Chapter 1

Introduction

This chapter covers the fundamentals of conducting polymers, including their historical development and literature survey on the recent progress. It also includes the scope of two-dimensional materials and metal nanoparticles. Moreover, it provides an overview of the basic principles of biosensors and their classifications. Additionally, the chapter explores the mechanisms behind various biosensors that utilize different bioreceptors and transducers. The importance of glucose and AF-B_1 detection have also been included in this chapter.

1.1. Materials

Materials in individual or composite form, remain the foundation of modern technology. They play a crucial role in almost all fields of science and engineering research [1]. They are broadly categorized into metals, polymers, ceramics, and composites, each with unique properties tailored for specific applications. Materials play a significant role in electrochemistry, influencing the stability, efficiency, and overall performance of electrochemical systems [2]. In applications such as batteries, fuel cells, sensors, and electrochemical reactions, the choice of electrode and electrolyte materials directly affects charge transfer, conductivity, and chemical stability [3]. For example, in energy storage devices like lithium-ion batteries, materials such as lithium cobalt oxide and graphite are used for their high charge storage capacity and stability. In electrochemical sensors, materials like gold nanoparticles, graphene, and conducting polymers enhance sensitivity and selectivity by providing large surface areas and catalytic properties. Additionally, in processes like water splitting or oxygen evolution reactions, the use of specialized materials, such as metal oxides or novel catalysts, can significantly lower activation energy and improve efficiency. Therefore, proper selection and engineering of materials is crucial for advancing technologies based on electrochemical principles.

1.1.1. Conducting polymers

The term "polymer" was introduced by Swedish chemist Jons Jacob Berzelius, who is also regarded as one of the founders of modern chemistry [4]. "Polymer" is derived from the Greek words "polys" (many) and "meros" (parts), meaning "many parts." Polymers consist of multiple repeating chemical units, known as "mers," connected together in a structure similar to beads on a string. Polymers have long been utilized as insulators in the form of plastic coatings in packaging, plastic bags and textiles etc. The breakthrough took place in

1977, when Alan J. Heeger, Alan G. MacDiarmid, and Hideki Shirakawa discovered that polymers, traditionally considered as insulators, could exhibit electrical conductivity under certain conditions [5]. They have observed the increase in conductivity of polyacetylene (PA) by 10^9-fold upon the oxidation using halogen vapour [6,7]. In 2000, they were awarded the Nobel Prize in Chemistry for the discovery and development of electronically conductive polymer [8-10]. The unexpected sudden discovery gave rise to a new category of conducting polymers known as "Synthetic Metals" [11]. The transition from insulating to conducting behavior in polymers was attributed to a process known as doping. In the nineteenth century, electrochemical and chemical methods for synthesizing π-conjugated polymers such as polyaniline, polypyrrole, and polythiophene were reported. Later, the concept of doping was applied to enhance their electrical properties [12-14]. In case of chemical synthesis, oxidizing agents like ammonium peroxydisulfate (APS) and ferric chloride ($FeCl_3$) are introduced into the monomer. The oxidizing agents initiate the polymerization reaction, resulting in the formation of the polymer. Electrochemical synthesis uses a standard three-electrode setup, with electrodes immersed in a solution containing both the supporting electrolyte and monomer. When an oxidation potential is applied to the working electrode, the monomer oxidises and forms a polymeric coating on the surface of the working electrode [15]. The unique chemical structure along with its excellent physical properties make them an attractive material for a variety of applications including photovoltaic devices [16], field effect transistors [17], electrochromic windows [18], actuators [19], super capacitors [20], chemical sensors [21], and biosensors [22]. Conducting polymers, also referred to as conjugated polymers, contain alternate single and double bonds along their polymer chains. This unique structure enables them to conduct electricity due to the presence of these alternating bonds along the polymer backbone [23]. The electronic structure of a material determines its electrical conductivity, as electrons move within specific energy levels known as bands. Band theory explains the electronic structure, classifying materials into conductors, semiconductors, and insulators based on their behavior. This concept of band theory, which links to the quantum theory of atomic structure, originated in the field of physical chemistry. In quantum mechanics, an isolated atom has distinct, sharply defined energy levels [24]. When an electron makes transition between these energy states, it produces spectral emission lines with narrow line widths. However, in solids, atoms are closely packed and cannot be considered as independent units. Instead, atoms with different electronic energy levels are chemically bonded, leading

to the overlap of their atomic orbitals with neighbouring atoms, forming molecular orbitals [25]. This overlap causes electrons in the same orbit to have varying energy levels. Consequently, electrons within the same orbit display distinct energy levels, resulting in a broadening of the sharp atomic energy states, forming an energy band [26]. At absolute zero temperature, electrons occupy the highest energy range known as the valence band, while the lowest unoccupied energy level is the conduction band. The energy gap between these two bands is referred to as the band gap (E_g) of the material. In conductors, the valence and conduction bands overlap, enabling free movement of electrons within the conduction band, which is a key characteristic of conductive materials. The band structures of insulators, semiconductors, and metals are typically illustrated in band diagrams (Figure 1.1). At the molecular level, the transport properties of conducting polymers (CPs) have been explored [27,28]. In conjugated conducting polymers, alternating single and double bonds along the polymer chain create a π-orbital system, allowing electrons to move freely from one end of the polymer to the other. The structure of organic polymers is typically based on aromatic rings or linear chains of carbon atoms, sometimes incorporating heteroatoms such as oxygen, nitrogen, or sulfur. The alternating single and double bonds along the polymer backbone result in sp^2 hybridization of the carbon atoms, involving one *s*-orbital and two *p*-orbitals [29]. In the carbon atom's ground state ($1s^2\ 2s^2\ 2px^1\ 2py^1$), the *2s* orbital overlaps with two of the *2p* orbitals, forming three sp^2 hybridized orbitals that are directed towards the corners of an equilateral triangle, each at a 120° angle. The remaining unhybridized p_z-orbital is perpendicular to the sp^2 hybridized plane.

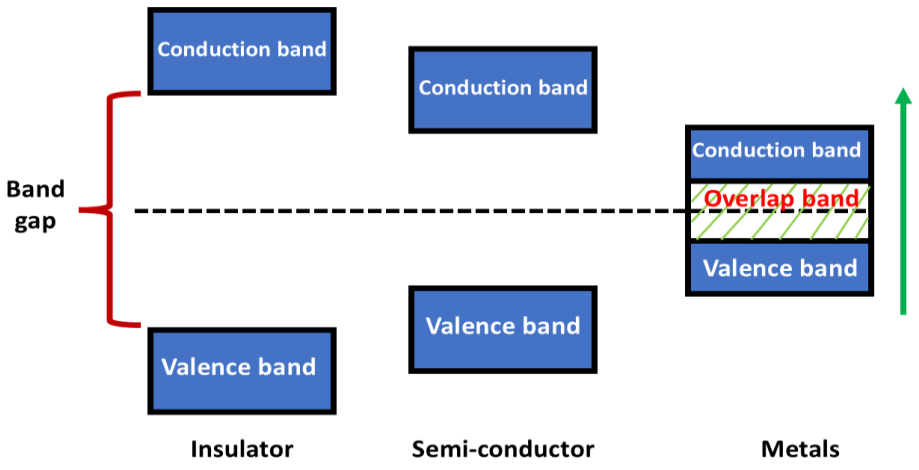

Figure 1.1: Diagrammatic representation of band gap in insulator, semiconductor and metal.

The head-on overlap of orbitals due to strong covalent bonding between two carbon atoms along the polymer chain axis forms strong σ (sigma) bonds, which are responsible for creating polymer chains. In contrast, the perpendicular p_z orbitals of two carbon atoms overlap side-by-side, forming π (pi) bonds. The continuous overlap of p_z orbitals causes the π-bond between the first and second carbon atoms to shift to between the second and third atoms. This attraction of π-electrons to the nuclei of neighbouring carbon atoms leads to delocalization of the π-electrons along the polymer chain [30,31].

The unique properties of conducting polymers arise from these delocalized electrons moving along the polymer backbone [32]. As a result, conjugated polymers can be doped to exhibit conductive or even metallic properties. The unhybridized p_z orbitals of one carbon atom overlap with those of another, forming two molecular orbitals: bonding (π) and anti-bonding (π*) orbitals. These molecular orbitals are shared between both atoms, with the bonding orbital having lower energy than the atomic orbitals of the individual carbon atoms, while the anti-bonding orbital has higher energy. The width of the individual energy bands formed by bonding and antibonding orbitals is known as the band width. The valence band (VB) is equivalent to the highest occupied molecular orbital (HOMO), while the conduction band edge corresponds to the lowest unoccupied molecular orbital (LUMO). The energy gap between the HOMO and LUMO is referred to as the band gap (E_g), typically ranging from 1.5 to 4 eV in conducting polymers. The weaker π-bonds in these polymers make them particularly susceptible to doping, which can be achieved through chemical or electrochemical oxidation and reduction processes. One schematic illustration of the HOMO and LUMO band formation in conducting polymers is provided in Figure 1.2.

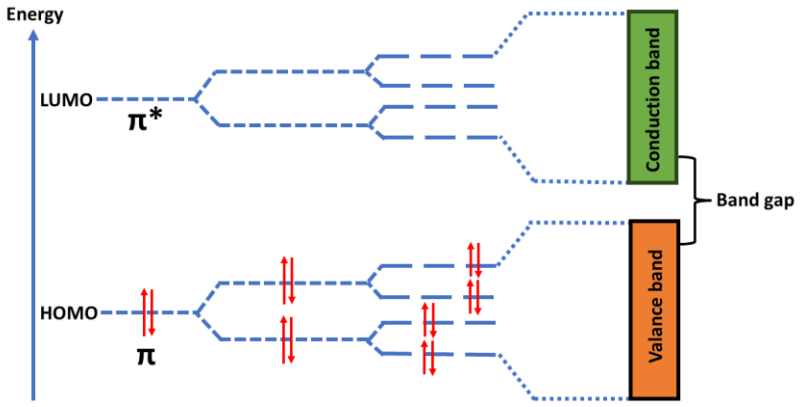

Figure 1.2: Schematic representation of band formation in conducting polymers.

In recent years, conducting polymers have been widely studied for their good conductivity, mechanical flexibility and cost-effective processing methods [33]. For these reasons, polymers can be used for energy-efficient and bioelectronic applications, such as supercapacitors, drug delivery systems, electrochemical sensors, organic transistors, flexible energy storage, touch panels and precision biosensors. Poly(3,4-ethylenedioxythiophene)-polystyrene sulfonate (PEDOT-PSS) and Polyaniline (PANI) have received appreciable consideration because of their outstanding properties including low redox potential, high conductivity, good processibility, biocompatibility and environment stability [34-36]. To be mentioned, they have extended π-conjugation along the polymer backbone. As a result of high conjugation, distinctly conjugated polymers can be rendered chemically, electrochemically and physically reversible, making them extremely fascinating as transducer materials as well as ideal matrix for immobilization in various sensing devices. Active layers of gas sensors were developed in the 1980s using conducting polymers and their derivatives [37]. The conducting polymer sensors operate at room temperature and have many advantages over most commercially available sensors based on metal oxides, which typically operate at high temperatures. Recently, conducting polymer nanostructures like nanoparticles, nanowires, nanotubes and nanosheets were used as biosensors for the detection of biomolecules [38]. As a result of their numerous characteristics, they are ideal for immobilizing biomolecules and facilitating rapid electron transfer in biosensor fabrication [39].

(a) Poly(3,4-ethylenedioxythiophene)- poly (styrenesulfonic acid) (PEDOT-PSS)

PEDOT, a fascinating derivative of polythiophene (PTh), was developed in the late 1980s at the Bayer AG research laboratories in Germany [40]. PEDOT is a *p*-type conducting polymer characterized by a moderate band gap of 1.5 eV, high conductivity of 300 S cm^{-1}, a low redox potential of -0.6 V, and excellent electrochemical stability [40]. However, the solubility of PEDOT is poor in many solvents. To address this issue, the Bayer AG research laboratories during 1990 introduced poly (styrenesulfonic acid) (PSS) with the positively charged PEDOT in order to produce a stable suspension named as PEDOT-PSS, illustrated in Figure 1.3. PEDOT-PSS is a prime and growing conducting polymer having great significance in elementary research and advanced applications [41,42]. PEDOT-PSS exhibits numerous advantages such as good film-forming ability, excellent thermal and electrochemical stability, high transparency in the visible range, especially the tunable and improved conductivity and biocompatibility [43-45].

