

## **CHAPTER 1**

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### **INTRODUCTION, AIMS AND OBJECTIVES**

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## **1.1. Introduction:**

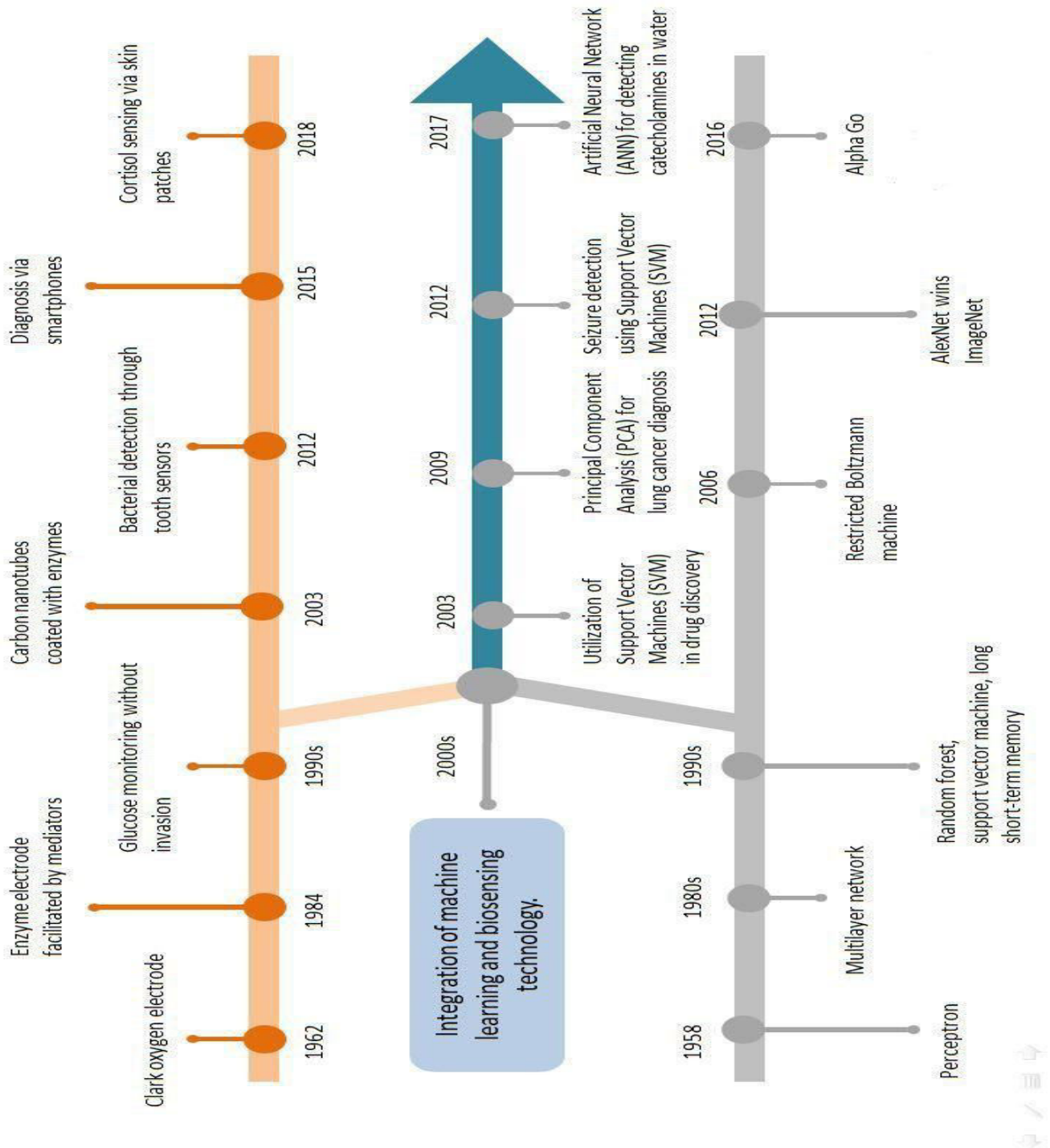
### **1.1.1 History:**

A biosensor is an analytical device, used for the detection of an analyte that combines a biological component with a physicochemical detector (Heineman et. al., 2006). According to the definition by the International Union of Pure and Applied Chemists, “A biosensor is a self-contained, integrated, analytical device, in which a biological recognition element (biochemical receptors, including enzymes, antibodies, antigens, peptides, DNA, aptamers or living cells) is retained in direct spatial contact with a transduction element (such as electrochemical, optical and mechanical transducers)”.

Biosensors were first reported by Leland Charles Clark Jr. in 1962, who conceived the idea of demonstrating the components of a biosensor along with a strategy to integrate a bioreceptor with a transducer device (Lee et al., 2021).

Biosensors were initially developed for point of care (POC) testing of biomolecular targets in the hope of extending clinical analysis from specialized laboratories to public settings, including hospitals, non-hospital nursing settings or home settings. Although various biosensors have been developed for the sensitive and selective detection of a range of disease-related molecules, clinical translation of biosensors remains limited owing to difficulties in integrating and miniaturizing biosensors into portable devices (Lambrianou et al., 2007).

Biosensors are attractive devices used in academia, industries, and research laboratories. They have the exceptional ability to recognize a biological event on a transducing device using a signal proportional to the analyte concentration to quantify a biological or biochemical response. Biosensors are used to monitor the presence of pathogens in physiological fluids such as blood, urine, saliva, tears, sweat, biomarkers for various diseases and environmental pollutants (Wang et al., 2001; Liu et al., 2020; Lee et al., 2019).

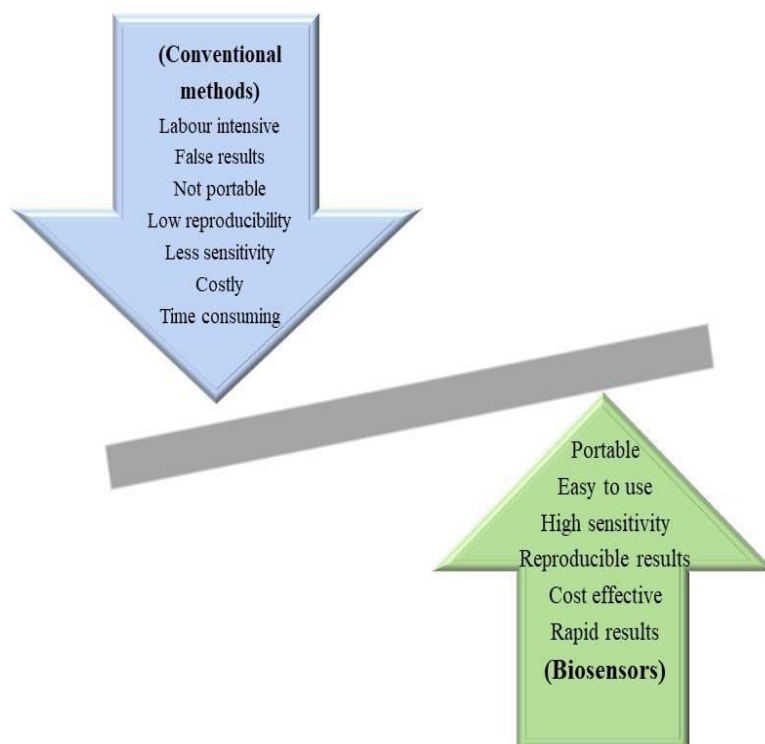


**Figure 1.1 Development of biosensors**

*Development of Prototypes of Biosensors for The Detection of Pathogens, Cancer Biomarkers and Environmental Pollutant*

### 1.1.2 What is the reason behind using biosensors as analytical devices?

Conventional analytical methods such as cell culture and colony count methods, physico-chemical techniques such as spectroscopy and chromatography, immunological tests such as ELISA, flow cytometry, nucleic acid based techniques such as PCR, despite being accurate with indisputable results, are confronted by many practical difficulties. These methods are known for being complex, laborious, time consuming, needing specialised technicians and high-end equipment (Jairath et al., 2015; Köse et al., 2011; Thakkar J.B., Ph.D. Thesis, April 2022; Papageorgiou et al., 2018; Torre et al., 2020; Zhao et al., 2014). Some of these techniques such as PCR, chromatography and spectroscopy are marred by interference from other species in the samples and require purified samples. Therefore, such methods of analysis can be carried out in sophisticated laboratories where the overall process is time consuming, ruling out the possibility of swift on-site testing and analysis (Torre et al., 2020). On the other hand, biosensors offer a possibility for rapid, on-site, continuous, sensitive and cost-effective testing.



**Figure 1.2 Advantages of biosensor over conventional methods**

Extensive research efforts spread over several decades and after numerous publications, the biosensor field can be split into two distinct categories. Firstly, there are complex, high-throughput, and expensive laboratory devices known for their ability to measure biological and analytical interactions accurately. Trained technicians in controlled laboratory environments primarily use these devices. Secondly, there are portable, affordable, and user-friendly biosensors designed for on-the-spot or for home use, intended for individuals without technical expertise. This classification highlights the differences in the two approaches, which include biosensor development, the variety of applications and end users (Turner et al., 2013).

Conventional Methods	Disadvantages of conventional methods	Advantages of biosensor
Pure culture method	<ul style="list-style-type: none"> <li>• Some serotypes are not distinguishing</li> <li>• Missed on specific media</li> <li>• Gave false negatives results</li> <li>• Increasing costs for further tests.</li> <li>• This requires at least 4/5 days to give results.</li> </ul>	<ul style="list-style-type: none"> <li>• Rapid</li> <li>• Continuous detection</li> <li>• Portable</li> <li>• Ease to use</li> </ul>
Nucleic acid-based techniques (PCR etc.)	<ul style="list-style-type: none"> <li>• Lack ability to distinguish between live and dead cells</li> <li>• Gives negative results</li> <li>• Not fast enough to respond bioterrorism events</li> <li>• Expensive</li> </ul>	<ul style="list-style-type: none"> <li>• No need of pure sample</li> <li>• Portable</li> <li>• Rapid</li> <li>• Cheap</li> </ul>
Physico-chemical methods	<ul style="list-style-type: none"> <li>• Do not permit site testing</li> <li>• Expensive</li> <li>• Require trained technician</li> <li>• Require specialised equipment's</li> <li>• Time consuming and tedious</li> </ul>	<ul style="list-style-type: none"> <li>• Only detect the analyte</li> <li>• Rapid</li> <li>• Cheap</li> <li>• No need of technicians</li> </ul>

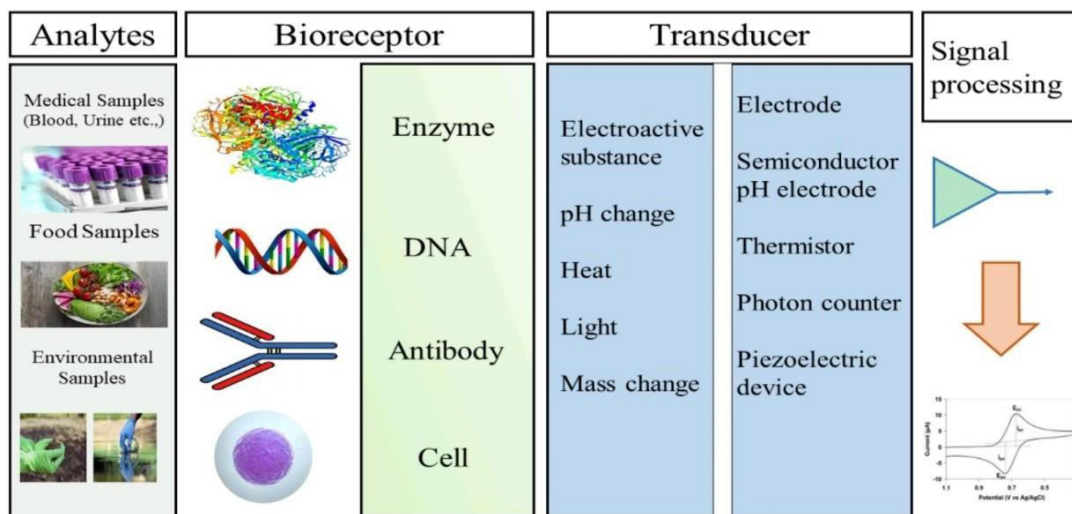
(spectroscopy, chromatography etc.)		<ul style="list-style-type: none"> <li>• Smaller size</li> <li>• On site field application</li> </ul>
Immunological methods (ELISA, IMS etc.)	<ul style="list-style-type: none"> <li>• Time consuming,</li> <li>• Labour intensive,</li> <li>• Provide limited information</li> <li>• Labeled reporter can sometimes alter the specific activity of the binding reagents</li> <li>• Gives false results.</li> </ul>	<ul style="list-style-type: none"> <li>• No labelling</li> <li>• Sometimes reusable</li> <li>• Substitute of traditional testing procedures</li> <li>• Able to catalyse large number of reactions</li> <li>• Significant cost in industries</li> <li>• Rapid</li> <li>• Cheap</li> </ul>

**Table. 1.1 Advantages of biosensor over conventional techniques.**

Biosensors have broken new ground in recent years, entering the fields of biology, medicine and environmental monitoring. Besides, their applications could be extended to high-throughput screening, homeland security, food safety and agriculture (Lee et al., 2021).

### **1.1.3 Designing of Biosensors and the Underlying Principles:**

Biosensors can come in various sizes, shapes and electrode materials. They can detect and evaluate viruses, pathogens, disease biomarkers and pollutants. They take the form of a small probe or an electronic device to generate an output on the indicator that can be used for quantification; the electronic device is responsible for communicating, recording, and detecting the changes in the physiological parameters of the biological or chemical components in the environment. The device includes (i) an analyte, (ii) biological material, (iii) a transducer, (iv) an electronic module, and (v) a display unit. When the tested material is introduced into an electrolytic solution, its constituents



**Figure 1.3 Components of a typical biosensor**

are recognized (alcohol, glucose, lactose, and ammonia). Biorecognition creates a signal creation during the interaction between the analyte and the biological components, while the transducer transforms a biorecognition signal into a quantifiable electrical form, which indicates the presence of a biological or chemical compound/ component in the solution. The analyte–bioreceptor interactions are proportional to the electrical or optical signals produced by the transducers, which can be connected to the cloud server to access and store the data; the output data can take the form of graphical, numerical or tabular analyses (Lee et al., 2021).

#### 1.1.4 Characteristics of Biosensors:

To develop a highly effective and capable biosensor system, certain requirements need to be met. Based on these specifications, the performance of the biosensors can be optimized for commercial uses.

(a) **Selectivity:** Selectivity is a crucial feature to consider when selecting a bioreceptor for a biosensor. A bioreceptor can detect a particular target analyte molecule in a sample which is an admixture of other species and unwanted contaminants (Pohanka, 2017).

(b) **Sensitivity:** Sensitivity is the ability of the biosensor to detect minimum amount of analyte that can be correctly detected/identified in a minimum number of steps (ng/mL or fg/mL). Many

times dilute solutions containing trace amounts of analyte have to be checked, to confirm the presence of certain environmental pollutants in the sample (Wang, 2008).

(c) Linearity: Linearity contributes to the accuracy of the measured results. The higher the linearity (straight line), the higher will be the detectable substrate concentration.

(d) Response time: The time that is taken for obtaining 95% of the results (Ferapontova and Gothelf, 2009).

(e) Reproducibility: Reproducibility is characterized by precision (similar output when the sample is measured more than once) and accuracy (capability of a sensor to generate a mean value closer to the actual value when the sample is measured every time). It is the ability of the biosensor to produce identical results whenever the same sample is measured more than once (Grieshaber et al., 2008).

(f) Stability: Stability is one of the key characteristics in biosensor applications where continuous monitoring is required. Stability is the extent of vulnerability to environmental disturbances inside and outside the biosensing device. The factors that affect stability are the affinity of the bioreceptor (the extent of binding of the analyte to the bioreceptor) and the degradation of the bioreceptor over time (Salimi and Noorbakhsh, 2009).

