# CHAPTER 1

# INTRODUCTION, AIMS AND OBJECTIVES

## 1.1. Introduction:

Biosensors are defined as analytical sensing devices measuring the target analyte in a given sample qualitatively and/or quantitatively. These integrated devices comprise of three elements: a) a biological recognizing element, identifies the analyte, b) transducer, converts the recognizing signal into detectable output and c) detector, the signal processing unit which amplifies and displays the output in easy to use format (**Figure 1.1**). The combination offers advantages of high specificity, sensitivity and selectivity of biological element in various analytical fields including research and technology ranging from medical diagnosis, food safety, drug discovery, process control, environmental monitoring, defense and security (Perumal & Hashim, 2014; A. P. F. Turner, 2013).

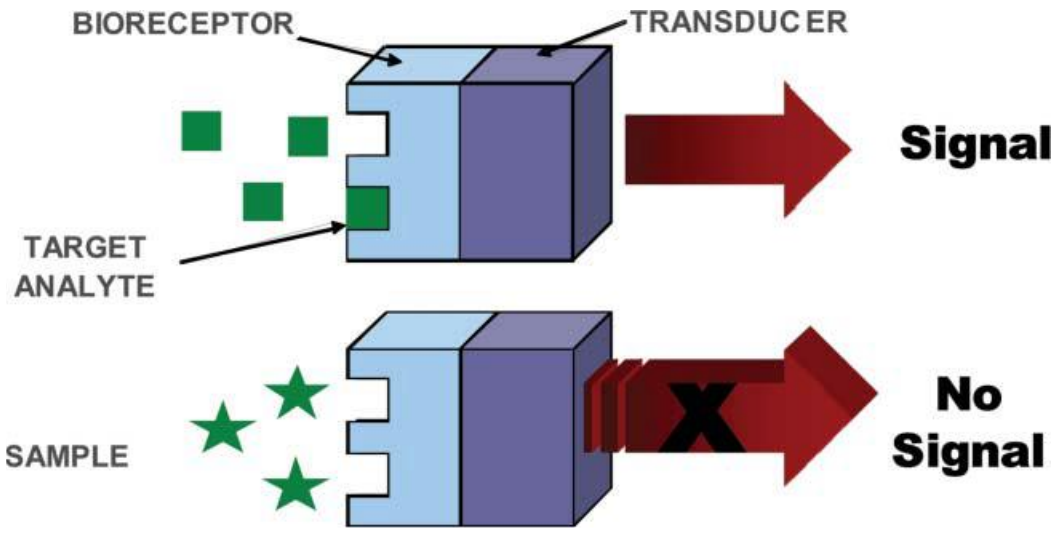


**Figure 1.1. Components of a typical biosensor** (Pejcic, Marco, & Parkinson, 2006)

## 1.2. Why biosensors?

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 are confronted with a list of pitfalls. There is no doubt that some of these techniques provide accurate and concluding results such as cell culture and colony counting, chromatography and PCR. But, these methods are also complex, laborious, time consuming requiring trained technicians and high throughput instrumentation (**Figure 1.2**) (Jairath, Singh, Dabur, Rani, & Chaudhari, 2015; Köse et al., 2011;

Opinion, 2011; Papageorgiou et al., 2018; Torre, Costa-Rama, Nouws, & Delerue-Matos, 2020; X. Zhao, Lin, Wang, & Oh, 2014). Some of these techniques such as PCR, chromatography, spectroscopy etc. encounter interference due to other species in the samples and requires sample purifications. In most of the conventional methods, the analysis has to be carried out in centralized laboratories where sample collection, testing, report preparation and delivery consume time (Torre et al., 2020). Conclusively, these methods do not offer feasibility for rapid and on-site testing analysis (**Figure 1.2**) (**Table 1.1**). Whereas biosensors offer rapid, on-site, continuous, sensitive and cost-effective testing (**Figure 1.2**). Despite decades of research and massive number of paper publications, the biosensor field is still found to be divided into two major sections: a) sophisticated, high throughput, expensive and laboratory hosted devices capable of precise and fast measurement of analytical and biological interactions/components used by technicians b) Handy, cheap, easy to use devices used by non-technicians for *in situ* or home analysis (A. P. F. Turner, 2013).

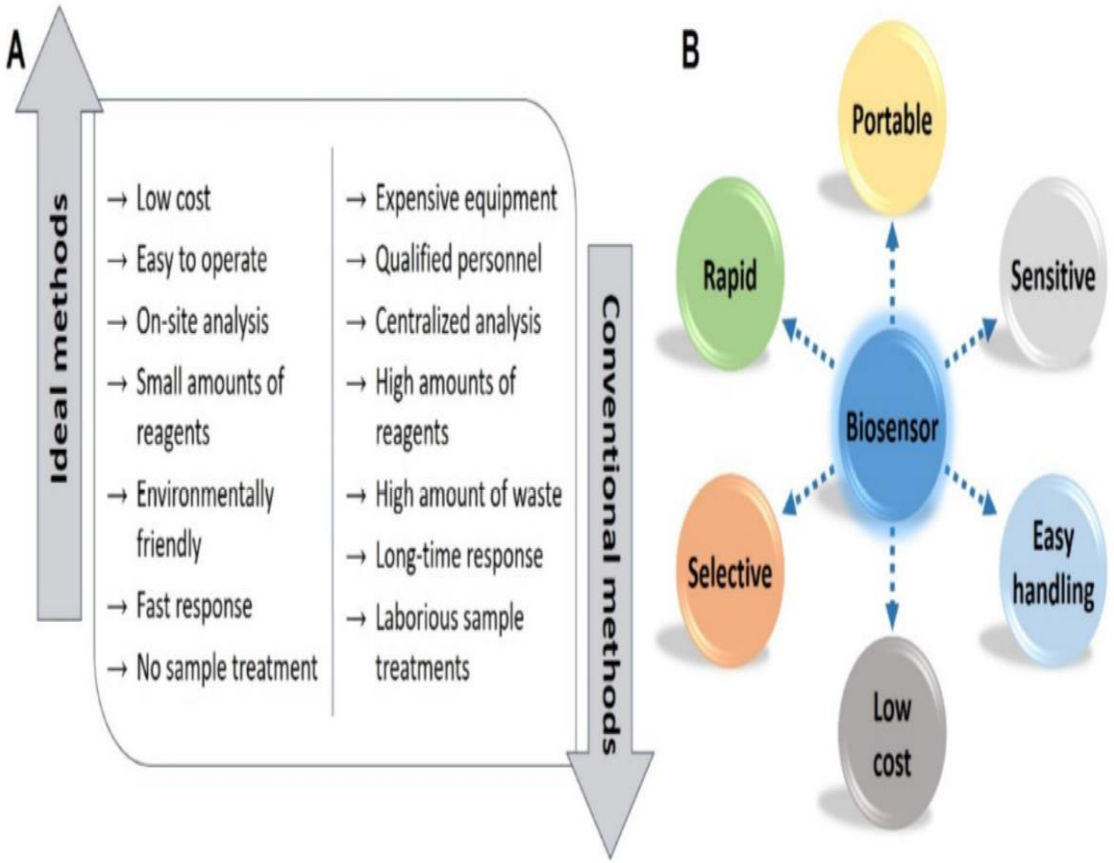


**Figure 1.2. Merits of biosensors over demerits of conventional methods** (Torre et al., 2020)(Mishra, Nunes, Souto, & Marty, 2018)

| Conventional Methods | Disadvantages of conventional methods | Advantages of biosensor |
|---|---|---|
| **Culture-Colony Method** | Time-consuming<br>Continuous detection not possible<br>Not portable | Rapid<br>Continuous detection<br>Portable |
| **Nucleic acid-based techniques (PCR etc.)** | Time consuming<br>Expensive<br>Require pure sample<br>Not portable | Rapid<br>Cheap<br>No need of pure sample<br>Portable |
| **Physico-chemical methods (spectroscopy, chromatography etc.)** | Interference due to other species<br>Time consuming<br>Expensive<br>Special Skills required | Only detect the analyte<br>Rapid<br>Cheap<br>No need of technicians |
| **Immunological methods (ELISA, IMS etc.)** | Time-consuming<br>Sometimes interference due to labels<br>Not reusable | Rapid<br>Easy<br>No labelling<br>Sometimes reusable |

**Table 1.1. Advantages of biosensors over conventional methods**

### 1.3. Applications of biosensors:

Biosensors offer various applications in the fields such as diagnostics, food and water analysis, environmental monitoring, pharmaceutical industry, defense etc. They propose enhanced sensitivity, portability with on-line sensing at lower economic costs (Mehrotra, 2016).

#### 1.3.1. Food monitoring and pathogen detection:

Food processing industry has to consistently monitor its food quality and safety measures throughout the processing period. Usually conventional techniques used during the processes such as chemical experiments, chromatography, spectroscopy etc. have their own limitations. These techniques are costly and time consuming causing fatigue to manpower. Continuous process measurements and monitoring in cost effective ways is always desired by food industry. Thus, simple, selective, inexpensive and on-line testing by biosensors seems to be a very convenient option (Scognamiglio, Arduini, Palleschi, & Rea, 2014).

Detection of pathogens in food is one of the most desired applications offered by biosensors. Faecal contamination in vegetables can be determined by checking the presence of *Escherichia coli* (Arora, Sindhu, Dilbaghi, & Chaudhury, 2011; Ercole, Del Gallo, Mosiello, Baccella, & Lepidi, 2003). 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. Ghasemi-varnamkhasti et al., in 2012 developed and electric tongue using tyrosinase and cobalt phthalcocynines as electron mediators to monitor aging of beers. The biosensor showed good capabilities when tested in beer sample bottles (Ghasemi-varnamkhasti et al., 2012).

### 1.3.2. Water and environmental monitoring:

Nowadays, continuous monitoring of water quality has become an essential need due to environmental concerns. Various anthropogenic activities such as industrial waste releases, mining of minerals and gases, burnings of non-renewable energy resources, excessive uses of pesticides and chemical fertilizer are the main reasons of abating quality of water (Bi et al., n.d.)(Singh et al., 2020). So, preventive actions are needed to be carried out such as controlled release of toxic compounds, handling the contaminating areas to sustain water qualities (Agrawal, Pandey, & Sharma, 2010; Singh et al., 2020). This can be achieved by continuously monitoring toxic elements in water samples. Traditional methods such as spectroscopy, titrations and chromatography have their own limitations such as they are expensive, laborious, low in sensitivity and are not portable. Biosensors serve here as a better option being portable, in-expensive, more sensitive and selective to monitor and detect water pollutants (Kovalchuk & Kovalchuk, 2008).

Waterborne diseases by bacteria cause about two million deaths as reported by World Health Organization. Speedy and on-site detection can prove a great help to reduce this death numbers. A paper based electrochemical biosensor was developed to detect total bacterial counts in water sample. Hydrophobic paper based screen printed carbon electrode was functionalized with carboxyl group-*Concanavalin A* complex to detect bacterial count. Linear range and detection limits of the biosensor were $10^3$-$10^6$ CFU/ml and $1.9\times10^3$ CFU/ml (Rengaraj, Cruz-Izquierdo, Scott, & Di Lorenzo, 2018). Cyanide is found to be most abundant pollutant in water. CAT/PANI/Pt electrode was developed to detect cyanides in water (Özcan & Aydin, 2016). The biosensor developed economic, simple and fast way of detecting cyanides in water with the detection time of only 6 min.

| Sr. No. | Field of application | Biosensor monitoring | References |
|---|---|---|---|
| **1** | Food monitoring and pathogen detection | Beer ageing monitoring using tyrosinase and cobalt phthalcocynines | (Ghasemi-varnamkhasti et al., 2012) |
| **2** | Food monitoring and pathogen detection | *Escherichia coli* detection in vegetables by urease-*E.coli* antibody complex | (Ercole et al., 2003) |
| **3** | Water and environmental monitoring | Total bacterial count in water by *Concanavalin A* | (Rengaraj et al., 2018) |
| **4** | Water and environmental monitoring | Cyanide detection by CAT/PANI/Pt electrode in water | (Özcan & Aydin, 2016) |
| **5** | Infections and disease detection | Piezoelectric-DNA biosensor for detection of Hepatitis B | (C. Y. Yao & Fu, 2014) |
| **6** | Infections and disease detection | Detection of miR-137 biomarker for Alzheimer's disease using doxorubicin | (Azimzadeh, Nasirizadeh, Rahaie, & Naderi-Manesh, 2017) |
| **7** | Toxin detection and defense use | Graphene oxide bound SNAP-25-GFP for detection of Botulinum neurotoxins | (Shi et al., 2015) |

**Table 1.2. Various applications of biosensors in different fields**

### 1.3.3. Infections and Disease detection:

Spread of various viruses such as SARS-CoV-2, Nipah, Hepatitis B, Avian influenza etc. are of global concern. To prevent the spread of these infections, their timely detection plays a key role. Biosensors propose very convenient tool being cheap, rapid and portable to detect these viruses (Singh et al., 2020). A group of researchers has published a piezoelectric biosensor based on DNA with the linear range of 0.02-0.14 µg/ml for the detection of Hepatitis B (C. Y. Yao & Fu, 2014). Alzheimer's disease (AD) is very prevalent type of dementia. Early detection of the disease using molecular biology approach is the traditional way of detection. Combining

electrochemistry with molecular biology showed a new approach to develop electrochemical biosensor for AD. Serum miR-137 was used as a biomarker for the detection of AD using doxorubicin as a receptor. The biosensor showed linearity from 5-750 fM with the limit of detection (LOD) as 1.7 fM (Azimzadeh et al., 2017). The biosensor proposed easy, convenient, portable and cost effective way for detecting Alzheimer's disease.