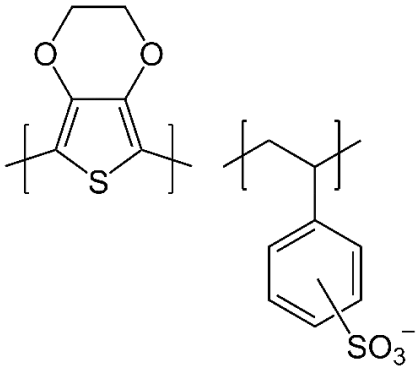

Figure 1.3: Chemical structure of PEDOT-PSS.

Because the dioxy group is present on the third and fourth positions of the thiophene ring, PEDOT-PSS has high stability where PSS acts as an additive that makes it soluble in water [46]. Nanocomposites based on PEDOT-PSS used in Li-ion batteries [47], supercapacitors [48], solar cell [49], electrocatalyst [50] and sensors [51] etc. Recent studies on PEDOT have shown that using dopant PSS can not only achieve good electrical properties but may also provide sufficient carboxyl groups for subsequent biofunctionalization [52]. PEDOT-PSS mixed polymer consists of conducting grains and insulating fibers composed of PEDOT rich grains interconnected by PSS rich fibers, demonstrating *p*-type ionic conductivity [53]. Electrochemical processes allow these ions to adsorb on the grains, which results a dual layer of electric charge at the interface among nanoscaled interconnected grains. Further, *p*-type PEDOT-PSS has a reversible modulation of hole density by compensating along ions obtained through an electrolyte, proving ideal for electrochemical sensing application [54].

(b) Polyaniline (PANI)

Polyaniline (PANI) is a heteroatomic conducting polymer belonging to the semi-flexible rod polymer group. PANI was first identified by John Lightfoot in the early 1860s and was known as aniline black [55]. But, the electrical characteristics of PANI were not investigated during that time. In 1986, MacDiarmid and colleagues chemically synthesized PANI by polymerizing aniline in an acidic aqueous solution using ammonium peroxydisulfate (APS) as an oxidizing agent [55,56]. They measured the conductivity of the resulting powdered PANI, which was approximately 3 S/cm. Since then, PANI has become one of the most widely researched conducting polymers. The favourable doping and de-doping properties of PANI, along with its environmental and thermal stability and relatively high electrical conductivity, have made it suitable for numerous applications. It

is typically synthesized through oxidative polymerization, which can be carried out using either chemical or electrochemical methods [57]. The optimal conditions for producing the polymer are achieved by oxidizing aniline in acidic aqueous solutions. Polyaniline possesses a complex molecular structure (Figure 1.4), initially proposed by MacDiarmid et al., with distinct oxidation states [56]. These states range from the fully reduced leucoemeraldine to partially oxidized protoemeraldine, emeraldine, nigraniline, and finally, the fully oxidized pernigraniline [58]. In 1910, Arthur G. Green and Arthur E. Woodhead first suggested the names for these oxidation states [59,60]. Depending upon the values of n (Again, $m = 1-n$), the different oxidation states of PANI is shown in Figure 1.5 [61,62]. For n=1, PANI exists in its fully reduced state, referred to as the leucoemeraldine form. At $n = 0.25$, it is in the protoemeraldine form. The emeraldine form, where PANI is half reduced and half oxidized, occurs when $n = 0.5$. For $n = 0.75$, PANI takes on a partially oxidized state known as nigraniline, while at $n = 0$, it reaches its fully oxidized state, called pernigraniline. Among these, the emeraldine base form is the most valuable because of its high stability at room temperature and it is highly soluble in polar solvents. The most stable form of PANI, emeraldine base contains imine (=N-) and amine (-NH-) groups in equal proportions. PANI nanostructures are a growing field of scientific interest with a wide range of applications [61-63]. PANI has recently gained considerable recognition because of its excellent electrical properties, good environmental and chemical stability, easy synthesis procedure and huge application. Additionally, PANI has a wide scale of tunable properties due to its flexibility of its structure, which can be applied to many fields including sensing, energy storage as well as electrocatalytic and electrochromic devices [64].

Now-a-days, PANI is widely used in electrochemical application due to its electrochemical stability and interesting electroactivity. PANI and its derivatives have been recognized as excellent immunosensor materials due to their high biocompatibility, easy modification, and excellent electrochemical properties [65,66].

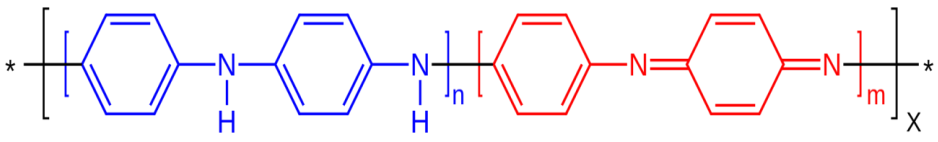

Figure 1.4: Structure of Polyaniline.

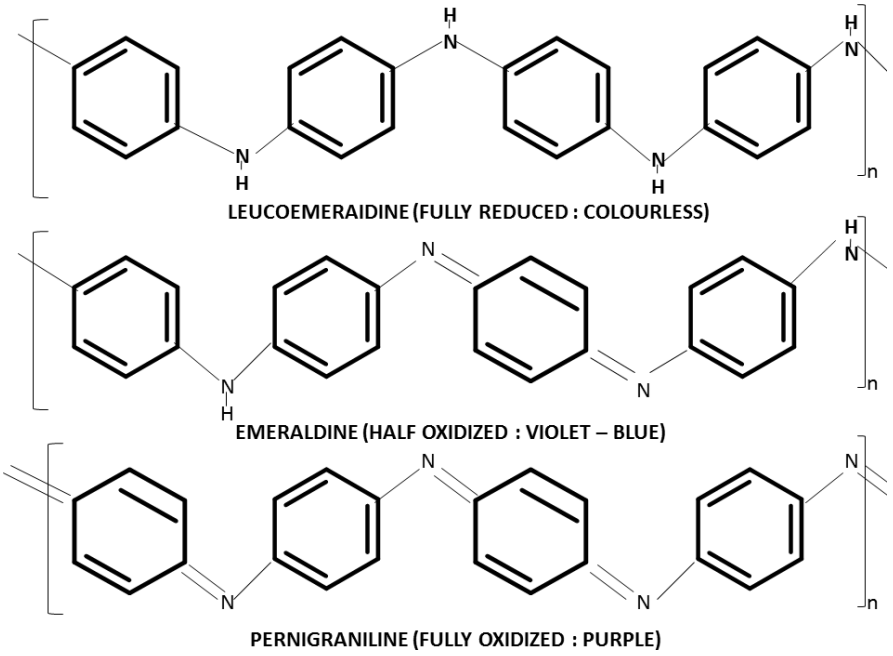

Figure 1.5: Structure of different oxidation states of polyaniline.

PANI's biocompatibility leads to the preservation of enzyme active sites while allowing analytes to permeate to enzyme catalytic sites [67-68]. Moreover, it can be used as an effective charge transfer agent in enzymatic or redox reactions which serve as a source for immobilizing biomolecules due to which PANI received a great deal of attention in biosensing and related applications [69].

1.1.2. Two dimensional layered nanostructures

Two dimensional (2D) materials are the class of substances with a thickness of just few atomic layers. 2D materials, a class of nanomaterials characterized by 1D spatial confinement [70]. Ideally, nanosheets, which can range from monolayer to few-layer forms, are considered 2D materials. A layered material, as the name implies, develops with a layer-by-layer structure. With confinement in one dimension, the system adopts a 2D planar configuration and can appear in either monolayer or few-layer forms. These materials have significant mechanical strength because of their strong in-plane bonding, and their weak van der Waals contact between the detachable stacked layers makes them ideal to develop novel, unique materials [70]. The structural and electronic behavior of a material can significantly change at the surface, and 2D nanomaterials exhibit the highest surface area-to-volume ratio, with a large number of atoms located on the surface. For example, atoms within the bulk of a crystal experience an isotropic environment in all

directions, whereas surface atoms tend to remain unsaturated, resulting in high chemical reactivity at the surface [71]. The concept of layered systems started years back when materials like limestone and graphite, abundant in nature, were first utilized practically. However, significant advancements in research began after the discovery of atomically thin, single-layer graphene. The discovery of graphene at the University of Manchester in 2004 opened up new possibilities for exploring layered 2D materials as a promising research area: an achievement that K.S. Novoselov and A.K. Geim earned the Nobel Prize in Physics [72]. There are numerous examples of layered materials that can be synthesized in a layer-by-layer fashion under suitable laboratory conditions. Notable examples include graphite and its derivatives, transition metal dichalcogenides (TMDCs), graphitic carbon nitride (g-C_3N_4), hexagonal boron nitride (h-B_3N_4), phosphorene (BP), MXenes, and metal oxides such as MoO_3 and WO_3 [73-75]. The exceptional structural and physico-chemical properties of these materials make them highly suitable for various applications, including catalysis, optoelectronics, energy storage, supercapacitors, sensors, and biomedical research [76-78]. 2D nanomaterials such as graphene and transition metal dichalcogenides, have spurred great interest in electrochemical biosensing field because of their innovative mechanical, physicochemical properties and multifunctionalities [79]. The rise of 2D materials as a new class of materials is gaining increasing interest because of their fascinating properties for development of biosensors and detection of biologically active molecules and different pollutants of the environment [80]. These materials show very high surface-to-volume ratio, allowing easy functionalization with different biomolecules. In addition to good environment and chemical stability with high conductivity, a high level of exfoliation can lead to beneficial electrochemical properties such as enhancement in the electron transfer kinetics (HET). As a result of these characteristics, as well as their biocompatibility, these two-dimensional materials are high in demand for the evolution of electrochemical sensors [81,82].

(a) Graphene oxide (GO)

In recent years, graphene along with its derivatives, namely graphene oxide (GO), have become increasingly attractive due to their peculiar properties [83]. Graphene possesses outstanding physical, optical, chemical and electronic properties like large specific surface area, good optical transparency, mechanical strength, as well as excellent thermal and electrical conductivity [84]. 2D graphene sheet is composed of *sp2*-hybridized carbon atoms where the atoms are ordered within hexagonal lattice [85].

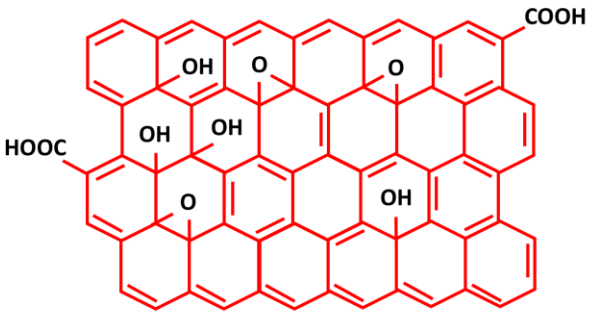

Figure 1.6: Schematic structure of GO.

Graphene oxide, a graphene derivative contains different functional groups of oxygen at the edges and basal planes of graphene oxide sheets [86], illustrated in Figure 1.6. The existance of these oxygen-carrying groups can be used as catalytic active centers for covalent/non-covalent modification which furnish prospective power to GO for various applications through chemical functionalization [84,85]. Aside from its low production cost and large scale, GO has many advantages over graphene. Furthermore, oxygen-carrying groups present in graphene oxide broaden the interlayer gap. Currently, significant advancement has been accomplished in the functionalization of graphene oxide [86]. The GO nanosheets have the advantage of being solvable in water along with polar solvents, which makes them useful for forming large-scale uniform films on a wide variety of substrates [84]. Alike graphene, GO also shows unique properties and due to its hydrophilic nature, it is possible to fabricate flexible and transparent nanosheets with biocompatibility. Biosensors can take advantage of the functional groups available on GO nanosheets to interact with an array of biomolecules [87].

(b) Transition metal dichalcogenides (TMDCs)

Transition metal dichalcogenides (TMDCs) account for a vast and essential family where MX_2 is the generalized formula for TMDCs, M represents a transition metal and X is a chalcogen [88]. MX_2 compounds are layered materials having strong and weak intralayer and interlayer bonding; respectively. It is well known that TMDCs have a wide range of electronic properties offering systems like, insulators, semimetals, semiconductors and metals [89]. To date, a total of 60 TMDCs have been investigated out of which about 40 having layered structures and that mostly of group 4 to 7 transition metals. Amidst all layered TMDCs, the members belonging to Group 6 (WS_2, WSe_2, MoS_2, $MoSe_2$) have special scientific and technological significance.

