOs), transition metal dichalcogenides (TMDs), hexagonal boron nitride (h-BN), etc. [Hu et al., 2019]. Nowadays, carbon-based 2D nanostructures find the dominating position in sensor applications. The huge surface area and sp^2-hybridized carbon are key characteristics of 2D carbon nanomaterials to be used in a sensor device. Analytes may interact through π-electrons of the aromatic carbon or via hydrogen bonding with functional groups present at the edge planes of carbon materials. Additional receptors may be introduced in order to improve the sensitivity and selectivity of the sensor device. Georgakilas et al. reported literature and explained the covalent and non-covalent functionalization of graphene [Georgakilas et al., 2012; Kirchner et al, 2020].

Analogous to graphene, other 2D nanomaterials with the thickness of a few layers have gained attention during the last decades, especially TMDs which comprise MoS_2, WS_2,

WSe_2, $MoSe_2$, etc. TMDs are defined as layered structured compounds with the formula MX_2. They are characterized by strong covalent bonding in-plane and have weak interlayer interactions. The monolayer of MX_2 has a transition metal atom (M) layer which is sandwiched between the layers of chalcogen atom (X = S, Te, or Se) with a thickness of 6–7 Å for each monolayer. TMD shows the layered dependent properties as the band gap changes with the number of layers. The surface area increases post exfoliation into single or few layers which enable easy immobilization of recognition elements onto their surface. In addition, 2D TMD nanomaterials exhibit fluorescence quenching properties, enabling them for fluorescence-based sensors. The tunable band gap and unique optical properties make TMDs a potential candidate to be explored for sensor applications [Hu et al., 2017].

Nowadays, scientists have adopted the technology of nanocomposite synthesis in order to improve the properties of pristine materials. Among them, noble metal nanoparticles decorated with inorganic two-dimensional nanomaterials have drawn great attention with significantly enhanced conductivity and efficient catalytic activity towards the fabrication of highly sensitive and selective sensors. These nanocomposites exhibit improved electrochemical and optical properties with better catalytic behavior than nanomaterials alone making them a potential candidate for application in the field of the sensor. The decoration of metal nanoparticles onto the surface of TMDs and carbon-based 2D nanomaterials like WS_2, MoS_2, graphene, GO, rGO resulted in improved sensitivity, catalytic activity, and surface-enhanced Raman scattering (SERS) [Cao et al., 2017; Lin et al., 2014].

Su et al. developed gold nanoparticles decorated MoS_2 nanocomposites-based probes for the quantification of the carcinoembryonic antigen. The developed probe showed

excellent performance with good stability, selectivity, and ultralow detection limit of 1.2 fg mL^{-1} suggesting that the developed immunosensor could be applied in the detection of CEA in real samples [Su et al., 2019]. Lan et al proposed a label-free colorimetric DNA sensor based on MoS_2, WS_2, $MoSe_2$, and WSe_2. The sensitivity was enhanced through a hybridization chain reaction. This developed sensor showed excellent selectivity and sensitivity with a low limit of detection [Lan et al, 2019]. Xu et al. reported a high-performance electrochemical sensor for acetaminophen based on graphitic carbon nitride-electrochemically deposited-poly (3,4-ethylene dioxythiophene). The developed sensor was found to be selective and reproducible in the concentration range 0.01 to 2 μM and 2–100 μM, with an ultra-detection limit of 34.28 nM [Xu et al., 2018].

1.7 Nanomaterials as artificial enzymes.

The term 'Artificial enzymes' was introduced first time by Ronald Breslow to define enzyme mimics. These materials open a new branch of biomimetic chemistry inspired by nature with the objective to imitate the principles of natural enzymes by utilizing alternative materials. Horseradish peroxidase (HRP) is a natural enzyme that acts as a biocatalyst and mediates several biological activities in living organisms by increasing the rate of biochemical reactions. Natural enzymes have found large applications in the field of the food industry, agrochemical production, sensors, and pharmaceutical processes [Pang et al., 2009; Wulff et al., 2002]. Natural enzymes bear several intrinsic limitations, such as low operational stability, purification, high costs of production, and sensitivity to environmental conditions [Ellis et al., 2009].