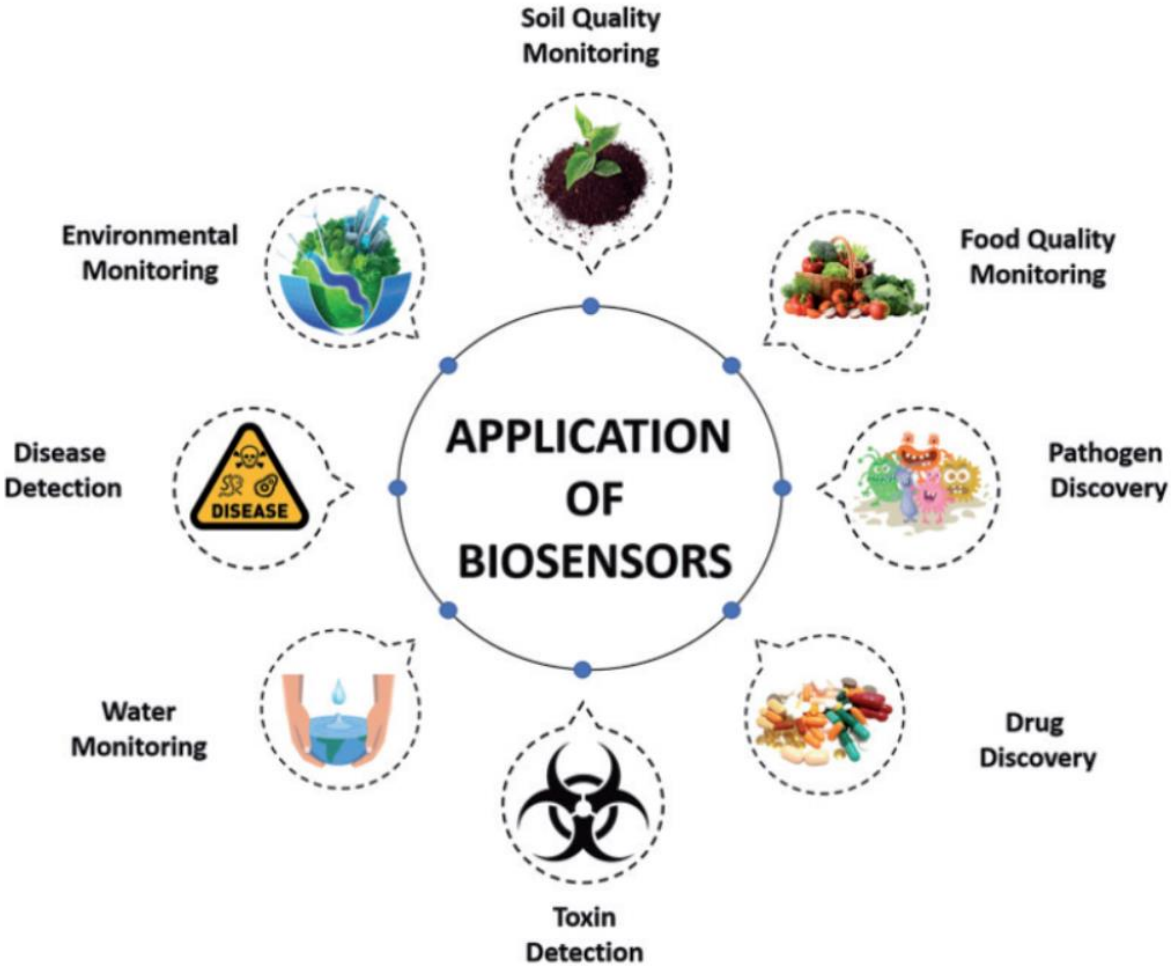


**Figure 1.3. Various applications of biosensors** (Singh et al., 2020)

### 1.3.4. Toxin detection and defense use:

The most promising and applicable field for biosensor can be seen its usages in defense at the time of biological attacks. During biowarefare (bacteria, toxin and viruses) attacks, biosensors can offer a help by rapidly, selectively and on-time detecting them. Various techniques such as enzyme assays, nucleic acid-based assays, anti-body based assays are combined in a typical biosensor fabrication procedure. Generally, nucleic acid based assays are more accurate and

specific compared to anti-body antigen assays. In such assays, samples are detected based on gene-specificity and do not require any amplification steps (Mehrotra, 2016)(Pohanka, 2019). In some cases, peptides along with enzymes are used to fabricate the sensor. Botulinum neurotoxins (BoNTs) is a bacterial protein and is very toxic to humans. It makes them potent biowarefare agent. Portable, easy to use and quick biosensors are needed for timely detection of the agent. Fluorescence resonance energy transfer (FRET) biosensor was developed by Shi and his team in 2015 for the detection of light chain of botulinum (BoNT-LcA) protease activity (Shi et al., 2015). A GFP conjugated SNAP-25 peptide was designed and synthesized by Shi and team. This designed complex was covalently immobilized on graphene oxide. BoNT-LcA tend to cleave SNAP-25-GFP and release GFP-conjugated fragment which can be sensed by fluorescence detector. The sensor showed linear range of 1 fg/ml - 1 pg/ml with 1 fg/ml as detection limit (Shi et al., 2015).

**1.4. Biosensors – from historical background to modern market:**

The historical background of biosensors takes us to beginning of 20$^{th}$ century. In 1906, M. Cremer validated that the electric potential arisen between the two sides of glass membrane dipped into the fluid is proportional to the amount of acid (Bhalla, Jolly, Formisano, & Estrela, 2016). Then, in 1909 "pH" as a concept of hydrogen ion concentration was introduced and following it, in 1922 pH meters were introduced. Meanwhile, from 1909 to 1922, the invertase enzyme was tested for its immobilization onto charcoal and aluminum hydroxide by Griffin and Nelson (Griffin, 1916). Biosensor as a concept was first proposed in 1962 by Leyland C. Clark, the father of biosensor as an "Enzyme electrode" and now it is popularly known as "Clark oxygen electrode" (Heineman & Jensen, 2006). He proposed that oxygen or hydrogen peroxide sensor could be used to build wide range of bioanalytical instruments by using immobilized enzyme. L. C. Clark also demonstrated that glucose level could be measured using enzyme electrode by measuring changes in current. Following this idea, Guilbault and Montalvo Jr. in 1969 made the first urea detector using potentiometric technique (Pasto, Richard Meyer, & Kang, 1967). But, the first ever commercial biosensor was launched in 1975 by Yellow Sprint Instruments. After 1990, the biosensor research field and commercial market has raced up with multidisciplinary areas. The concept of biosensor bridges the fundamentals of physics, chemistry and biology with biotechnical fields such as electronics, nanotechnology, medicine etc. Only from 2005 to 2015, 84000 reports are published in the field of biosensor (Bhalla et al., 2016). The classic example of breakthrough

of biosensor can be seen as glucometer which measures glucose level in human samples (A. P. F. Turner, 2013).

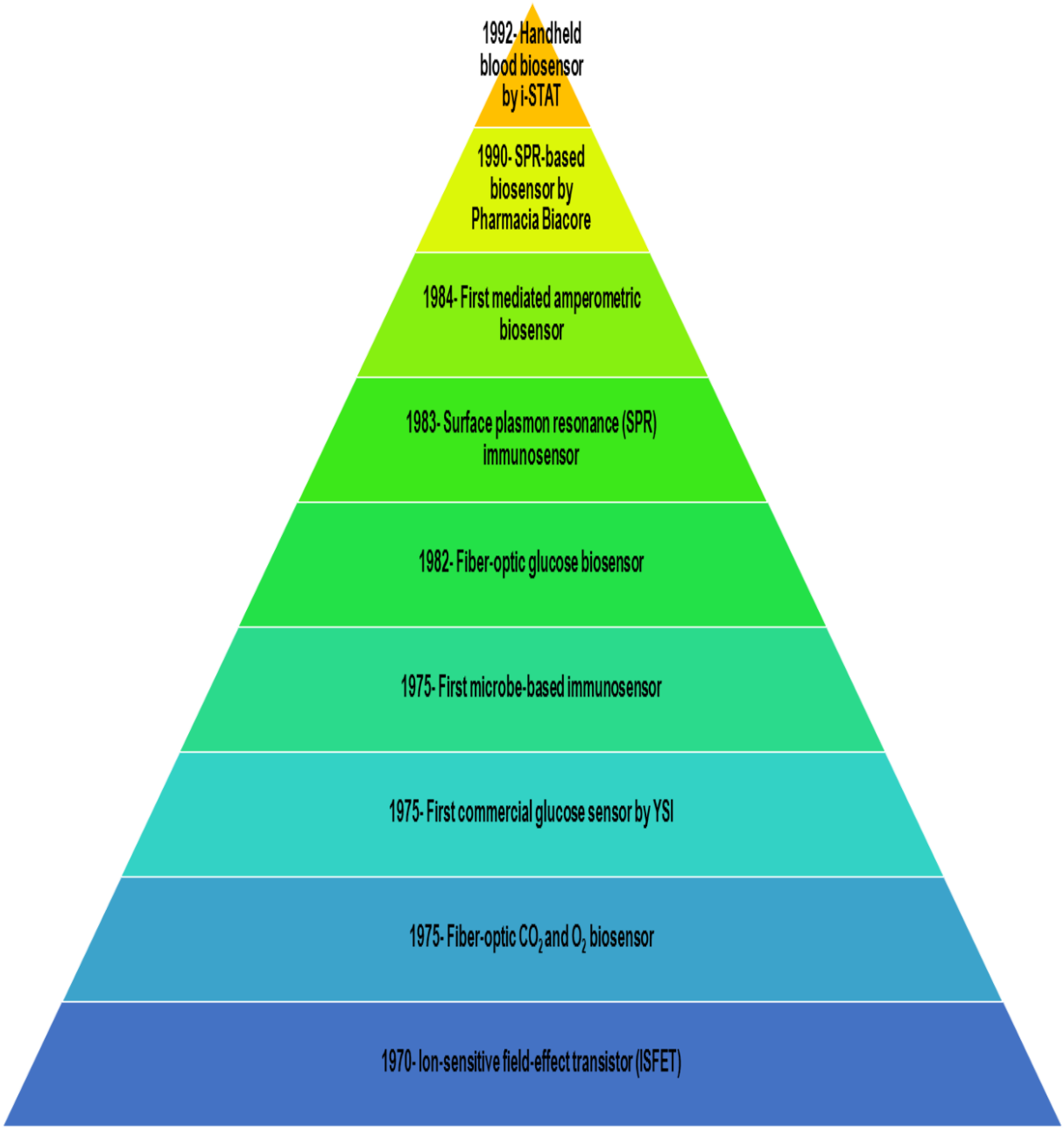


**Figure 1.4. Important breakthroughs in the field of biosensor during 1970-1992**

(Adapted from (Bhalla et al., 2016); (Bergveld, 1970) (Vestergaard et al., 2015) (Yoo & Lee, 2010) (Suzuki et al., 1975) (Z. Yang, Peng, Wang, & Liu, 2010) (Schultz et al., 1982) (Liedberg, Nylander, & Lunström, 1983) (Cass et al., 1984))

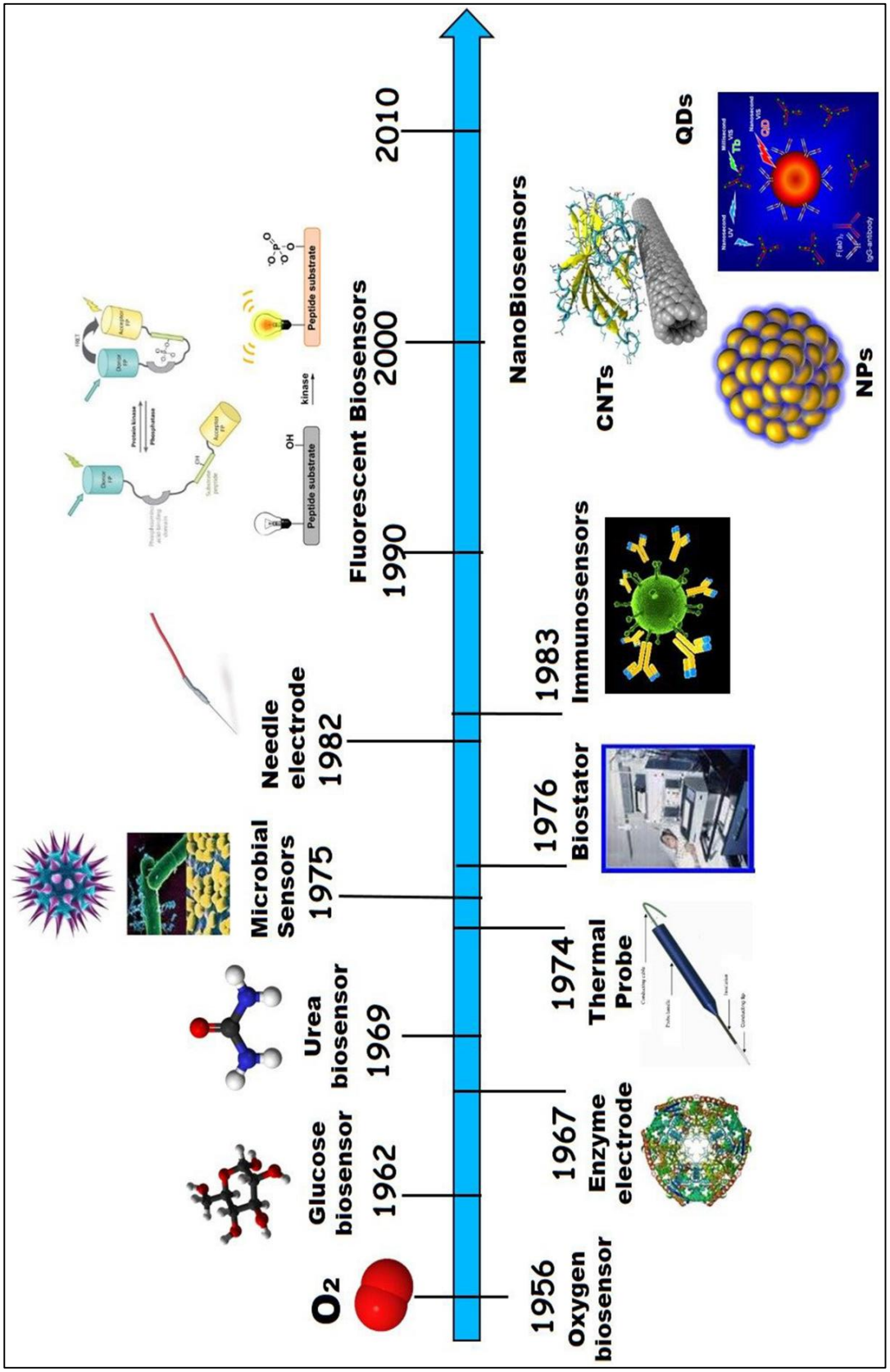


**Figure 1. 5. Biosensor development over the time from 20th century to 21st century** (Adapted from Tilmaciu & Morris, 2015)

From the past 20 years, the healthcare and biosensor market has witnessed a great boom due to increase of aging population and increase of prevalence of diabetes and other diseases. The technological advancements in biosensor field offers point-of-care and self-testing options, making it more convenient to both patients and hospital administration. Nowadays, even government and global companies are encouraging its use (Socorro-Leránoz, Santano, Del Villar, & Matias, 2019). Apart from healthcare, other fields also realize its benefits such as research labs, robotics, industrial processes, defense, environmental monitoring, veterinary and agriculture testing etc. (Socorro-Leránoz et al., 2019) (**Figure 1.3**). By the end of 2017, India alone holds a share of 13% in global biosensor market. According to a survey, the global biosensor market is supposed to reach USD 35,729.14 Million by 2025 (Data retrieved from: https://www.marketresearchfuture.com/sample_request/1228 on Date:19th February, 2022; Report code: MRFR/HC/0720-CR). The above market predictions are based on existing types of biosensors (electrochemical, optical, thermal and piezoelectric biosensors), type of end use (point-of-care, home diagnostics, research labs, food industry, security and biodefence) and applications (medical and food testing, environmental and agricultural testing, industrial process testing) (Data retrieved from: https://www.marketresearchfuture.com/sample_request/1228 on Date:19th February, 2022; Report code: MRFR/HC/0720-CR).

The beginning of 21st century marked the development of a broad range of biosensors for different applications, *in vitro* and *in vivo*. Due to a wide range of receptors and transducers being used nowadays to develop the biosensors, the field has now started being divided based on the type of transducer or receptor used (Tîlmaciu & Morris, 2015; A. P. F. Turner, 2013; Pandey & Malhotra, 2019).