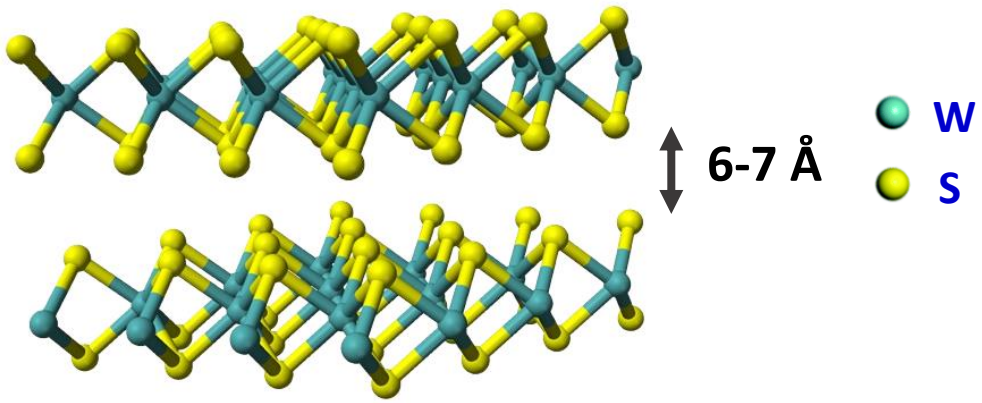

Figure 1.7: Schematic layered structure of WS_2.

Owing to their size and dimensionality, these TMDCs are used in many technologically important fields, such as electronics and optoelectronics, catalysis, energy storage devices, solid lubrication, sensors, etc [90]. The reason for their dominance is their outstanding electrochemical equity, including fast electron transfer kinetics (HET) potentials and a controllable band gap [90]. Furthermore, they hold less cytotoxicity in contrast with other 2D material like Graphene, making them suitable for biosensors. In the development of environmental and biomedical biosensors, TMDCs, with their intriguing properties, have been emerging as suitable material [91]. Among all, tungsten disulfide (WS_2) is a highly promising material for the development of biosensing devices because of its fascinating properties (Figure 1.7). Bulk WS_2 has an indirect band gap of 1.35 eV, but when reduced to a monolayer form, it transforms into a direct band gap semiconductor with a band gap of 2.0 eV [92,93]. In its monolayer form, the tungsten atoms are sandwiched between two sulfur atom layers, held together by strong covalent bonds. The atomically thin layers of WS_2 are stacked via weak van der Waals forces (with an interplanar separation of 6-7 Å), making it relatively easy to exfoliate into monolayer or few-layer nanosheets using techniques such as micro-mechanical exfoliation, intercalation-assisted exfoliation, and liquid phase exfoliation [94,95].

1.1.3. Noble metal nanoparticles

Noble metal nanoparticles are known for their inert behavior, showing exceptional resistance to corrosion and oxidation in humid conditions, as well as exhibiting lower toxicity compared to transition metal nanoparticles [96]. These unique characteristics have made noble metal nanoparticles highly versatile, with applications ranging from catalysis

to nano-biosensing and plasmonics. Noble metal nanoparticles include elements such as Au, Ag, Rh, Pt, Ir, Ru, and Pd. Common synthesis methods for these nanoparticles include chemical reduction, photochemical reduction, thermal decomposition, and electrochemical deposition [97]. Nanoparticles, including Au, Ag, Pd, and Pt, have garnered significant research attention due to their unique physicochemical properties, which distinguish them from their bulk forms [98]. Among all, AuNPs and AgNPs are the most extensively researched metal nanoparticles, owing to their unique chemical, physical, and biological properties [99]. Gold (Au) and silver (Ag) nanoparticles offer significant advantages over other nanomaterials, including excellent stability, biocompatibility, high conductivity, low cytotoxicity etc. [100,101]. Nanoparticles possess key characteristics such as high surface-to-volume ratio, monodispersity, and strong adsorption capabilities [102]. Among these, the surface-to-volume ratio is particularly crucial, as it enables analytes to move swiftly through nanoparticle-based sensors, thereby enhancing both catalytic and sensing activities [103]. This high adsorption allows nanoparticles to absorb and interact effectively with chemical species or biomolecules. Additionally, the adsorption and interaction of nanoparticles with analytes significantly influence the optical and electrical properties of the sensors [104]. These exceptional properties make nanoparticles ideal for use in the design of colorimetric, fluorescent, and electrochemical sensors. In electrochemical biosensor fabrication, their large surface areas allow for the stable immobilization of biomolecules while preserving their bioactivity. The conductivity of nanoparticles enhances electron transfer between biological components and the electrode surfaces. Furthermore, their stability and biocompatibility enable easy conjugation with various biomolecules, chemical groups, and polymer materials. Research has shown that incorporating noble metal NPs like Au and Ag can profoundly augment the electrochemical responses of fabricated sensors.

1.2. Biosensors: Principles, components and types

Sensors are crucial in our everyday lives, offering vital insights into the physical and chemical conditions of systems or processes. They deliver real-time information, enabling prompt and accurate diagnosis of any potential issue. A biosensor is a chemical sensing device that combines biologically active components, such as enzymes, tissues, microorganisms, antibodies, or nucleic acids, with a suitable transducer to selectively detect the concentration of a target analyte. The detection process involves two main steps:

the recognition step, where the biological element identifies the analyte in either a solution or the atmosphere, and the transducing step, where the signal is converted for measurement [105,106]. Bioreceptors can be categorized based on the biorecognition elements they contain. These elements may be biological molecules, such as antibodies, nucleic acids, proteins, enzymes, or living biological systems like tissues, cells, or whole organisms [107]. When a bioreceptor interacts with its target analyte, it triggers a biological response, which the transducer converts into measurable electrical signals for quantitative analysis. The bioreceptor within a biosensor offers a high degree of specificity and selectivity but is sensitive to environmental changes such as temperature, along with fluctuation in solution properties like pH, ionic strength, and also toxicity ［108］. The performance of the biosensor, including its sensitivity, analyte detection, linearity, and shelf life, is directly linked to the activity of the bioreceptor［109］. To preserve this activity, factors such as surface area, matrix porosity, with other surface properties must be carefully considered. Additionally, the method of immobilizing the biological component is critical and varies depending on the type of bioreceptor used［110］.

Biosensors are generally classified into three generations ［111］. The first generation, known as mediator-less biosensors, is based on Clark's biosensors, in which the biologically active molecule is either attached or encapsulated in a membrane coupled to the transducing surface. The diffusion of the reaction product across the membrane-transducer interface generates a detectable electrical signal. Second-generation biosensors use a specific mediator for the transmission of the signal from the active position of the bioreceptor to the transducer, with the goal of improving sensitivity. The mediator must have a lower redox potential than other electroactive compounds in the solution. Third-generation, or direct biosensors, rely on bioelectrocatalysis, where the bio-component is an integral part of the sensor, eliminating the need for a mediator. In this case, the bioactive agent is directly linked to the transducer, allowing the sensor to measure the signal directly. Conducting polymer-based biosensors belong to this third generation, where redox polymers facilitate signal propagation [111].

1.3. Types of biosensors based on different transducers

Based upon transduction of the biological response, biosensors are classified as electrochemical, optical, calorimetric, piezoelectric, colorimetric etc.

1.3.1. Electrochemical biosensor

An electrochemical biosensor operates by measuring the electrical properties generated from the interaction between the immobilized biomolecule and the target analyte. The electrochemical transducer extracts information from biological systems, with the intensity of the measurable signal being directly proportional to the concentration of the detected compound. Typically, a biochemical reaction produces a current signal which is known as amperometric. Additionally, the reaction may alter the oxidation or reduction potential known as potentiometric or affect the conductivity of the reaction medium which is named as conductometric. Changes in resistance or reactance between the electrodes and the electrolyte (impedimetric) can also serve as the electrochemical signal [112-114]. Electrochemical sensors typically employ a three-electrode system immersed in an electrolyte, consisting of a reference electrode, counter electrode, and working electrode. The reference electrode maintains a stable potential at the working electrode, ensuring that the electrochemical reactions being studied are unaffected by fluctuations in reference potential. Common reference electrodes include calomel and Ag/AgCl, while platinum (Pt) wires are often used as counter electrodes due to their inertness, completing the circuit by allowing current flow without affecting the reference potential. The working electrode is where the key electrochemical reactions, such as oxidation and reduction, occur, producing a measurable current or potential that reflects the process being studied. Common working electrodes include glassy carbon, carbon paste, and gold/Pt [115].

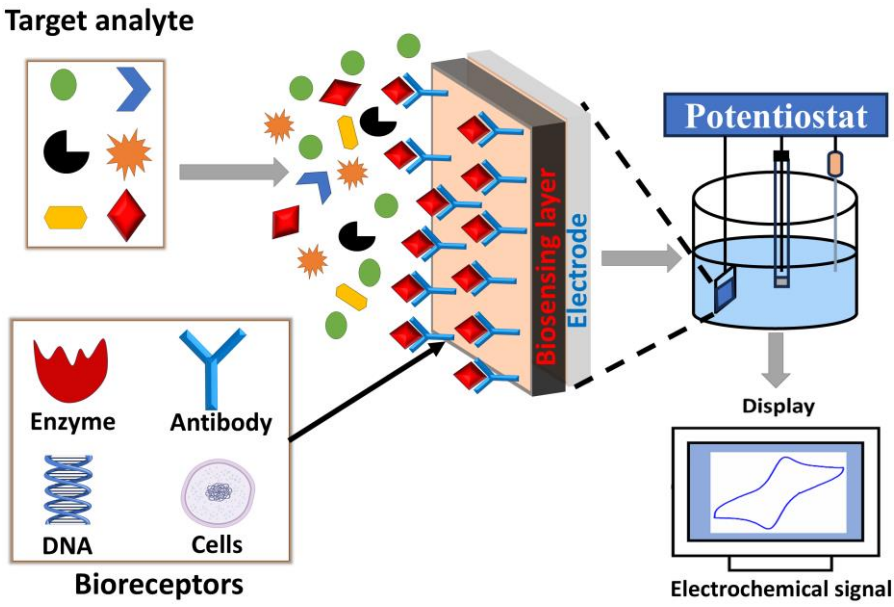

Figure 1.8: Schematic representation of an electrochemical biosensor setup.

However, traditional electrodes face challenges like high overpotentials, surface fouling, slow kinetics, low sensitivity and limited selectivity. Bare electrodes are particularly prone to fouling due to the adsorption of analyte molecules or reaction byproducts, which affects the stability of the electrode for repetitive usage. To address these issues, modifying the electrode surface with suitable materials offers a promising strategy to improve stability and enhance overall electrochemical performance.

1.3.1.1. Amperometric biosensor

The most common approach to electrochemical detection involves measuring the current generated during a biochemical reaction. The magnitude of the current is directly proportional to the concentration of the target analyte. commonly, three-electrode systems are employed for this process. The performance of the working electrode is crucial in amperometric sensors, and significant efforts have been made to design electrodes that offer high sensitivity and stability. Noble metals and various carbon-based materials are often used as solid electrodes in this method. In amperometric sensing, the redox-active site within the enzyme layer produces a current signal typically in the nA to μA range, which can be directly correlated to the concentration of the target of interest. The applied potential is set at a level where the analyte generates oxidation or reduction current, acting as the driving force for electron transfer. Mediators in the biochemical reaction enhance electron transfer by actively participating in the redox process [116].

1.3.1.2. Potentiometric biosensor

A potentiometric transducer measures the potential at which a biochemical reaction occurs between the analyte and the biocatalyst. Potentiometric biosensors operate by applying a voltage to an electrode system in a solution, resulting in current flow. The oxidation or reduction potential measured during the electrochemical reaction provides information about the specific reaction and analyte involved. The potential difference between two electrodes is recorded using a high-impedance voltmeter, with minimal current passing through the system [117,118]. The response time of potentiometric sensors is generally fast, and they offer long-term stability, making them suitable for continuous monitoring applications. Potentiometric sensors are widely used in fields such as environmental monitoring, medical diagnostics, and industrial process control.

1.3.1.3. Impedimetric biosensor

Impedimetric biosensors work by measuring the resistance to charge flow caused by biochemical interactions at the working electrode. In this technique, an alternating current (AC) signal is applied across a range of frequencies, generating a current through the bioelectrode. It is commonly used to study bio-affinity interactions, where the binding of an antigen to the electrode surface increases impedance, which is detected through impedimetric transduction. The impedance, or resistance to current flow between the working and counter electrodes, is directly related to the amount of analyte present on the sensor surface. The Electrochemical Impedance Spectroscopy (EIS) method enables label-free detection of the target analyte [119,120].