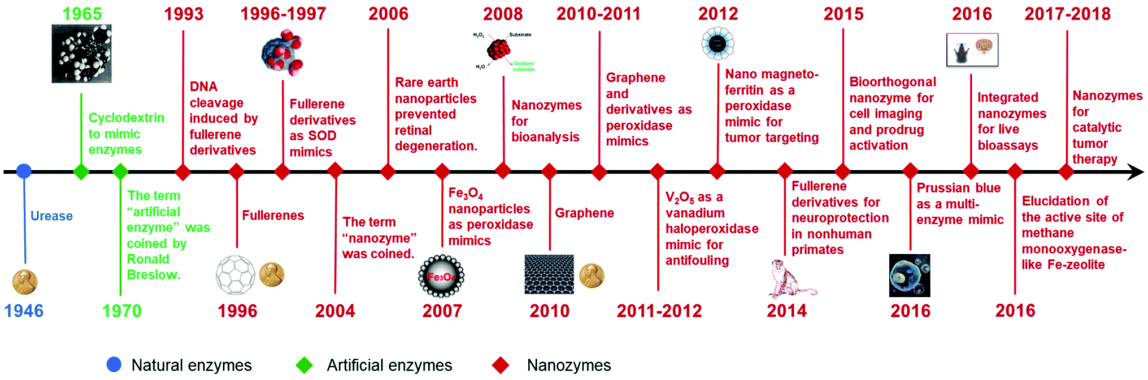


Figure 1.5. A brief timeline for the artificial enzymes (natural enzymes are also listed for comparison [Wei et al., 2008].

Therefore, in order to overcome the above limitations, the development of artificial enzymes in the field of sensors is very crucial [Wei et al., 2008]. A brief timeline for the artificial enzymes development during the last decades is shown in Figure 1.5. Recently, with the development of nanoscience and nanotechnology, the development of new functional nanomaterials and metal organic frameworks with intrinsic catalytic properties and enzyme mimetic behavior became a matter of prime concern. During the past two decades, scientists have greatly investigated artificial enzymes and established them as a highly stable, sensitive, and low-cost alternative to natural enzymes in sensor applications. Metal complexes, cyclodextrins, polymers, porphyrins, biomolecules, nanomaterials, and supramolecules have been very much explored to mimic natural enzymes in the context of structural and functional similarity. The artificial enzymes are empowered with several advantages over the natural enzymes, like easy and controlled synthesis, low cost, tunable catalytic activity, and high stability against harsh environmental conditions, etc. [Wei et al., 2008; Gao et al., 2007]. These nanomaterials-based nanozymes catalyze the oxidation of a peroxidase chromogenic substrate 3,3',5,5' tetramethylbenzidine (TMB), in presence of electron acceptor O_2 or H_2O_2 to give the oxidized blue-colored product (Figure 1.6). The nanozymes are termed peroxidase when

H_2O_2 acts as electron acceptor and termed oxidase when oxygen act as electron acceptor.

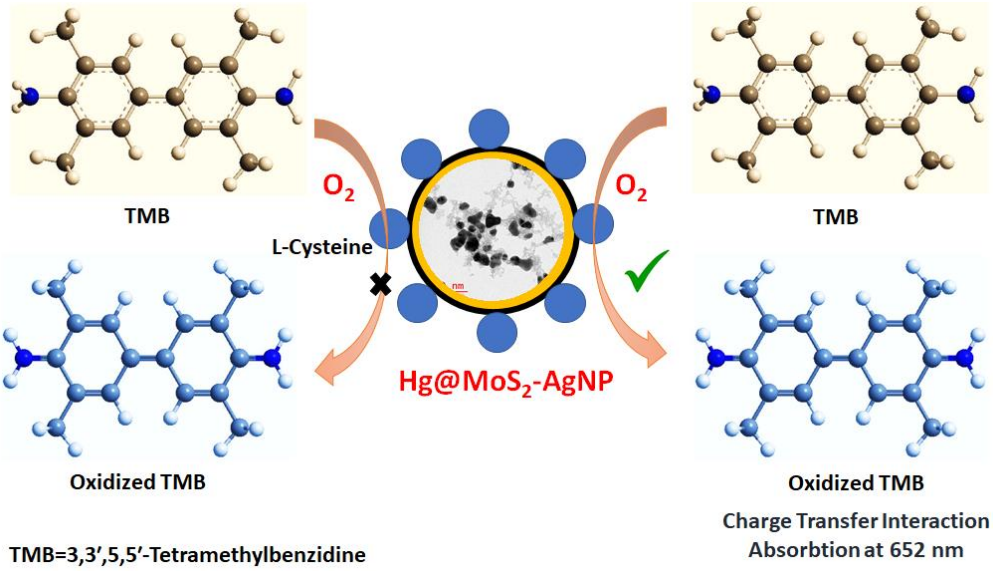


Figure 1.6 Schematic illustration of oxidation reaction of TMB in the presence of O_2 catalyzed silver nanoparticles.