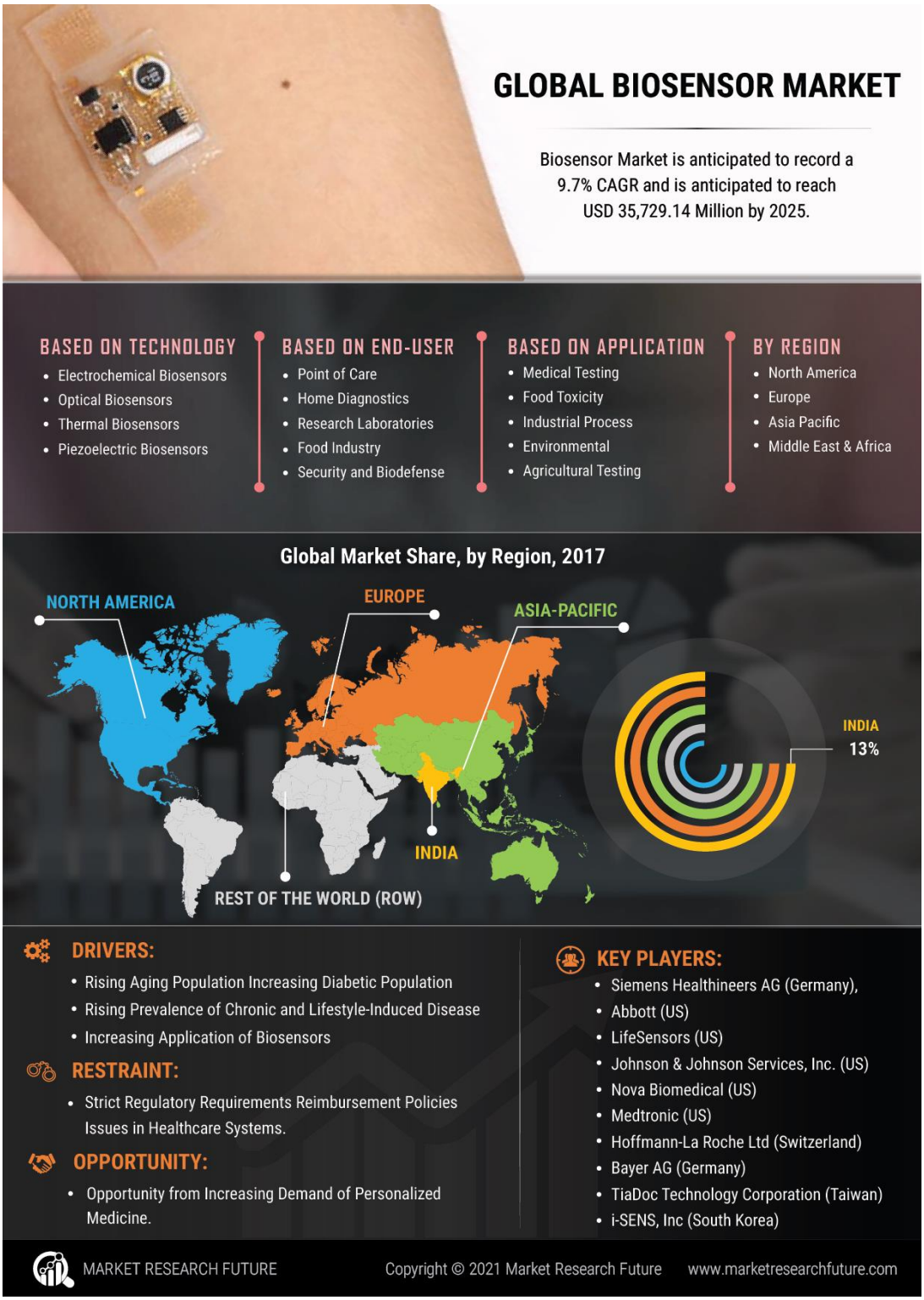


**Figure 1.6. A detailed forecast on global biosensor market by "Market Research Future"** (Retrieved from: https://www.marketresearchfuture.com/sample_request/1228 on Date:19th February, 2022; Report code: MRFR/HC/0720-CR)

## 1.5. Types of biosensors:

Based on signal transduction system and bioreceptor for biological signaling, biosensors can be divided into many types (**Figure 1.7**) (Perumal & Hashim, 2014).

### 1.5.1. Types of biosensors based on bioreceptors:

Bioreceptor component of a biosensor has significant role of recognizing the analyte specifically. Selectivity and sensitivity majorly depends on type of bioreceptor used to fabricate a sensor. Bioreceptor plays a distinguished role by thwarting the inference when tested for sample identification (Lowe, 2008). 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 (**Figure 1.7**) (Perumal & Hashim, 2014). Each of the type is discussed shortly in next section.

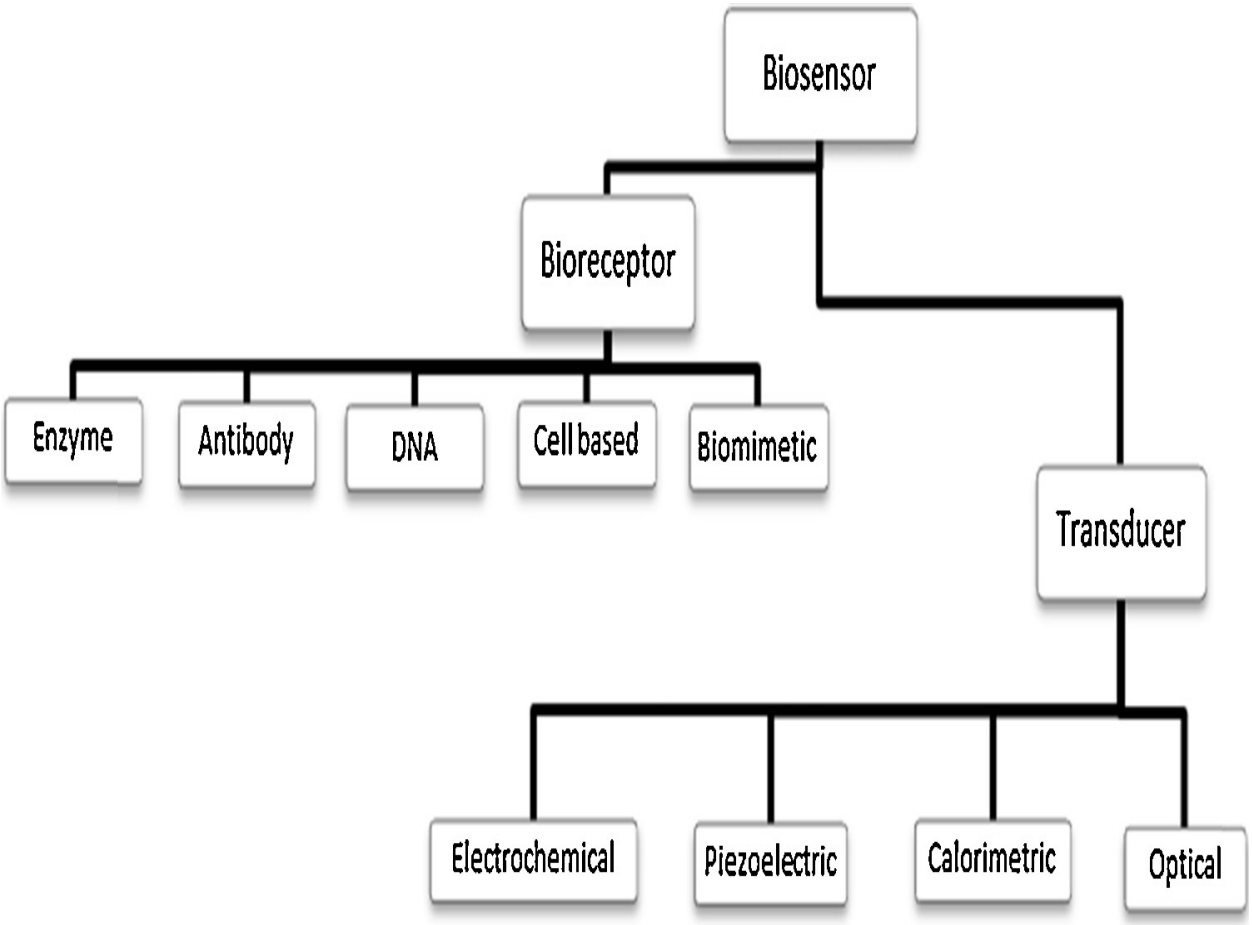


**Figure 1.7. Biosensors categorized into different classes and subclasses** (Adapted from Perumal & Hashim, 2014)

**1.5.1.1. Enzymatic biosensor:**

Enzymatic biosensors were the first which were introduced in 1962 by Clark and Lyons. They used soluble enzyme onto the electrode which could sense glucose level using change in current (Perumal & Hashim, 2014). Since then, the enzymatic biosensors containing a proficient catalyst have massively penetrated the global market for different applications. Enzymes can uniquely recognize and bind with the substances to develop a product. This property is very useful to make enzyme based analytical devices (Zourob, 2010a). Here in case of enzymatic biosensors, the upper most sensing layer is made up of enzyme closely bound to a transducer. This kind of sensor works on specific binding efficiency of the enzyme with its particular substrate from a sample matrix and its ability to convert the substrate into product (Morrison, Dokmeci, Demirci, & Khademhosseini, 2007; Perumal & Hashim, 2014). The lock and key model for enzyme and substrate explains the high specificity and success of this kind of biosensors. These properties make biosensors to go up to lower detection limits making it more superior than other techniques (Zourob, 2010a; Perumal & Hashim, 2014). Though, the enzymatic sensors are found to be most successful ones, their performance is influenced by several crucial factors such as: substrate concentrations, presence of inhibitors (competitive & non-competitive), temperature and pH (Perumal & Hashim, 2014; Tan, 2006). Though, Glucose oxidase (GOx) and Horseradish peroxidase (HRP) are more frequently used enzymes according to the literature survey, pesticides, triglycerides and heavy metals are also found to be detected using several enzymes (Chouteau, Dzyadevych, Durrieu, & Chovelon, 2005; Kartal, Kilinç, & Timur, 2007; Ma, Cheong, Weng, Tan, & Shen, 2018; Narang & Pundir, 2011; Solanki et al., 2009)(**Table 1.3**). Biological receptors used in enzyme based biosensors are catalysts only which does not get consumed after its use. Thus, these biosensors are compatible for their long reuse. Detection range and life of the enzymatic biosensor is majorly influenced by stability of the enzyme attached closely with the transducer (Marquette & Blum, 2006; Norsuzila Ya'acob et al., 1989).

**1.5.1.2. Antibody based biosensor:**

Antibody based biosensors offer the possibility of replacing the conventional methods giving more accurate and faster results. This kind of biosensor was first introduced in 1950, opening the door for immune-diagnosis (Zourob, 2010b). Here, antigen/antibody are used as a bioreceptor component which is close in contact with the transducer to make diagnostic kit (Conroy, Hearty, Leonard, & Kennedy, 2009; Perumal & Hashim, 2014). Though, an antibody

(Immunoglobulin Ig) has "Y" shaped structure containing heavy and light chains, some of the antibodies generated in humans are also dimeric or pentameric. The later structures are joined by joining protein (J-chain) and diasulphide bonds (Wood P., 2006; Pohanka & Skládal, 2008; Perumal & Hashim, 2014). Each heavy and light chain contains constant and variable region in its structure. Variable part selectively binds with the particular antigen based on its specificity towards the antigen (Conroy et al., 2009; Zourob, 2010b). This specificity towards antigens is totally based on amino acid side chains of antibodies. When an immunosensor is fabricated using antigen/antibody as a bioreceptor, the biding with its target (antibody/antigen) is very specific and stable (Fowler, Wong, Brian Halsall, & Heineman, 2008). Majorly, two types of transduction methods are used while fabricating an immunosensor: electrochemical transduction and optical transduction. In case of optical transduction, so far mostly conventional radioimmunoassay was used to fabricate the sensor proposing low sensitivity, short-life and discarding problems in general. Though, some immunoassay kits launched nowadays use visible color changes by particular enzymes coupled into it, they also propose several drawbacks such as high ratio of false positive/ negative results and lowered stability-sensitivity. Electrochemical transduction method has advantages over these issues caused by optical mode of detection. Use of electrochemical transduction method makes the immunosensors fast, cheap, simple and stable (Fowler et al., 2008; Perumal & Hashim, 2014). Due to recent developments in technology, now optical transducers are also considered equally important as they are successful in market (Shankaran, Gobi, & Miura, 2007; Bhatta, Stadden, Hashem, Sparrow, & Emmerson, 2010a). Conventional diagnostic techniques suffer from some shorts comings such as non-portability, low sensitivity, being lengthy, need of technicians etc. Immunosensors being rapid, portable and cheap are becoming promising tools for diagnosis of early stage cancers (Ushaa, Madhavilatha, & Rao, 2011). Ramírez et al., proposed the application of immunosensors (Ramirez et al., 2009) in public health (fast and cost effective diagnosis), food analysis, clinical diagnosis, environmental monitoring and pathogen detection (M.L., K.E., P.K., & J.C., 2014; Skládal, Dos Santos Riccardi, Yamanaka, & Da Costa, 2004; Skottrup, Nicolaisen, & Justesen, 2008; Holford, Davis, & Higson, 2012; Barton et al., 2009; Socorro-Leránoz et al., 2019; Braiek et al., 2012) (**Table 1.3**).