(g) Regeneration Ability: Ability to regenerate the biosensor for multiple uses, improving cost-effectiveness. (Luo et al., 2006).

### **1.1.5 Types of biosensors:**

Based on signal transduction system and bioreceptor for biological signalling, biosensors can be divided into many types (Perumal and Hashim, 2014).

#### **1.1.5.1 Types of Biosensors based on bioreceptors:**

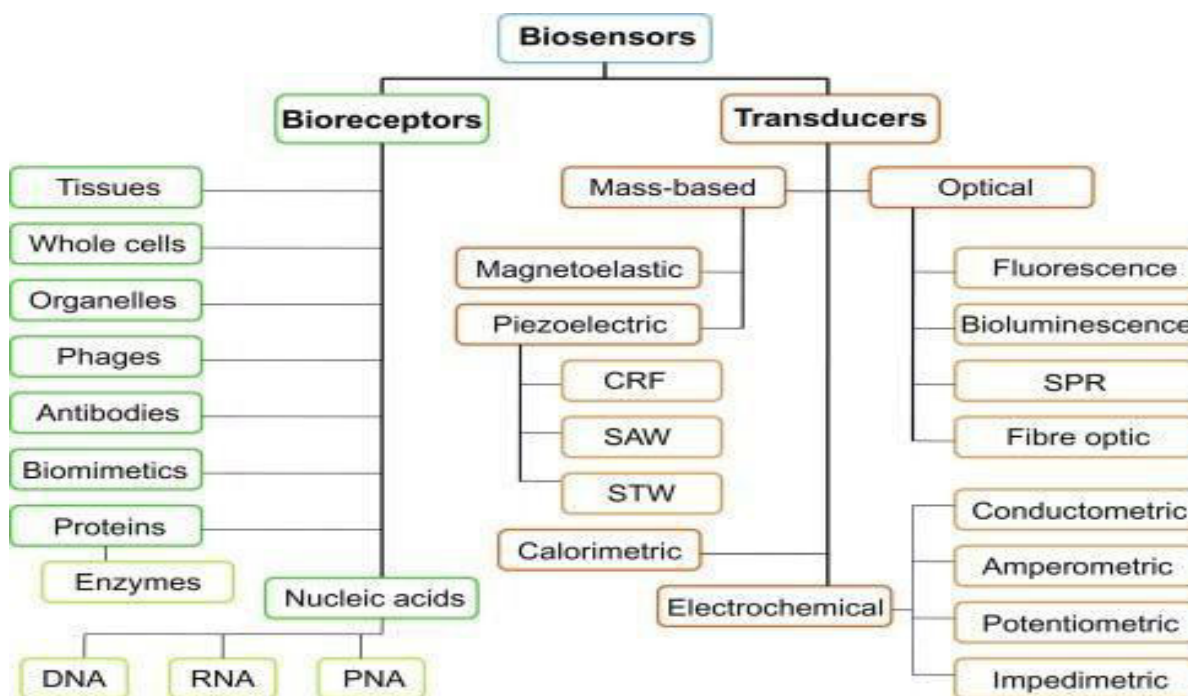
Biosensors are classified as catalytic or non-catalytic, based on the principle of biorecognition. The interaction between analyte and bioreceptor in a catalytic biosensor results in a unique biochemical reaction generating a product. Tissues, whole cells, bacteria, and enzymes are all included under this type of biosensor. The ligand to the analyte is irreversibly coupled to

the receptor in a non-catalytic biosensor, and no new biochemical reaction product is formed during the interaction of the ligand and the analyte. To identify the target, these sensors interact with nucleic acid, antibodies, and cell receptors.

The classification of biosensors into catalytic and non-catalytic types based on bioreceptors highlights the different mechanisms by which these sensors interact with analytes.

### Catalytic Biosensors:

In catalytic biosensors, the interaction between the analyte and the bioreceptor results in a unique biochemical reaction product. This type of biosensor can utilize various bioreceptors, including tissues, whole cells, bacteria, and enzymes. The interaction leads to a biochemical reaction that produces a specific product. This product can be detected and quantified, providing a measurable signal. Enzyme-based biosensors fall under this category, where the enzyme catalyses a reaction with the analyte, resulting in a detectable product.



**Figure 1.4** Flowchart showing the various types of biosensors based on their transducers and biological recognition elements (Aniruddha et al., 2019)

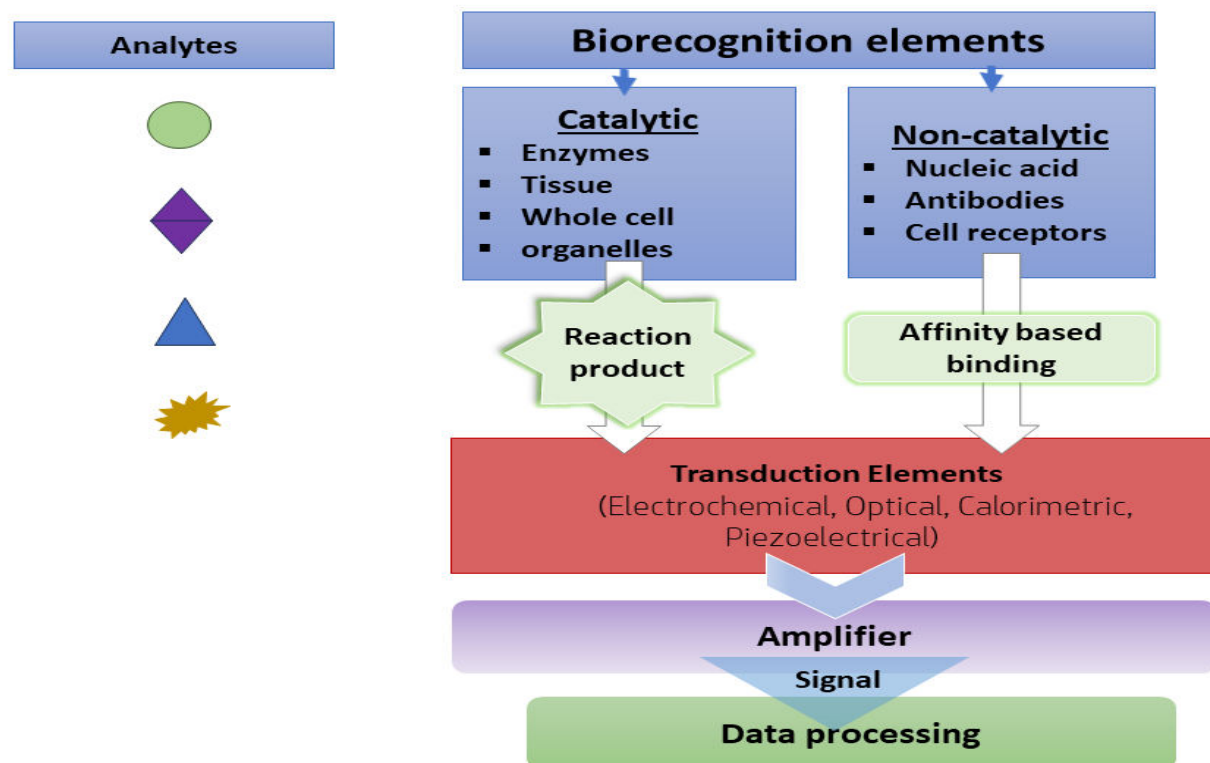
## Non-Catalytic Biosensors:

In non-catalytic biosensors, the analyte is irreversibly coupled to the receptor, but no new product due to a biochemical reaction is formed during the interaction.

**Bioreceptor Examples:** Non-catalytic biosensors interact with various bioreceptors such as nucleic acids, antibodies, and cell receptors.

**Coupling Mechanism:** The interaction involves the binding or coupling of the analyte to the receptor without the occurrence of a chemical reaction leading to a new product.

**Target Identification:** These biosensors are commonly used for identifying specific targets, such as nucleic acids in DNA biosensors, antigens in antibody-based biosensors, or cell surface receptors in cell-based biosensors. Example: DNA biosensors, where the binding of the analyte to complementary DNA sequences is detected without involving any enzymatic reaction.



**Figure 1.5 Schematic Diagram of Biosensor Interaction Mechanisms: Catalytic vs. Non-Catalytic**

*Development of Prototypes of Biosensors for The Detection of Pathogens, Cancer Biomarkers and Environmental Pollutant*

In summary, the catalytic and non-catalytic classification of biosensors is based on how the bioreceptor interacts with the analyte and whether a biochemical reaction product is formed during this interaction. These distinctions allow for the design and application of biosensors tailored to different detection needs and target analytes (Madhusudan et al., 2022).

Based on the use of biological receptor, biosensors can be divided into five different types: Enzyme based biosensor, antibody-based biosensor, DNA based biosensor, Cell based biosensor and biomimetic biosensor (Perumal and Hashim, 2014). Each of the type is discussed shortly in the next section.

#### **1.1.5.1.1 Enzymatic biosensors:**

Enzymes are biocatalysts that can increase the rate of biochemical reactions in the bio-domain. Methods that are used in the recognition of the analyte include: (i) the enzyme metabolizing the analyte and obtaining the concentration by determining the catalytic conversion of the analyte; (ii) the analyte restraining or stimulating the enzyme where the concentration of the analyte is linked to reducing the formed enzymatic product; (iii) monitoring the changes in enzyme characteristics (Simonian et al., 2001). Jinal et al. (2019) developed an organophosphorus pesticide biosensor by directly immobilizing acetylcholine esterase (AChE) onto carboxylic acid-functionalized multiwalled carbon nanotubes (MWCNTs) via amide bonds. This approach enabled the sensitive detection of organophosphorus pesticides, with a linear detection range between 10 nM and 50 nM, and detection limits of 0.1 nM and 500 nM. The sensor demonstrated high sensitivity for pesticide monitoring.

#### **1.1.5.1.2 Antibody based Biosensors:**

Antibodies have been employed for several decades in the biomedical studies, and these sensors can be coated with antibodies or ligands that can affect antibody–antigen interactions. These are further divided into two categories: (i) non-labelled and (ii) labelled. (Skottrup et. al., 2008). Ercole and his team developed urease-antibody conjugate based biosensor which can detect *E. coli* by the changes in pH due to ammonia production from urease *E. coli* antibody complex (Ercole et al., 2003) in vegetables.

### **1.1.5.1.3 Nucleic acid or DNA based biosensor:**

A nucleic acid-based biosensor employs an oligonucleotide as the sensing element, with a known sequence of bases. Nucleic acid biosensors can be used to detect DNA/RNA fragments of either biological or chemical origin. In its application, DNA/RNA is the analyte and it is detected through the annealing reaction (this kind of biosensor is also called a genosensor). Genosensors result from the integration of a sequence-specific probe and a signal transducer. The probe immobilized onto the transducer surface, acts as the biorecognition molecule and recognizes the target DNA or RNA. The main steps to make a genosensor are probe selection and immobilization, development and detection of the hybridization reaction (Palchetti and Mascini 2008). Once the target nucleic acid has been captured onto the sensor surface, a range of different approaches can be used for transducing the biorecognition event; these can be broadly divided into label-free and label-based schemes. The detection technique of the sequence-selective DNA hybridization has been developed through various methods such as optical (Ferguson et al., 1996) electrochemical (Mikkelsen, 1996) and piezoelectric transduction modes (Palecek and Jelen, 2002). Among all the methods, electrochemical method has attracted the attention of many researchers because of its high sensitivity, low cost, and compatibility with microfabrication technology (Eunkyung et al., 2003). Nucleic acid molecules can be classified as DNA, RNA, peptide nucleic acid (PNA) and aptamers (Castaneda et al., 2021).

#### **DNA based biosensors:**

DNA based biosensors utilize DNA strands as recognition elements and their design can vary based on the detection method and application. These biosensors can detect specific DNA sequences, mutations or hybridization events (Wang and Liu, 2007). DNA is composed of two complementary strands forming a double helix, held together by hydrogen bonds between complementary nucleotide bases (adenine-thymine, guanine-cytosine and adenine-uracil). The sequence of nucleotide bases provides the inherent specificity of DNA. DNA biosensors rely on the selective hybridization between target DNA or RNA sequences and complementary DNA probes. Many a time arrays of DNA probes immobilized on a surface are employed for parallel detection of multiple targets. PCR based biosensors utilize polymerase chain reaction (PCR) for target amplification before detection. Electrochemical DNA sensors exploit changes in electrical

properties upon interaction of the analyte with DNA. The DNA biosensors are employed in genetic analysis, including the detection of specific DNA sequences, mutations, and single nucleotide polymorphisms (SNPs). These biosensors are used for the identification of pathogens, viruses, and bacteria based on their nucleic acid signatures. DNA biosensors play a role in cancer diagnostics by detecting specific genetic markers associated with cancer. DNA probes can be labelled with fluorophores for fluorescence-based detection, allowing real-time monitoring of hybridization events. DNA sensors can be integrated into electrochemical platforms for sensitive and selective detection. Changes in colour or absorbance indicate the presence of the target DNA sequence. Achieving high sensitivity is crucial for reliable detection of low-abundance target sequences. Ensuring high specificity is important to avoid false-positive results. The complexity of biological samples may impact the performance of DNA biosensors (Paleček, 2002; Wang, 2006; De la Rica and Stevens, 2012).

### **RNA based biosensors:**

RNA based biosensors employ RNA molecules for specific target recognition. They can be designed to detect RNA sequences or monitor RNA-protein interactions (Zhang and Zuo, 2020). These biosensors leverage the unique properties of RNA, including its ability to fold into structures and interact with target molecules. Natural RNA elements that undergo conformational changes upon binding specific ligands serve as regulatory elements. Artificially selected RNA sequences bind to specific targets with high affinity and specificity. Small regulatory RNAs (sRNAs), RNA molecules involved in post-transcriptional regulation, often bind to target mRNAs. Riboswitches formed due to conformational changes in response to ligand binding can be transduced into measurable signals. Binding of target molecules induces structural changes in the aptamer, which can be detected through various means. Interaction of sRNAs with target mRNAs, which have influence over gene expression, can be monitored. RNA based biosensors can be designed to detect small molecules, such as metabolites or drugs. Aptamers can be engineered to bind to specific proteins, enabling protein detection. RNA based biosensors are also used for the identification of pathogens based on their nucleic acid sequences. Fluorophores can be incorporated into RNA molecules, as target binding can be followed by monitoring the changes in fluorescence. RNA can be integrated into electrochemical platforms for sensitive detection and changes in absorbance can serve as indication for the presence of the target. RNA-based biosensors can be designed for a

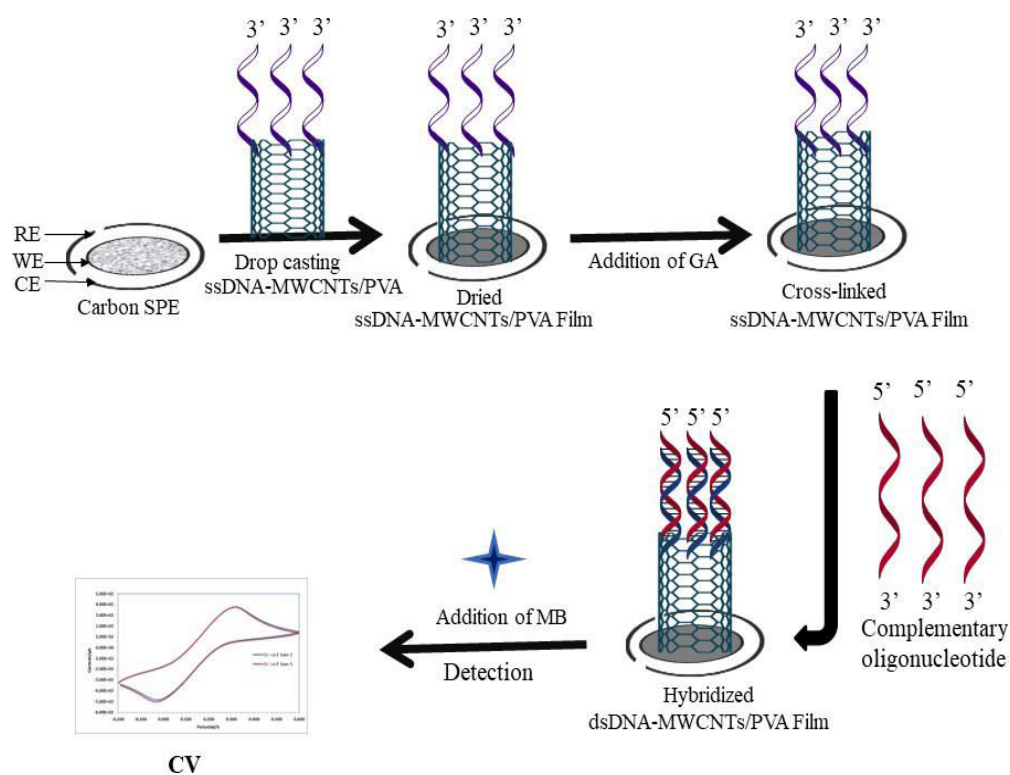
wide range of targets, including small molecules, proteins and nucleic acids. However, the susceptibility of RNA to degradation makes careful design and stabilization strategies inevitable. Ensuring high specificity is crucial to avoid cross-reactivity. Strategies for delivering RNA molecules to the target environment may be needed (Breaker, 2012; Ellington and Szostak, 1990; Mandal and Breaker, 2004).