Several studies have proven that metal nanomaterials are a potential candidate for enzyme mimic [He et al., 2011]. In the case of metal nanoparticles, gold nanoparticles with either positive or negative charges on their surface, show excellent oxidase and peroxidase mimetic activity. Wang et al. have investigated the origin of the peroxidase-like activity of gold nanoparticles [Wang et al., 2012]. Carbon-based nanomaterials like graphene and their derivatives, carbon nanotubes (CNTs), and fullerene show great potential as enzymes mimic their different applications [Gao et al., 2014]. Researchers have developed a colorimetric method for the detection of glucose using GO–COOH with peroxidase mimetic behavior activity in presence of glucose oxidase and chromogenic substrate (TMB). Enzyme-mimetic with TMDs such as MoS_2 and WS_2 have developed as significant tools for the colorimetric technique owing to their easy synthesis, high stability, controllable structure, and composition, as well as tunable

catalytic activity [Nirala et al., 2018; Lin et al., 2014]. There are scientific communities who are doing extensive research to design new avenues to synthesize some nanozymes possessing significant mimicking properties and tolerance to physical conditions that can be applied to fabricate sensitive and selective platforms for sensing.

1.8 Immobilization of biomolecules on nanomaterials

One of the great challenges in the development of nanomaterial-based sensors is the immobilization or fuctionalizations of the bio-specific units on such nanomaterials. Thus, the method utilized to immobilize enzymes or other receptors is a key factor in developing a reliable sensor [Holzinger et al, 2014]. The immobilization technique should be adopted such that structure, function, and biological activity of receptor biomolecules should be preserved without leaching. The immobilization techniques are represented in Figure 1.7. There are two crucial immobilization techniques i.e. physical and chemical immobilization. The selection is based on the chosen biorecognition element, physiochemical environment, analyte, and transducer.

1.8.1 Physical immobilization

In this technique, receptors are reversibly attached to the transducers without the involvement of any chemical bond. Depending on the kind of interaction, physical immobilization can take place by (a) physical adsorption, and (b) physical entrapment. In the physical adsorption technique, receptor biomolecules are immobilized on the outer surface of nanomaterials modified transducers through weak electrostatic forces like van der Waals forces, electrostatic interactions, an ionic bond, and hydrogen bond. This technique is extensively applied in enzyme-based biosensors. The benefits of using this technique include its economical and easy process without affecting the biological and catalytic activity of the biorecognition element. The physical adsorption technique

is prone to changes in several factors like temperature, pH, and ionic strength. It is linked through a very weak interactions resulting in poor stability [Martinkova et al., 2017; Nguyen et al., 2017; Sassolas et al., 2012; Naresh et al., 2021]. In physical entrapment, bioreceptors are physically entrapped inside the 3D matrices through covalent or non-covalent interactions. The entrapment of biorecognition element in a polymer matrix is achieved by mixing receptors with a monomer solution and followed by polymerization by changing experimental conditions. The polymers often used for entrapment are gelatin, cellulose, polyacrylamide, modified polypropylene, alginate, acetate phthalate, polydimethylsiloxane, etc. [Martinkova et al., 2017; Nguyen et al., 2017; Sassolas et al., 2012; Naresh et al., 2021].