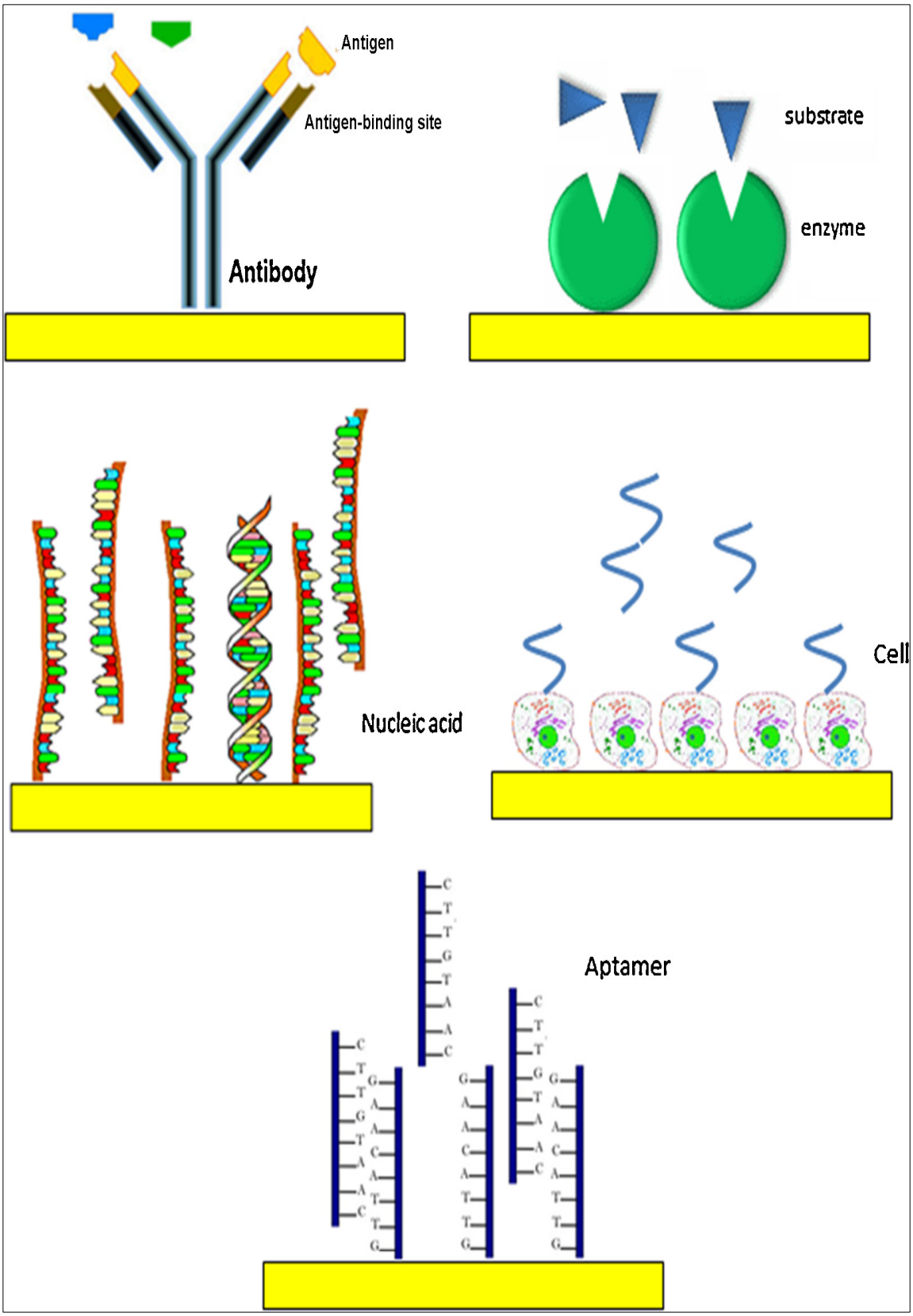


**Figure 1.8. Different types of biosensors based on bioreceptors** (Perumal & Hashim, 2014)

**1.5.1.3. Nucleic acid/DNA based biosensor:**

Amongst the most specific biosensors towards its analyte, nucleic acid or DNA based biosensors are found to be very prominent. In early 1950s, use of nucleic acid sequence as bioreceptor was introduced and now has become very popular due to its specificity (A. Liu et al., 2012)(**Figure 1.8**). This technique has overcome the short comings such as high cost, being lengthy and with a hazardous procedure offered by other techniques such as electrophoretic separations and radio iso-tropic detection (Pejcic et al., 2006). Use of specific nucleic acid sequences as bioreceptors contributes to its specificity towards target in a sample matrix. When DNA sequences or fragments are used as bioreceptors, they are term as DNA probes or DNA primers. These receptors are easily synthesizable and specific towards their complementary sequences (Teles & Fonseca, 2008). These receptors are usually designed to target specific proteins or non-macromolecular compounds. The specificity is usually driven through stable hydrogen bonds between two helical strands of nucleic acids (Joseph Wang, 1998; Norsuzila Ya'acob et al., 1989). When ssDNA probe binds with its complementary ssDNA, it forms dsDNA and produces biochemical changes which are amplified by the transducer to a detectable signal. While fabricating the DNA based biosensors, the most important step to be considered is immobilization of the nucleic acid strand onto its support. Most common linkers used to immobilize ssDNA are biotin and thiol (Perumal & Hashim, 2014). Sensitivity of the biosensor is totally based on lined up nucleotide strands which are exposed properly to their target sequence. These nucleotide strands may be sensitive to DNA, RNA or any peptide sequences (Monošík, Streďanský, & Šturdík, 2012; Norsuzila Ya'acob et al., 1989). Short nucleic acid sequesnces having potential of hybridizing with specific target area of the complementary sequence cause conformational changes when provided with chemical buffering systems along with the target sequence. This causes hybridization of the complementary strands and minimize non-specific binding (Lucarelli, Tombelli, Minunni, Marrazza, & Mascini, 2008; Norsuzila Ya'acob et al., 1989). The nucleic acid biosensors can be regenerated and reused by changing the compositions and concentrations of washing solutions and giving the heating treatments. This kind of biosensors show very high specificity which can detect even single molecule in a complex mixture (Perumal & Hashim, 2014). Nucleic acid sensors propose various applications in diagnostics such as bacterial, virus and physiological disease detection (Lui, Cady, & Batt, 2009; Chua, Yean, Ravichandran, Lim, & Lalitha, 2011; Thuy et al., 2012; Yeh, Chang, Lin, Chang, & Lin, 2012) (**Table 1.3**). DNA-electrochemical biosensors have

large potential to penetrate the global market owing to high sensitivity and selectivity and being rapid and cost effective (A. Liu et al., 2012).

**1.5.1.4. Cell based biosensor:**

In cell-based biosensors, living cells are used as the bioreceptors to detect intracellular and extracellular microenvironment and physiological parameters. Detectable responses are generated in response to interactions between the stimuli and cell (Ziegler, 2000) (**Figure 1.8**)**.** Bacterial or fungal cells are used as bioreceptors to detect target molecules or monitor surrounding microenvironments. Sometimes, specific proteins present in cells are also used as bioreceptor to detect target analyte (Zourob, 2010b). Living cell biosensors are different from other biosensors where extracted components of living cells are used rather than living cells as bioreceptors. Due to this reason, cells based biosensors offer several advantages and disadvantages as well. 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 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 majorly 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 & Hashim, 2014; Shimomura-Shimizu & 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 does not cause cell death. This kind of sensors have long shelf life and are cheap as active cells are not needed to get isolated. In this context, cell based sensors are superior to enzyme based biosensors (Zourob, 2010a). Cell based sensors can be thought to be future of nano-diagnostics due to their special characteristics (Shinde, Fernandes, & Patravale, 2012). Cell based biosensors find applications mainly in medical field such as detection of a disease and drug-analyte discovery, pharma industry, environmental monitoring, food quality assessment (Veiseh, Veiseh, Martin, Bertozzi, & Zhang, 2007; Banerjee & Bhunia, 2009; Z. Yang et al., 2010; T. H. Wang, Hui, & Deng, 2010; Bohrn et al., 2012; Q. Liu et al., 2012; Melamed, Elad, & Belkin, 2012) (**Table 1.3**).

**Table 1.3. Uses of different receptors for detection of various analytes**

| Sr. No. | Receptor type | Analyte | Fabrication chemistry | Linear range | Detection limit | Reference |
|---|---|---|---|---|---|---|
| **1** | Enzyme | Organophosphorus pesticide | GCE/AuNP/mercaptom ethamidophos/ mercaptohexanol/AChE | 0.1-1500 ng/mL | 0.019-0.077 ng/mL | (G. Zhao, Zhou, Wang, Shen, & Zhao, 2021) |
| **2** | Enzyme | Organophosphorus pesticide | CS/KbE/Au/cCNTs–cGR/GCE | - | 3 ng/L | (Tao et al., 2022) |
| **3** | Aptamer | Organophosphorus pesticide | BSA/AuNPs-DTN/GCE | 0.0001-10,000 ng/mL | 0.07 and 0.8 pg/mL | (Jiansen Li et al., 2022) |
| **4** | Aptamer | Organophosphorus pesticide | MoS2- ssDNA | 2–10 µg/L | 0.018 µg/L | (Radhakrishnan & Kumar, 2022) |
| **5** | Antibody | Organophosphorus pesticide | Anti-ChlT-mAB/ ChlT/SPE | 0.01–10 µg/L | 22.4 ng/L | (Surribas, Barthelmebs, & |
| **6** | Antibody | Organophosphorus pesticide | Anti-IgG-HRP/IMD/mAb-IMD/SPE | 50-10000 pM | 24 pM | (Pérez-Fernández, Mercader, Checa-Orrego, De La |
| **7** | Cell based | Organophosphorus pesticide | poly(2-hydroxyethyl methacrylate) (pHEMA)/ *Anabaena* | 0.01–0.75 µg/L | 0.117 µg/L | (W. Gao et al., 2011) |

| Sr. No. | Receptor type | Analyte | Fabrication chemistry | Linear range | Detection limit | Reference |
|---|---|---|---|---|---|---|
| **8** | Cell based | Organophosphorus pesticide | C. vulgaris/BSA/GA/ Pt. electrode | - | 10 ppb | (Chouteau et al., 2005) |
| **9** | Biomimetic | Organophosphorus pesticide | Thiamethoxam-MIP/Au/rGO/SPCE | 0.5–3.0 µmol/L | 0.5 µmol/L | (Peng et al., 2021) |
| **10** | Biomimetic | Organophosphorus pesticide | Gold electrode/Polytyramine/ | 5–100 µM | 4.61 µM | (El-Kady & Kaner, 2013) |
| **11** | Aptamer | SARS-CoV-2 | Nanoprobes/BSA/MCH /Dual-aptamer/GE | 0.025 to 50 ng m/L | 8.33 pg m/L | (J. Tian et al., 2021) |
| **12** | Aptamer | SARS-CoV-2 | Aptamer/AuNPs/ SPCE | 10 pM – 25 nM | 1.30 pM (66 pg/mL) | (Abrego-Martinez et al., 2022) |
| **13** | Antibody | SARS-CoV-2 | SARS-CoV-2 antibodies (IgG & IgM)/(GO)- | 1 to 1000 ng/mL | 0.11 ng/mL | (Yakoh et al., 2021) |
| **14** | Antibody | SARS-CoV-2 | Gold nanoparticles/anti-N protein mAb | - | $10^{-18}$ M | (Murugan, Bhatia, Sai, & |

**Continue…**

**Table 1.3. Uses of different receptors for detection of various analytes**

| Sr. No. | Receptor type | Analyte | Fabrication chemistry | Linear range | Detection limit | Reference |
|---|---|---|---|---|---|---|
| **15** | Aptamer | SARS-CoV-2 | GQH DNAzyme + HRP/Dualaptamer/Au@Pt /MIL-53(Al) nanoprobes | - | 8.33 pg/mL | (J. Tian et al., 2021) |
| **16** | Enzyme | Triglycerides | Lipase/NPs/GKNPs/GPO NPs/PG | 0.1 mM–45 mM | 0.1 nM | (Narwal & Pundir, 2017) |
| **17** | Enzyme | Triglycerides | Lipase/ GKNPs-GPONPs/Au electrode | 10-500mg/dL | 1.0 µg/ml | (Pundir & Aggarwal, 2017) |
| **18** | Aptamer | *Staphylococcus aureus* | Electrode/HCR nanowires/3WJ-NEASA | - | 1.2 × $10^1$ CFU/mL | (Peng et al., 2021) |
| **19** | Aptamer | *Staphylococcus aureus* | MCH/aptamer/gold electrode | 0.5 ng/mL - 500 ng/ mL | 0.17 ng/mL | (Xiong, Shi, Liu, Lu, & You, 2018) |
| **20** | Antibody | *Staphylococcus aureus* | Carboxyl magnetic particles/Chicken anti-protein A IgY/ | 5.0 × 102 CFU/mL - 5.0 × 104 CFU/mL | 1.1 × $10^2$ CFU/mL | (Yun Zhang et al., 2019) |
| **21** | Antibody | *Staphylococcus aureus* | Ab-HMS-GCE | 10-20 × $10^3$ CFU/mL | 11 CFU/mL | (Yun Zhang et al., 2019) |

**Continue…**

**Table 1.3. Uses of different receptors for detection of various analytes**

**1.5.1.5. Biomimetic biosensor:**

The name biomimetic biosensor itself gives its definition of having synthetic or artificial bio-receptor which can mimic the natural bio-receptor or biosensor. This kind of biosensors include molecularly imprinted synthetic receptors and synthetic aptamers as receptors (Morrison et al., 2007;Gai et al., 2008; Mosbach, 1994) (**Figure 1.8**). Molecular imprinting was first reported in 1994 by Mosbach and his team and continued to advance technologically, specially in 3D printing of particular analytes/objects. In molecular imprinting, 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 template. After polymerization, the template analyte is washed off by several washing solutions which generates its complementary cavities when washed off. These cavities are complementary to the target analyte template in its size, shape and spatial arrangement of chemical functional groups. When target analyte comes in contact with the imprinted polymer material, it re-binds with the target analyte specifically for detection (Mosbach, 1994). Molecular imprinting approach offer cost effective, sturdy and specific receptors for 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 (A. Turner, Minunni, Brys, Tombelli, & Mascini, 2006; Zourob, 2010a). 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. Though, aptamers have much more simple chemical structures, the manufacturing cost are still high and sometimes nonspecific recognition are the drawbacks of nucleic acid-based biosensors. *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, 2010a). Both molecularly imprinted and aptamer based biosensors offer their applications majorly in clinical diagnostics such as bacterial, viral and other disease detection (Cohen, Starosvetsky, Cheruti, & Armon, 2010; Pang, Cheng, Li, Lu, & Zhang, 2005; Parmpi & Kofinas, 2004; X. Xue, Pan, Xie, Wang, & Zhang, 2009; Strehlitz,

Nikolaus, & Stoltenburg, 2008; Torres-Chavolla & Alocilja, 2009; Weng, Huang, & Lee, 2012; Y. X. Wang, Ye, Si, & Ying, 2012) (**Table 1.3**).

### 1.5.2. Types of biosensors based on transducers:

Biosensors are majorly divided into four types based on their method of transduction they use. Transducer is a very important component in a biosensor which converts the chemical, biological or physical response into detectable signal with low interference and high sensitivity (Lowe, 2008). Depending on the literature survey, transducer based biosensors are of Electrochemical, Optical, Calorimetric and Piezoelectric types (**Figure 1.9**)(**Table 1.4**)(Perumal & Hashim, 2014). Apart from types of transducers, biosensors can be categorized as labelled and label free biosensors. Label free biosensors are gaining more popularity than earlier one (Pejcic et al., 2006; Perumal & Hashim, 2014). A brief description about each type is given below along with their working mechanism.

#### 1.5.2.1. Electrochemical biosensor:

An electrode is used for as a transduction component in electrochemical biosensor (**Figure 1.9**). 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 (Norsuzila Ya'acob et al., 1989; The, 1999; Thévenot, Toth, Durst, & Wilson, 2001). Electrochemical biosensors are best opted for detecting glucose concentrations, specific DNA strand hybridization or protein and drug detection by DNA aptamers. Electrical biosensors measure the change in potential caused by interaction between the analyte and transducer surface. The change in electrical potential is usually measured in proportional to the concentration of particular analyte in the sample by quantitative biosensor (Norsuzila Ya'acob et al., 1989). Thus, current flowing amid the electrodes is generated as an outcome of redox reactions carried out by electroactive analyte in the sample. Electrochemical biosensors are thought to be more superior than optical biosensors due to advantages offered by them and thus are prominently used for sensing applications. This kind of biosensors are fast responsive, stable, highly sensitive, portable, cost-effective and interference proof (Koyun, Ahlatcolu, & Koca, 2012; Lazcka, Campo, & Muñoz, 2007; Mungroo & Neethirajan, 2014; Norsuzila Ya'acob et al., 1989; J. Wang et al., 1997). Electrical biosensors can be further subdivided based on measuring electrical parameters: potentiometric, conductometric, amperometric, impedimetric (Huet et al., 2010).