### **PNA based biosensors:**

Peptide Nucleic Acid (PNA) biosensors use synthetic PNA molecules as recognition elements. PNA offers enhanced stability and specificity in nucleic acid detection (Singh et al., 2019). Peptide Nucleic Acid (PNA) based biosensors utilize synthetic PNA molecules as sensing elements for the detection of specific DNA or RNA sequences. PNA is a type of nucleic acid analogue with a neutral peptide backbone, making it resistant to enzymatic degradation and offering unique advantages in biosensing applications. PNA has a backbone composed of repeating N-(2-aminoethyl) glycine units, which is neutral and uncharged. Nucleobases (adenine, thymine, cytosine, and guanine) are attached to the backbone, allowing sequence-specific hybridization with complementary DNA or RNA. The sequence specificity of PNA allows for highly selective binding to target nucleic acid sequences. PNA exhibits high stability due to resistance against nucleases and proteases, making it suitable for various biological environments. PNA shows a strong binding affinity to DNA or RNA targets, often surpassing the binding affinity of natural DNA-DNA or RNA-DNA interactions. PNA-based biosensors offer high specificity, reducing the likelihood of false-positive or false-negative results. PNA-based biosensors are widely used for genetic analysis, including the detection of specific DNA or RNA sequences, mutations in the sequences and single nucleotide polymorphisms (SNPs). These biosensors find applications in clinical diagnostics for identifying disease-related genetic markers and pathogens. PNA-based biosensors are employed in environmental monitoring for the detection of specific nucleic acid sequences indicative of environmental contaminants. PNA probes can be labelled with fluorophores for fluorescence-based detection, allowing real-time monitoring of hybridization events. PNA probes can be immobilized on electrodes for electrochemical sensing, providing a quantitative measurement of nucleic acid targets. The synthesis of PNA molecules is more complex than traditional DNA or RNA synthesis. PNA-based biosensors may be more expensive to produce compared to conventional nucleic acid-based biosensors. (Nielsen, 1993; Nielsen, 1994; Gothelf, 2010).

**Aptamer based biosensor:**

Aptamers, first raised by two research groups independently in 1990, are single-stranded DNA (ssDNA) or RNA sequences obtained through systematic evolution of ligands by events exponential enrichment (SELEX) that can fold into secondary and three-dimensional shapes, conditions. Unlike antibodies that require biological systems to be generated, aptamers can be chemically synthesized, are stable in a pH range of 2 to 12, and have certain thermal refolding capabilities. A further benefit of aptamers is that they can be chemically modified according to the detection criteria chosen for the target molecule (Kumar et al., 2019). By an *in vitro* selection mechanism, SELEX (Systematic Evolution of Ligands by EXponential enrichment), aptamers can be isolated from oligonucleotides libraries (Stoltenburg et al., 2007; Tuerk et al., 1990). Several SELEX variants have recently been established, including cell-SELEX, capillary electrophoresis-based SELEX, microfluidics-SELEX, FACS-based SELEX, microtiter plate-SELEX, magnetic bead SELEX, and *in vivo* SELEX (Kaur and Shorie, 2019). Optical, electrochemical, and piezoelectric techniques are the most frequently used ones in biosensors.



**Figure 1.6 An aptamer based electrochemical biosensor**

Depending on different transduction techniques, these biosensors are further categorized as labelled or label-free aptasensors. Surface plasmon resonance (SPR) is the most commonly used method for label-free optical sensors, whereas fluorescent dyes (fluorescein) are used for label-based optical aptasensors (Hainik et al., 2018). For tracking biological systems in real-time, fluorescent nanoparticles (NPs), such as quantum dots (QDs), provide many benefits over regular fluorescent dyes. To identify targets, such as cancer cells, bacterial spores, and proteins, aptamer-QD conjugates were used (Chu et al., 2006; Ikanovic et al., 2007). Starno et al. demonstrated detection of thrombin protein using aptamer capped NIR PbS QDs to, based on selective charge transfer, within 1 min of exposure and with a detection limit of ~1 nM (Starno et al., 2006). Gold nanoparticles (GNPs) have demonstrated interesting absorption characteristics that vary depending upon their aggregation state. Furthermore, GNPs are more biocompatible, easier to bioconjugate, and less toxic than QDs ((Arya et al., 2019). Feng et al. fabricated an electrochemical impedance spectroscopy (EIS)-based biosensor for L-Arg detection, which exhibited acceptable performance in the linear range of 0.1 pM – 0.1 mM with a detection limit of 0.01 pM (Feng et al., 2021)

#### **1.1.5.1.4 Cell based biosensor:**

Living cells express a wide variety of molecules (receptors) in different proportions, hence cells can not only yield quantitative response to specific stimuli in a given condition but they can also help in quantitatively analysing more than one analyte, while requiring less effort and expenses. This was the primary motivation behind utilization of cells as bioreceptors in the earlier days (Corcoran and Rechnitz, 1985). Cell-based biosensors enable analysis and monitoring of drug-ligand interactions, effect of bioactive agents, environmental toxicity studies and others in a more physiologically relevant way (Cevenini et al., 2016; Liu et al., 2014; Pan et al., 2015). With the use of suitable substrates, it is possible now to use cell-based biosensors for in situ (ex vivo in some cases) monitoring with living cells in their native environment. Different cell types such as bacteria, yeast, fungi, algae and other eukaryotes including fish, rat and human cells have been utilized for biosensor fabrication (Brennan et al., 2016; Liu et al., 2014; Su et al., 2011)

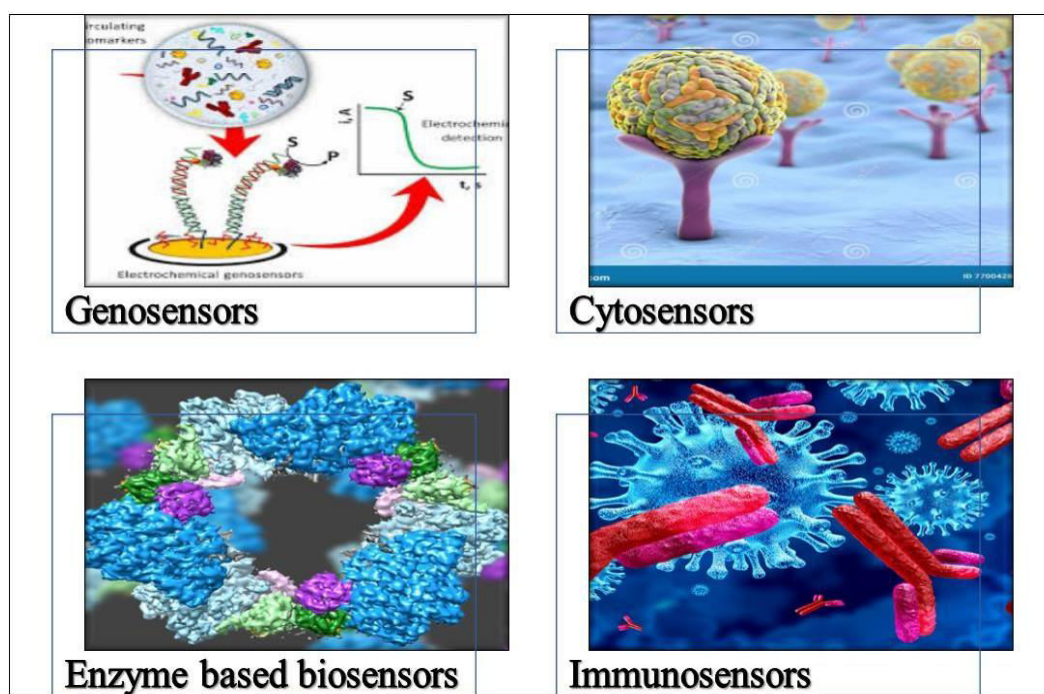
Many different types of microorganisms have been utilized for biosensor fabrication over the years, including bacteria, photosynthetic algae (Tahirbegi et al., 2017; Tsopela et al. 2014, 2016) and yeast cells (Pham et al., 2013; Pham et al., 2015; Vopálenská et al., 2015) with prominent applications in environmental monitoring and antimicrobial susceptibility testing. Some of these biosensors exploit the natural ability of a particular microorganism to sense a specific analyte while several others are dependent on the introduction of specifically designed genetic constructs in a host microorganism for the detection of bioavailable analytes. Animal cells of higher eukaryotes differ from microbial cells in terms of their nutritional requirements, growth rate and many morphological and structural characteristics. Thus, biosensors based on animal cells face altogether different types of design and fabrication challenges. These biosensors have important applications in the fields of food safety, disease modelling, and drug toxicity assessment. Electrical cell substrate impedance sensing (ECIS) and light addressable potentiometric sensing (LAPS) are the two most prominently used transduction techniques for animal cell based biosensors. ECIS, developed by Giaever and Keese (1993), utilizes gold interdigitated electrodes (IDEs) for monitoring of impedance (or, capacitance) with changes in cell density (Giaever and Keese, 1993). LAPS, on the other hand, is a semiconductor based chemical sensor with an electrolyte insulator semiconductor configuration (EIS). This technique is most prominently used by microphysiometers, devices designed for measuring more than one metabolic parameter at a given time. Other than these electrochemical devices, the quartz crystal microbalance (QCM) has found applications in animal cell based biosensors. Cells immobilized on to the support must be preserved alive for a very long period in the natural environmental conditions with limited physical and chemical parameters. Thus, sensitivity and detection limit of the biosensor totally depends on the natural microenvironment where the cells or their components are preserved for a long time. Major shortcoming faced by this kind of biosensor is stability of the cell which is influenced by sterility, biocompatibility, life-cycle etc. Cell based sensors offer multiple receptor sites which also become the reason for its poor specificity or selectivity sometimes (Perumal and Hashim, 2014; Shimomura-Shimizu and Karube, 2010). Even after facing such issues, cell based sensors are still thought to be very exciting by researchers as they can distinguish inhibitors very rapidly and are tolerant to a narrow range of temperature and pH fluctuations which do not cause cell death. This kind of sensors have long shelf life and are cost effective as active cells are not needed to be isolated repeatedly. In this context, cell based sensors are superior to enzyme based biosensors

(Zourob, 2010). Cell based sensors can be the future of nano-diagnostics due to their special characteristics (Shinde et al., 2012). Cell based biosensors find applications mainly in medical field for the detection of a disease, drug discovery, pharma industry, environmental monitoring and food quality assessment (Veiseh et al., 2007; Banerjee and Bhunia, 2009; Yang et al., 2010; Wang et al., 2010; Bohrn et al., 2012; Q. Liu et al., 2012; Melamed et al., 2012).

#### **1.1.5.1.5 Biomimetic biosensor:**

Biomimetic sensors are devices that replicate biological receptors and enzymes with extraordinary accuracy and sensitivity by taking their cues from nature's effective mechanisms. Some of the recognition components used in biomimetic sensors are aptamers, molecularly imprinted polymers (MIPs) and immunomimetic sensors (Morrison et al., 2007; Gai et al., 2008; Mosbach, 1994).

Molecular imprinting was first reported by Mosbach and his team and continued to advance technologically, especially in 3D printing of particular analytes/objects (Mosbach et al., 1994). In molecular imprinting, a particular analyte to be detected is “frozen” by a mixture of monomers, co-polymers and cross-linkers. The premix is then allowed to polymerize around that particular analyte called a template. After polymerization, the template analytes are washed off by several washing solutions, which leave behind their complementary cavities when washed off. These cavities are complementary to the target analytes in their size, shape and spatial arrangement of chemical functional groups. When the imprinted polymer material comes in contact with the target analyte, it binds with the target analyte in the cavities present in the template specifically for detection (Mosbach, 1994).



**Figure 1.7 Different types of biosensors based on bioreceptors**

Molecular imprinting approach offers cost effective, sturdy and specific receptors in biosensor fabrication. Aptamers as artificial nucleic acid strands were first introduced in 1990s and continued to grab attention for developing modern day biosensors. Aptamers are chemically synthesized long nucleic acid strands which act similar to antibodies. Aptamers sometimes show tremendous specificity towards their targets as the biorecognition elements compared to antibodies (Turner et al., 2006; Zourob, 2010). Aptamers can be designed and synthesized to recognize specific peptides, proteins, oligosaccharides and other amino acids as well. Aptamer based biosensors are highly comparable with antibody based biosensors. Aptamers are highly specific to their targets, small in size and less complex in structures. In silico designing and optimization of aptamers offers the solution to the above limitations. Aptamers offer a variety of advantages as receptor in the biosensors such as small size, specificity, modification and immobilization capabilities, reusability (Zourob, 2010). Both molecularly imprinted and aptamer based biosensors offer their applications majorly in clinical diagnostics for the detection of

bacterial, viral and other diseases (Cohen et al., 2010; Pang et al., 2005; Parmpi and Kofinas, 2004; Xue et al., 2009; Strehlitz et al., 2008; Torres-Chavolla and Alocilja, 2009; Weng et al., 2012; Wang et al., 2012). Though aptamers have much simpler chemical structures, the manufacturing costs are still high. Further, in nucleic acid based biosensors nonspecific recognition is encountered with some aptamers. Research in the area is focused on overcoming these two problems.

### 1.1.5.2 Types of Biosensors based on transducers

The transducer converts the biochemical interactions into measurable electronic signals. Transducer is a very important component in a biosensor which converts the chemical, biological or physical response into a detectable signal with low interference and high sensitivity (Lowe, 2008). According to their operating principle, transducers are broadly categorized as electrochemical, optical, thermal/ calorimetric, gravimetric, magnetic, piezoelectric and resonant types (Perumal and Hashim, 2014).

#### 1.1.5.2.1 Electrochemical biosensors:

An electrode is used as a transduction component in an electrochemical biosensor. IUPAC in 1999, declared that an electrochemical biosensor is an integrated device which is used for quantitative or semi-quantitative detection of particular analyte using a receptor placed in close contact with electrical transducer on electrode surface (Ya'acob et al., 1989; Thevenot, 1999; Thévenot et al., 2001). The electrochemical transducers monitor electric potential or electric current changes caused by electrons or ions during a biochemical reaction of the biorecognition element (mostly enzyme) with the analyte. A standard electrochemical sensor is composed of two (reference and working) or three (reference, working and counter) electrodes.