1.8.2 Chemical immobilization

In chemical immobilization technique, there is covalent interaction between biorecognition elements functional groups and the nanomaterials modified transducer. The chemical immobilization technique is of two types based on the type of bonding, (a) covalent cross-linking and (b) direct covalent binding [Martinkova et al., 2017; Nguyen et al., 2017; Sassolas et al., 2012; Naresh et al., 2021]. In the cross-linking process, multi-functional reagents are used which act as a linking agent between the biorecognition element and transducer. The optimal conditions are needed for linking like temperature, ionic strength, and pH. It is facilitated with strong attachment, little response time, and better catalytic activity. The advantages are stronger chemical binding, reduced leakage of enzymes, and the property to tune the environment for receptors using stabilizing agents. The entrapment involves the bonding with protein molecules which may denature the enzymes which is a limitation of this technique. Direct covalent binding is a widely applied immobilization technique, where

biomolecules acting as the receptor is strongly bonded and attached to the nanomaterials or transducer surface. The advantages of direct covalent binding include less chance of leakage, a firm linkage between the recognition element and surface, stable to environmental changes. The major disadvantages of this technique are the application of harsh chemicals and incapability to regenerate the developed matrix [Naresh et al.; 2021].

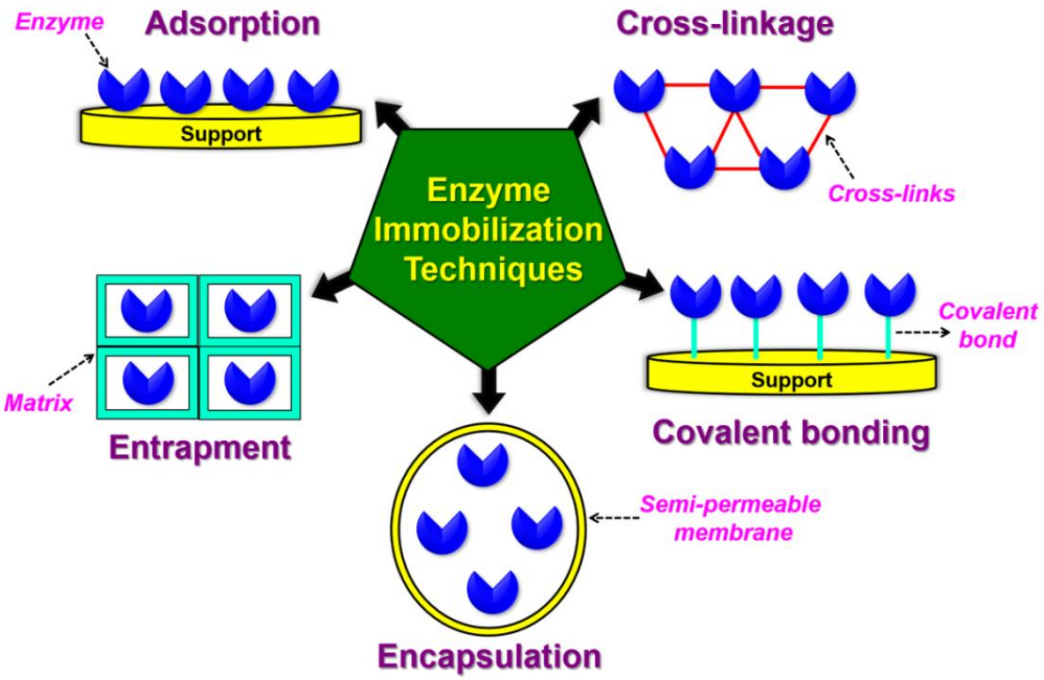


Figure 1.7 Schematic representation of immobilization methods [Naresh et al.; 2021].

Table1.1 Interactions and Importance of immobilization methods

Immobilization method	Type of Interaction	Advantages
Adsorption	Electrostatic Hydrophobic Vanderwaal forces	• Simple and easy • Limited loss of activity
Entrapment	Entrapment	• No chemical reaction between the monomer and enzyme that could affect activity • Several types of enzymes can be immobilized within same polymer
Covalent	Covalent bonding	• Stable • Short response time • No diffusion barrier
Affinity based	Support and affinity tag on a protein sequence	• Controlled and oriented immobilization

Biomolecules can also be immobilized with nanomaterials by covalence, adsorption and entrapment to design a sensitive sensor. Nanomaterials have fascinating immobilization surfaces with large surface area [Luo et al., 2006]. Furthermore, direct adsorption of the enzymes on the bulk metal surfaces often results in the denaturation of proteins and loss of activity which can be improved if enzymes are primarily adsorbed on metal nanomaterials prior to being electrodeposited onto the surface of the electrode [Peng et al., 2005]. Table 1.1 shows the types of interactions and advantages for different immobilization techniques.