- **Potentiometric biosensors:**
  - ✓ In response to a particular voltage applied, current flows though 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: Working and reference electrodes.
  - ✓ Not very popular for biosensor fabrications.
  - ✓ According to literature, used to detect cancerous cells and bacterial species.
    (N. Hu et al., 2013; Norsuzila Ya'acob et al., 1989)
- **Conductometric biosensors:**
  - ✓ Upon electrochemical reaction in a sample solution, ions and electrons are produced causing change in resistance or conductance of the solution. This change in electrical conductance is measured by conductometric biosensor.
  - ✓ When biological receptor is used to fabricate the electrode, low signal to noise ratio makes it impractical for biosensing applications.
  - ✓ No need of reference electrode, cost-effective and miniaturized system.
  - ✓ Useful in affinity interactions detection.
    (Korotkaya, 2014; Norsuzila Ya'acob et al., 1989; Perumal & Hashim, 2014)
- **Amperometric biosensors:**
  - ✓ Change in potential during oxidation-reduction reactions, when receptor and analyte react it is sharply measured. The best example of a successful amperometric biosensor is Clark oxygen electrode.
  - ✓ The sensor is made up of three electrode system (Working, Reference and Counter electrodes).
  - ✓ Most commonly used electrochemical technique for fabricating biosensors.
  - ✓ Proposes high sensitivity for electroactive species in a given test sample specially for biological tests.
  - ✓ Direct and indirect detection of bacterial species is performed using amperometric biosensors.
    (Brooks, Mirhabibollahi, & Kroll, 1992; N. Nakamura, Shigematsu, & Matsunaga, 1991; Norsuzila Ya'acob et al., 1989; Perumal & Hashim, 2014)

- **Impedimetric biosensors:**
  - ✓ Change in impedance upon reaction between receptor-analyte in a given test sample is measured.
  - ✓ The biosensor is composed of three electrode system (working, reference and counter electrodes).
  - ✓ Offers high detection sensitivity.
  - ✓ Detection of bacterial species in a given clinical sample, continuous monitoring food quality and other industrial processes by such kind of sensors are reported in literature (Norsuzila Ya'acob et al., 1989; P & S, 1996; Perumal & Hashim, 2014).

**1.5.2.2. Optical biosensor:**

Optical biosensors are rapidly growing for their demand due to the applications in food security and safety, industrial processes and environmental monitoring, drug detection and defense (Bhatta et al., 2010a; Bhatta, Stadden, Hashem, Sparrow, & Emmerson, 2010b; Johansson, Kromer, Sroka, & Stepp, 2008; Md Muslim, Ahmad, Heng, & Saad, 2012; Perumal & Hashim, 2014; Tsoka, Gill, Brookman, & Hoare, 1998; Védrine, Leclerc, Durrieu, & Tran-Minh, 2003). Optical biosensors are most proficiently used in research and diagnosis nowadays (Caygill, Blair, & Millner, 2010; Perumal & Hashim, 2014). Sometimes electrical and optical transduction methods are combined to fabricate the biosensor electrode, therefore it is also known as "Optical electrode" or "Optrode" (Biran, Yu, & Walt, 2008; Perumal & Hashim, 2014). Based on the type of spectroscopy used to fabricate the electrode, optical biosensor has been subdivided into different classes (Abdulhalim, Zourob, & Lakhtakia, 2008; Perumal & Hashim, 2014). Optical biosensors provide label free and online detection method (Bhatta et al., 2010b, 2010a; Di Francia, La Ferrara, Manzo, & Chiavarini, 2005; Fan et al., 2008; Pohanka, 2019). Surface plasma resonance or fluorescence based biosensors are gaining more popularity (Caygill et al., 2010; Perumal & Hashim, 2014). Types of optical transduction methods are described very shortly below:

- **Surface plasma resonance (SPR):**
  - ✓ Uses electromagnetic waves to detect the interaction between target analyte and receptor.
  - ✓ Changes in refractive index upon interaction with analyte is recorded at the biosensor surface. This changes causes deviation in the transmission of surface plasmon waves.

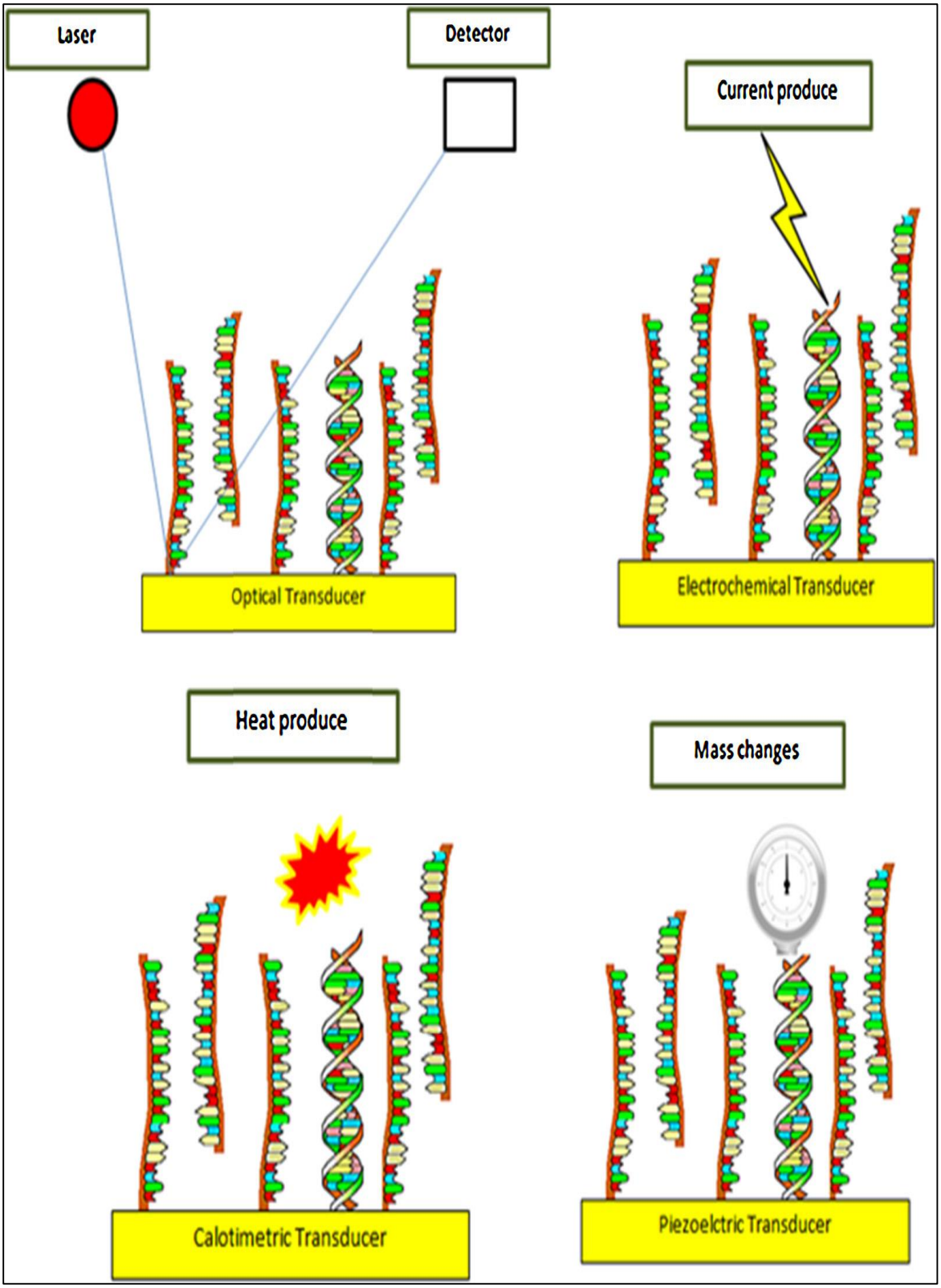


**Figure 1.9. Different types of biosensors based on use of transducer** (Perumal & Hashim, 2014)

- ✓ Bioreceptors such as enzymes, antigens, antibodies, nucleic acids can be incorporated in biosensor fabrication.
- ✓ Real time interaction detection is possible due to label-free integrated sensing systems provided by SPR without using radioactive substances or fluorescence.
- ✓ The results are of low specificity due to non-specific binding. Sometimes these results are wrongly correlated to biosensor performance.
- ✓ When any analyte interacts with the receptor, SPR measures the mass of the analytes. Thus, small anayltes (2kDa ≤) would give very small difference in SPR pattern making it less convenient for small analyte detection.
- ✓ SPR finds applications in environmental monitoring, pathogen detection, nucleic acid detection, drug detection and in pharma industry.

  (Caygill et al., 2010; de Mol & Fischer, 2010; Endo, Yamamura, Kerman, & Tamiya, 2008; Fan et al., 2008; Homola, Yee, & Myszka, 2008; Mannelli et al., 2006; Merwe, 2001; H. Nakamura et al., 2008; Nguyen, Tanious, & Wilson, 2007; Park, Hyun, Lee, Lee, & Ko, 2009; S. & Wu, 2010; Shankaran et al., 2007; Skottrup et al., 2008; Špringer, Piliarik, & Homola, 2010)

- **Chemiluminescence:**
  - ✓ Chemical reaction produces an energy and emits light. It is generally known as chemiluminescence. In such chemical reactions, molecules go to ground (relaxed) state from exited state and release energy and yield luminescence as a byproduct.
  - ✓ Luminescence generated by interaction between analyte and receptor is sensed by photomultiplier tube (PMT).
  - ✓ Chemiluminescence is gaining popularity due to simple instrumentation, high sensitivity, specificity and speed.
  - ✓ Drawbacks of chemiluminescence includes less quantitative accuracy, short shelf-life and lack of continuous detection technique.
  - ✓ Chemiluminescence is more compatible with immunoassays and nucleic acid based assays.
  - ✓ Chemiluminescence finds its applications in virus, bacterial and drug detection, environmental, pharmaceutical and food detection.

(Atias et al., 2009; Ding, Zhong, & Zhang, 2008; Dodeigne, Thunus, & Lejeune, 2000; Gámiz-Gracia, García-Campaña, Huertas-Pérez, & Lara, 2009; Guo, Yang, Zhang, & Zheng, 2013; Holford et al., 2012; Kricka, 2003; Lara, García-Campaña, & Aaron, 2010; D. Li, 2015; M. Liu, Lin, & Lin, 2010; Pejcic et al., 2006; Z. Zhang, Zhang, & Zhang, 2005)

- **Fluorescence:**
  - ✓ Fluorescence is also a kind of luminescence where energy applied to go to excited state is provided in the form of electromagnetic radiation. It is also called as photoluminescence or phosphorescence. Thus, florescence needs external light source to initiate transition of atoms.
  - ✓ Mostly receptors used in biosensor do not possess inherent properties of fluorescence. Fluorochrome is attached to biosensors to produce light during the interactions between receptor and analyte.
  - ✓ Mostly used with antibody and nucleic acid based assays.
  - ✓ Shortcomings of fluorescence based biosensors include complex instrumentation and low possibility for continuous online monitoring.
  - ✓ According to literature, fluorescence so far has been used for research and clinical diagnostics and in environmental monitoring.

    (Abdulhalim et al., 2008; Berdat, Marin, Herrera, & Gijs, 2006; Daly & McGrath, 2003; Dodeigne et al., 2000; Grubor, Shinar, Jankowiak, Porter, & Small, 2004; Hong & Kang, 2006; Lee, Thompson, Hall, Fulton, & Wong, 1993; Moghissi, Stringer, & Dixon, 2008; Ramanathan, Pape, & Schwartz, 2005; Schultz et al., 2008; Védrine et al., 2003)

- **Optical fibre:**
  - ✓ Optical fibres are also called as “Optrodes” are popular due to their high sensitivity features.
  - ✓ Contain three major parts in an integral chip: a light source, a recognition receptor element mounted on to the optical fibre surface and the optical fibre which transmits the light and also works as a detector measuring the change in transmission of light signals upon biochemical interaction between receptor and analyte.

- ✓ Optical fibre based biosensors carry some desirable advantages such as high sensitivity, small size, portability, low detection limit, high specificities etc.
- ✓ Even this kind of sensors are thought to be more advantageous over electrochemical biosensors, such as they provide freedom from interference posed by electromagnetic waves, are flexible and suitable for real time detection. Thus, it is advantageous to use them for single molecule detection and remote sensing.
- ✓ Such sensors also carry some disadvantages such as interference due to ambient light source and low stability.
- ✓ Usually such sensors find their application in investigating antibodies, nucleic acids, pathogens and viruses by combining optical fibre with other optical method for desirable results.

  (Amin, Kulkarni, Kim, & Park, 2012; Atias et al., 2009; Biran et al., 2008; Brogan & Walt, 2005; Caygill et al., 2010; Cecchini, Manzano, Mandabi, Perelman, & Marks, 2012; Huang et al., 2009; H. S. Jang et al., 2009; Leung, Shankar, & Mutharasan, 2007; L. Zhang, Lou, & Tong, 2011)

**1.5.2.3. Piezoelectric biosensor:**

Piezoelectric effect is an outcome of the balanced interaction amongst mechanical and electric systems on non-centric crystals. This effect was fist observed by Curie brothers on 19$^{th}$ century (KATZIR, 2003; Perumal & Hashim, 2014). Piezoelectric biosensors use oscillating crystalline material which resonates at natural resonance frequency. This kind of biosensors are generally made up of two elements: receptor and transducer. In such sensors, the transducers comprise of piezoelectric materials such as quartz and the receptors are coated with piezoelectric materials. Here, the coated receptors vibrate/oscillate at a natural frequency controlled by external electrical stimuli producing current. When a specific analyte binds with the receptor, it causes changes in the frequency and thus causes change in current which collate to the analyte mass (KATZIR, 2003). Piezoelectric sensor further can be divided into two types: Bulk wave (BW) and Surface acoustic wave (SAW). Piezoelectric sensors are not so popular due to low sensitivity and specificity and calibration issues (Nicu et al., 2005; Perumal & Hashim, 2014). Such sensors find their applications in fabrication of microelectromechanical systems (MEMS) being portable, label free, simple and offering on-site detection. Such sensors find their applications for virus, protein,

nucleic acid and pathogen detection using immunoassays (Nicu et al., 2005; Perumal & Hashim, 2014; Serra, Gamella, Reviejo, & Pingarrón, 2008; C. Yao et al., 2008).