The electrochemical biosensor is a sensing device which causes transduction of the biochemical reactions to electronic signals. In electrochemical sensing devices, the electrode is employed as a solid support for electron movement and immobilization of biomolecules (Ahlawat et al., 2023). Electrochemical sensors are able to detect various biomolecules in the human body such as glucose, cholesterol, uric acid, lactate, DNA, haemoglobin, blood ketones, and others.

Electrochemical biosensors generate electronic signals by transducing biochemical interactions between a target analyte and a biorecognition element immobilized on the electrode surface. These interactions, such as enzyme-substrate reactions, antibody-antigen binding, or DNA hybridization, induce changes in electrochemical properties, including potential, current, impedance, or conductivity, at the electrode interface. Depending on the transduction principle, these changes are measured as specific signals: potentiometric biosensors monitor variations in electric potential due to ion activity, amperometric sensors detect current from redox reactions, impedimetric sensors evaluate changes in system impedance, and voltammetric sensors analyze current responses to applied potentials. The electrode serves as a solid support for biomolecule immobilization and electron transfer, enabling sensitive detection of electroactive species generated during the reaction. This signal is amplified and processed, correlating its magnitude with analyte concentration. For example, in glucose biosensors, glucose oxidase catalyzes glucose oxidation, producing hydrogen peroxide, which generates a measurable current upon oxidation at the electrode surface. Such mechanisms underpin the high sensitivity and selectivity of electrochemical biosensors in detecting biomolecules, making them pivotal in diagnostics, environmental monitoring, and food safety.

Electrochemical biosensors exhibit high sensitivity and selectivity in detection. Based on the transduction principle, electrochemical biosensors are categorized as: (a) potentiometric, (b) amperometric, (c) impedimetric, (d) conductometric, and (e) voltammetric (Grieshaber et al., 2008).

**(a) Potentiometric biosensors:**

In response to a particular voltage applied, current flows through the electrode set up due to electroactive species present in the sample. Change in ionic concentration and pH is measured in response to receptor-analyte interaction. The biosensor is made up of two electrodes, namely working and reference electrodes. The arrangement is not very popular for biosensor fabrications. According to literature, such sensors can be used to detect cancerous cells and bacterial species (Hu et al., 2013; Ya'acob et al., 1989).

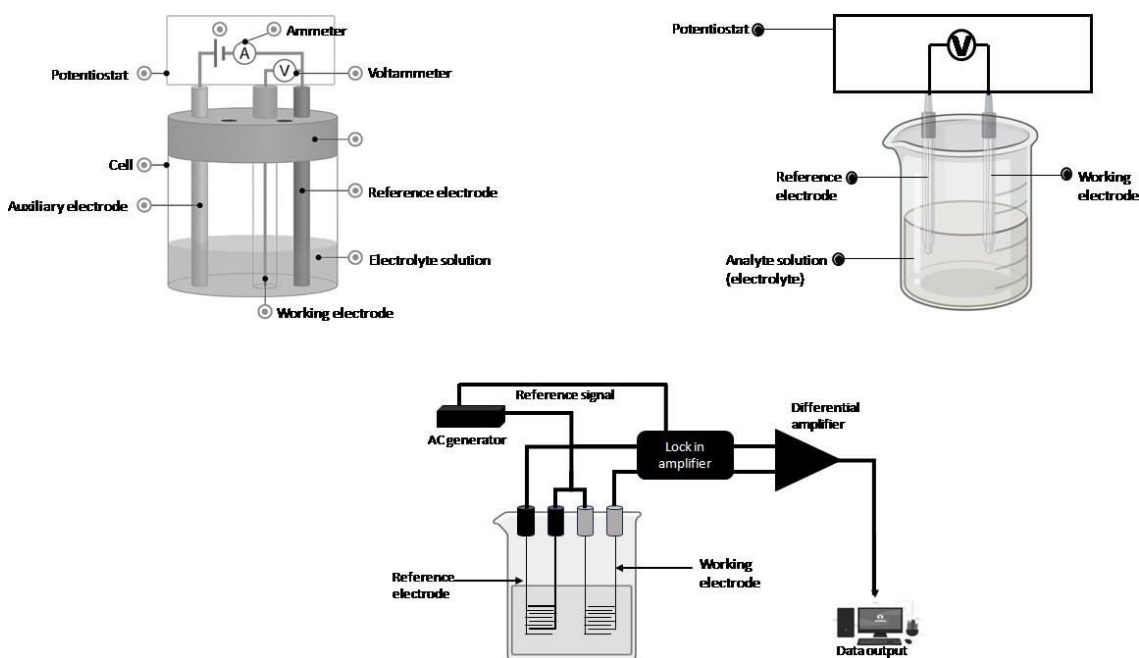
**(b) Conductometric biosensors:**

Upon electrochemical reaction in a sample solution, ions and electrons are produced causing decrease in resistance or increase in conductance of the solution. This change in electrical

conductance is measured by conductometric biosensor. When biological receptor is used to fabricate the electrode, often low signal to noise ratio makes it impractical for biosensing applications. No need of reference electrode in this system. It is cost-effective and can be miniaturized. Conductometric biosensors are useful in the detection of affinity interactions (Korotkaya, 2014; Ya'acob et al., 1989; Perumal and Hashim, 2014).

**(c) Amperometric biosensors:**

In certain chemical reactions, when receptor and analyte undergo oxidation-reduction reactions a sharp change in potential occurs, that is measured by an amperometric biosensor. The best example of a successful amperometric biosensor is Clark's oxygen electrode. The sensor is made up of three electrode system (working, reference and counter electrodes). This is the most commonly used electrode arrangement for fabricating biosensors, as it provides high sensitivity for electroactive species in a given test sample specially for biological tests. Amperometric biosensors make direct and indirect detection of bacterial species possible (Brooks et al., 1992; Nakamura et al., 1991; Ya'acob et al., 1989; Perumal and Hashim, 2014).



**Figure 1.8 Schematic diagram of (a) amperometric/voltammetric (b) potentiometric (c) conductometric**

#### **(d) Impedimetric biosensors:**

Change in impedance upon reaction between receptor and analyte in a given test sample is measured using impedimetric biosensor. The biosensor is composed of three electrode system (working, reference and counter electrodes). The high sensitivity of detection made it feasible to detect bacterial species in clinical samples, continuous monitoring food quality and other industrial processes as reported in literature (Ya'acob et al., 1989; Silley and Forsythe, 1996; Perumal and Hashim, 2014).

#### **(e) Voltammetric biosensors:**

Voltammetric biosensors operate based on the measurement of electrical current resulting from electrochemical reactions occurring at the sensor surface. These biosensors utilize electrochemical reactions, such as oxidation or reduction of analytes, occurring at the electrode surface. A voltage is applied to the working electrode, and the resulting electrical current, which is proportional to the concentration of the analyte, is measured. Biomolecular binding events at the

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sensor surface can modulate the electrochemical reactions, leading to changes in the measured current, enabling the detection of specific analytes. These biosensors are used for the detection of biomarkers for diseases such as cancer, diabetes, and infectious diseases (Wang, 2008), detection of pollutants, heavy metals, and toxins in water, air, and soil samples (Zou et al., 2019), rapid detection of pathogens, toxins, and chemical contaminants in food and beverages (Bansal et al., 2017), monitoring of fermentation processes, enzyme kinetics, and cell growth in bioprocessing industries (Ritzoulis et al., 2019) and high-throughput screening of drug candidates, studying drug-receptor interactions, and evaluating drug efficacy and toxicity (Dincer et al., 2019).

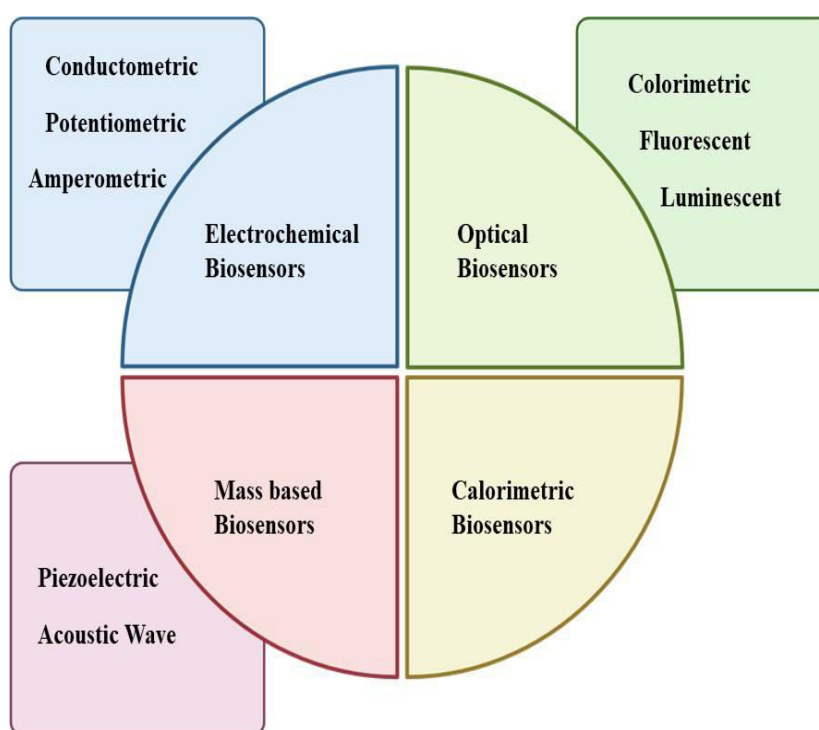
#### **1.1.5.2.2 Optical Biosensor:**

Optical biosensors utilize the interaction of light with biological molecules to detect and quantify specific analytes. Various optical techniques are employed for biosensing, providing high sensitivity and specificity. Surface Plasmon Resonance (SPR) biosensors measure changes in refractive index at a metal-dielectric interface, allowing real-time monitoring of biomolecular interactions (Homola, 2008). In fluorescence based biosensors, fluorescent molecules are used as labels for biomolecular interactions, with changes in fluorescence indicating the presence of analytes (Wolfbeis, 2015). In fiber optic biosensor, fiber optics transmit light to sensing regions where interactions with analytes occur, and changes are detected optically (Wolfbeis, 2013). Photonic crystal biosensors manipulate light to create resonance conditions that are sensitive to changes in refractive index due to biomolecular interactions (Cunningham and Qavi, 2010). In interferometric biosensors, Interference patterns are generated by the interaction of light waves, and changes are measured to detect biomolecular binding events (Piliarik and Sandoghdar, 2014).

In Raman spectroscopy biosensors, Raman scattering provides molecular fingerprinting, and changes in Raman spectra indicate binding events in biosensing (Schlücker, 2014). Optical coherence tomography (OCT) biosensors combine low coherence interferometry and depth resolved imaging for label free detection of biomolecular interactions (Wang et al., 2006).

### 1.1.5.2.3 Piezoelectric based Biosensor:

Piezoelectric biosensors leverage the piezoelectric effect, which involves the generation of an electric charge in response to mechanical stress, for the detection of biological interactions. These sensors are widely used due to their high sensitivity, real-time monitoring capabilities, and versatility. Piezoelectric biosensors typically utilize a quartz crystal microbalance (QCM), where binding events on the crystal surface cause changes in its resonance frequency (Rantala and Laitinen, 2010).



**Figure. 1.9** Types of biosensors based on transducers.

The quartz surface is modified with specific receptors (e.g., antibodies, aptamers) for selective binding to target analytes (Dincer et al., 2011). The initial use of piezoelectric crystals for sensing applications dates back to the 1960s. The development of Quartz crystal microbalance (QCM) technology in the 1960s laid the foundation for piezoelectric biosensors. Early applications focused on using QCM for thin film deposition monitoring in material science. In the 1980s, researchers began exploring the use of piezoelectric devices as immunosensors. The

immobilization of antibodies onto the crystal surface enabled the selective detection of antigens. This era marked the transition from generic sensing applications to the development of biosensors for specific biological interactions. During the 1990s and 2000s, the commercialization of piezoelectric biosensors gained momentum. Companies started offering QCM-based biosensors for various applications, including medical diagnostics and environmental monitoring. Advancements in microfabrication technologies led to the miniaturization of piezoelectric sensors, allowing for portable and point-of-care devices. The 21st century witnessed an expansion of applications for piezoelectric biosensors. Researchers explored DNA hybridization detection, enzyme-based assays, and other biomolecular interactions. Integration of nanomaterials enhances the performance of piezoelectric biosensors, offering improved sensitivity and detection limits (Adhikari et al., 2015). Recent developments focus on the integration of piezoelectric biosensors into point of care testing devices. These devices aim to provide rapid and on-site detection of various analytes, contributing to personalized healthcare (BelBruno, 2019). Ongoing research focuses on improving the selectivity, sensitivity, and multiplexing capabilities of piezoelectric biosensors. Integration with advanced technologies, such as microfluidics and signal processing, is further enhancing their performance.

#### **1.1.5.2.4 Calorimetric biosensor:**

Calorimetric biosensors rely on the measurement of heat changes associated with biochemical reactions to detect and quantify biological analytes. This approach provides valuable information about binding events and reactions occurring on sensor surfaces. Calorimetry has a long history, with the study of heat changes dating back to the early 20<sup>th</sup> century. Isothermal Titration Calorimetry (ITC) emerged as a powerful technique for studying biomolecular interactions by measuring heat changes during titration (Velázquez-Campoy and Freire, 2005). Integration of calorimetric principles into biosensor platforms gained prominence in the 21<sup>st</sup> century (Andersson et al., 2006). Microcalorimetry techniques, such as microscale thermophoresis, have been developed for biosensing applications (Jerabek-Willemsen et al., 2011). Calorimetric biosensors find applications in drug discovery, studying molecular interactions critical for pharmaceutical development (Francoeur et al., 2015). Ongoing research focuses on enhancing the sensitivity and specificity of calorimetric biosensors (Zhao et al., 2020).

Advancements in the design of calorimetric biosensors aim to make them more portable and applicable for point of care diagnostics (Nahavandi et al., 2014). In essence, calorimetric biosensors are adaptable instruments that improve the accuracy and comprehensiveness of data in many scientific domains, eventually augmenting our comprehension of basic biological and chemical processes.

#### **1.1.5.2.5 Magnetic biosensors:**

Magnetic biosensors utilize paramagnetic or supra-paramagnetic particles, or crystals, to detect biological interactions. Examples could be coil-inductance, resistance, or other magnetic properties. It is common to use magnetic nano or microparticles. On the surface of such particles are the bioreceptors that can be DNA (complementary to a sequence or aptamers) antibodies, or others. The binding of the bioreceptor will affect some of the magnetic particle properties that can be measured by AC susceptometry (Strömber et al., 2014), a Hall effect sensor (Liu et al., 2011) and a giant magnetoresistance device (Huang et al., 2017).

#### **1.1.5.2.6 Resonant biosensors:**

Resonant biosensors are a type of biosensors that utilize the principles of resonance to detect and analyse biological interactions. These sensors are designed to measure changes in resonance frequency or other resonant properties caused by the binding of biological molecules, such as proteins or DNA, to a sensing surface. They can provide information on the mass and other characteristics of the bound molecules, enabling sensitive detection and quantification. The change in resonance is correlated with the presence and concentration of specific analytes. The transduction methods used in resonant biosensors vary, but they often involve measuring changes in frequency, impedance or other resonant properties. Common techniques include surface acoustic wave (SAW) sensors, quartz crystal microbalance (QCM) sensors and microcantilever sensors. SAW resonators utilize piezoelectric crystals to generate acoustic waves on the surface of a material. The binding of molecules to the surface induces changes in the wave's velocity or amplitude, which can be detected and correlated with the mass changes (Morgan and Straser, 1995). QCM sensors use a quartz crystal coated with a thin sensing layer. The binding of molecules causes a change in the crystal's resonance frequency, and this change is proportional to the mass

of the bound molecules (Sauerbrey, 1959). Microcantilever sensors involve a cantilever beam with a surface coating that interacts with target molecules. The binding of molecules induces a stress or bending in the cantilever, leading to changes in its resonance frequency (Thundat et al., 2005).