1.9 Motivation and Objective of the thesis:

Nanotechnology in the field of sensors has played a pivotal role in designing advanced sensors with a low detection limit for the analytes as they have a large surface area that

provides ease of functionalization and better biocompatibility, sensitivity and also selectivity. Nanotechnology has made possible the miniaturization of devices into portable sensors. There are several diseases like Tuberculosis, Dengue, and Cancer, which if not identified at an early stage, may become severe and life-threatening. So, there is a crucial demand for portable, cheap, and reliable sensors that can be used to identify them. Motivated by these facts, we are introducing an effective electrochemical technique to quantify a very lethal disease biomarker i.e. Dengue biomarker 'NS1'. After the identification of the diseases, there are challenges to monitoring the drug level as there is a narrow effective window for their action. Higher levels of the drugs may have several side effects and even results in organ failures, while low level makes them impotent and ineffective. In the thesis, we are introducing a very facile method with a portable device to quantify the anti-Tuberculosis drug 'Isoniazid'. Not only diseases but there are several biomolecules like cholesterol and L-cysteine, glucose whose discrepancy may cause several fatalities like cardiac failure, diabetes, and many more. So, at present-day, we need regular monitoring of our health which motivated us to fabricate sensors to identify levels of biomolecules. In our thesis, we have presented the sensors for L-cysteine and cholesterol.

Recent studies have shown the future promises of carbon and transition metal-based nanomaterials e.g. GO, g-C_3N_4, MoS_2 nanosheet, $MoSe_2$ nanosheet, MoS_2 quantum dots and their composites for the development of efficient sensors. Prussian blue analogues (PBAs) are considered a prime class of metal-organic frameworks (MOFs) with the general formula of $A_xM_y[M'(CN)_6]$. zH_2O (where M and M′ represent transition metal elements and A denotes an alkali metal ion). PBAs have extensive

application in the field of energy storage, hydrogen evolution, oxygen reduction, and biosensing because of their high surface areas and good electron-transport property.

Considering these facts and futuristic applications of nanomaterials in the field of sensors, and bio-mimic. The major objective of the thesis is:

- To synthesize nanomaterial, their nanocomposites, and characterization for their sensing applications by using various techniques.
- To investigate physical, chemical, electrochemical, and catalytic properties of synthesized nanomaterials and nanocomposites for fabrication of sensors of biomolecules (cysteine, cholesterol), antigens (Dengue Virus antigen, NS1), and drug (isoniazid), and developing portable kits for onsite detection of target analytes.

Further, the thesis is focused on the following sub-objectives as given below:

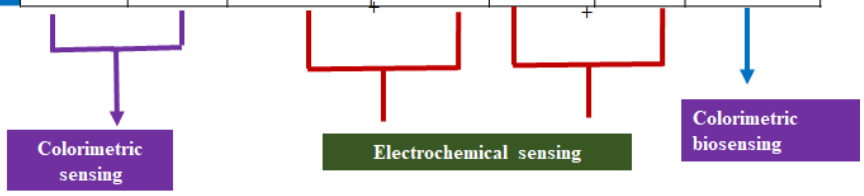
- Facile synthesis of graphitic carbon nitride and its nanocomposite with gold nanorod. Development of impedimetric immunosensor for the NS1 Dengue biomarker based on the gold nanorod decorated graphitic carbon nitride modified electrode.
- Synthesis of molybdenum diselenide nanoflower ($MoSe_2$) and its nanocomposites Au-$MoSe_2$ by decorating gold on its surface. Further development of Au-$MoSe_2$ modified electrode for the electrochemical detection of free cholesterol.
- Synthesis of a nanocomposite from MoS_2 quantum dots and silver nanoparticles and stimulated by mercury(II) and utilizing them as a robust oxidase mimetic for the development of a colorimetric technique for detection of L-cysteine
- Facile synthesis of Cu-Fe Prussian blue analog nanocube and its characterization. Further investigation of its intrinsic oxidase mimetic behavior for the non-invasive colorimetric detection of isoniazid in human urine.