**1.5.2.4. Calorimetric based biosensor:**

First ever enzyme electrode introduced by Clark and his team has inspired many researchers to develop calorimetric biosensors as mostly all the chemical or biochemical reactions cause either absorption or release of heat during the process (Xie, Ramanathan, & Danielsson, 1999). This shift in heat can be measured suing thermistor or thermopile. Thermistor is usually made up of metal oxides and thermopile is made up of ceramic conductors (Cooper, 2003). This release or absorbance of heat has driven scientist to innovate calorimetric biosensor (2016; Xie et al., 1999; L. M. Ahmad, Towe, Wolf, Mertens, & Lerchner, 2010). In typical calorimetric biosensor, when analyte binds with or interacts with the receptor it consumes or produces heat and thus changes temperature (**Figure 1.9**). This shift in temperature is seen to be proportional to the concertation of substrate consumed or product released (Xie et al., 1999). Enzyme thermistor (ET) is prominently used by scientists worldwide for many applications (Yakovleva, Bhand, & Danielsson, 2013). This kind of sensors also offers possibilities for miniaturization and integration into microfluidic devices along with high sensitivity and stability (L. M. Ahmad et al., 2010; Yuyan Zhang & Tadigadapa, 2004). Majorly, this kind of sensors are best to investigate biomolecule interactions (Cooper, 2003). Calorimetric biosensors find applications in DNA hybridization detection and in food and environmental monitoring (Buurma & Haq, 2008; Kirchner et al., 2012; Maskow, Wolf, Kunze, Enders, & Harms, 2012; Paul, Hossain, Yadav, & Kumar, 2010; Watterson, Piunno, & Krull, 2002; Xi, Kumar, Dosen-Micovic, & Arya, 2010).

| Sr. No. | Receptor Transducer | Analyte | Fabrication chemistry | Linear range | Detection limit | Reference |
|---|---|---|---|---|---|---|
| **1** | Electrochemical | Organophosphorus | GCE/nanogold/mercapto methamidophos/ mercaptohexanol/AChE | 0.1-1500 ng/mL | 0.019-0.077 ng/mL | (G. Zhao et al., 2021) |
| **2** | Electrochemical | Organophosphorus | BSA/AuNPs-DTN/GCE | 0.0001-10,000 ng/mL | 0.07 and 0.8 pg/mL | (Jiansen Li et al., 2022) |
| **3** | Optical | Organophosphorus | poly(2-hydroxyethyl methacrylate) (pHEMA)/*Anabaena torulosa*/cellulose membrane | 0.01–0.75 µg/L | 0.117 µg/L | (W. Gao et al., 2011) |
| **4** | Calorimetric | Organophosphorus | MoS2-ssDNA | 2–10 µg/L | 0.018 µg/L | (Radhakrishnan & Kumar, 2022) |
| **5** | Electrochemical | SARS-CoV-2 | nanoprobes/2019-nCov-NP/BSA/MCH/Dual-aptamer/GE | 0.22 pM | 8.33 pg/mL | (J. Tian et al., 2021) |
| **6** | Electrochemical | SARS-CoV-2 | S-protein/Aptamer/AuNPs-modified SPCE | 10 pM – 25 nM | 1.30 pM (66 pg/mL) | (Abrego-Martinez et al., 2022) |
| **7** | Optical | SARS-CoV-2 | gold nanoislands (AuNIs)/DNA | - | 0.22 pM | (*Qiu 2020*) |

**Table 1.4. Uses of different receptors for detection of various analytes**

| Sr. No | Receptor Transducer | Analyte | Fabrication chemistry | Linear range | Detection limit | Reference |
|---|---|---|---|---|---|---|
| **8** | Optical | SARS-CoV-2 | gold nanoparticles/anti-N protein mAb | - | $10^{-18}$ M | (Murugan et al., 2020) |
| **9** | Electrochemical | Triglycerides | Lipase NPs/GKNPs/GPONPs/PG | 0.1–45 mM | 0.1 nM | (Narwal & Pundir, 2017) |
| **10** | Electrochemical | Triglycerides | LIPNPs/GKNPs/GPONPs/ Au | 0.11–5.65 mM | 0.001mM | (Pundir & Aggarwal, 2017) |
| **11** | Optical | Triglycerides | Pectin-CaCl2-$Cl_2$/lipase | 100-400 mg/dL | 15 mg/dL | (Hasanah, Md Sani, Heng, Idroes, & Safitri, 2019) |
| **12** | Electrochemical | *Staphylococcus aureus* | SEB (Staphylococcal enterotoxin B)/MCH/aptamer/Gold electrode) | 0.5- 500 ng/mL | 0.17 ng/mL | (Xiong et al., 2018) |
| **13** | Calorimetric | *Staphylococcus aureus* | Electrode/HCR nanowires/3WJ-NEASA | - | 1.2 × $10^1$ CFU/mL | (Peng et al., 2021) |

**Continue…** **Table 1.4. Uses of different receptors for detection of various analytes**

## 1.6. Types of nanomaterials:

Nanotechnology is the combination of science and engineering dealing with synthesis, characterization and application of materials with their dimensions in nanometer scale. Although the term nanoparticles came into use only in the recent times, we find its roots in ancient science in fourth and ninth century. Artisans in Rome used nanoparticles to decorate cups and pots. The first scientific description of nanomaterials and their optical properties was given by Michael Faraday in 1857 (Sutarlie, Ow, & Su, 2017). Nanomaterials with their diameters less than 100 nm have gained much more attention in various fields like electronics, biomedical engineering, diagnostics, industrial and environmental monitoring mostly due to their favorable properties. In recent years, nanomaterials are found to be more suitable for analytical and sensing applications due to their special properties such as high surface area and mechanical strength, ease of functionalization and labeling, surface plasma resonance and high electrical conductance. Biosensors fabricated in combination with nanoparticles are of great importance for onsite detection of microorganisms, mycotoxins, antigens, viruses, DNA, pesticides, metabolites, biomarkers, proteins and other molecules (Sutarlie et al., 2017). There is also a need for comparing the performance of various nanoparticles and sensor fabrication chemistry with respect to a given analyte for improving the design of electrochemical biosensors (**Table 1.4**). The vast diversity in nanomaterials, molecular recognition elements and analytes raised questions like: (i) is there any particular nanoparticle or sensor fabrication chemistry specific for each target analyte, and (ii) how to fabricate the best suitable nanosensor for onsite application. Therefore, this review endeavors to give insights on properties of various nanoparticles. Numerous varieties of nanomaterials with their unique intrinsic properties are used widely as signal transducers in different biosensors as capture probes or are functionalized with various recognizing receptors through different chemical reactions. This section highlights the nine different types of nanoparticles as classified by Sutarlie and coworkers (Sutarlie et al., 2017). They are namely magnetic nanoparticles, carbon nanotubes, metal nanoparticles, graphene, carbon nanodots, quantum dots, graphene quantum dots, silica nanoparticles and upconversion nanoparticles (UCNPs) (**Figure 1.10**).

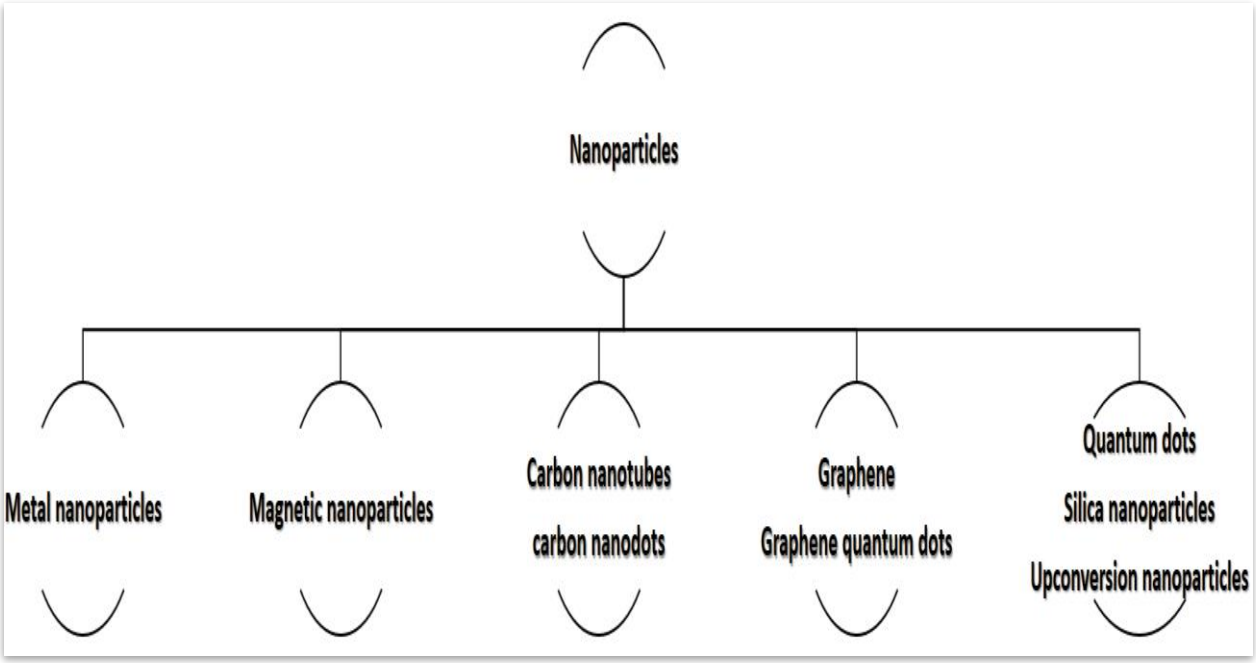


**Figure 1.10. Various types nanoparticles**

### 1.6.1. Metal nanoparticles:

Metal nanoparticles exist in a variety of sizes and shapes, which influence some of the optical properties such as localized surface plasmon resonance (LSPR). Further, changes in dielectric constant and particle aggregation lead to color change in metal nanoparticles (Hutter & Fendler, 2004). Metal nanoparticle can either enhance or quench emission of the fluorophores in the vicinity and with overlapping excitation or emission spectra (Lakowicz, 2005). Silver and gold nanoparticles are commonly used in biosensor fabrication. Gold nanoparticles enhance as well as quench emission of fluorophores depending on whether their diameter is greater or less than 80nm respectively, through the phenomena of FRET (Forster resonance energy transfer) and NSET (Nanomaterial surface enhancement transfer). Metal nanoparticles display SERS (Surface enhanced Raman scattering), a key detection technique (Qian & Nie, 2008) and high electric conductance. Thus, metal nanoparticles are used in Raman spectroscopic, electrochemical, colorimetric and fluorimetric applications (Sutarlie et al., 2017).

### 1.6.2. Magnetic nanoparticles:

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 (Y. Chen et al., 2015; Lu, Salabas, & Schüth, 2007). Smaller MNPs (with their diameter <20 nm) exhibit an inherent property of

superparamagnetism, that is they show magnetic properties only under the influence of external magnetic field (Y. Chen et al., 2015). The superparamagnetic behavior does not allow aggregate formation by magnetic nanoparticles, which comes handy in applications involving capture and separation of target molecules. Hence, MNPs can be used in the light independent technique of magnetic relaxometry for the detection of analyte molecules, especially when the latter are colored or fluorescent (Sutarlie et al., 2017).

**1.6.3. Carbon nanotubes (CNTs) and Carbon nanodots (CDs):**

Carbon nanotubes find their applications in the field of biosensors and mostly in electrochemical biosensors due to their properties such as higher electrical conductivity, mechanical strength, increased surface area for labelling, ease of labelling and inherent peroxidase like behavior. Increased conductivity of CNTs is due to free movement of electrons in $sp^2$-hybridization C-atoms, arranged in a basic lattice structure of hexagon. There are two types of CNTs: SWCNTs and MWCNTs. CNTs apart from conductivity also show photoluminescence and act as acceptors in FRET experiments (Gupta, Murthy, & Prabha, 2018; Pérez-López & Merkoçi, 2012; W. Yang et al., 2010).

Carbon dots (CDs) generally have their diameter around 10 nm and are described as quasi-spherical shaped nanomaterials. They are either crystalline or amorphous in nature (Baker & Baker, 2010; Himaja, Karthik, & Singh, 2015; H. Li, Kang, Liu, & Lee, 2012; Miao et al., 2015). CDs are preferred as fluorescent probes and FRET donors. CDs are less toxic and biodegradable as they are synthesized from renewable resources and their surfaces are amenable to modification and functionalization (Himaja et al., 2015; Kotchey, Zhao, Kagan, & Star, 2013).

**1.6.4. Graphene and Graphene quantum dots (GQDs):**

Graphene is composed of 2D-sheets of carbon. Graphene possesses high conductivity same as CNTs despite the striking difference between the structures. Graphene can act as fluorescence quencher, peroxidase catalyst and as an enhancer of Raman signals and photoluminescence. Graphene is used in the construction of portable nano-electrodes. Graphene is oxidized to graphene oxide for better functionalization (Pérez-López & Merkoçi, 2012; W. Yang et al., 2010).

Graphene's lattice structure with some layers make Graphene quantum dots and they range from 3 to 20 nm of diameters (Luo et al., 2015; Shen, Zhu, Yang, & Li, 2012; Wu et al., 2014; X. T. Zheng, Ananthanarayanan, Luo, & Chen, 2015). GQD shows tunable fluorescence which can be adjusted according to excitation wavelength and depend on chemical functional groups, size

and surface functionalization. Being photo stable and less toxic, GQDs are strongly recommend as good fluorescent probes and FRET donors (Baker & Baker, 2010; Luo et al., 2015; Shen et al., 2012).

**1.6.5. Quantum dots, Silica nanoparticles and Upconversion nanoparticles:**

Quantum dots (QDs) having a core-shell structures are nanoparticles made up of semiconductor materials belonging to groups 12-16, 13-15 or 14-16 of periodic table (Vasudevan, Gaddam, Trinchi, & Cole, 2015). QDs are good FRET donors and this property is tunable according to the semiconductor's band gap. This band gaps vary according to composition and size of the QDs (Algar, Tavares, & Krull, 2010; Esteve-Turrillas & Abad-Fuentes, 2013; J. Y. Kim, Voznyy, Zhitomirsky, & Sargent, 2013). Distinct intrinsic properties of QDs include: narrow emission band, more photostability, more quantum yield compared to organic fluorophores (Medintz, Uyeda, Goldman, & Mattoussi, 2005).

Lanthanide ions (Er3+, Tm3+, Ho3+, Yb3+) are doped into nanocrystals such as silica of the size of 100 nm to make luminescent upconversion nanoparticles or UCNPs (G. Chen, Qiu, Prasad, & Chen, 2014; Jiao Chen & Zhao, 2012; Haase & Schäfer, 2011). In upconversion process, infrared light used for excitation leads to emission of NIR or visible light with higher energy and shorter wavelength. Interestingly, emission wavelength of UNCPs can be varied and fine-tuned by changing the combinations of dopant and the host crystal material, and by varying the size and morphology of the nanoparticles (Sun, Wang, & Yan, 2014; Zhou, Liu, Feng, Sun, & Li, 2015). Infrared light due to its longer wavelength compared to radiation of UV-visible range has greater penetrability and does not cause photo damage. Since UNCPs can be excited by infrared light, they can be used safely for in vivo applications as biosensors, fluorescence probes (F. Wang, Banerjee, Liu, Chen, & Liu, 2010; Zhou et al., 2015).