Resonant biosensors find applications in various fields, including medical diagnostics, environmental monitoring and drug discovery. They are particularly useful for studying biomolecular interactions, such as antigen-antibody binding, protein-protein interactions, and DNA hybridization. Resonant biosensors offer high sensitivity and real time monitoring capabilities. They can provide label-free detection, eliminating the need for fluorescent or radioactive labels, and are capable of detecting small changes in mass and molecular interactions. Challenges associated with resonant biosensors include the need for careful surface functionalization, optimization of experimental conditions and potential interference from non-specific binding. Thus, resonant biosensors are powerful tools in the field of biosensing, leveraging the principles of resonance to achieve high sensitivity and specificity in detecting and quantifying biological interactions. They play a crucial role in advancing our understanding of molecular interactions and have practical applications in various research and diagnostic settings.

### **1.1.6 Applications of Biosensors:**

#### **1.1.6.1 Food monitoring and pathogen detection:**

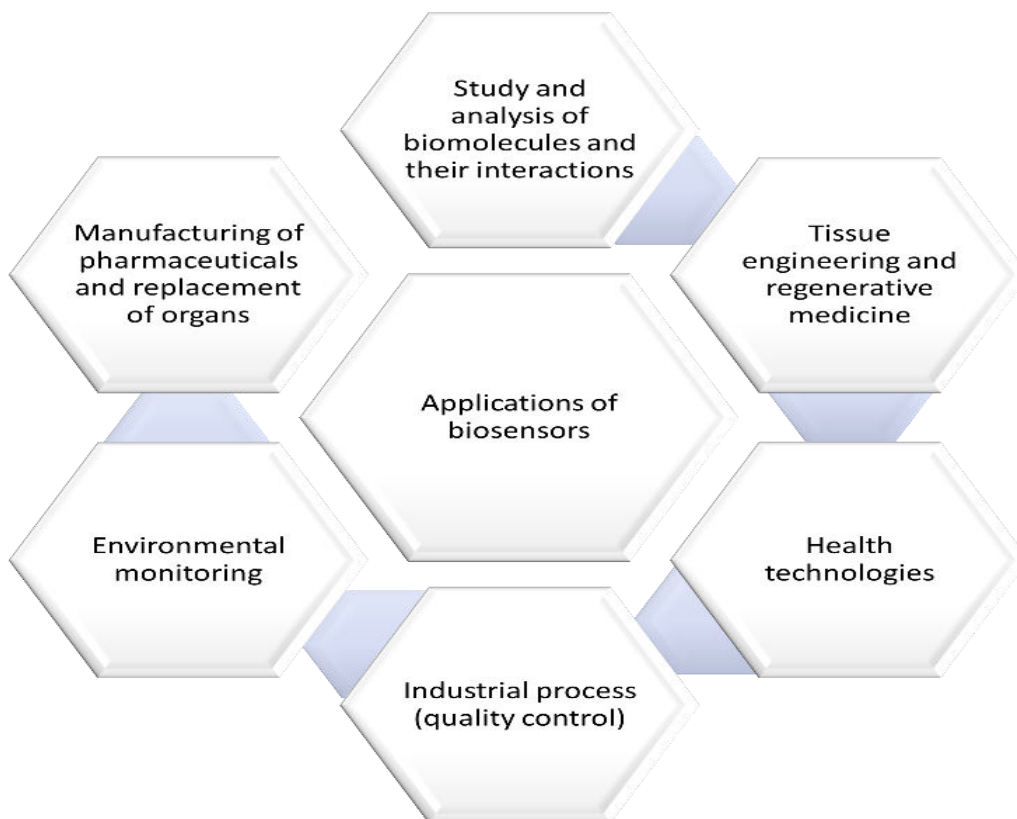
Monitoring the quality and safety of food products ensures that they meet regulatory standards and are free from contaminants and prevents the spread of foodborne illnesses (Jay et al., 2005; Hoorfar, 2011; Wilson, 1997). Biosensors can potentially detect the presence of foodborne pathogens quickly and with high sensitivity (Bhunia, 2014). Immunosensors are employed to detect allergens in food products, addressing concerns related to food allergies (Peltre and Blanca, 2007).

### **1.1.6.2 Water and environmental monitoring:**

Biosensors play a significant role in water and environmental monitoring, providing rapid and sensitive detection of various contaminants. Utilizing biosensors to detect and monitor waterborne pathogens such as bacteria (e.g., *Escherichia coli*, *Salmonella typhimurium*) and viruses, ensuring the safety of drinking water (Bhalla et al., 2016; Zourobot et al., 2009), detecting and quantifying heavy metals (e.g., lead, mercury) and various chemical contaminants in water sources (Randviir et al., 2013), Campos and Boaventura, 2008). Bioindicators, which are a type of biosensors, are used to assess the overall water quality and to gauge the health of aquatic ecosystems (Escudero and Silva, 2010; Haiss et al., 2007). Besides, employing biosensors for real-time monitoring of key water parameters such as pH, dissolved oxygen and conductivity (Wanekaya and Chen, 2006; Karube and Suzuki, 2009), they can also be put to use to detect and monitor harmful algal blooms, which can produce toxins that impact aquatic ecosystems and water quality (Chen et al., 2017; Pohanka, 2017).

### **1.1.6.3 Infections and Disease detection:**

Biosensors have been widely applied in the field of pathology for the detection of infections and disease, offering rapid, sensitive and specific detection of pathogens and biomarkers associated with various diseases (Gubala et al., 2012). They are employed for the rapid and accurate detection of infectious agents, including bacteria, viruses, and fungi. Biosensors for viruses such as influenza, HIV and Zika aid in the early diagnosis and management of viral infections (Malhotra and Kumar, 2016; Chen et al., 2017). In the case of bacterial infections, biosensors can be used for the specific detection of bacterial pathogens and in the monitoring of antibiotic resistance (Pohanka and Skládal, 2009; Shrivastava et al., 2017). Biosensors play a crucial role in the early detection of cancer by identifying specific biomarkers associated with various types of cancers. In all these cases the biomarkers may include proteins, nucleic acids, or other molecules (Kaushik et al., 2016; Damborský et al., 2016).



**Figure 1.10 Applications of biosensor in different areas of specialization**

Biosensors are designed for point of care testing, enabling rapid and decentralized diagnosis of infectious diseases. These devices are portable and user-friendly (Kaushik et al., 2017; Wang et al., 2018). Further, biosensors are employed for the detection of biomarkers associated with neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, providing early diagnostic capabilities (Chandra et al., 2017; Shende and Minić, 2019).

#### **1.1.6.4 Toxin detection and defense use:**

Biosensors play a crucial role in toxin detection and defence applications, providing rapid and sensitive detection of various toxins in different environments. Biosensors are used to detect biological toxins produced by microorganisms, including bacteria, fungi and algae. This includes toxins such as bacterial toxins, mycotoxins, and algal toxins (Palchetti and Mascini, 2008; Wang et al., 2018). Biosensors are employed for monitoring environmental toxins, including pollutants

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and contaminants such as heavy metals, pesticides, and industrial pollutants in air, water and soil (Turner, 2013; Pohanka and Skládal, 2009). Biosensors are used in the food industry to detect toxins such as mycotoxins, pesticides, and contaminants, ensuring food safety and quality (Wang, 2006). Biosensors are utilized for the detection of chemical warfare agents, providing a rapid and specific means of identifying toxic substances (Anik et al., 2019). They are also employed in defence applications to detect biological toxins used in bioterrorism or warfare, providing early warning and protection (Rusling et al., 2017; Ivnitski et al., 1999). Biosensors are applied in agriculture to detect plant toxins, ensuring the safety of crops and preventing the consumption of contaminated produce (Krejcová et al., 2016; Arduini et al., 2013).

### **1.1.7 Challenges:**

The utilization of biosensors for various applications comes with its own set of challenges. These challenges can be diverse and may include issues related to sensitivity, specificity, stability, reproducibility, and realworld implementation. Achieving high sensitivity is crucial for biosensors, especially when dealing with low concentrations of analytes. Improving detection limits is a constant challenge in biosensor development (Ronkainen et al., 2010; Turner, 2013). Ensuring biosensors can selectively recognize and respond to the target analyte in the presence of potential interferents is a challenge, particularly in complex sample matrices (Wilson and Nie, 2006; Ferapontova, 2010). Achieving consistent and reproducible results is a challenge in biosensor development. Variability in fabrication, environmental conditions and biological components can affect reliability (Cevenini et al., 2016; Erdem et al., 2017). Ensuring the compatibility of biosensor components with biological systems and maintaining stability over time are challenges that impact the long-term performance of biosensors (Liu et al., 2010; Chinnadayya et al., 2019). Integrating biosensors into small, portable devices and ensuring miniaturization without compromising performance pose engineering challenges (Kaushik and Choi, 2019; Yoon and Kim, 2018). Developing biosensors that are cost-effective for widespread use, especially in resource-limited settings, is a challenge that impacts accessibility (Malhotra et al., 2016; Hussain et al., 2014). Addressing these challenges is essential for advancing the field and realizing the full potential of biosensors in various practical applications.

### **1.1.8 Future prospects:**

The future of biosensors holds immense promise, driven by ongoing research, technological advancements, and interdisciplinary collaborations. Biosensors have enormous potential for various applications in healthcare, environmental monitoring, food safety, and beyond.

#### **1.1.8.1 Miniaturization and wearable biosensors:**

Miniaturization of biosensors enables their integration into wearable devices for continuous health monitoring. Wearable and implantable biosensors offer real-time data collection of physiological parameters such as glucose levels, heart rate, and biomarkers indicative of disease states, enabling personalized healthcare and disease management. Future developments may focus on enhancing sensor performance, biocompatibility, and wireless connectivity, enabling seamless integration into everyday life and healthcare management (Kim et al. 2019; Bandodkar and Wang, 2014; Wang, 2018).

#### **1.1.8.2 Integration with Artificial Intelligence (AI):**

Integration of biosensors with AI algorithms enhances data analysis, pattern recognition, and predictive modeling. AI-driven biosensors enable real-time monitoring, decision-making, and personalized health recommendations. Future scope includes the development of AI-driven biosensors capable of autonomous decision-making, continuous learning, and adaptation to dynamic biological environments (Kaushik and Solanki, 2013; Esteva et al. 2019; Gao et al., 2016).

#### **1.1.8.3 Environmental Monitoring:**

Biosensors play a critical role in environmental monitoring by detecting pollutants, pathogens, and toxins in air, water, and soil. Advanced biosensor technologies contribute to pollution control, resource management, and sustainable development. Efforts towards designing biosensors with enhanced sensitivity, selectivity, and portability for on-site detection, as well as integration with IoT platforms for real-time surveillance and data sharing are promising (Gamella and Pingarrón, 2020; Grieshaber et al., 2008; Vaddiraju et al., 2010).

#### **1.1.8.4 Food Safety and Quality control:**

Biosensors are employed in agriculture and food production to monitor soil quality, crop health, and food safety parameters. Real-time monitoring of pesticides, contaminants, and allergens ensures food security and regulatory compliance. Additional prospects encompass the advancement of biosensors with improved specificity, multiplexed detection capabilities, and compatibility with complex food matrices for rapid and reliable screening (Sharma et al., 2020; Campàs and Prieto-Simón, 2015; Liu and Lin, 2007).

### **1.2 Nanomaterials:**

#### **1.2.1 Introduction:**

While the concept of nanotechnology, as we understand it today, emerged in the 20<sup>th</sup> century, there are instances in the ancient world where people unknowingly interacted with nanoscale materials. However, it is important to note that the ancients did not have a scientific understanding of nanotechnology, and their interactions were often unintentional. Some medieval stained glass windows contain gold and silver nanoparticles (Short, 2011). Artisans in medieval times used metal salts to create vibrant colours in glass. When these windows were examined using modern techniques, it was discovered that the nanoparticles were responsible for the unique optical properties and colours observed. The legendary Damascus steel, used for forging high quality blades in the Middle East during the medieval period, is believed to contain carbon nanotubes and nanowires (Wadsworth and Sherby, 1980). These nanoscale structures could contribute to the exceptional strength and sharpness of the blades. The Romans, during the 1st century AD, were adept glassmakers (Jackson et al., 2013). Some Roman glassware exhibits nanoscale features, such as the famous Lycurgus Cup. This cup changes colour when illuminated from different angles, a phenomenon attributed to nanoparticles of silver and gold dispersed in the glass (Freestone et al., 1994). Ancient pottery, particularly from Islamic civilizations, often contained glazes with metal nanoparticles. These nanoparticles could enhance the colours and appearance of the pottery (Carboni and Whitehouse, 2001; Pradell et al., 2009). While these instances demonstrate the unintentional use of nanoscale materials in ancient times, it is crucial to recognize that the ancients did not possess a scientific understanding of the nanoscale, while manipulating materials at that level. Nanotechnology, as a scientific discipline, emerged much later with advancements in

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microscopy and the development of new tools for manipulating and characterizing materials at the nanoscale.

### **1.2.2 Types of Nanomaterials:**

Nanomaterials are materials with dimensions at the nanometer scale, typically ranging from 1 to 100 nanometers. At this scale, materials exhibit unique and often enhanced properties compared to their bulk counterparts. Nanomaterials play a crucial role in various fields, including nanotechnology, materials science, electronics, medicine, and environmental science.

#### **1.2.2.1. Metal nanoparticles:**

Metal nanomaterials, characterized by their unique properties at the nanoscale, have gained immense significance across various fields. These materials, typically ranging from 1 to 100 nanometers, exhibit distinct physical, chemical and electronic characteristics compared to their bulk counterparts. Metal nanoparticles are integral components in biosensors, leveraging their unique properties to enhance sensing capabilities. Gold and silver nanoparticles, renowned for their surface plasmon resonance (SPR), enable label-free detection and real-time monitoring of biomolecular interactions (Lee et al., 2007). The high surface to volume ratio of metal nanoparticles facilitates efficient biomolecule immobilization, contributing to heightened sensitivity in biosensors (Haiss et al., 2007). Certain metal nanoparticles, such as gold and platinum, possess catalytic activity, allowing for signal amplification and the detection of low analyte concentrations (Dreaden et al., 2012). The biocompatibility and chemical stability of metal nanoparticles, particularly gold and silver, make them suitable for biological applications in biosensors (Zhang et al., 2016). Additionally, the versatility of metal nanoparticles in various detection methods, including colorimetry, fluorescence, and electrochemistry enhance their utility in biosensor design (Wang and Chen, 2015). These nanoparticles play a pivotal role in advancing biosensing technologies, finding applications in medical diagnostics, environmental monitoring and beyond.