1.3.1.4. Conductometric biosensor

In conductometric biosensor, changes in the overall conductivity of a solution appears due to the changes in ionic species generated during a bio-recognition event. A conductometric transducer detects this change in electrical conductance, which increases as the concentration of the target analyte rises. When the analyte interacts with a biorecognition element, such as an antibody, it produces or consumes ions, affecting the ionic content of the solution and thus its conductance. However, conductometric transducers tend to have relatively low sensitivity. This method works by applying an AC across two metal electrodes and measuring the conductance, which reflects the analyte concentration [121]. To improve sensitivity, the electrode surface can be modified with various nanomaterials. These modifications enhance sensor performance by increasing the surface area for ion interaction and improving ion transport properties.

1.3.2. Optical biosensor

Optical biosensors detect analytes by measuring the light absorbed or emitted during catalytic or affinity-based reactions. This optical detection can be classified into methods such as reflection, infrared, absorption, fluorescence, raman, and resonance, depending on the type of biochemical reaction. Surface plasmon resonance (SPR) and fluorescence are the most commonly used techniques due to their high sensitivity and selectivity in detecting biochemical interactions. In SPR, a metal layer (typically gold) serves as the substrate, where bioactive molecules like antibodies or enzymes are immobilized. When the analyte flows over the receptor, the refractive index near the metal surface changes,

and this is detected by the SPR transducer as a variation in light intensity. The resulting signal corresponds to changes in the refractive index of the medium [122-125]. Fluorescence-based biosensors, on the other hand, monitor changes in the frequency of incident electromagnetic radiation. These sensors are advantageous for *in vivo* applications as they are non-electrical and can detect multiple analytes by utilizing different wavelengths [126].

1.3.3. Calorimetric biosensor

A calorimetric transducer utilizes a fundamental aspect of biological interactions, known as expulsion or adsorption of heat. The endothermic or exothermic reaction between a biocomponent and its target analyte causes a temperature change in the reaction medium. The experimental setup is designed with immobilized enzymes kept in a small, enclosed column, with temperature sensors placed at both the entrance and exit points. In this sealed system, up to 80% of the heat generated can be attributed to the temperature change in the sample stream. The amount of heat absorbed or produced is directly proportional to the number of molecules involved in the reaction and the molar enthalpy. Thermistors are typically used to measure the temperature change before and after the interaction, and the difference in enthalpy is calibrated to the analyte concentration. Calorimetric biosensors are commonly employed for detecting pesticides and bacteria [127].

1.3.4. Piezoelectric biosensor

Piezoelectric biosensor known as mass-based biosensors operate on the principle of sound vibrations, or acoustics [128]. These sensors function by coupling a biological element to a piezoelectric material. Common piezoelectric substances such as quartz, lithium niobate, oriented zinc oxide, aluminum nitride, and tourmaline are generally used to coat metal electrodes. The piezoelectric crystal vibrates at a specific frequency, which determines the transducer's sensitivity. When biomolecules attached to the sensor interact with the analyte, mechanical vibrations occur, which are then transformed into an electrical signal. As the mass increases due to complex formation, the oscillation frequency shifts, with the electrical signal directly corresponding to this change [128].

1.3.5. Colorimetric biosensor

A colorimetric sensor is a type of sensor that detects changes in the color of a solution or surface due to a chemical reaction [129]. These sensors work based on the principle that

the presence or concentration of an analyte causes a visible color change, which can be quantified by measuring the intensity of light absorbed or transmitted by the sensor. In a typical colorimetric sensor, the interaction between the target analyte and a color-changing reagent or indicator produces a distinct color shift. The degree of color change is directly related to the concentration of the analyte. These sensors are widely used due to their simplicity, cost-effectiveness, and ability to provide quick, visual results.

1.4. Enzyme/Antibody based electrochemical biosensors

1.4.1. Enzyme and antibody

(i) Enzyme:

Enzymes, which act as biological catalysts, accelerate biochemical reactions and are frequently utilized as biomaterials for developing biosensors. These enzymes exhibit high specificity for their target analyte and facilitate a particular reaction without being consumed in the process [130,131]. The catalytic activity of enzymes occurs in a specialized region of the protein known as the active site. Enzymes can be reused as long as this active site remains functional, though their activity may sometimes diminish over time. Some enzymes, like glucose oxidase, require a co-factor, such as FAD, which plays a key role in the redox reaction by gaining or losing electrons [132]. The enzyme-analyte interaction typically follows the lock and key theory.

The Lock and Key mechanism, first proposed by Emil Fischer in 1894, explains how enzymes interact with a single substrate [133]. In this model, the enzyme is likened to a lock, and the substrate to a key, shown in Figure 1.9. The process begins when the substrate binds to the enzyme’s active site. This precise binding leads to the exchange of electrons between the redox-active site of the enzyme and the substrate, ultimately producing a product. A perfectly sized substrate (key) fits into the enzyme's active site (lock), while substrates that are too small or too large may not bind properly. This specific fit, where only substrates with a matching structure can bind, ensures high specificity of the enzyme. The activity of an enzyme is influenced by several factors, including the pH of the solution, temperature, and substrate concentration. Since enzymes are zwitterionic, the interaction between the amino acids available in the active site of enzymes and the substrate depends on their electrostatic properties and spatial arrangement.

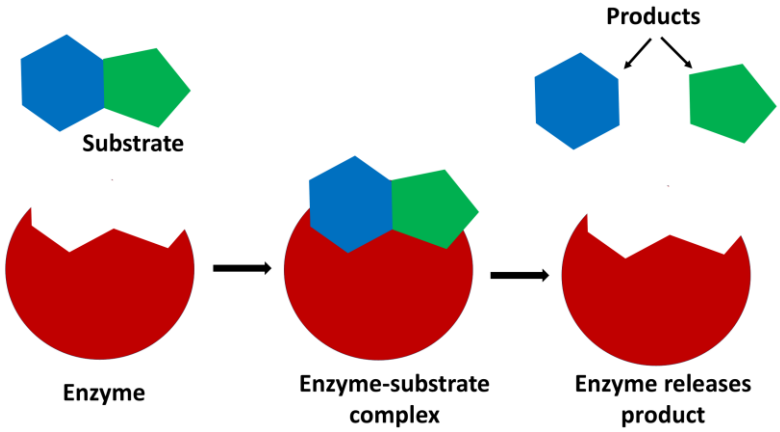

Figure 1.9: Schematic representation of interaction between enzyme and substrate.

In acidic conditions, the amino acid groups carry positive charges, while in basic conditions, they become negatively charged. Each enzyme has a specific optimal pH that maintains its structural configuration [134]. Temperature is another critical factor affecting enzyme kinetics; higher temperatures increase the frequency of effective collisions between the enzyme and substrate. However, if the temperature exceeds a certain threshold, the thermal energy can disrupt the forces that stabilize the three-dimensional structure of the enzyme, leading to denaturation [135].

(ii) Antibody:

Antibodies are the most diverse class of protein, composed of millions of distinct amino acids and comprising for about 20% of total plasma proteins, collectively known as immunoglobulins (Ig) [136]. The properties of antibodies are determined by their structure, which includes a constant region and a variable region. The tail section of the antibody, known as the F_c region, is a uniform protein fragment that facilitates interactions with cell surface receptors [137]. Antibodies can be simplified into Y-shaped molecules, each featuring two identical binding sites for analytes/antigens. The variable ends, called the F_{ab} regions, contain unique configurations that allow them to bind specifically to distinct targets. These F_{ab} regions, often referred to as the "arms" of the antibody, have diverse amino acid sequences that differentiate various antibody molecules, making each one specific to a particular epitope [137]. Schematic representation of an antibody is illustrated in Figure 1.10. Unlike enzymes, antibodies do not act as catalysts; instead, they bind reversibly to their specific antigens. Based on their interaction with epitopes, antibodies are classified as either monoclonal or polyclonal.

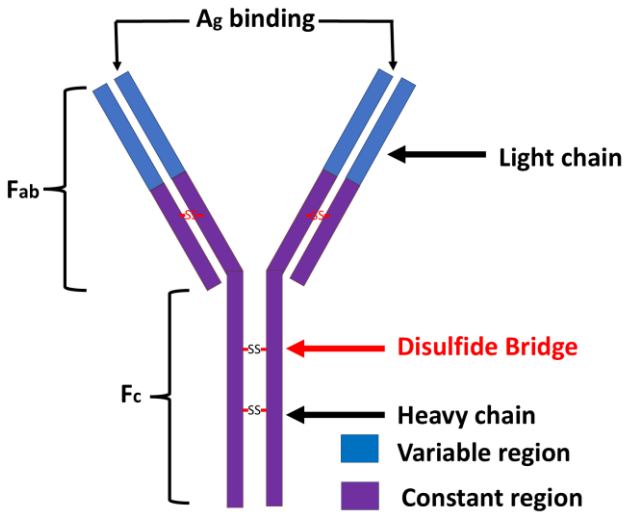

Figure 1.10: Schematic representation of an antibody.

Monoclonal antibodies are derived from a single source (such as one mouse) and target a single epitope, making them highly specific and reducing the possibility of cross-reactivity. In contrast, polyclonal antibodies respond to multiple epitopes on an antigen, resulting in a heterogeneous response. The chemical structure of antibodies plays a key role in their properties, such as binding specificity, versatility, and biological activity. A schematic representation of interaction between antibody with the target analyte is shown in Figure 1.11. Typically, antibody-antigen interactions are non-covalent, with rare exceptions, and occur when the two molecules collide randomly. The binding site of an antibody known as paratope, attaches to a specific part of the antigen, known as the epitope, through non-covalent bonds. For fruitful binding, the attractive forces between the molecules must compensate any repulsive forces in order to overcome zeta potential. At a molecular level, long-range forces such as ionic and hydrophobic interactions facilitate the binding when the epitope is several nanometers away from the antibody [138]. As water molecules are displaced, these forces overcome the hydration energy, drawing the epitope and paratope closer together. At very close distances, hydrogen bonds, van der Waals forces, and electrostatic forces between oppositely charged amino acids contribute to the biochemical interaction [139].

Antibody affinity refers to the strength of the reversible interaction between a single antigenic site and the F_{ab} portion of the antibody [140]. This affinity is influenced by factors such as valency and how well the antigen fits into the binding groove of the antibody. The sensitivity of an immunoassay is directly related to the antibody's affinity for its antigen. Affinity can be further classified into intrinsic affinity and functional affinity.

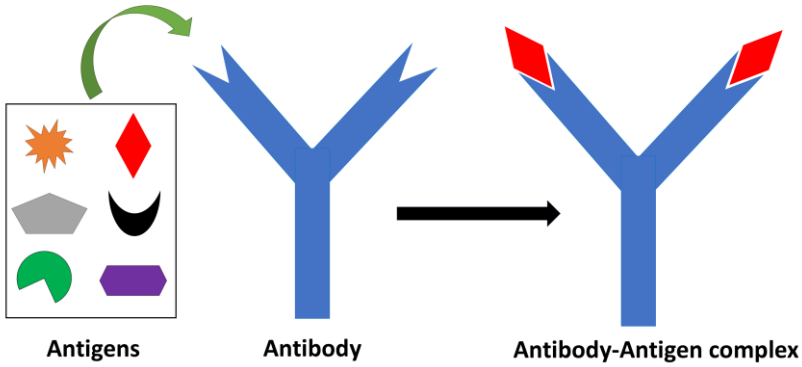

Figure 1.11: Schematic representation of interaction between antibody with the target analyte.

Intrinsic affinity measures the strength of interaction between a monovalent epitope and the paratope of an antibody, typically determined by the equilibrium association constant. Functional affinity, on the other hand, describes the interaction between an intact antibody and a multivalent antigen [141].

Several factors influence the activity of antibodies and their interaction with antigens. The pH of the surrounding medium significantly affects antibody affinity, with extreme acidic or alkaline conditions strongly inhibiting the antibody-antigen reaction. At very high or low pH levels, antibodies may undergo conformational changes, disrupting their binding to antigens. The optimal pH range for most antibodies, where maximum affinity is observed, lies between 6.5 and 8.2; beyond this range, results tend to become unreliable [142]. The duration of the antibody-antigen interaction is also crucial. Sufficient time is needed for the reaction to occur, but excessively long incubation can lead to dissociation of the antibody-antigen complex. Moreover, the sensitivity of these reactions can be enhanced in the presence of some low ionic strength saline solutions [143].

1.4.2. Preparation of enzyme electrodes

Electrochemical transducers based on enzyme can be prepared through various physical and chemical immobilization methods. When enzymes are free in a solution with the substrate, they cannot be recovered post-reaction, limiting their practical use [144]. Immobilization aims to achieve (1) high specificity without altering the structure of the enzyme, (2) better enzyme stability against temperature, pH, ionic strength, and redox potential, and (3) the ability to immobilize multiple biological components on a single matrix [144].