1.10 Benefits of the proposed materials for sensing applications

The various functional nanomaterials like molybdenum disulfide quantum dots (MoS_2-QDs), graphitic carbon nitride (g-C_3N_4), molybdenum diselenide nanosheet ($MoSe_2$), and their composites have been synthesized with metal nanoparticles for the enhancement of their catalytic properties and stability like MoS_2-QDs stabilized silver nanoparticles, gold nanorod decorated g-C_3N_4 nanosheet, gold nanoflower decorated $MoSe_2$ nanosheet, and synthesis of transition metals based metal-organic frameworks e.g. Cu-Fe Prussian blue analogue nanocube (Cu-Fe-PBA-NC). Further, the synthesized nanocomposites have been exploited for the sensing of different biological and chemical species like Dengue biomarker NS1, L-cysteine, cholesterol, and isoniazid drug. We have explored the typical properties of nanomaterials and their composites in the present work for the development of a sensor prototype (Table 1.2).

Table 1.2: Typical properties of nanomaterials used in sensing.

	MoS_2 QDs	Ag NPs	Au NRs	g-C_3N_4	AuNps	$MoSe_2$	Cu-Fe-PBA-NC
High Surface Area	✓	✓	✓	✓	✓	✓	✓
Highly Functionalized							
Biocompatible	✓	✓	✓	✓	✓	✓	✓
Conducting		✓	✓		✓	✓	✓
Stability	✓		✓	✓	✓	✓	✓
Quantum size (1-10 nm)	✓						
Electro-catalytic	✓	✓	✓	✓	✓	✓	✓



g-C_3N_4 is a metal-free semiconductor that contains a graphitic layered structure. In addition, the high N content due to graphitic N and pyridinic species introduce the g-C_3N_4 a potential candidate for electrocatalytic reactions. The structure comprises repeated aromatic heptazine units, that are connected via tertiary amines. g-C_3N_4 bears unique optical, electrochemical, electronic, and physiochemical properties, which makes them good 2D nanomaterials in the field of bioimaging applications [Pang et al., 2009; Pham et al., 2021], photocatalytic degradation of pollutants [Li et al., 2020], optoelectronics [Liu et al., 2020]. The electrochemical properties of g-C_3N_4 are little explored due to large contact resistance, and poor conductivity. The abundant coordinating sites in g-C_3N_4 for the metals that can act as catalytic active sites in the electrocatalytic process prompted us to improve the electrochemical properties of g-C_3N_4 of by making nanohybrid with gold nanorod (AuNRs). The nanohybrid not only improved its conductivity but also assisted in immobilizing the antibody. Further, we have developed an immunosensor for a very lethal disease "Dengue".

Among transition metal dichalcogenides, $MoSe_2$ is found to be analogous to MoS_2 and exhibits good potential in a similar field of applications. The conductivity of the $MoSe_2$ is comparatively greater than MoS_2 due to the intrinsic metallic nature of Se (5×10^{-28} sm^{-1} for S and 1×10^{-3} Sm^{-1} for Se). Further, as per the reports, the incorporation of the gold nanoparticles into $MoSe_2$ enhances its electrical and optical performances considerably [Wu et al., 2020; Eftekhari et al., 2017]. We have decorated the $MoSe_2$ with gold nanoflower utilizing its reducing nature without any aid of a reducing agent. The synthesized nanocomposites exhibited better electrochemical properties and eases the immobilization of enzymes for fabricating the sensor. We have exploited this nanocomposite to fabricate the biosensor of cholesterol.

The greater molar extinction coefficient of silver nanoparticles makes them as a good candidate for optical sensors. However, bare AgNPs are unstable and prone to aggregation, creating complications in practical applications. In our work, we have functionalized the surface of the nanoparticles with MoS_2-QDs which not only assisted in stabilizing the sol but also induced the mimicking property to it. We have utilized functionalized silver nanoparticles for developing the sensors for an important biomolecule, L-Cysteine. PBAs have drawn great attention in the field of hydrogen evolution, oxygen reduction, biosensing, and energy storage due to their good electron-transport property and high surface area. In our work, Cu-Fe Prussian blue analog nanocube (CuFe-PBA-NC) was synthesized which exhibited intrinsic oxidase mimetic property. The CuFe-PBA-NC has good affinity toward chromogenic substrate with considerably better enzyme velocity. These properties have motivated us to fabricate a colorimetric sensor of anti-Tuberculosis drug, isoniazid (INH).