#### **1.2.2.2 Magnetic nanoparticles:**

Magnetic nanoparticles (MNPs) possess unique properties, such as superparamagnetism, high magnetic moment, large surface area, and biocompatibility. These characteristics make them

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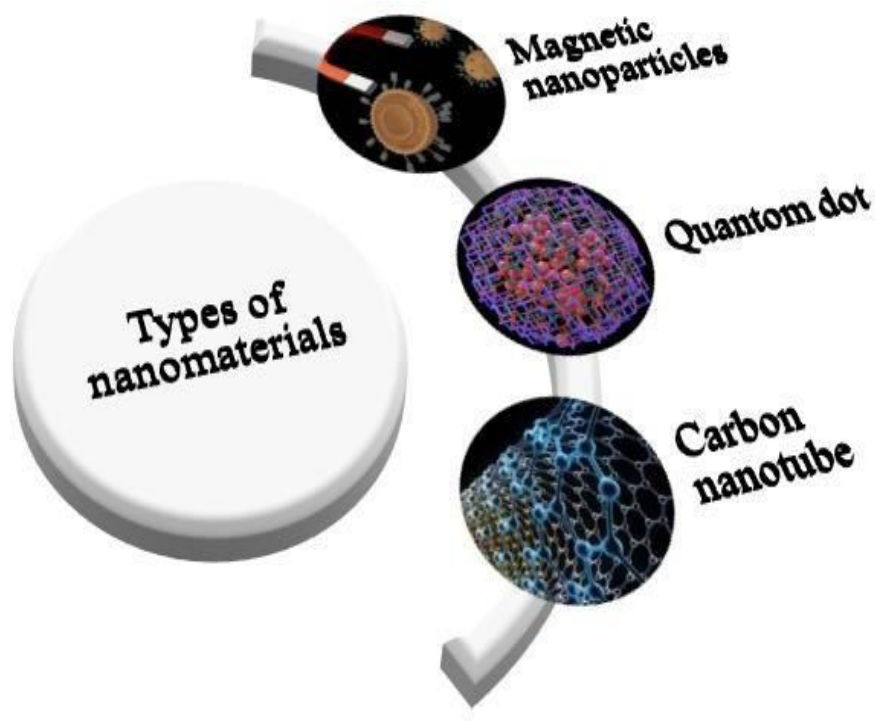
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highly valuable for applications in biosensors, particularly in enhancing sensitivity and enabling efficient target separation and concentration. Magnetic nanoparticles (MNPs) may be of pure iron, iron oxide or iron alloys. Magnetism of the particles is determined by factors such as size and shape of the magnetic nanoparticles and composition of the materials making them (Chen et al., 2015; Lu et al., 2007). Various techniques are employed for the synthesis of magnetic nanoparticles. Co-precipitation, sol-gel methods, and thermal decomposition are common approaches that allow control over nanoparticle size, shape, and surface characteristics, influencing their performance in biosensing applications. Magnetic nanoparticles find versatile applications in biosensors, including biomedical imaging, wherein they serve as contrast agents in magnetic resonance imaging (MRI) for improved visualization (Gupta and Gupta, 2005). Functionalized magnetic nanoparticles enable targeted drug delivery, enhancing therapeutic efficacy (Wang et al., 2001). Magnetic Hyperthermia is utilized in cancer treatment by generating heat in response to an alternating magnetic field (Pankhurst et al., 2009). Functionalized magnetic nanoparticles are integrated into biosensors for efficient target molecule separation, improving detection sensitivity (Lee et al., 2007). They are also applied in the removal of pollutants from water through magnetic separation for sustainable water purification (Zhou et al., 2013).

### **1.2.2.3 Carbon nanotubes (CNTs) and Carbon nanodots (CNDs):**

Carbon nanotubes (CNTs) are cylindrical structures composed of carbon atoms arranged in a hexagonal lattice forming single-walled (SWCNTs) or multi-walled (MWCNTs) configurations. Their unique properties, such as high surface area, excellent electrical conductivity, and biocompatibility, make them promising materials for biosensors. CNTs have been utilized in various biosensing applications, including electrochemical and optical biosensors. They enhance sensitivity and selectivity, allowing for the detection of specific biomolecules with improved performance (Patolsky et al., 2006; Kong et al., 2000). Carbon nanodots (CNDs) are zero-dimensional carbon nanoparticles with diameters typically less than 10 nanometers. They possess unique optical properties, including fluorescence, making them suitable for biosensing applications. CNDs are known for their biocompatibility, low toxicity, and ease of functionalization. They find applications in fluorescence-based biosensors, where their optical properties are exploited for the detection of biomolecules. (Zhou et al., 2012; Yang et al., 2009).

The exceptional surface area of CNTs provides a large platform for the immobilization of biomolecules, enhancing the sensitivity of biosensors. This property facilitates efficient interaction with target analytes (Wang, 2005). CNTs exhibit excellent electrical conductivity, enabling their use in electrochemical biosensors. Changes in electrical properties can be monitored to detect and quantify specific biomolecules (Kong et al., 2000). CNTs demonstrate biocompatibility, making them suitable for interfacing with biological systems without causing significant harm. This property is essential for biosensors used in medical applications (Kam and Dai, 2005). The remarkable mechanical strength of CNTs ensures the stability and durability of biosensor platforms, making them resilient under various environmental conditions (Coleman et al., 2006). CNTs can be easily functionalized with various molecules, allowing for surface modifications that enhance the specificity and selectivity of biosensors (Wang, 2005). CNTs exhibit unique optical properties, such as fluorescence, which can be harnessed in optical biosensors for sensitive detection and imaging of biomolecules (Zhou et al., 2012). CNT-based biosensors offer the capability of multiplexed detection, allowing simultaneous analysis of multiple analytes in complex biological samples (Kong et al., 2000). CNT-based biosensors often demonstrate rapid response times, enabling real-time detection of target analytes, which is crucial for applications requiring timely diagnostic information (Wang, 2005).



**Figure 1.11 Types of nanomaterial**

#### **1.2.2.4 Graphene and Graphene quantum dots (GQDs):**

Graphene's remarkable surface area and electrical conductivity make it an excellent platform for the immobilization of biomolecules, enhancing the sensitivity of biosensors (Novoselov et al., 2005). Graphene's biocompatibility and flexibility enable its integration into biosensing platforms, ensuring compatibility with biological systems without causing harm (Yang et al., 2013). The ease of functionalization allows the tailoring of graphene-based biosensors, adding specific functionalities for selective detection. GQDs exhibit quantum confinement effects due to their small size, leading to tunable optical properties, making them suitable for fluorescent biosensors (Zhu et al., 2011). GQDs demonstrate biocompatibility and low toxicity, essential for their integration into biosensing applications without adverse effects (Zheng et al., 2015). High photostability ensures reliable fluorescence signals, while the presence of functional groups facilitates bioconjugation, enhancing specificity (Liu et al., 2010). Graphene and GQDs find applications across various biosensing platforms, including electrochemical, optical, and fluorescent detection methodologies (Chen et al., 2010). The unique properties of graphene and

GQDs contribute to their effectiveness in biosensing applications, offering diverse opportunities for advanced and sensitive detection methodologies in biomedical and environmental research.

### **1.2.2.5 Quantum dots, Silica nanoparticles and Upconversion nanoparticles:**

Quantum dots (QD) are semiconductor nanocrystals with unique optical and electronic properties due to quantum confinement effects. They exhibit size-dependent tuneable emission spectra, high quantum yield, and exceptional photostability. QDs find applications in bioimaging, drug delivery, and sensors due to their fluorescence properties. In electronics, QDs are used in displays and solar cells for their efficient light emission and absorption (Bruchez Jr. et al., 1998; Michalet et al., 2005). Silica nanoparticles are composed of a silica (SiO<sub>2</sub>) shell, providing a versatile platform for functionalization and drug encapsulation. They are known for their biocompatibility, low toxicity, and stability. Silica nanoparticles are extensively used in drug delivery systems, where their porous structure allows controlled release of therapeutic agents. They find applications in imaging, sensing and as carriers for targeted delivery in biomedical applications (Slowing et al., 2008; Lu et al., 2010). Upconversion nanoparticles are capable of converting lower-energy photons into higher-energy ones. They exhibit anti-Stokes emission, allowing for background-free imaging. UCNPs are used in bioimaging, especially in deep-tissue imaging, due to their ability to overcome tissue autofluorescence. They find applications in photodynamic therapy and as contrast agents in biomedical imaging (Wang and Liu, 2018; Chen et al., 2014). Upconversion nanoparticles (UCNPs) have gained significant attention in the field of biosensors due to their unique optical properties, such as anti-Stokes emission, photostability, and tunable emissions in the visible and near-infrared (NIR) range. These characteristics make UCNPs well-suited for various biosensing applications.

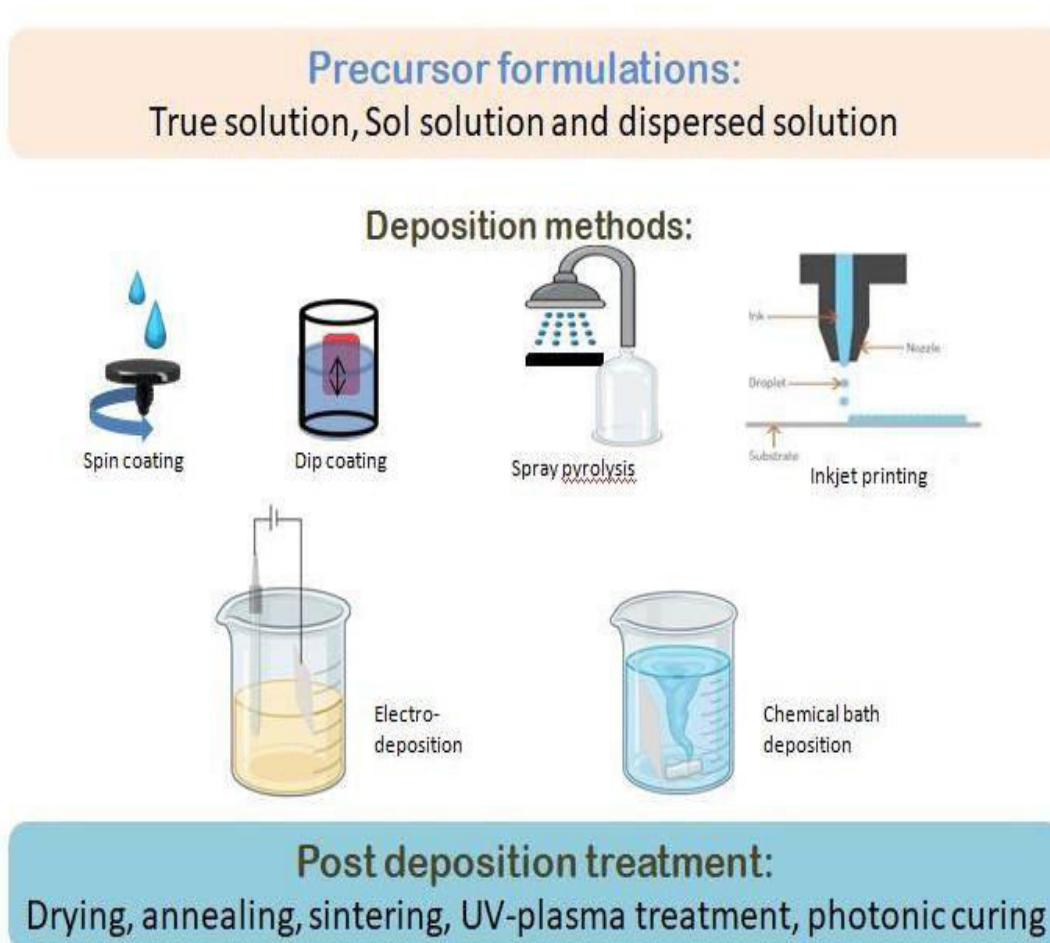
### **1.2.3 Deposition techniques for Nanomaterials:**

To enhance conductance, it is essential to apply an appropriate nanomaterial coating on the surface of biosensors based on nanomaterials. The initial and crucial step for improved biosensor performance involves the application of nanomaterial coatings or depositions. Different deposition methods have been developed to efficiently create a matrix using nanomaterials. The selection of a deposition technique is critical to ensure proper contact between the electrode matrix and the

incoming nanomaterial (Ahmad et al., 2018). The primary goals of putting nanoparticles onto a supporting surface matrix are to boost a biosensor's analytical capabilities, provide a large surface area, and create a location where the target receptor may be quickly rendered immobile. Therefore, proper nanomaterial deposition plays a crucial part in defining the sensor's level of stability, sensitivity, reproducibility, and selectivity. This section briefly gives an insight about deposition methods of nanomaterials (Ahmad et al., 2018).

### 1.2.3.1 Coating based deposition methods

#### 1.2.3.1.1 Drop casting method



**Figure 1.12** Different types of coating based deposition methods for nanomaterials.

The drop-casting method is a straightforward and cost-effective technique widely employed for depositing nanomaterials onto electrodes. In this process, a small volume of a nanomaterial suspension is applied to the electrode surface, and as the solvent evaporates, a thin

layer of nanomaterials is left behind. The simplicity of the method makes it user-friendly and accessible, requiring minimal equipment. However, achieving uniform nanomaterial distribution and precise control over layer thickness can be challenging. Despite these limitations, drop-casting finds extensive use in sensor fabrication and device prototyping, offering a rapid and convenient approach for the initial stages of research and development. This technique's versatility makes it applicable to a broad range of nanomaterials and electrode materials, providing a practical solution for the creation of thin films in various applications. Recent progress in drop casting methods continues to contribute to material fabrication advancements, making it a valuable tool in nanotechnology research and development (Zhang et al., 2016).

#### **1.2.3.1.2. Dip coating method:**

The dip coating method is a widely utilized and cost-effective technique for depositing nanomaterials onto electrodes. In this process, the electrode is submerged into a solution containing the desired nanomaterials, and upon withdrawal, a thin and uniform film adheres to the substrate. While dip coating offers simplicity and versatility, allowing for the coating of complex-shaped substrates, challenges such as limited control over film thickness and potential issues with large-scale homogeneity should be considered. Despite these challenges, dip coating remains a popular choice in sensor fabrication and thin-film deposition due to its ease of use and applicability to diverse nanomaterials. Post-deposition treatments or additional processing steps can be employed to enhance the adhesion and properties of the resulting nanomaterial layer. While dip coating provides a convenient and cost-effective approach, achieving precise and uniform thicknesses can be challenging. This limitation may impact the reproducibility and consistency of the coated layers, particularly in applications where highly controlled film thickness is crucial (Zhang et al., 2016).

#### **1.2.3.1.3 Spin coating method:**

The spin coating method is a widely employed and precise technique for depositing nanomaterials onto electrodes. In this process, a nanomaterial solution is dispensed onto the electrode, which is then spun at high speeds. Centrifugal force spreads the solution uniformly, resulting in a thin, homogenous film as the solvent evaporates. Spin coating offers excellent control

over film thickness, making it advantageous for applications requiring precision. However, it is primarily suitable for planar substrates, and achieving uniformity on complex surfaces may be challenging. The versatility and ease of implementation make spin coating a preferred choice in various fields, including microelectronics and sensor fabrication. Post-coating treatments can be applied for further optimization. The method's effectiveness in achieving controlled film thickness contributes to its widespread use in nanomaterial-based research and development (Ohring, 2002).