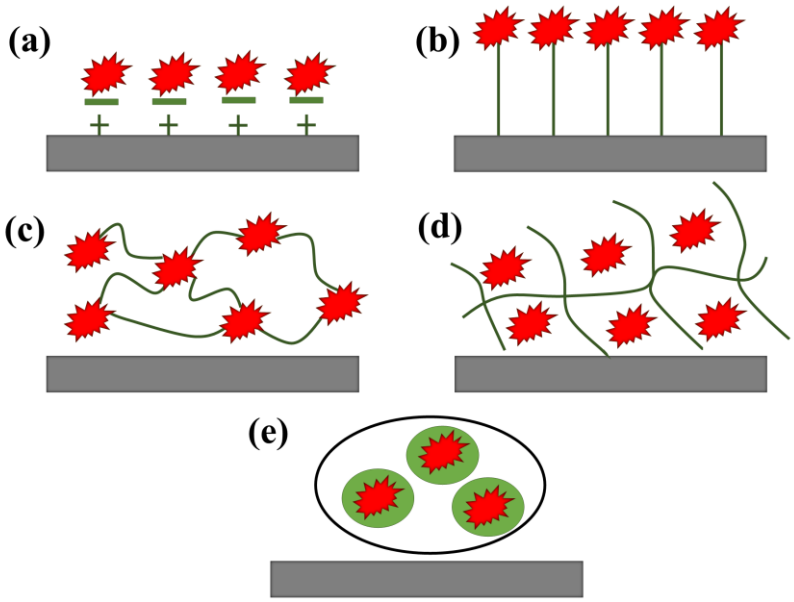

Figure 1.12: The diagrammatic representation of (a) adsorption, (b) covalent bonding, (c) cross linking, (d) entrapment, and (e) encapsulation of immobilization of enzyme.

Adsorption, being the simplest method of enzyme immobilization, involves attaching enzyme proteins to a water-insoluble matrix [145]. Different physical adsorption techniques (Figure 1.12) are used, such as static processes where the enzyme solution contacts the matrix without agitation and dynamic batch processes where the matrix is stirred with the enzyme.

(a) Physical adsorption: Physical adsorption relies on a combination of van der Waals forces, hydrogen bonding, hydrophobic interactions, and ionic forces to bind biomaterials to the sensor surface [146]. Substrates like cellulose, silica gel, hydroxyapatite, collodion, glass, and collagen are commonly known for their ability to adsorb biological components [147]. Although this method is easy, the forces involved are relatively weak, making it possible for biomolecules to detach or lose stability over time.

(b) Covalent bonding: In covalent binding, the sensor surface is modified to introduce reactive groups that allow for the attachment of biological materials. In enzymatic biosensors, this is achieved by targeting functional groups on the enzyme that do not interfere with its catalytic activity. Functional groups such as hydroxyl, thiol, carboxyl, amino, and imidazole groups in the matrix facilitate this covalent immobilization [148]**.** Carriers for covalent coupling include carbohydrates, protein carriers, synthetic agents, and amino group-bearing matrices. A key advantage of covalent binding is the minimal enzyme leakage during practical applications [148].

(c) Cross-linking: In the cross-linking method, enzymes are linked to the matrix using bifunctional or polyfunctional agents such as hexamethylene di-isocyanate, glutaraldehyde 1,5-difluoro 2,4-dinitrobenzene, diazonium salts, and bisdiazobenzidine-2,2'-disulphonic acid which create intermolecular bonds between the biocatalyst and the solid support [149]. Among these, glutaraldehyde is the most commonly used cross-linking agent in biosensor applications, as it forms bonds with the lysine amino groups present in the enzymes.

(d) Entrapment: Another significant immobilization technique is entrapment, which involve physical trapping of enzymes within a porous matrix without directly attaching to the protein. The enzymes are stabilized inside the matrix through covalent or non-covalent bonding, and the process typically involves polymerizing a monomer solution with the biocatalyst [150]. Matrices like chitosan, cellulose acetate, collagen, or polyacrylamide are commonly used, and the pore size of these matrix can be adjusted to minimize immobilized enzyme leaching. However, the potential leakage of biological components can limit the effectiveness of this method.

(e) Encapsulation: This method involves the use of a porous encapsulation matrix, such as lipid bilayers which is formed around the biological material, aiding its attachment to the sensor. Other encapsulation approach involves using the sol-gel method to immobilize biological molecules within glass, ceramics, and other inorganic materials [151]. In the sol-gel process, biomolecules are allowed to get entrapped in a porous matrix like a polymeric oxo-bridged SiO_2 network. These matrices, being optically transparent, enable optical monitoring of chemical interactions. The sol-gel process can be carried out at room temperature (300 K), which helps in protecting biomolecules from denaturation. While this method provides high thermal stability for immobilized biomolecules, challenges remain, such as achieving reproducible pore sizes in the sol-gels. Other issues, including diffusion limitations within the porous network, brittleness of the glassy matrix, and inconsistencies in preparation procedures, need to be resolved for routine applications [152].

1.4.3. Preparation of antibody based electrodes

Like enzymes, the immobilization of antibodies can be carried out through hydrophilic or hydrophobic interactions with a solid matrix. However, immobilized antibodies often exhibit random orientation, which can lead to denaturation and reduced binding

capabilities [153]. The alignment of antibodies on a solid surface can be "head-on," "side-on," or "lying down." Non-specific interactions or random orientations can cause denaturation at the binding sites of antibody. The simplest method for immobilizing antibodies is physical adsorption, but its weak binding limits practical application [154]. Covalent coupling of antibodies to the substrate surface is regarded as the most reliable immobilization method. Antibodies contain lysine residues with primary amine groups. The presence of carboxyl groups across the surface of antibody due to the existence of aspartate and glutamate residues. These amine and carboxyl groups are targeted for covalent bonding with the substrate, typically achieved using carbodiimide chemistry, which involves EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) and succinimidyl esters like NHS (N-hydroxysuccinimide). Thiol groups from cysteine residues can also participate in the covalent bonding of antibodies with the substrate. Additionally, glycosylation in the *Fc* region provides another target for effective immobilization. The biotin–avidin/streptavidin interaction is another important method used for antibody immobilization [155]. Again, Glutaraldehyde is a dialdehyde with two highly reactive aldehydic groups which can bind covalently with different functional groups such as hydroxyl, amine, phenol, thiol etc. [156]. The reactive –CHO group of glutaraldehyde reacts with the amino group of the antibody and binds it covalently.

1.5. Sensing activity of an electrochemical biosensor

Electrochemical sensors are capable of detecting and quantifying a particular analyte or target molecule within a sample through electrochemical reactions. There are a few parameters that determine the performance of a sensor, such as sensitivity, linearity, selectivity, detection limit, and reliability.

- **Sensitivity**: Sensitivity reflects the sensor's ability to detect and measure the concentration of the analyte. A high sensitivity means that the sensor can detect small changes in analyte concentration with significant signal amplification. Sensitivity refers to the ratio of change in the output signal to the change in analyte concentration and can be determined from the slope of the linear regression curve.
- **Linearity**: The linearity of a sensor can be measured by plotting the relationship between the value of its output signal and its analyte concentration. The range of analyte concentration over which the sensor displays minimal deviation from this straight line is known as linear range. It is desirable to have high linearity in most

applications since it simplifies calibration and ensures accurate measurements. The calibration equation is the equation of a straight line corresponding to the linear range, which is called a linear equation.

- **Selectivity**: Selectivity measures the sensor's ability to differentiate the target analyte from other potentially interfering substances in the sample. An ideal sensor will have minimal response to interfering substances, ensuring accurate and reliable detection of the target analyte.
- **Limit of Detection (LOD)**: The LOD refers to the lowest concentration of the analyte that a sensor is capable of detecting with a high degree of precision. It is a critical parameter for determining the trace amounts of the analyte. The LOD can be calculated using the formula:

$$LOD = 3.3 \times \frac{\sigma_y}{m} \; ;$$

Where m is the slope of the linear calibration curve and σ_y is the standard deviation of the y-intercept.

- **Repeatability**: Repeatability refers to the sensor's ability to produce identical results consistently under the similar test conditions. High repeatability is essential for achieving precise and reliable measurements. To assess this feature, sensing experiments can be repeated while keeping all other parameters constant. In electrochemical sensing, multiple electrodes with the same composition are used to conduct the experiments several times. Good repeatability is indicated by consistent quantitative measurements and a low standard deviation, demonstrating the sensor's reliability in generating stable results across multiple trials

1.6. Statement of problem and scope of the thesis

There exist serious concerns on diseases associated with elevated levels of pressure, albumin, blood glucose, etc. The conditions imposed by Diabetes mellitus is considered to be a deadly one. Globally, approximately 537 million people are affected by diabetes, leading to millions of deaths each year as a consequence of this disease according to statistics from the International Diabetes Federation (IDF), 2021 [157]. If not examined properly, abnormal glucose level in human body leads to serious difficulties such as blindness, kidney failure, heart attack, stroke and many more (WHO 1016) [158].

Glycemic control, in managing blood glucose levels in diabetic patients, has long been recognized as beneficial. Continuous glucose monitoring can reduce diabetes-induced complications by a great deal, allowing diabetics to maintain a healthy lifestyle while avoiding the costly, and sometimes fatal, complications associated with late-stage diabetes [159,160]. Since the introduction of self-testing electrochemical meters for monitoring blood sugar in the 1980s and their widespread use during the 1990s, glucose monitoring technology has advanced significantly. Till now several techniques have been considered for accurate and continuous monitoring of glucose level such as chromatography, polarimetry and spectroscopy technique [159]. However, these techniques suffer from certain limitations. These assays are expensive and demand specialised people, making them unaffordable for poor developing nations where the risk of disease and outbreak is highest [161]. In order to tackle separation and interference issues, these procedures also demand knowledge, time, and experience. To defeat these obstacles, simpler, user friendly, highly sensitive and selective biosensing device is highly desirable [162].

Detecting glucose in real samples like blood, saliva, sweat or urine is crucial for understanding the metabolic health condition of a person and is often used to diagnose and monitor conditions such as diabetes and stress-related blood sugar [163]. A huge amount of research focuses on blood glucose detection because of its key role in managing diabetes [164]. Methods range from traditional approaches like finger-pricking and drawing blood to more advanced continuous glucose monitoring (CGM) systems [165,166]. However, invasive methods like finger pricks have some significant drawbacks. The most noticeable is the pain and discomfort caused by repeated pricking, which can lead to skin irritation and soreness. For people who need frequent glucose testing, this discomfort can discourage regular monitoring, making it harder to manage their condition effectively [167]. In addition, invasive techniques can increase the risk of infection, especially in individuals with weak immune systems or slow healing, which is common in people with diabetes. The need for needles, lancets, and test strips also makes these methods less convenient and more expensive over time. Furthermore, many people, especially children or those afraid of needles, find these procedures anxiety-inducing, making long-term use difficult. These issues show the growing need for more patient-friendly, non-invasive glucose monitoring options. Non-invasive methods, such as detecting glucose in saliva, offer a painless and convenient alternative, making it easier for patients to monitor their glucose levels regularly and manage their health more effectively [168].

On the other hand, Mycotoxins are natural toxic contaminants that can be found in food and feed resulting from the growth of fungal organisms on grain, seed, or any other agricultural products during storage and transportation [169]. Animals and humans can be adversely affected by these toxic substances upon consumption. Among all mycotoxins, Aflatoxin (AF) is the most dangerous and toxic in naturally occurring substances [170]. Although AFs are available in 18 different types, only 6 are generally regulated for food control purposes due to their high toxicity which are the most prevalent in the environment [170,171]. To be specific, AF-B_1, AF-B_2, AF-G_1 and AF-G_2 are the four main aflatoxins, and AF-M_1 and AF-M_2 are two other metabolites of aflatoxins [172]. As one of the most toxic aflatoxins, AF-B_1 has been listed as a Group I human carcinogen by the IARC, an international agency for cancer research determines that this substance may cause cancer in liver, lung, stomach etc. [173]. It is also worth noting that, in addition to their potential as carcinogens, AFs are also teratogens and mutagens in proportions as follows: AF-G_2<AF-B_2<AF-M_2<AF-G_1<AF-M_1<AF-B_1 [174]. Because of the impact of AF-B_1, a great variety of foods and beverages can become contaminated, including peanuts, corn, wheat, barley, cottonseed, spices, dried fruits, cereals, soy products etc. [175-178]. Notably AF-B_1 is prone to acute toxicity with a wide distribution area and chemical stability, which makes it difficult to remove, so the best way to control is in prevention [179]. To detect AF-B_1 in a rapid and sensitive manner, there exist an urgent need to develop a reliable method. Till date, various analytical assays have been reported for detecting Aflatoxin B_1, like: ELISA, HPLC, TLC and many more [172]. It should be noted that although chromatography-based methods have the advantage of being robust, sensitive, and selective, they are typically dependent on well-equipped laboratories, a lengthy pre-treatment of samples, and skilled operators, which limits their application to serve as a means of routine diagnostic purposes. As a result of these disadvantages, various electrochemical biosensors have been suggested so far to detect AF-B_1 to overcome this obstacle.