Silica nanoparticles (SiNPs) having higher porosity compared to any other nanomaterials, can encapsulate many fluorophores, giving rise to higher fluorescence intensities. The fluorophores are more stable to light and other environmental factors. Further, silica matrix prevents fluorescence quenching and fluorophore aggregation (Burns, Ow, & Wiesner, 2006; Burns, Sengupta, Zedayko, Baird, & Wiesner, 2006).

| Sr. No. | Analyte | Type of analyte | Fabrication chemistry | Detection range | Reference |
|---|---|---|---|---|---|
| **1** | Urea | Metabolite | CdS QDs–MIPs/Au | $1.0\times10^{-12}$M | (Lian, Liu, Chen, & Sun, 2012) |
| **2** | Creatinine | Metabolite | Cu/SPCE | $0.0746\times10^{-6}$ M | (Raveendran et al., 2017) |
| **3** | Paraoxon | Pesticide | Ag-Ag NPs/AChE | 2 ppb | (Q. Zheng et al., 2016) |
| **4** | $Zn^{+2}$, $Cu^{+2}$ and $Fe^{+2}$ | Metal ions | EtC4/APTMS/ITO | 9.88 pg/L ($Zn^{+2}$), 8.33 µg/L ($Cu^{+2}$), 1.15 µg/L ($Fe^{+2}$) | (Ruslan et al., 2017) |
| **5** | *Staphylococcus aureus* | Microorganism | Ab-*S. aureus*-MPA/Au | 10 CFU/ml | (Braiek et al., 2012) |
| **6** | HER2 | Disease Biomarker | NFG/AgNPs/PANI | 2 cells/ml | (Salahandish et al., 2018) |

**Table 1.5. Uses of various nanoparticles for detection of various analytes**

## 1.7. Deposition techniques for nanomaterials:

Nanomaterial based biosensors should be coated properly on its surface with nanomaterials for its better conductance. Coating or deposition of nanomaterials is very critical step for improved biosensor performance. Different deposition methods are developed to successfully prepare a nanomaterial based matrix. The deposition technique should be chosen such that it makes sure appropriate contact between the upcoming nanomaterial and electrode matrix is present (R. Ahmad et al., 2018). The main motive of deposing nanomaterials onto supportive surface matrix is to provide high surface area, increase the analytical performance of a biosensor and to provide a site where receptor of choice can easily be immobilized. Thus, appropriate deposition of nanomaterials has an important role in determining in deciding how much stable, sensitive, reproducible and selective the sensor would be. This section briefly gives an insight about deposition methods of nanomaterials (R. Ahmad et al., 2018). **Figure 1.11** gives schematic representation of various deposition methods onto electrode surface (R. Ahmad et al., 2018).

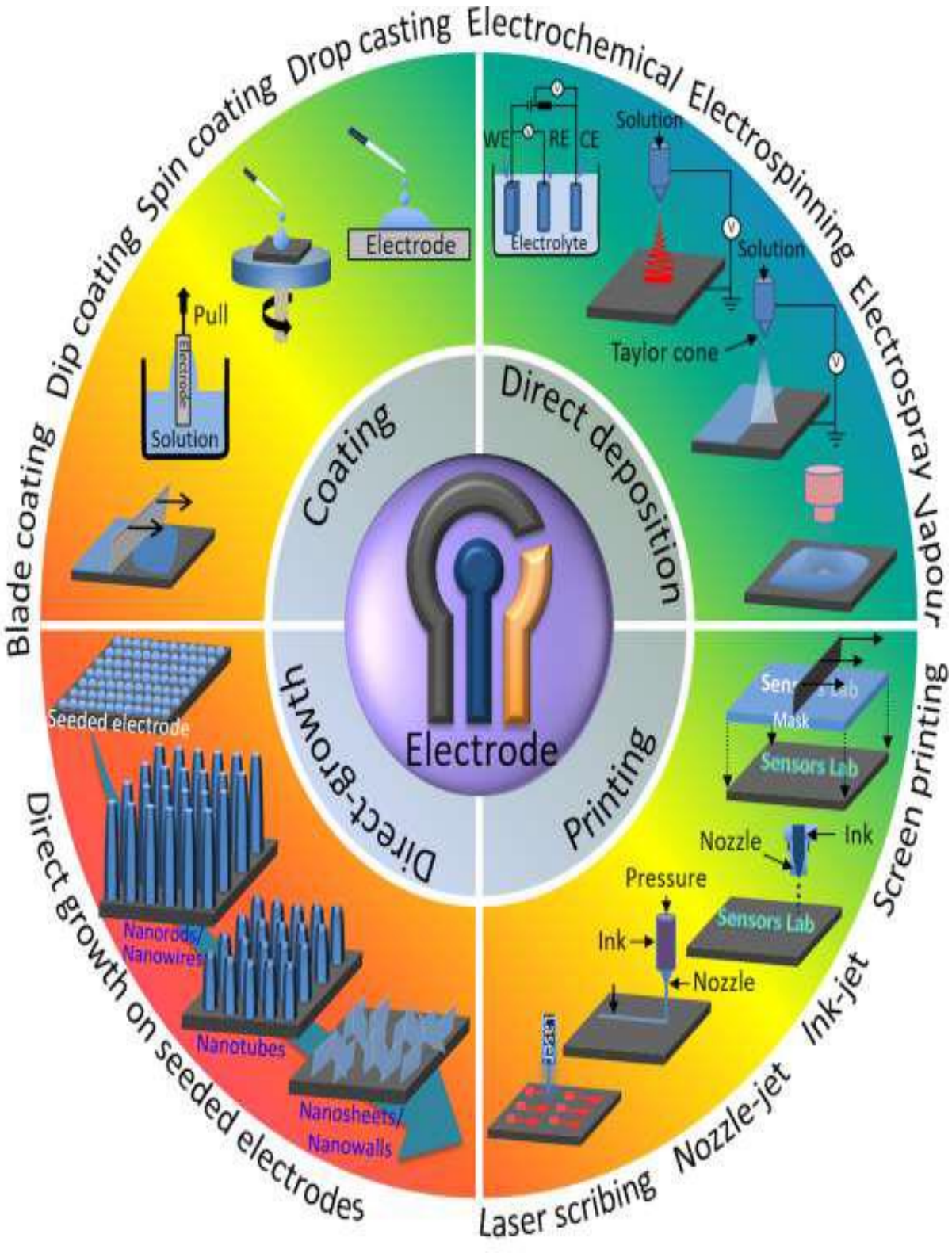


**Figure 1.11. Different deposition techniques for nanomaterials onto sensing support material for biosensor fabrication** (R. Ahmad et al., 2018)

### 1.7.1. Coating based deposition methods:

There are mainly four types of nanomaterial based coating methods to modify the electrode surface: drop casting, spin coating, blade coating and dip coating. These methods do not require any high throughput instruments thus decrease the manufacturing cost of sensor fabrication. The main condition for coating based technique is that it can be only performed with soluble or solution processible materials (R. Ahmad et al., 2018). Four types of coating methods are listed below and explained in Figure **1.11** (R. Ahmad et al., 2018).

- **Drop-casting method:**
  - ✓ Generally called as "manual deposition" technique.
  - ✓ Easy to use, simple and provide high surface area due to porosity of the membrane.
  - ✓ Lower fabrication cost.
  - ✓ Uses membranes, polymers or binders with nanomaterials in an appropriate ratio to immobilize firmly on supporting matrix. Usually, for this purpose sonicator or mortar pastel is used.
  - ✓ Since, to control pore size of the membrane, uniformity and thickness is difficult, only used to demonstrate the working principle of biosensor chemistry.
  - ✓ Parameters to be taken care for desired results in this kind of coating are the ratio of nanomaterials and polymer/binder, evaporating temperature, mixing technique, amount of suspension/slurry.
    (R. Ahmad et al., 2018; Bitsch et al., 2014; Chan, Eng, Pumera, & Webster, 2015; Ibrahim et al., 2017)
- **Dip coating method:**
  - ✓ The name itself justifies that it's a simple process approaching use of sol-gel/nanomaterial mixture to coat the sensing surface.
  - ✓ It ensures uniformity on the surface and large surface area convergence.
  - ✓ Parameters to be taken care while performing the dip coating to get best performance of the sensor are: binder-nanomaterial concentration, time of dipping, withdrawal speed, types of forces at slurry-electrode surfaces.
  - ✓ Creates thin uniform layer of coating on the surface.

- ✓ Not suitable for great quantity of enzyme loading.
- ✓ Increases the wastage of coating material by coating onto undesirable surface of the supporting material and is time consuming.
- ✓ Homogenous dispersion of nanomaterials and wettability of supporting matrix are crucial parameters for efficient dip coating.
- ✓ Most of the nanomaterials could not be dip coated so the technique is least used for biosensor fabrication.

  (R. Ahmad et al., 2018; Mikani, Rahmanian, Karimnia, & Sadeghi, 2017; Rahman, Musarraf Hussain, & Asiri, 2017; Yin et al., 2017)

- **Spin coating method:**
  - ✓ In spin coating, nano-polymer mixture is dropped and then is subsequently spread very rapidly by spinning the electrode surface. The remaining extra mixture is spun out of the electrode surface by centrifugation.
  - ✓ Here, thinness of the film is decided by centrifugal speed, concentration of nanoparticle and polymer in the mixture and viscosity of the mixture.
  - ✓ It is superior than drop/dip-coating methods to coat the electrode as its controlled process and provides uniform thickness all over the electrode surface.
  - ✓ It is useful to coat only small planner electrode surface area and it also causes waste of coating material during the process.
  - ✓ After coating, the film has to solidify (Crystalize) and remain annealed onto the electrode surface. During this solidification process, the solvent is evaporated and additives are removed. For this purpose, usually thermal and vapor based techniques are used. These techniques make the coated film un-annealed and less popular for electrode coating.
  - ✓ Mostly used for nano-polymer and meta oxide film coatings.

    (R. Ahmad et al., 2018)

- **Blade coating method:**
  - ✓ The name “Blade coating” itself says that blade is used to coat the coating slurry on to large electrode surface area.
  - ✓ The technique is fast, cost-effective, easy to operate even at low temperatures with controlled thickness and uniformity.

- ✓ Thickness of the coated film by blade coating technique is greatly influenced by wettability of the coating surface, surface tensions between coating slurry and electrode surface, viscosity of the coating slurry and speed of coating.
- ✓ The technique is prominently used in manufacturing of batteries, fuel cells, solar cells, printing, textiles and ceramic industry.
- ✓ The technique is least applicable for biosensor fabrications as electrodes offer very smaller working area for coating nanomaterials which is difficult to perform by blade coating.

(R. Ahmad et al., 2018; Luo et al., 2015; H. Yang & Jiang, 2010)

### 1.7.2. Direct deposition methods:

Direct deposition techniques have been used frequently to fabricate biosensors (Benzigar et al., 2018; Panda, Katz, & Tovar, 2018). Five different types of direct deposition techniques can be seen to be used to coat the electrodes are: vapor deposition, electrospray, electrochemical, sputtering and electrospinning (**Figure 1.11**). These kind of techniques offer less steps to be carried out for deposition compared to coating based techniques. All the direct deposition techniques are briefly described with their pros and cons below (R. Ahmad et al., 2018).

- **Electrodeposition:**
  - ✓ In electrodeposition technique, three electrode system is dipped in the solution to be coated and required current is supplied to the configured system. Thus, in this process metal ions acquires oxidized state if present in the solution while pre-polymer mixture gets polymerized onto the surface of the working electrode.
  - ✓ Various modes of electrodeposition using electrochemical techniques are: potentiometry, cyclic voltammetry, chronoamperometry, pulsed-potentiometry and square wave-potentiometry.
  - ✓ Uniformity and thinness of the film can be controlled using electrochemical and other parameters such as pH, temperature, potential and current.
  - ✓ The technique is cost-effective, easy to carry out and fast.
  - ✓ The technique is famous for metal, metal oxides and polymer coating on working electrode of biosensor.
  - ✓ Major limitations of the technique include need of conductive electrode surface and skilled personnel for technical set up and handling.

(R. Ahmad et al., 2018; Suginta, Khunkaewla, & Schulte, 2013; Wei, Wu, Yang, & Liu, 2016)

- **Electrospinning deposition:**
  - ✓ Electrospinning is highly used technique for coating nanomaterials and nano-polymer mixtures on working electrode surfaces. The technique is simple and widely accepted.
  - ✓ Electrospinning is one step procedure, easy to implement, fast. The technique also offers application specific alterations such as increased surface active area and porosity, altered chemical and morphological features.
  - ✓ The drawbacks of the technique include usage of lethal solvents, lowered catalysis in polymer based electrospinning, need of calcination and difficulty to work with very small nanoparticles (<10 nm).
  - ✓ The technique is majorly used for sensing devices, tissue engineering, environmental monitoring, purification of air and water, textiles, encapsulating drugs, drug delivery and surface coating.

    (R. Ahmad et al., 2018; J. I. Kim, Hwang, Aguilar, Park, & Kim, 2016; Shrestha, Ahmad, Shrestha, Park, & Kim, 2017; J. Xue, Xie, Liu, & Xia, 2017)
- **Electrospray deposition:**
  - ✓ Electrospray is similar to electrospinning deposition where coating of metallic or nonmetallic nanostructures are performed over surfaces by nozzle spray. Thus, formed nano/polymer films are embedded via electrostatic forces on the surface.
  - ✓ During deposition of nano-films, speed of deposition, size and charge of coating slurry onto the surface can be controlled electrically.
  - ✓ The technique avoids the use of vacuum and operates best at room temperature.
  - ✓ Drawbacks associated with the technique involve need of high surface area, ink instability and optimization of other parameters such as distance between the spraying object and substrate surface, pressure, viscosity of the mixture, type of nozzle tip and solvent etc.
  - ✓ Use of this technique is highly recommended for commercial and scientific purposes such as nanotechnology, micromachining, material sciences and microelectronics.

(R. Ahmad et al., 2018; Chen, Mao, Lu, & Yu, 2010; D. Liu, Rahman, Ge, Kim, & Lee, 2017; Ruecha, Rangkupan, Rodthongkum, & Chailapakul, 2014; Wu et al., 2014)

- **Vapor deposition:**
  - ✓ The technique offers two types of vapor deposition on substrate surface: Physical vapor deposition (PVD) and Chemical vapor deposition (CVD).
  - ✓ The technique provides very precise thin films with desired morphology by using atomic layer deposition and sputter tactics. Highly replicable coatings of the electrodes offered make the technique useful for commercial biosensor market.
  - ✓ 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 of functionalization of the surface for the attachment of receptors and enzyme leaching.

    (R. Ahmad et al., 2018; Dominik et al., 2017; Muñoz-Rojas & Macmanus-Driscoll, 2014; Srivastava et al., 2012; Wingqvist, 2010)

### 1.7.3. Printing based deposition methods:

Printing based deposition methods can be used to coat both flexible and non-flexible surfaces (R. Ahmad et al., 2018). 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. Printing based methods are of four types: screen printing, ink-jet printing, nozzle-jet printing and laser-scribing process of deposition (**Figure 1.11**). These methods find their applications in sensing devices, batteries, electronics and photonics (R. Ahmad et al., 2018; G. Hu et al., 2018; Prevatte et al., 2016; D. 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 (R. Ahmad et al., 2018).