#### **1.2.3.1.4 Blade coating method:**

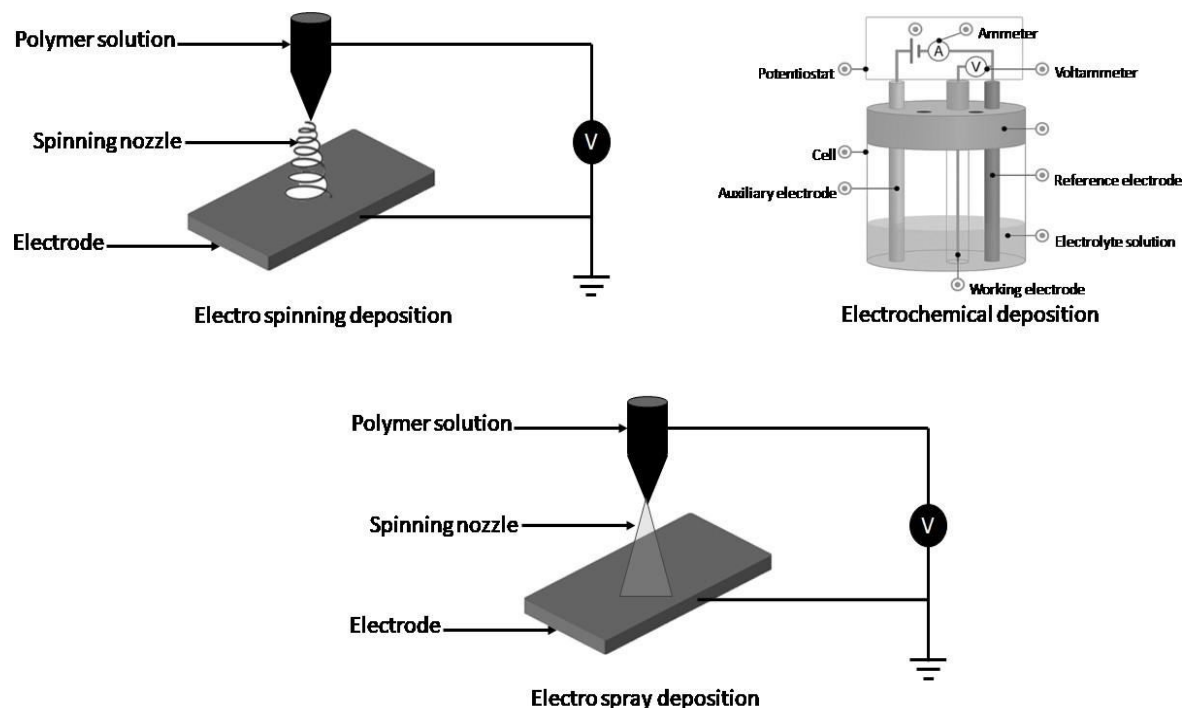
The blade coating method stands as a promising technique for depositing nanomaterials onto electrodes with a focus on simplicity and scalability. In this process, a blade or a doctor blade is used to spread a nanomaterial suspension onto the electrode surface, ensuring an even and controlled coating. Blade coating offers advantages in terms of cost-effectiveness, ease of operation, and suitability for large-scale production. However, achieving precise control over film thickness can be challenging compared to some other deposition methods. The simplicity and potential for high-throughput fabrication make blade coating particularly attractive for applications such as flexible electronics and energy devices. While it may require optimization for specific nanomaterials and substrates, blade coating holds promise as a versatile and accessible method for depositing nanomaterials on electrodes (Kim et al., 2019).

#### **1.2.3.1.2 Direct deposition methods**

##### **1.2.3.2.1 Vapor deposition method:**

Vapor deposition techniques play a crucial role in the fabrication of biosensors by allowing precise control over thin film deposition. Some vapor deposition techniques commonly used in biosensor fabrication are: (i) Chemical vapor deposition (CVD) (Lee and Lee, 2016), (ii) Physical vapor deposition (PVD) (Bergmann et al., 2013), (iii) Atomic layer deposition (ALD) (Shin and Lee, 2018), (iv) Metal-organic framework (MOF) deposition (Park and Kim, 2018) and (v) Pulsed laser deposition (PLD) (Kuchmizhak et al., 2019). Sometimes, use of high temperature makes the techniques inconvenient to coat the surface substrates. Other disadvantages offered by vapor deposition techniques include less convenience to coat small surface areas, need offunctionalization of the surface for the attachment of receptors and enzyme leaching (Ahmad et

al., 2018; Dominik et al., 2017; Muñoz-Rojas and Macmanus-Driscoll, 2014; Srivastava et al., 2012; Wingqvist, 2010). However, these vapor deposition techniques provide precise control over film thickness, uniformity and composition, making them crucial in the development of advanced biosensors.



**Figure 1.13** Different types of direct deposition methods for nanomaterials.

### 1.2.3.2.2 Electrodeposition method:

Electrodeposition allows a controlled deposition of materials onto a substrate. Various methods can be used for electrodeposition. In electrochemical deposition (ECD) method, materials are electrodeposited onto a conductive substrate through an electrochemical reaction. This method is widely used for fabricating biosensor electrodes and immobilizing biomolecules (Wang, 2005). Pulse Electrodeposition employs pulses of current during the deposition process, allowing better control over the film properties. It offers improved control over the deposition process for sensitive biosensor interfaces (Park et al., 2006). Deposition is controlled by maintaining a constant current during the electrochemical process in galvanostatic deposition method. It is suitable for creating controlled and uniform coatings in biosensor fabrication

(Ramanavicius and Ramanaviciene, 2016). In potentiostatic deposition, deposition is controlled by maintaining a constant potential during the electrochemical process. It allows for precise control over the composition and structure of deposited films (Ensafi et al., 2012). Electrodeposition is carried out within a template to control the morphology of the deposited material in template assisted method. It is useful for creating nanostructured materials for enhanced biosensor performance (Zeng et al., 2015).

#### **1.2.3.2.3 Electrospinning deposition:**

A polymer solution containing the desired sensing material is prepared. This solution is often a combination of a polymer matrix and the electrochemically active component (Li and Xia, 2004). The polymer solution is loaded into a syringe, and a high voltage is applied to create a charged jet of polymer solution (Reneker and Chun, 1996). The electrostatic repulsion forces cause the charged jet to stretch and thin into nanofibers, which are collected on a substrate to form a nanofibrous mat (Huang et al., 2003). The nanofibrous mat is coated onto the electrode surface, providing a high surface area for the immobilization of biomolecules (Gao et al., 2013). Biomolecules, such as enzymes or antibodies, can be immobilized onto the electrospun nanofibers to impart specificity to the biosensor (Vashist, 2017). The electrospun nanofiber-based electrode is characterized using techniques such as SEM, TEM, and electrochemical measurements to assess its structure and electrochemical performance (Pham et al., 2019). Electrospinning produces nanofibrous mats with high surface areas, enhancing the sensitivity of the biosensor. The porosity of electrospun nanofibers can be controlled, allowing for optimized mass transport in electrochemical reactions. Electrospinning is versatile and can be applied to various polymers and sensing material. Electrospun nanofibers provide an ideal platform for the immobilization of biomolecules, enhancing the biosensor's specificity.

#### **1.2.3.2.4 Electrospray deposition:**

A solution containing the electroactive material or biomolecules is prepared. This solution is typically a mixture of the sensing material and a solvent (Sill and von Recum, 2008). The solution is loaded into a syringe, and an electric field is applied to produce a fine spray of charged droplets (Wu et al., 2007). The charged droplets are directed towards the electrode surface, forming

a thin film as the solvent evaporates (Yang et al., 2002). The solvent evaporates, leaving behind a thin film of the electroactive material on the electrode surface. Additional biomolecules, such as enzymes or antibodies, can be immobilized onto the deposited film to confer specific recognition capabilities (Wang, 2008). The resulting electrode is characterized using techniques such as SEM, AFM, and electrochemical measurements to assess film morphology and electrochemical performance. Electrospray deposition produces a uniform coating on the electrode surface, ensuring consistent performance. The thickness of the deposited film can be controlled by adjusting the electrospray parameters, allowing for tuneable sensor characteristics. Electrospray allows for high-resolution deposition, particularly beneficial for miniaturized or microfabricated biosensors.

### **1.2.3.3 Printing based deposition methods:**

The number of established printing processes has significantly increased due to decades of intensive study in the field of printing technology. In addition to the significant contributions made by screen printing (Zhang et al., 2021) and inkjet printing (Bai et al., 2021) other methods have also been studied for the fabrication of biosensing, including 3D printing (Distler and Boccaccini, 2020), gravure printing (Reddy et al., 2011), reverse offset printing (Nagamine and Tokito, 2019), flexographic printing (Assaifan et al., 2016), e-beam, photo, and probe-based lithographies (Smolyarova et al., 2022; Liu et al., 2021) and laser printing (Bordbar et al., 2021). The primary goals of the printing techniques that have been created are: greater data density; ease of use; flexibility; miniaturization; high throughput; high precision; high resolution; distant applicability; portability; and cost-effective manufacturing procedures for mass production. The method offers pilot scale productions with cost effective means. In printing based methods, nanomaterials with or without polymers are made to form printable liquid ink. Thus, the method operates at low temperature with fast printing approach for soft electronics and other applications. These methods find their applications in sensing devices, batteries, electronics and photonics (Ahmad et al., 2018; Hu et al., 2018; Prevatte et al., 2016; Wang et al., 2018). The main advantage of printing based methods is that they can also be used directly to coat the predesigned substrates without any binders unlike in coating based methods and the printing properties also can be controlled (Ahmad et al., 2018).

However, the biggest disadvantage of screen printing is its subtractive nature, which results in a large amount of precious ink wastage (Kant et al., 2021). This, in return, is both costly and environmentally dangerous. Moreover, screen printing is only limited to high-viscosity inks and is a contact-based printing technique. It requires skilled operators and is costly in small scale production. Another limitation of screen printing is its confinement to flat surfaces, which restricts its use for biosensor fabrication (Yanget al., 2021).

#### **1.2.3.3.1. Screen printed electrodes:**

A specialized ink is prepared, containing biomolecules, nanoparticles, or other sensing materials. A mesh screen, typically made of polyester or stainless steel, is coated with a stencil defining the biosensor pattern. The prepared ink is applied to the screen, and a squeegee is used to force the ink through the openings in the stencil onto the substrate. The printed substrate is subjected to a drying or curing process to fix the biomolecules or sensing materials onto the surface. For more complex biosensors, multiple layers of different inks can be applied sequentially. The fabricated biosensor undergoes characterization using analytical techniques, and its performance is tested. (Su and Mo, 2018). The advantages of this method include cost-effectiveness for mass production, versatility in terms of applicability to various substrates, including flexible and unconventional materials, scalability to large scale production due to its simplicity, speed and adaptability as it can be used to print a variety of biomolecules and materials, making it useful for different biosensor designs.

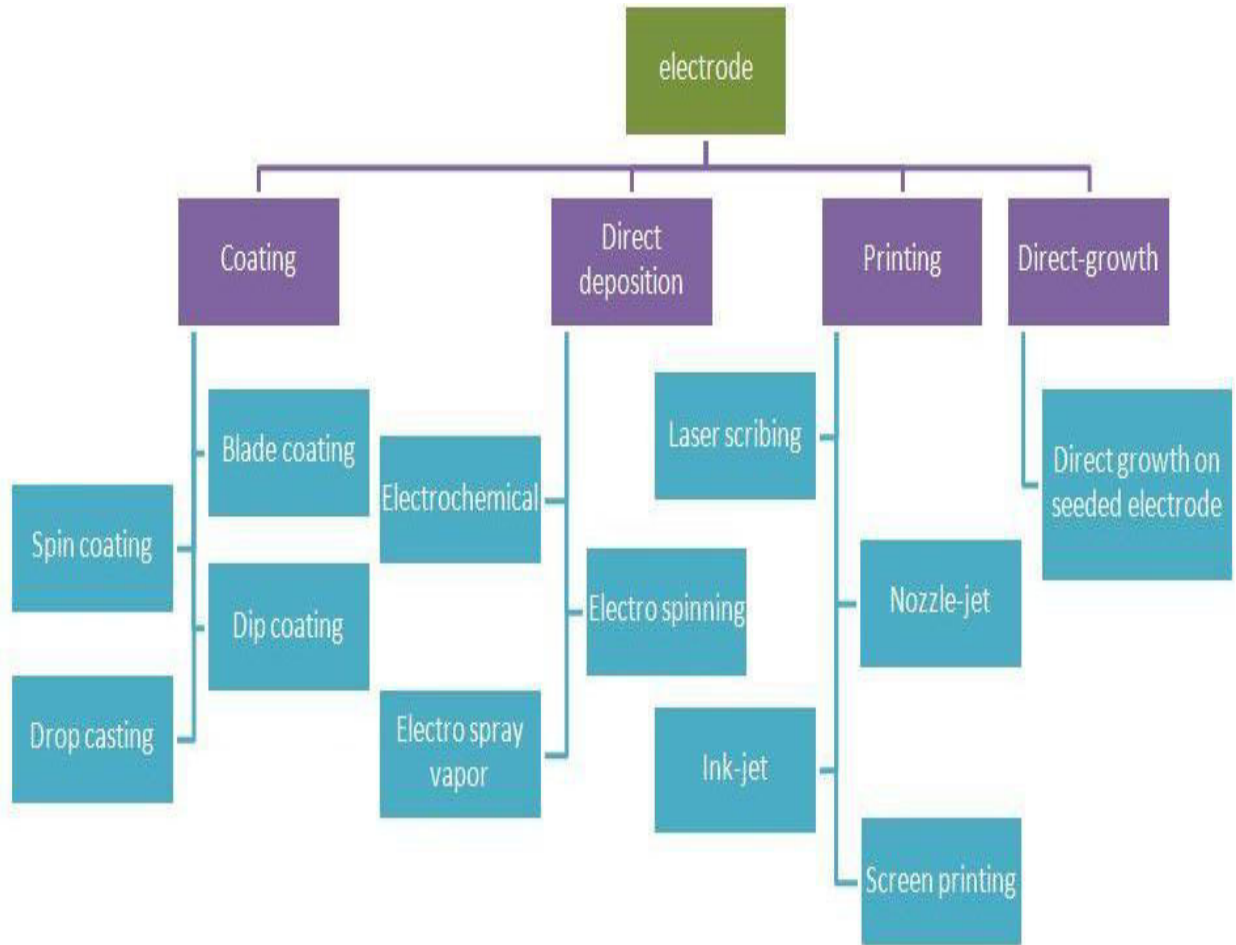
#### **1.2.3.3.2 Inkjet technology:**

The drop-on-demand nature of inkjet printing technology enables the printing of materials only when required, reducing the wastage of both the electronic and biological inks, while at the same time offering rapid processing, high resolution, and repeatability (Ghorbani and Rabbani, 2021). Inkjet printing technology allows for the use of a wide variety of inks and for low-temperature printing (Kosmala et al., 2011) that supports the use of thermally unstable compounds (Hiraoka et al., 2006). The non-contact, maskless, and direct writing capability of inkjet printing enables or allows for (i) combinatorial studies of various devices (Sun and Jabbour, 2002), (ii) CAD data modifications, without the need to change the master template, (iii) a low risk of

contamination, (iv) precise spatial control, (v) the use of both solid and flexible substrates, and (vi) the printing of functional materials on 2.5D and 3D surfaces (Trotter et al., 2020). Inkjet-printing can also be employed as an etch-dispensing process that allows for the use of very small portions of the pre-deposited materials at levels not achievable by its close competitors (Trotter et al., 2020). One of the major advantages of inkjet printing is the ability to deposit the materials in very small volumes that contain a high loading of nanomaterials or delicate biological materials, at very high speeds (Castrejón-Pita et al., 2013).

#### **1.2.3.3.3. Nozzle-jet printing:**

Nozzle-jet printing is a precision-based technique for fabricating biosensors, particularly in inkjet printing. Specific inks containing biomolecules or sensing materials are prepared for the printing process. The printing system consists of a nozzle or jet that ejects droplets of ink onto the substrate. The desired biosensor pattern can be achieved by controlling the droplets of ink are ejected. The printed substrate subjected to drying or curing to fix the biomolecules or sensing materials in place. For complex biosensors, multiple layers of different inks may be printed sequentially. The fabricated biosensor is characterized using various analytical techniques, and its performance is tested (Unger et al., 2000). Nozzle-jet printing offers high precision in depositing droplets, enabling fine details in biosensor patterns. The technique allows for customization, facilitating the creation of tailored biosensor designs. Nozzle-jet printing minimizes material wastage, making it efficient for resource utilization. Enables rapid prototyping of biosensors due to its ability to create intricate patterns quickly (Unger et al., 2000).



**Figure 1.14 Methods of deposition of nanomaterials**

Here, the properties of the printed surface can be controlled by various parameters such as temperature and height of nozzle, speed, diameter and pressure of the nozzle drive. The technique is well known for deposition of hydrogen on surfaces but nowadays nanomaterial deposition is also tried with the same technique. The nozzle jet printing is less used compared to inkjet method as it only prints objects with smaller surfaces (Ahmad et al., 2018).