Conducting polymers are widely explored in the field of electrochemical sensing due to their inherent conductivity, biocompatibility, large surface area and good redox activity with easy synthesis strategy. To date, few studies have explored PEDOT-PSS and PANI as an electrode material for application in electrochemical sensors and biosensors. For the detection of xanthine (XA), Khan et al. developed a biosensor based on glassy carbon electrode modified with PEDOT-PSS and gold nanoparticles

(GCE/PEDOT-PSS/AuNPs) [180]. The sensor demonstrated sensitive and selective detection of XA, among other interferents such as hypoxanthine (HXA) and uric acid (UA), with a limit of detection (LOD) of 30 nM. It also yielded satisfactory result for XA detection in real samples, including fish and meat samples. An amperometric sensor based on screen printed carbon electrodes (SPCEs) with AuNP/PEDOT-PSS was developed by Phongphut et al. for the detection of triglyceride (TG) with a response time of 30 s within a wide dynamic range from 0 to 531 mg/dL, having a detection limit of 7.88 mg/dL [181]. An effective electrochemical sensor was proposed by Manivannan et al. for detection of chlorogenic acid in real samples by using zinc oxide (ZnO) covered PEDOT-PSS and glassy carbon electrode [182]. The sensing platform showed a wide linearity in between 0.03 μM – 476.2 μM towards oxidation of chlorogenic acid with a very high sensitivity 29.38 $\mu A \mu M^{-1} cm^{-2}$ and LOD was found to be 0.02 μM in soft drink along with coffee powder [182]. Kergoat et al. fabricated PEDOT-PSS/platinum nanoparticle composites for detection of neurotransmitters of human body (known as Glutamate and Acetylcholine) and achieved linearity in the range 0.9–14 μM for Glutamate. For both Glutamate and Acetylcholine detection they have found a low LOD of 5 μM [183]. Using the electrospray technique, Liu et al. developed PEDOT-PSS/graphene nanoplatelet (GNP) composites on an FTO electrode for detecting dopamine in the presence of interferents like uric acid and ascorbic acid in aqueous solutions. The bio-electrode exhibited enhanced selectivity with a LOD of 105 nM (S/N = 3) and a sensitivity of 27.7 $\mu A\ \mu M^{-1}\ cm^{-2}$ [184]. Wong et al. fabricated a highly sensitive rGO/PEDOT-PSS/GCE sensor for the selective and simultaneous detection of nimesulide and piroxicam [185]. Utilizing square wave voltammetry (SWV), the sensor detected these compounds in the micromolar concentration range, achieving limits of detection of 100 nM for piroxicam and 2.4 nM for nimesulide. The device provided reliable results when analyzing river water and pharmaceutical samples.

The development of glucose sensors based on various materials has been reported over the years. Without utilizing any external redox probe, a micromolar sensitive glucose sensor was demonstrated by Kanakamedala et al. by using an electrochemical transistor based on PEDOT-PSS that can detect glucose in the concentration range, 10 mM [186]. Senel et al. synthesized a glucose sensor relying on polypyrrole/chitosan/AuNP composite. The obtained sensor displayed reproducible sensitivity of 0.58 μA/mM with linearity in between 1 to 20 mM and LOD 0.068 mM [187]. Zahed et al. developed a PEDOT-PSS-

based flexible electrochemical biosensor for glucose detection, incorporating 3D stable porous laser-induced graphene (LIG) modified with platinum-palladium nanoparticles (Pt@Pd). The sensor exhibited high selectivity, a wide linear range from 10 μM to 9.2 mM, and a detection limit of 3 μM [188]. It was found that, incorporation of 2D TMDCs nanostructures have high potential to synergistically enhance the electrochemical performance as well as sensing activity of the sensor. Rohaizad et al. developed a WS_2-based glucose sensor that demonstrated a linear range from 180 to 770 μM and a LOD of 82.6 μM [189]. Authors have shown that tungsten dichalcogenides (WS_2, WSe_2) are superior to their molybdenum counterparts (MoS_2, $MoSe_2$) [189]. Jeong et al. reported a glucose biosensor based on a composite of MoS_2 and graphene sheets, which exhibited linearity in the range of 2 to 20 mM, a LOD of 290 μM, and a sensitivity of 3.36 μA mM^{-1} for glucose oxidation [190]. Further, Miao et al. developed a highly stable and reproducible biosensor for detecting glucose with gold nanoparticles- polyaniline and polyvinylpyrrolidone- nanocomposite over glassy carbon electrode [191]. The sensor exhibited a good linearity with the low LOD of 0.1 μM, and achieved as high as sensitivity of 9.62 μA mM^{-1} cm^{-2} [191]. Still there exist a huge scope to improve the analytical parameters of the glucose sensors.

A highly sensitive immunosensor was developed by Shi et al. to detect AF-B_1 with Graphene/Polyaniline nanohybrid enriched with AuNPs [185]. The sensor exhibited good sensitivity and linearity (0.05 to 25 ng/mL) with LOD 0.034 ng/mL for detecting AF-B_1 [192]. The use of piezoelectric quartz crystal microbalance (QCM) was reported by Spinelle et al. for detection of AF-B_1 within the range from 0.5 to 10.0 ppb [193]. Jin et al. utilized a QCM to detect AF-B_1 in artificially adulterated milk samples, achieving detection within a concentration range of 0.01 to 10.0 ng mL^{-1} [194]. The SPR-based sensor demonstrated a sensing range of 3.0 to 98.0 ng mL^{-1} and exhibited satisfactory reproducibility [195]. Colorimetric approach for AF-B_1 detection was reported based on interaction of gelatin functionalized AuNPs mediated by a specific enzymatic reaction. The authors observed linearity within the range of 10.0–140.0 pg mL^{-1} with a LOD of 4.0 pg mL^{-1} [196]. Setlem et al. developed a fluorescent aptasensor for detecting AF-B_1, utilizing graphene oxide-mediated quenching. The sensor achieved a LOD of 20 ppb and demonstrated linearity over a range of 0.2 to 200.0 ppb [197]. Using AuNPs, Xu et al. fabricated a DNA based sensor and achieved a LOD of 2.3 nM [198]. Dehaghani et al. designed an aptasensor using AuNPs and carbon nanodots, combined with two redox

probes, for the simultaneous detection of AF-B_1 and Ochratoxin A via an electrochemical strategy. The sensor achieved a concentration range from 1.0×10^{-2} to 100.0 ng mL^{-1}, with detection limits of 5.2×10^{-3} ng mL^{-1} for AF-B_1 and 4.3×10^{-3} ng mL^{-1} for Ochratoxin A. [199]. Another aptasensor was developed Dehaghani et al. by decorating AuNPs on the surface of nickel-based (Ni) metal-organic framework (MOF) nanosheets and incorporating a ferrocene redox probe. This aptasensor achieved a LOD of 1.0×10^{-3} ng mL^{-1} and demonstrated a concentration range from 5.0×10^{-3} to 150.0 ng mL^{-1} for AF-B_1 detection [200]. Using a GO/AuNP composite platform, Dadmehr et al. developed another aptasensor for detecting AF-B_1 [201]. The sensor exhibited a linear response within the range of 0.5 to 20.0 pg mL^{-1} and achieved a LOD of 0.1 pg mL^{-1}. Optical waveguide laser mode spectroscopy has also been used in both competitive and direct immunoassays by Adanyi et al. to detect AF-B_1 and ochratoxin-A entities [202]. Analysis of AF-B_1 in real samples of wheat flour and barley displayed the LOD values of approximately 10.0 and 0.5 ng mL^{-1}; respectively. While optical sensors provide high sensitivity, their high costs and bulkiness often make electrochemical sensors the preferred choice for many practical applications [195,202]. Continued advance research is needed to develop more effective detection, prevention, and detoxification methods to ensure food safety and protect human and animal health from the adverse effects of AF-B_1.

Composite conducting polymers with 2D nanostructures and metallic nanoparticles, as indicated in the literature, can notably enhance the stability and catalytic activity of the hybrid nanosystems, thereby leading to a more effective detection mechanism.

Being motivated from the above aspects, the following objectives have been set to address the key points for this thesis:

1. To employ physicochemical techniques for fabricating conducting polymers (PEDOT-PSS, PANI/PVA)

2. To incorporate 2D nanomaterials (GO, TMDCs) into conducting polymer followed by surface anchoring with metallic Au nanoparticles

3. To immobilize specific biomolecules (anti-Aflatoxin B_1, mouse IgG, glucose oxidase) on the surface of the conducting polymer deposited ITO electrodes via covalent linkers

4. To develop and evaluate electrochemical biosensors for enzymatic detection of glucose and Aflatoxin B_1

Based upon the aforementioned goals, the entire work of the thesis has been organized into seven chapters. The glucose sensor is primarily used in the medical sector, especially for diagnosing and managing diabetes by continuously monitoring blood glucose levels. It typically employs an enzymatic reaction, where glucose oxidase catalyzes glucose to produce a measurable electrical signal which directly correlates with glucose concentration in the blood. This simple technology is vital for patients who require precise and timely information to manage their health condition effectively. On the other hand, the AF-B_1 sensor is devoted to ensuring food safety. This sensor uses antibodies to identify and quantify AF-B_1, providing critical information to prevent food contamination and protect human health. A glucose sensor for diagnostic purposes and an AF-B_1 sensor for food safety are two independent biosensors, each serving distinct and critical roles in their respective fields. Both sensors operate independently, with the glucose sensor focused on human health diagnostics and the AF-B_1 sensor on food safety, highlighting their applications in different domains. Chapter 4, 5 and 6 include two parts: (A) and (B) for the respective systems. Part A explains the development of a glucose sensor while part B shows the fabrication of AF-B_1 sensor. Each developed sensor demonstrates practical applicability by being tested with real-life samples.

1.7. References

[1] Miracle, D. B. Metal matrix composites–from science to technological significance. *Composites science and technology*, 65:2526-2540, 2005.

[2] Chen, K., Xue, D. Materials chemistry toward electrochemical energy storage. *Journal of Materials Chemistry A*, 4:7522-7537, 2016.

[3] Guo, C. X., Li C. M. Recent advances in soft materials to build and functionalize hard structures for electrochemical energy storage and in situ electrochemical molecular biosensing. *Advanced Functional Materials,* 26:8824-8853, 2016.

[4] Jensen, W.B. The origin of the polymer concept. *Journal of chemical education*, 85:624, 2008.

[5] Inzelt, G. Rise and rise of conducting polymers. *Journal of Solid State Electrochemistry*, 15:1711-1718, 2011.

[6] Shirakawa, H., Louis, E.J., MacDiarmid, A.G., Chiang, C.K. and Heeger, A.J. Synthesis of electrically conducting organic polymers: halogen derivatives of polyacetylene, (CH)x. *Journal of the Chemical Society, Chemical Communications,* 16:578-580, 1977.

[7] Pohl, H. A. Engelhardt, E. H. Synthesis and characterization of some highly conjugated semiconducting polymers. *Journal of Physical Chemistry*, 66:2085, 1962.

[8] Qiu, F., Hou, T., Huang, D., Xue, Z., Liang, D., Li, Q., and Lin, W. Evaluation of two high-abundance protein depletion kits and optimization of downstream isoelectric focusing. *Molecular Medicine Reports*, 12(5):7749-7755, 2015.

[9] Zhang, W., Wang, R., Luo, F., Wang, P., and Lin, Z. Miniaturized electrochemical sensors and their point-of-care applications. *Chinese Chemical Letters*, 31(3):589-600, 2020.

[10] Sun, Y., Li, Y., Wang, N., Xu, Q. Q., Xu, L., and Lin, M. Copper-based Metal-organic Framework for Non-enzymatic Electrochemical Glucose. *Electroanalysis*, 30(3):474-478, 2018.

[11] Shirakawa, H. Nobel Lecture: The discovery of polyacetylene film-the dawning of an era of conducting polymers, *Reviews of Modern Physics*, 73:713-718, 2001.

[12] Wernet, W., Monkenbusch, M., and Wegner, G. A new series of conducting polymers with layered structure: Polypyrrole n-alkylsulfates and n-alkylsulfonates. *Die Makromolekulare Chemie, Rapid Communications*, 5(3):157-164, 1984.