- **Screen printing:**
  - ✓ In this method, ink is transferred in the designed pattern on to the substrate surface (electrode) by a stencil mesh. This mesh permeate ink only on desired area to coat

the electrode. This stencil mesh is either made up of metal wire mesh, fabric or other synthetic wires.

- ✓ The printing ink used is generally made up of metal/metal oxide nanoparticles combined with polymers increasing the surface area. Thus, printing based deposition technique gifts high performance devices.
- ✓ Screen printing is famous since a very long time for its roll-to-roll printing deposition with very high efficiency. Thus, it provides deposition on large surface area in cost-effective, simple and speedy manner.
- ✓ The technique is used to manufacture solar cells, electronic circuits, transistors and electrodes. Screen printed electrodes in bioelectronics are used mainly in food and medical industry.
- ✓ The technique offers direct deposition of a few nanomaterials only. Mostly the nanomaterials are supposed to be dispersed into solvents or polymers. Some efforts have been made to coat the enzyme by mixing it directly with nanomaterials without any polymer/binder. But, this has resulted in aggregation of enzyme and nanoparticles offering uneven coating of the surfaces.
- ✓ Even in some ink preparations, additives or stabilizer are added apart from polymer/binder to make stable ink as nanomaterials tend to aggregate.
- ✓ The method proposes printing of films with comparatively high thickness which limits its use in soft electronics.
- ✓ Screen printing also suffers from wastage of ink and contamination issues.
  (R. Ahmad et al., 2018; Alonso-Lomillo, Domínguez-Renedo, & Arcos-Martínez, 2010; Chu, Peng, & Jin, 2017; Cinti & Arduini, 2017; Hughes et al., 2016; Jaiswal & Tiwari, 2017; Kang, Lee, & Cho, 2013; Secor & Hersam, 2015; Suikkola et al., 2016; Taleat, Khoshroo, & Mazloum-Ardakani, 2014; Tong, Sun, & Yang, 2018; W. Xu, Li, Xu, & Wang, 2018)

- **Inkjet printing:**
  - ✓ In inkjet printing, small nozzle with jetting mode is used to print the surface of the surface/electrode with controlled speed and drop size (up to 5-10 nm).

- ✓ The technique operates totally on non-contact mode avoiding any kind of cross contaminations even on pre-treated surface, reduces the wastage of coating ink and produces highly conductive surfaces.
- ✓ Generally, metals and their oxides, carbon nanomaterials and conductive polymer mixtures are extensively used to coat the surfaces using inkjet method.
- ✓ In most of the cases, surfactants, additives, solvents, polymers, membranes are preferred to control surface tensions, wettability, rheology, viscosity and stability of the ink.
- ✓ The technique is used to mass produce batteries, transistors, sensors etc.
- ✓ The method offers high reproducibility with rapid, easy and cost-effective means.
- ✓ Timely maintenance and cleaning is required to make sure that nozzle is not choked.
- ✓ The technique is not compatible with dry ink usages.

  (Abadi, Mottaghitalab, Bidoki, & Benvidi, 2014; R. Ahmad, Vaseem, Tripathy, & Hahn, 2013; R. Ahmad et al., 2018; Gabardo & Soleymani, 2016; M. Gao, Li, & Song, 2017; Karim et al., 2017; Khan, Maddaus, & Song, 2018; Jia Li, Rossignol, & Macdonald, 2015; Majee, Liu, Wu, Zhang, & Zhang, 2017; Mattana & Briand, 2016; Moya, Gabriel, Villa, & Javier del Campo, 2017; Raut & Al-Shamery, 2018; Salim & Lim, 2017)

- **Nozzle-jet printing:**
  - ✓ Nozzle jet printing is recommended with high viscosity inks where pressure is applied throughout the large sized nozzle to print the substrate surface. 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.

    (R. Ahmad et al., 2018; Bhat, Ahmad, Yoo, & Hahn, 2017; Bhat et al., 2019; T. S. Jang et al., 2018)
- **Laser scribing:**

- ✓ The technique avoids masking the surface and uses laser to scribe graphite oxide. Here, graphite oxide gets reduced with laser and arranges itself on the surface with reduced graphene oxide (rGO).
- ✓ The technique is easy to perform, rapid, cost-effective and offers large surface area, enhanced conductivity and porosity.
- ✓ The technique is very useful to fabricate lab-on-disc which can be filled in sensors, capacitors or multifunctional devices.
- ✓ The technique is highly used to fabricate paper-based devices as it offers flexibility and disposability in just 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.

  (R. Ahmad et al., 2018; Das et al., 2016; El-Kady & Kaner, 2013; W. Gao et al., 2011; Hou, Zhao, Bi, & Lu, 2017; C. Hu et al., 2015; W. Li et al., 2017; H. Liu, Li, Kaner, Chen, & Pei, 2018; Nayak, Kurra, Xia, & Alshareef, 2016; H. Tian et al., 2014; Weng et al., 2012; G. Xu, Aydemir, Kilmartin, & Travas-Sejdic, 2017; Yi et al., 2018)

### 1.7.4. Direct growth deposition methods:

In direct growth deposition technique, nanomaterials ranging from 1D to 3D are directly grown and tailored on to the surface of substrate electrode in an organized and precise means. Types of various direct growth methods such as thermal and hydrothermal deposition, anodization, template and chemical deposition methods are used to grow nanorods, nanowires, nanoplates, nanodendrites, nanoneedles and nanoflowers on electrodes areas (R. Ahmad et al., 2018). Here, the main advantage of using this method includes controlled coating over the surface area with desired morphology. The coating method is successful in coating large surface area providing direct contact between nanomaterials and electrode without any binder/polymer. Thus, wide-open

catalytic sites offer high electrical conductance and electron transfer process for enzymatic reactions to take place with improved stability. Thus, this method makes sensing applications very feasible (R. Ahmad et al., 2018). The method is very simple and can control the morphology of the coated electrode by controlling various crucial parameters such as pH, type of template, pressure, concentration of solution, time of growth and temperature set up, type and concentrations of additives etc. (R. Ahmad et al., 2018). This method is more popular for bulk production. Keeping in mind that nanomaterials provide platform for immobilization of bioreceptors, generate and amplify signals, the deposition techniques are wisely chosen as they greatly influence the performance of the sensor (R. Ahmad et al., 2018).

**1.8. Aims and Objectives:**

The need for research to develop and explore biosensor field comes from the shortcomings suffered by the conventional techniques. The major goal of developing new prototypes of biosensors can be expressed as enhancing the detection capacity, sensitivity and reducing the detection time and cost. Biosensors find their applications from medical diagnostics to environmental and industrial processes, drug and toxin detection, water and food analysis and defence. However, sensors used nowadays also have some limitations in terms of specificity, selectivity, detection limit, range of detection, artefacts, high cost and invasive nature. Idea of the study is to develop prototypes of sensors which can overcome the drawbacks offered by both conventional methods and available sensors. The study focuses on advancement, fabrication, characterization and standardization of biosensors. Fabrication or development of biosensor involves a challenge to decide target analyte to be detected and to investigate how target would interact with its biological molecules (receptor element). Once this has been established, the following steps need to be carried out very carefully for fabricating a successful biosensor (Norsuzila Ya'acob et al., 1989):

- **Selection of biological receptor:**

  Type of biological receptor chosen contributes mainly to the selectivity and sensitivity of a biosensor. Thus, stable and high affinity receptor for an analyte is strongly recommended. Before choosing any receptor for a particular analyte, its advantages and disadvantages should be known in detail (Norsuzila Ya'acob et al., 1989; Grieshaber, MacKenzie, Vörös, & Reimhult, 2008; Chaubey & Malhotra, 2002; Saha, Agasti, Kim, Li, & Rotello, 2012).

- **Selection of immobilization method:**

  Any biological receptor chosen for the biosensor fabrication requires its attachment on to the surface of a transducer to efficiently recognize the analyte. The process of attaching receptor is called as immobilization. Various means of techniques are used for immobilizing a receptor such as entrapment, adsorption, covalent attachment, micro encapsulation and cross linking (Morales & Halpern, 2018; Korotkaya, 2014; Norsuzila Ya'acob et al., 1989).

- **Selection of transducer:**

  Transducer has a major role in determining the sensitivity level of a biosensor. Use of right transducer greatly enhances sensitivity of a sensor while inappropriate transducer may compromise the sensitivity of a sensor (Sassolas, Blum, & Leca-Bouvier, 2012; Morales & Halpern, 2018; Norsuzila Ya'acob et al., 1989).

Our lab has previously optimized glucose biosensor for its optimum glucose oxidase immobilization on to Polyvinyl alcohol-Multiwalled carbon nanotube membrane using drop-casting method (PVA-MWCNTs membrane). The fabricated glucose biosensor was suggested to be used for its industrial application (Gupta, Prabha, & Murthy, 2016). This study focuses on the development and standardization of biosensors to detect organophosphorus pesticide, triglycerides, SARS-CoV-2 and *Staphylococcus aureus*. The study has used optical and electrochemical methods to develop these biosensors using enzymes (enzyme-assay), aptamers (nucleic acid assay) and polymers (molecular imprinting) as receptors. For electrochemical biosensors, nanoparticles were used to immobilize receptors by drop-casting method. Apart from selecting receptor, transducer and immobilization method, certain parameters should meet for its desired performance while designing a biosensor (Norsuzila Ya'acob et al., 1989).

The study has raised several key questions while designing biosensors for its practical use:

- Is the biosensor specific?
- 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?

- What is the linear range of biosensor?

  (Norsuzila Ya'acob et al., 1989)

**Specific objectives of this thesis are Designing and characterization of the following biosensors:**

1. **Acetylcholine esterase enzyme doped multiwalled carbon nanotubes for the detection of organophosphorus pesticide using cyclic voltammetry**
2. **Design and Characterization of conductive Nano-PEI-lipase film based biosensor for efficient electrochemical detection of triglycerides**
3. **Whole bacterial cell macromolecular imprinting in polyacrylamide gel slab: Towards optical detection and removal of target bacteria**
4. **Electrochemical biosensor for cyclic voltammetric detection of Nsp3 protein gene of SARS-CoV-2 using DNA-Chip**

The above objectives are separately described in detail in four different chapters of this Ph.D. thesis.

**Chapter 2** focuses on fabrication, standardization and characterization of organophosphorus pesticide biosensor using the electrochemical technique cyclic voltammetry. The study focuses on use of carbon nanotubes (MWCNTs) to dope acetylcholine esterase enzyme (AChE) directly without using any binder, polymer or cross-linker. Glassy Carbon Electrode was layered with carboxylic acid modified multiwalled carbon nanotubes (MWCNTs) followed by doping with AChE. Carboxylic groups of MWCNTs were found to get interacted with amine group of AChE to form amide bond in FT-IR analysis. The biosensor performance depends on activity and immobilization of the AChE on to the electrode. The biosensor principally works on inhibition of the active sites of AChE. The enzyme AChE when exposed to the substrate acetylthiocholine iodide (ATChI), forms the product thiocholine which further gets oxidized. This oxidation of thiocholine releases electrons which can be measured in the form of current. But, when the biosensor is first exposed to organophosphorus pesticide, it blocks the enzyme activity and thus decreases the current. This decrease of current magnitude can be calculated as inhibition percentage. Thus, inhibition can be seen in proportion to the concentration of the pesticide. The research focuses here on optimization and standardization of organophosphorus pesticide biosensor. The chapter focuses on optimization and standardization of the concentration of

MWCNTs, AChE and ATChI, response time, regeneration procedure, stability and concentration of organophosphorus pesticide. 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 triglyceride detection using cyclic voltammetry. Enzyme assay method using lipase as an enzyme was used to coat the electrode. Glassy carbon electrode was coated by drop casting rGO. It was then followed by MWCNTs-COOH cast-off to the surface. Then, another layer of polyethyleneimine-$TiO_2$ nanoparticles was dropped off onto it. Lipase was then layered onto the surface, following it glutaraldehyde was added for cross-linking. Lipase thus bound covalently hydrolyses triglycerides into glycerol, fatty acids and protons. This release of protons causes change in pH of the solution. This change is directly correlated to the concentration of triglycerides. Cyclic voltammetry was used to measure the current changes. The fabricated biosensor showed successful detection of triglycerides with acceptable linear range. Here, the focus of the research is to propose new fabrication chemistry for biosensors to detect triglyceride and standardize the biosensor for its practical uses which can be adapted for miniaturization. The research showed sufficient enzyme immobilization and was tested for real sample testing. The fabrication chemistry can be opted to immobilize any kind of enzyme.

**Chapter 4** focuses on possibility of development of optical biosensor for detection of *Staphylococcus aureus* using polyacrylamide gel and molecular imprinting. *Staphylococcus aureus* was used as the template to imprint polyacrylamide gel. *Staphylococcus aureus* bacteria was first entrapped in polyacrylamide gel and then was removed by lysing using SDS and NaOH. Entrapment and removal of the bacteria was confirmed by Gram's staining. Cavities formed after removal of the bacteria was assumed to be complementary to the removed *Staphylococcus aureus* bacteria. The bacteria in suspension were then allowed to get re-adsorbed on the imprinted cavities. Thus, the imprinted acrylamide gel was assumed to re-adsorb *Staphylococcus aureus* bacteria. Removal of the bacteria from its suspension when exposed to the imprinted polyacrylamide gel, was measured using change in optical density of the suspension at 15 min. interval time at 600 nm in spectrophotometer. Optimized incubation time of the imprinted gel to remove *Staphylococcus aureus* and specificity of the imprinted cavities towards the bacteria was checked in this chapter. So far, lots of papers were published which are focused on creation of imprinted cavities in

polyacrylamide gel for *Staphylococcus aureus*. But, to our knowledge molecularly imprinted biosensors are still not launched in the market due to their lack of specificity issues. The study here aims to develop molecularly imprinted polyacrylamide gel as a proof of concept for the detection of *Staphylococcus aureus*.

**Chapter 5** is focused on the development of electrochemical biosensor for detection of Nsp3 protein gene of SARS-CoV-2 using novel aptamer sequences. Focus of the research here is to develop rapid, cost-effective and specific electrochemical kit for Covid-19 detection. A label free electrochemical oligonucleotide-chip for detecting Nsp3 gene was fabricated using novel ssDNA aptamer designed in the laboratory. The aptamer was designed using NCBI/Primer-BLAST as a tool. Designed ssDNA aptamer was functionalized with carboxylic group of MWCNTs via ester bond. Polyvinyl alcohol (PVA) along with glutaraldehyde was used as a matrix to embed and hold functionalized MWCNTs on screen printed carbon electrode chip (SPE). The fabricated chip was allowed to hybridize with the complementary ssDNA chip. Methylene blue here was used as a redox marker which can intercalate between hybridized dsDNA on to the chip. Thus, when hybridization with complementary ssDNA occurs on the electrode chip, current increases. This increase in the magnitude of current is seen as directly proportional to the concentration of complementary strand. Detection limit, linear range and specificity were optimized. The study finds its scope to eliminate cDNA amplification step of RT-PCR. Thus, the strategy has the potential to replace qPCR.