#### 1.2.3.3.4. Laser Scribing:

The substrate (commonly a thin film) is prepared for laser scribing, ensuring that it is compatible with laser treatment. A laser scribing system is configured, including parameters such as laser wavelength, intensity, and focus. The laser is precisely directed to scribe or ablate material from the substrate, creating patterns or circuits for biosensors. After laser scribing, biomolecules

or sensing materials may be deposited onto the patterned substrate. The biosensor is subjected to drying or curing processes to fix biomolecules or sensing materials onto the substrate. The fabricated biosensor is characterized using analytical techniques, and its performance is tested (Yang et al., 2011). The technique is highly used to fabricate paper-based devices as it offers flexibility and disposability in just a single step method. The method is compatible with small size devices and also offers high reproducibility. Laser scribing based devices are more sensitive after subsequent coating of enzyme/polymer/metal. The technique finds its applications in the detection of food contaminants, toxins, metabolites and proteins. The method suffers the drawback that not all the graphite oxides are converted to reduced graphene oxide and graphite oxides are soluble in water. So, the electrodes fabricated with this technique are not very stable in aqueous solutions. (Ahmad et al., 2018)

#### **1.2.3.3.5. Direct growth deposition methods:**

The direct growth deposition technique represents a sophisticated method for precisely synthesizing nanomaterials directly onto substrate electrodes, encompassing a spectrum orchestrates an organized and controlled growth process, leveraging various direct growth methods to achieve tailored nanomaterial structures. The key direct growth techniques involved include: (a) Thermal and Hydrothermal Deposition which utilizes heat or hydrothermal conditions to induce the growth of nanomaterials on the substrate electrode. This method enables controlled and temperature-dependent growth of various nanostructures. (b) Anodization which involves the controlled oxidation of a metal surface to generate nanomaterial structures. This method has a benefit that it offers precise control over nanomaterial morphology and structure through electrochemical processes. (c) Template-Assisted Methods utilizes templates or scaffolds to guide the growth of nanomaterials into specific shapes. It allows for the creation of well-defined nanostructures with tailored properties. (d) Chemical Deposition Techniques involves chemical reactions that lead to the deposition of nanomaterials on the electrode surface. This method provides flexibility in choosing precursor materials, leading to a wide variety of nanomaterial compositions (Ahmad et al., 2018).

These methods collectively enable the growth of diverse nanostructures, such as nanorods, nanowires, nanoplates, nanodendrites, nanoneedles, and nanoflowers, directly on the electrode

surfaces. The notable advantage of employing the direct growth deposition approach is the precise control it affords over the coating of the electrode surface, allowing for the creation of nanomaterials with specific morphologies tailored to the desired application. Importantly, this method excels in coating large surface areas, facilitating direct contact between the nanomaterials and the electrode without the need for binders or polymers. This feature enhances the efficiency and performance of the resulting nanomaterial-based electrodes in various applications, such as sensors, catalysts, and energy storage devices (Ahmad et al., 2018).

### **1.3. Aims and Objectives:**

The necessity for advancing research in biosensors field arises from the limitations faced by traditional methods. The primary aim of developing novel biosensor prototypes is to increase detection efficiency, sensitivity, simultaneously reducing detection time and costs. Biosensors exhibit wide-ranging functionality, from medical diagnostics to environmental monitoring and industrial processes, incorporating drug and toxin screening, water and food analysis, and applications in defense. Despite this, the sensors utilized nowadays shows some drawbacks in terms of specificity, selectivity, detection thresholds, detection ranges, expensive and invasive properties. Purpose of the study is to develop prototypes of biosensors capable of addressing limitations in in traditional methodologies and currently available biosensor technologies. The primary focus of this study is on advancing, producing, characterizing, and standardizing biosensors. Fabricating or developing biosensors poses a challenge in selecting the target analyte for detection and investigating how the target would interact with its receptor (biological molecules). Once this has been established, the subsequent steps must be executed with great care to fabricate a successful biosensor (Ya'acob et. al., 1989):

#### **Essential elements in the biological receptor selection process:**

The biological receptor chosen significantly affects the selectivity and sensitivity of the biosensor. Thus, it is advisable to choose a receptor that is stable and exhibits high affinity for the analyte. Before making a decision on the receptor for a specific analyte, it is important to thoroughly understand its advantages and disadvantages. (Grieshaber et. al., 2008; Chaubey and Malhotra, 2002; Ya'acob et. al., 1989).

### **Essential elements in the Selection of immobilization method:**

In the fabrication of biosensors, it is crucial to immobilize the chosen biological receptor onto the surface of a transducer to facilitate the recognition of the analyte. Multiple approaches are utilized for immobilizing a receptor, which include entrapment, adsorption, covalent attachment, microencapsulation, and cross-linking (Korotkaya et. al., 2014; Ya'acob et. al., 1989).

### **Essential elements in the Selection of transducer:**

The sensitivity level of a biosensor is largely influenced by the choice of transducer. Opting for the right transducer can significantly increase sensitivity of the sensor, whereas selecting an unsuitable transducer may reduce the sensitivity. (Morales and Halpern 2018; Ya'acob et al., 1989).

Our laboratory has previously developed various biosensors for glucose, Organophosphorus pesticide, Triglycerides, *Staphylococcus aureus* and SARS-CoV-2. Both optical and electrochemical methodologies were employed in the study to fabricate biosensors, specifically focusing on the immobilization of enzymes (enzyme-assay), aptamers (nucleic acid assay), and polymers (molecular imprinting) as receptors onto a polyvinyl alcohol-multiwalled carbon nanotube membrane using the drop casting method (Gupta, Prabha, & Murthy, 2016; Thakkar et. al., 2019; Thakkar et al., 2023; Jinal Thakkar's thesis 2022). These biosensors were recommended for industrial applications. The primary aim of this study is the development and standardization and testing of biosensors for the detection of hydroquinone pollutant, lactose, PupE gene (For tuberculosis), BRCA1 gene (breast cancer), triglycerides and SARS-CoV-2. In this study, electrochemical methods were employed to develop biosensors utilizing enzymes (enzyme-assay) and aptamers (nucleic acid assay) as receptors. For electrochemical biosensors, nanoparticles were used to immobilize receptors through the drop-casting method. In designing biosensors, it's crucial to consider various parameters alongside the selection of the receptor, transducer, and immobilization method to achieve the desired performance (Ya'acob et. al., 1989).

In the process of designing biosensors for practical application, several critical questions have been raised:

- **Is the biosensor specific?**
- **What is the linear range?**
- **What is the sensitivity of the biosensor?**
- **Is the biosensor stable?**
- **What is the detection limit of the biosensor?**
- **Is the biosensor reusable or reproducible?**
- **What is the response time of the biosensor?**
- **What is the detection range of the biosensor?**

The specified objective of this thesis involves the designing, characterization, and testing of the following biosensors:

- **Novel Electrochemical biosensor based on immobilized fungal laccase for the detection of hydroquinone**
- **Development of lactose biosensor using immobilized  $\beta$ -Galactosidase and Galactose Oxidase with Multi-Walled Carbon Nanotubes and CuO-TiO.**
- **DNA aptamer based biosensor for the detection of *Mycobacterium tuberculosis*.**
- **Testing of Covid-19 real samples of Nsp3 gene of SARS-CoV-2 using DNA chip.**
- **Testing of triglyceride real samples using conductive Nano-PEI-lipase based biosensor.**
- **Electrochemical detection of breast cancer related BRCA1 gene using DNA chip.**

#### **1.4. Brief introduction to chapters**

The Ph.D. thesis is divided into six separate chapters, with each one explaining one of the above-mentioned objectives in detail.

**Chapter 1** is an introduction to biosensors and nanomaterials. Biosensors are analytical devices that combine a biological sensing element with a transducer to detect and quantify a target analyte. They offer rapid, sensitive, and selective detection of a wide range of substances, *Development of Prototypes of Biosensors for The Detection of Pathogens, Cancer Biomarkers and Environmental Pollutant*

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making them invaluable tools in various fields including medical diagnostics, environmental monitoring, and food safety. Biosensor design typically involves selecting a biorecognition element (such as enzymes, antibodies, or nucleic acids) that interacts specifically with the target analyte, coupled with a transducer that converts the biological response into a measurable signal. Biosensors come in various types, including enzymatic, immunosensors, DNA biosensors, and cell-based biosensors, each tailored to different applications. Despite their widespread use, biosensors face challenges such as stability, reproducibility, and interference from complex sample matrices. The integration of nanomaterials into biosensors enhances their performance by providing unique properties such as high surface area, conductivity, and biocompatibility. Nanomaterials are structures with dimensions at the nanoscale, offering unique properties and applications across various fields. They can be classified into nanoparticles, nanowires, nanotubes, and nanostructured surfaces, each with distinct characteristics and functionalities. Nanomaterials can be synthesized using bottom-up approaches like chemical vapour deposition, sol-gel methods, and electrospinning, or top-down techniques such as lithography and etching. Deposition methods play a crucial role in controlling the size, shape, and properties of nanomaterials, enabling precise engineering for specific applications in electronics, catalysis, medicine, and energy storage. Ongoing research in nanomaterial synthesis and deposition techniques holds promise for continued advancements in material design, leading to innovations in nanotechnology and beyond. Nanomaterials like nanoparticles, nanowires, and nanotubes serve as sensing elements, amplifying signals and improving sensitivity and selectivity in detecting biomolecules. Functionalized nanomaterials enable specific recognition of target analytes, while their compatibility with biological systems minimizes interference and enhances biocompatibility. Furthermore, nanomaterials facilitate miniaturization, enabling the development of portable and cost-effective biosensing devices for various applications in healthcare, environmental monitoring, and food safety. Despite challenges in reproducibility and scalability, ongoing research continues to explore novel nanomaterial-based biosensing platforms, paving the way for advanced diagnostics and personalized medicine.

**Chapter 2** is dedicated to the fabrication, standardization, and characterization of a biosensor for hydroquinone pollutant detection, employing the electrochemical technique cyclic

voltammetry. The study focuses on utilizing carbon nanotubes (MWCNTs) to incorporate laccase enzyme via crosslinking. Screen-printed electrodes were coated with carboxylic acid-modified multiwalled carbon nanotubes (MWCNTs), followed by laccase doping. The interaction between the carboxylic groups of MWCNTs and the amine group of laccase, forming an amide bond. The biosensor's performance is based on the activity of Laccase enzyme immobilized on the electrode. When an enzyme is exposed to hydroquinone substrate, it forms p-benzoquinone + 2H<sub>2</sub>O + 2H<sup>+</sup> + 2e<sup>-</sup>. Current produced due to this reaction was measured by the fabricated electrode. Thus, the sensor was exposed to various concentrations of hydroquinone pollutant and current was measured before and after its exposure. The research focuses on optimization and standardization of hydroquinone biosensor. The chapter focuses on optimization and standardization of the regeneration procedure, stability and concentration of hydroquinone pollutant. At the end of optimization procedures, the biosensor was also tested against real samples proving its practical application aspect.

**Chapter 3** proposes novel fabrication chemistry and standardization of the electrochemical biosensor for lactose detection using cyclic voltammetry. Enzyme assay method using lipase as an enzyme was used to coat the electrode. Screen printed carbon electrode was coated by drop casting MWCNTs-COOH to the surface. Then, followed by layer of polyethyleneimine-TiO<sub>2</sub>/CuO nanoparticles was dropped off onto it. β-galactosidase-galactose oxidase was then layered onto the surface, following it glutaraldehyde was added for cross-linking. This study explores the enzymatic hydrolysis of lactose sugar into glucose and galactose, accompanied by the release of protons, which in turn causes a shift in the solution's pH. This pH change directly correlates with the concentration of lactose present in the solution. To measure the current changes cyclic voltammetry was utilized. The developed biosensor demonstrated effective detection of lactose within a suitable linear range. The primary focus of the research is to propose novel fabrication chemistry for biosensors capable of detecting lactose and to standardize the biosensor for practical applications, with a particular prominence on adaptability for miniaturization. The study successfully confirmed enzyme immobilization and real sample testing. Moreover, the proposed fabrication chemistry offers flexibility, allowing for the immobilization of various enzymes.

**Chapter 4** is focused on creating an electrochemical biosensor to detect the PupE gene of *Mycobacterium tuberculosis*, using innovative aptamer sequences. The aim is to develop a quick, cost-effective, and highly specific electrochemical kit for TB (tuberculosis) detection. The biosensor, designed as a label-free electrochemical oligonucleotide-chip, targets the PupE gene and is fabricated using newly developed single-stranded DNA (ssDNA) aptamers synthesized in the laboratory. These aptamers are designed using NCBI/Primer-BLAST software. To enhance the biosensor's functionality, the ssDNA aptamers are linked to multi-walled carbon nanotubes (MWCNTs) through ester bonds formed with carboxylic groups. A matrix made up of polyvinyl alcohol (PVA) and glutaraldehyde is used to immobilize the functionalized MWCNTs onto a screen-printed carbon electrode chip (SPE). This approach allows for the development of a versatile biosensor platform capable of detecting TB-related genetic material with high sensitivity and specificity. Following the fabrication of the chip, it was allowed to a complementary single-stranded DNA (ssDNA) chip to hybridize. Methylene blue was employed as a redox marker, capable of intercalating between the hybridized double-stranded DNA (dsDNA) on the chip. So, when hybridization with the complementary ssDNA occurs on the electrode chip, there is an increase in the current. This increase in current magnitude is directly proportional to the concentration of the complementary strand. The study optimized parameters such as detection limit, linear range, specificity, and reproducibility. Moreover, the research aims to streamline the process by eliminating the complementary DNA (cDNA) amplification step of reverse transcription-polymerase chain reaction (RT-PCR). So, this strategy offers a more efficient and cost-effective approach for tuberculosis detection.

**Chapter 5** is focused on the DNA-chip was designed for the electrochemical detection of a non-structural protein Nsp3 gene of SARS-CoV-2. To create this chip, a single-stranded DNA oligonucleotide probe representing the sequence from the gene of the non-structural protein was covalently attached to functionalized multi-walled carbon nanotubes (MWCNTs). The resulting ssDNA-MWCNT complex was mixed with polyvinyl alcohol (PVA) polymer and applied as a coating on a screen-printed carbon electrode. This film was crosslinked using glutaraldehyde (Jinal Thakkar, Ph.D. thesis 2022). However, Validation of performance of the electrode with real samples remained to be established. This study focused on real sample analysis by using previously made DNA-chip.

**Chapter 6** focused on the applied analysis of real samples using a previously developed TGA biosensor. This biosensor was designed for the electrochemical detection of triglycerides (TGA). To enhance its performance, the study utilized a combination of reduced graphene oxide nanoparticles, carbon nanotubes, titanium oxide nanoparticles, and polyethyleneimine. The fabrication process was optimized to increase enzyme loading on the sensor, consequently improving its detection limit. The electrode after fabrication was characterized and standardized using cyclic voltammetry. However, real sample analysis was not completed and needed to be accomplished (Jinal Thakkar's thesis, 2022). In the present study real sample analysis was done using sunflower oil, coconut oil and groundnut oil (Thakkar et al., 2023).

**Chapter 7** focuses on detection of the BRCA1 gene of breast cancer using novel aptamer. A label-free electrochemical biosensor for the BRCA1 gene associated with breast cancer. Using newly developed single-stranded DNA aptamers, designed with NCBI/primer-BLAST, the biosensor's performance improved by attaching them to multi-walled carbon nanotubes. These were then drop casted onto a screen-printed carbon electrode chip using a polyvinyl alcohol and glutaraldehyde. This method allowed to develop a biosensor capable of detecting BRCA1 gene with high sensitivity and specificity. After fabricating the chip was allowed it to hybridize with a complementary single-stranded DNA. Methylene blue is used as a redox indicator, allowing detection by intercalating between the hybridized double-stranded DNA on the chip. The increase in current upon hybridization directly correlated with the concentration of the target DNA. Additionally, our research aims to simplify the detection process by eliminating the need for complementary DNA amplification, offering a more efficient and cost-effective approach.