[13] Cui, C. X., and Kertesz, M. Two helical conformations of Polythiophene, polypyrrole, and their derivatives, *Physical Review B*, 40(14):9661 9670, 1989.

[14] Groenendaal, L., Jonas, F., Freitag, D., Pielartzik, H and Reynolds, J.R. Poly(3,4-ethylenedioxythiophene) and Its Derivatives: Past, Present, and Future. *Advanced Materials*, 12(7):481-494, 2000.

[15] Biallozor, S. and Kupniewska, A. Conducting polymers electrodeposited on active metals. *Synthetic metals*, 155(3):443-449, 2005.

[16] Pei, J., Yu, W. L., Huang, W., and Heeger, A. J. A novel series of efficient thiophene-based light-emitting conjugated polymers and application in polymer light-emitting diodes. *Macromolecules*, 33(7):2462-2471, 2000.

[17] Hangarter, C. M., Bangar, M., Mulchandani, A., and Myung, N. V. Conducting polymer nanowires for chemiresistive and FET-based bio/chemical sensors. *Journal of Materials Chemistry*, 20(16):3131-3140, 2010.

[18] Coskun, Y. E. N. E. R., Cirpan, A., and Toppare, L. E. V. E. N. T. Construction of electrochromic devices using thiophene based conducting polymers. *Journal of materials science*, 42:368-372, 2007.

[19] Naka, Y., Fuchiwaki, M., and Tanaka, K. A micropump driven by a polypyrrole-based conducting polymer soft actuator. *Polymer international*, 59(3):352-356, 2010.

[20] Snook, G. A., Kao, P., and Best, A. S. Conducting-polymer-based supercapacitor devices and electrodes. *Journal of power sources*, 196(1):1-12, 2011.

[21] Lei, W., Si, W., Xu, Y., Gu, Z., and Hao, Q. Conducting polymer composites with graphene for use in chemical sensors and biosensors. *Microchimica acta*, 181:707-722, 2014.

[22] Gerard, M., Chaubey, A., and Malhotra, B. D. Application of conducting polymers to biosensors. *Biosensors and bioelectronics*, 17(5):345-359, 2002.

[23] Batchelder, D. N. Colour and chromism of conjugated polymers. *Contemporary physics*, 29(1):3-31, 1988.

[24] Orton, J.W. The story of the semiconductor, Oxford University Press Inc, New York. 2004.

[25] Atkins, P.W., Paula, J.D. Physical Chemistry, Oxford University Press, Canada. 2002.

[26] Nordlander, P., and Tully, J. C. Energy shifts and broadening of atomic levels near metal surfaces. *Physical Review B*, 42(9):5564. 1990.

[27] Stafstrom, S. and Chao, K.A. Polaron-bipolaron–soliton doping in polyacetylene. *Physical Review B*, 30:2098-2103, 1984.

[28] Bredas, J.L. and Street, G.B. Polarons, bipolarons, and solitons in conducting polymers. *Accounts of chemical research*,18:309-315, 1985.

[29] Bakhshi, A., Kaur, A. and Arora, V. Molecular engineering of novel low band gap conducting polymers. *Indian Journal of Chemistry, Sect A*, 51(1):57, 2012.

[30] Ravichandran, R., Sundarrajan, S., Venugopal, J.R., Mukherjee, S. and Ramakrishna, S. Applications of conducting polymers and their issues in

biomedical engineering. *Journal of the Royal Society Interface*, 7:S559-S579, 2010.

[31] Wise, D.L., Wnek, G.E., Trantolo, D.J., Cooper, T.M., Gresser, J.D. and Marcel, D. Electrical and Optical Polymer Systems: Fundamentals, Methods and Application, pages 1031-1040. CRC Press: Boca Raton, FL, USA, 1998.

[32] Salaneck, W.R., Friend and R.H. and Bredas, J.L. Electronic structure of conjugated polymers: Consequences of electron-lattice coupling. *Physics reports,* 319(6):231-251, 1999.

[33] Sharma, S., Sudhakara, P., Omran, A. A. B., Singh, J., and Ilyas, R. A. Recent trends and developments in conducting polymer nanocomposites for multifunctional applications. *Polymers*, 13(17):2898, 2021.

[34] Nardes, A. M., Kemerink, M., De Kok, M. M., Vinken, E., Maturova, K., and Janssen, R. A. J. Conductivity, work function, and environmental stability of PEDOT: PSS thin films treated with sorbitol. *Organic electronics*, 9(5):727-734, 2008.

[35] Seekaew, Y., Lokavee, S., Phokharatkul, D., Wisitsoraat, A., Kerdcharoen, T., and Wongchoosuk, C. Low-cost and flexible printed graphene–PEDOT: PSS gas sensor for ammonia detection. *Organic Electronics*, 15(11):2971-2981, 2014.

[36] Aziz, S., Chang, D. E., Doh, Y. H., Kang, C. U., and Choi, K. H. Humidity sensor based on PEDOT:PSS and zinc stannate nano-composite. *Journal of Electronic Materials*, 44:3992-3999, 2015.

[37] Bai, H., and Shi, G. Gas sensors based on conducting polymers. *Sensors*, 7(3):267-307, 2007.

[38] Ahuja, T., and Kumar, D. Recent progress in the development of nano-structured conducting polymers/nanocomposites for sensor applications. *Sensors and Actuators B: Chemical*, 136(1):275-286, 2009.

[39] Odaci, D., Kayahan, S. K., Timur, S., and Toppare, L. Use of a thiophene-based conducting polymer in microbial biosensing. *Electrochimica Acta*, 53(12):4104-4108, 2008.

[40] Kim, N., Petsagkourakis, I., Chen, S., Berggren, M., Crispin, X., Jonsson, M.P. and Zozoulenko, I. Electric transport properties in PEDOT thin films. In Conjugated Polymers, pages 45-128, ISBN: 9780429190520. CRC Press, 2019.

[41] Groenendaal, L., Jonas, F., Freitag, D., Pielartzik, H., and Reynolds, J. R. Poly (3, 4-ethylenedioxythiophene) and its derivatives: past, present, and future. *Advanced materials*, 12(7):481-494, 2000.

[42] Yue, R., and Xu, J. Poly (3, 4-ethylenedioxythiophene) as promising organic thermoelectric materials: A mini-review. *Synthetic metals*, 162(11-12):912-917, 2012.

[43] Ismailova, E., Doublet, T., Khodagholy, D., Quilichini, P., Ghestem, A., Yang, S. Y., ... and Malliaras, G. G. Plastic neuronal probes for implantation in cortical and subcortical areas of the rat brain. *International journal of nanotechnology*, 9(3-7):517-528, 2012.

[44] Ouyang, J. "Secondary doping" methods to significantly enhance the conductivity of PEDOT:PSS for its application as transparent electrode of optoelectronic devices. *Displays*, 34(5):423-436, 2013.

[45] Richardson-Burns, S. M., Hendricks, J. L., and Martin, D. C. Electrochemical polymerization of conducting polymers in living neural tissue. *Journal of neural engineering*, 4(2):L6, 2007.

[46] Lee, C. P., Lai, K. Y., Lin, C. A., Li, C. T., Ho, K. C., Wu, C. I., ... and He, J. H. A paper-based electrode using a graphene dot/PEDOT:PSS composite for flexible solar cells. *Nano Energy*, 36:260-267, 2017.

[47] Higgins, T. M., Park, S. H., King, P. J., Zhang, C., McEvoy, N., Berner, N. C., ... and Coleman, J. N. A commercial conducting polymer as both binder and conductive additive for silicon nanoparticle-based lithium-ion battery negative electrodes. *Acs Nano*, 10(3): 3702-3713, 2016.

[48] Cheng, T., Zhang, Y. Z., Zhang, J. D., Lai, W. Y., and Huang, W. High-performance free-standing PEDOT: PSS electrodes for flexible and transparent all-solid-state supercapacitors. *Journal of Materials Chemistry A*, 4(27):10493-10499, 2016.

[49] Wang, H., Wang, J., Hong, L., Tan, Y. H., Tan, C. S., and Rusli. Thin film silicon nanowire/PEDOT: PSS hybrid solar cells with surface treatment. *Nanoscale Research Letters*, 11:1-10, 2016.

[50] Zhang, M., Yuan, W., Yao, B., Li, C., and Shi, G. Solution-processed PEDOT: PSS/graphene composites as the electrocatalyst for oxygen reduction reaction. *ACS applied materials & interfaces*, 6(5):3587-3593, 2014.

[51] Wang, Z., Xu, J., Yao, Y., Zhang, L., Wen, Y., Song, H., and Zhu, D. Facile preparation of highly water-stable and flexible PEDOT: PSS organic/inorganic composite materials and their application in electrochemical sensors. *Sensors and Actuators B: Chemical*, 196:357-369, 2014.

[52] Collazos-Castro, J. E., Hernandez-Labrado, G. R., Polo, J. L., and Garcia-Rama, C. N-Cadherin-and L1-functionalised conducting polymers for synergistic stimulation and guidance of neural cell growth. *Biomaterials*, 34(14):3603-3617, 2013.

[53] Sun, K., Zhang, S., Li, P., Xia, Y., Zhang, X., Du, D., ... and Ouyang, J. Review on application of PEDOTs and PEDOT: PSS in energy conversion and storage devices. *Journal of Materials Science: Materials in Electronics*, 26:4438-4462, 2015.

[54] Fan, X., Nie, W., Tsai, H., Wang, N., Huang, H., Cheng, Y., ... and Xia, Y. PEDOT: PSS for flexible and stretchable electronics: modifications, strategies, and applications. *Advanced Science*, 6(19):1900813, 2019.

[55] Genies, E. M., Boyle, A., Lapkowski, M., and Tsintavis, C. Polyaniline: A historical survey. *Synthetic metals*, 36(2):139-182, 1990.

[56] Chiang, J. C., and MacDiarmid, A. G. 'Polyaniline': protonic acid doping of the emeraldine form to the metallic regime. *Synthetic Metals*, 13(1-3):193-205, 1986.

[57] MacDiarmid, A. G. Polyaniline and polypyrrole: where are we headed?. *Synthetic Metals*, 84(1-3):27-34, 1997.

[58] Rasmussen, S. C. The early history of polyaniline: discovery and origins. *Substantia*, 1(2):99-109, 2017.

[59] Green, A. G., and Woodhead, A. E. CCXLIII.—Aniline-black and allied compounds. Part I. *Journal of the Chemical Society, Transactions*, 97:2388-2403, 1910.

[60] Rasmussen, S. C. Early history of conductive organic polymers. In *Conductive Polymers*, pages 1-22. CRC Press, 2018.

[61] Albuquerque, J. E., Mattoso, L. H. C., Balogh, D. T., Faria, R. M., Masters, J. G., and MacDiarmid, A. G. A simple method to estimate the oxidation state of polyanilines. *Synthetic Metals*, 113(1-2):19-22, 2000.

[62] Bagheri, H., Ayazi, Z., and Naderi, M. Conductive polymer-based microextraction methods: a review. *Analytica chimica acta*, 767:1-13, 2013.

[63] Komathi, S., Gopalan, A. I., Muthuchamy, N., and Lee, K. P. Polyaniline nanoflowers grafted onto nanodiamonds via a soft template-guided secondary nucleation process for high-performance glucose sensing. *RSC advances*, 7(25):15342-15351, 2017.

[64] Dhand, C., Das, M., Datta, M., and Malhotra, B. D. Recent advances in polyaniline based biosensors. *Biosensors and bioelectronics*, 26(6):2811-2821, 2011.

[65] Wang, L., Liu, N., and Ma, Z. Novel gold-decorated polyaniline derivatives as redox-active species for simultaneous detection of three biomarkers of lung cancer. *Journal of Materials Chemistry B*, 3(14):2867-2872, 2015.

[66] Wang, L., Feng, F., and Ma, Z. Novel electrochemical redox-active species: one-step synthesis of polyaniline derivative-Au/Pd and its application for multiplexed immunoassay. *Scientific reports*, 5(1):16855, 2015.

[67] Kausaite-Minkstimiene, A., Mazeiko, V., Ramanaviciene, A., and Ramanavicius and A. Enzymatically synthesized polyaniline layer for extension of linear detection region of amperometric glucose biosensor. *Biosensors and Bioelectronics*, 26(2):790-797, 2010.

[68] Prabhakar, P. K., Raj, S., Anuradha, P. R., Sawant, S. N., and Doble, M. Biocompatibility studies on polyaniline and polyaniline–silver nanoparticle coated polyurethane composite. *Colloids and Surfaces B: Biointerfaces*, 86(1):146-153, 2011.

[69] Lai, J., Yi, Y., Zhu, P., Shen, J., Wu, K., Zhang, L., and Liu, J. Polyaniline-based glucose biosensor: A review. *Journal of Electroanalytical Chemistry*, 782:138-153, 2016.

[70] Zhang, R., and Cheung, R. Mechanical properties and applications of two-dimensional materials. Two-dimensional Materials-Synthesis, Characterization and Potential Applications. Rijeka, Crotia: InTech, 219-246, 2016.

[71] Rafiei-Sarmazdeh, Z., Zahedi-Dizaji, S. M., and Kang, A. K. Two-Dimensional Nanomaterials. London, UK: IntechOpen, 2019.

[72] Novoselov, K. S., Geim, A. K., Morozov, S. V., Jiang, D. E., Zhang, Y., Dubonos, S. V., ... and Firsov, A. A. Electric field effect in atomically thin carbon films. *science*, 306(5696):666-669, 2004.

[73] Das, S., Robinson., J. A., Dubey, M., Terrones, H. and Terrones, M., Beyond graphene: progress in novel two-dimensional materials and van der Waals solids. Ann. *Annual review of materials research*, 45:1–27, 2015.

[74] Fawaz, M., Bahadur, R., Dharmarajan, N. P., Yang, J. H., Sathish, C. I., Sadanandan, A. M., ... and Vinu, A. Emerging trends of carbon nitrides and their hybrids for photo-/electro-chemical energy applications. *Carbon*, 214:118345, 2023.

[75] Yang, L., Chen, W., Yu, Q. and Liu, B., Mass production of two-dimensional materials beyond graphene and their applications. *Nano Research*, 1-15, 2020.

[76] Wang, H., Yuan, X., Zeng, G., Wu, Y., Liu, Y., Jiang, Q., and Gu, S. Three dimensional graphene based materials: Synthesis and applications from energy storage and conversion to electrochemical sensor and environmental remediation. *Advances in colloid and interface science*, 221:41-59, 2015.

[77] Jin, H., Guo, C., Liu, X., Liu, J., Vasileff, A., Jiao, Y., ... and Qiao, S. Z. Emerging two-dimensional nanomaterials for electrocatalysis. *Chemical Reviews*, 118(13):6337-6408, 2018.

[78] Karthick Kannan, P., Shankar, P., Blackman, C., and Chung, C. H. Recent advances in 2D inorganic nanomaterials for SERS sensing. *Advanced Materials*, 31(34):1803432, 2019.

[79] Hossain, N., Mobarak, M. H., Mimona, M. A., Islam, M. A., Hossain, A., Zohura, F. T., and Chowdhury, M. A. Advances and significances of nanoparticles in semiconductor applications–A review. *Results in Engineering*, 19:101347, 2023.

[80] Khan, K., Tareen, A. K., Aslam, M., Wang, R., Zhang, Y., Mahmood, A., ...and Guo, Z. Recent developments in emerging two-dimensional materials and their applications. *Journal of Materials Chemistry C*, 8(2):387-440, 2020.

[81] Chia, X., Ambrosi, A., Sofer, Z., Luxa, J., and Pumera, M. Catalytic and charge transfer properties of transition metal dichalcogenides arising from electrochemical pretreatment. *ACS nano*, 9(5):5164-5179, 2015.

[82] Kaplan, A., Yuan, Z., Benck, J. D., Rajan, A. G., Chu, X. S., Wang, Q. H., and Strano, M. S. Current and future directions in electron transfer chemistry of graphene. *Chemical Society Reviews*, 46(15):4530-4571, 2017.

[83] Liu, Y., Yu, D., Zeng, C., Miao, Z., and Dai, L. Biocompatible graphene oxide-based glucose biosensors. *Langmuir*, 26(9):6158-6160, 2010.

[84] Huang, X., Yin, Z., Wu, S., Qi, X., He, Q., Zhang, Q., ... and Zhang, H. Graphene-based materials: synthesis, characterization, properties, and applications. *small*, 7(14): 1876-1902, 2011.

[85] Compton, O. C., and Nguyen, S. T. Graphene oxide, highly reduced graphene oxide, and graphene: versatile building blocks for carbon-based materials. *small*, 6(6):711-723, 2010.

[86] Dreyer, D. R., Park, S., Bielawski, C. W., and Ruoff, R. S. The chemistry of graphene oxide. *Chemical society reviews*, 39(1):228-240, 2010.

[87] Xu, J., Wang, Y., and Hu, S. Nanocomposites of graphene and graphene oxides: synthesis, molecular functionalization and application in electrochemical sensors and biosensors. A review. *Microchimica Acta*, 184:1-44, 2017.

[88] Wilson, J. A., and Yoffe, A. D. The transition metal dichalcogenides discussion and interpretation of the observed optical, electrical and structural properties. *Advances in Physics*, 18(73):193-335, 1969.

[89] Jariwala, D., Sangwan, V. K., Lauhon, L. J., Marks, T. J., and Hersam, M. C. Emerging device applications for semiconducting two-dimensional transition metal dichalcogenides. *ACS nano*, 8(2):1102-1120, 2014.

[90] Chhowalla, M., Shin, H. S., Eda, G., Li, L. J., Loh, K. P., and Zhang, H. The chemistry of two-dimensional layered transition metal dichalcogenide nanosheets. *Nature chemistry*, 5(4):263-275, 2013.

[91] Li, X., Shan, J., Zhang, W., Su, S., Yuwen, L., and Wang, L. Recent advances in synthesis and biomedical applications of two-dimensional transition metal dichalcogenide nanosheets. *Small*, 13(5):1602660, 2017.

[92] Li, N., Liu, Z., Hu, S., and Wang, H. Structural and electronic properties of effective p-type doping WS_2 monolayers: A computational study. *Solid State Communications*, 269:58-63, 2018.

[93] Jiang, L., Gallagher, J. D., Senaratne, C. L., Aoki, T., Mathews, J., Kouvetakis, J., and Menendez, J. Compositional dependence of the direct and indirect band

gaps in Ge1− ySny alloys from room temperature photoluminescence: implications for the indirect to direct gap crossover in intrinsic and n-type materials. *Semiconductor Science and Technology*, 29(11):115028, 2014.

[94] Wang, S., Ren, C., Tian, H., Yu, J., and Sun, M. MoS_2/ZnO van der Waals heterostructure as a high-efficiency water splitting photocatalyst: A first-principles study. *Physical Chemistry Chemical Physics*, 20(19):13394-13399, 2018.

[95] Lee, J. Y., Shin, J. H., Lee, G. H., and Lee, C. H. Two-dimensional semiconductor optoelectronics based on van der Waals heterostructures. *Nanomaterials*, 6(11):193, 2016.

[96] Pan, M., Yang, J., Liu, K., Yin, Z., Ma, T., Liu, S., ... and Wang, S. Noble metal nanostructured materials for chemical and biosensing systems. *Nanomaterials*, 10(2):209, 2020.

[97] Azharuddin, M., Zhu, G. H., Das, D., Ozgur, E., Uzun, L., Turner, A. P., and Patra, H. K. A repertoire of biomedical applications of noble metal nanoparticles. *Chemical Communications*, 55(49):6964-6996, 2019

[98] He, L., Liu, Y., Liu, J., Xiong, Y., Zheng, J., Liu, Y., and Tang, Z. Core–shell noble-metal@ metal-organic-framework nanoparticles with highly selective sensing property. *Angewandte Chemie International Edition*, *52*(13):3741-3745, 2013.

[99] Zhao, X., Zhao, H., Yan, L., Li, N., Shi, J., and Jiang, C. Recent developments in detection using noble metal nanoparticles. *Critical Reviews in Analytical Chemistry*, 50(2):97-110, 2020.

[100] Daniel, M. C., and Astruc, D. Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chemical reviews*, 104(1):293-346, 2004.

[101] Wang, J., Yang, L., Boriskina, S., Yan, B., and Reinhard, B. M. Spectroscopic ultra-trace detection of nitroaromatic gas vapor on rationally designed two-dimensional nanoparticle cluster arrays. *Analytical Chemistry*, 83(6):2243-2249, 2011.

[102] Guo, S., and Wang, E. Synthesis and electrochemical applications of gold nanoparticles. *Analytica chimica acta*, 598(2):181-192, 2007.

[103] Nie, S., and Emory, S. R. Probing single molecules and single nanoparticles by surface-enhanced Raman scattering. *science*, 275(5303):1102-1106, 1997.

[104] Aslan, K., Badugu, R., Lakowicz, J. R., and Geddes, C. D. Metal-enhanced fluorescence from plastic substrates. *Journal of fluorescence*, 15:99-104, 2005.

[105] Van Dorst, B., Mehta, J., Bekaert, K., Rouah-Martin, E., De Coen, W., Dubruel, P., ... and Robbens, J. Recent advances in recognition elements of food and environmental biosensors: A review. *Biosensors and Bioelectronics*, 26(4):1178-1194, 2010.

[106] Rodriguez-Mozaz, S., Lopez de Alda, M. J., and Barcelo, D. Biosensors as useful tools for environmental analysis and monitoring. *Analytical and bioanalytical chemistry*, 386:1025-1041, 2006.

[107] Vo-Dinh, T. and Cullum, B. Biosensors and biochips: advances in biological and medical diagnostics. *Fresenius Journal of Analytical Chemistry*, 366(6-7):540-51, 2000.

[108] Naresh, V., and Lee, N. A review on biosensors and recent development of nanostructured materials-enabled biosensors. *Sensors*, 21(4):1109, 2021.

[109] Sadik, O. and Wallace, G.G. Pulsed amperometric detection of proteins using antibody containing conducting polymers. Analytica Chimica Acta, 279:209-212, 1993.

[110] Evtugyn, G.A., Budnikov, H.C. and Nikolskaya, E.B. Sensitivity and selectivity of electrochemical enzyme sensors for inhibitor determination. *Talanta*, 46:465-484, 1998.

[111] Castillo, J., Gaspar, S., Leth, S., Niculescu, M., Mortari, A., Bontidean, I., ... and Csoregi, E. Biosensors for life quality: Design, development and applications. *Sensors and Actuators B: Chemical*, 102(2):179-194, 2004.

[112] Lazanas, A. C., and Prodromidis, M. I. Electrochemical impedance spectroscopy─ a tutorial. *ACS Measurement Science Au*, 3(3):162-193, 2023.

[113] Lazcka, O., Del Campo, F.J., and Munoz, F.X. Pathogen detection: A perspective of traditional methods and biosensors. *Biosensors and Bioelectronics*, 22:1205-1217, 2007.

[114] Wang, J., Rivas, G., Cai, X., Palecek, E., Nielsen, P., Shiraishi, H., Dontha, N., Luo, D., Parrado, C., Chicharro, M., Farias, P. and Valera, F.S. DNA

electrochemical biosensors for environmental monitoring: a review. Analytica Chimica Acta, 347:1-8, 1997.

[115] Wenjing, H., Yaoming, X., Gaoyi, H. and Haihan, Z. Electropolymerization of polypyrrole/multi-wall carbon nanotube counter electrodes for use in platinum-free dye-sensitized solar cells, *Electrochimica Acta*,190:720-728, 2016.

[116] Bhalla, N., Jolly, P., Formisano, N., and Estrela, P. Introduction to biosensors. *Essays in Biochemistry*, 60(1):1-8, 2016.

[117] D'Souza, S.F. Microbial biosensors. *Biosensors & Bioelectronics*, 16:337–353, 2001.

[118] Gunalan, K., Warren, D. J., Perry, J. D., Normann, R. A., and Clark, G. A. An automated system for measuring tip impedance and among-electrode shunting in high-electrode count microelectrode arrays. *Journal of neuroscience methods*, 178(2):263-269, 2009.

[119] Yang, L., and Bashir, R. Electrical/electrochemical impedance for rapid detection of foodborne pathogenic bacteria. Biotechnology Advances, 26(2):135-150, 2008.

[120] Carpick, R.W., Sasaki, D.Y., Marcus, M.S., Eriksson, M.A., and Burns, A.R. Polydiacetylene films: A review of recent investigations into chromogenic transitions and nanomechanical properties. *Journal of Physics: Condensed Matter*, 16 (23):R679-R697, 2004.

[121] Soto, A. G., Jaffari, S. A., and Bone, S. Characterisation and optimisation of AC conductimetric biosensors. *Biosensors and bioelectronics*, 16(1-2):23-29, 2001.

[122] Watts, H.J., Lowe, C.R., and Pollard-Knight, D.V. Optical biosensor for monitoring microbial cells. *Analytical Chemistry*, 66:2465-2470, 1994.

[123] Damborsky, P., Svitel, J., and Katrlik, J. Optical biosensors. *Essays in Biochemistry*, 60(1):91-100, 2016.

[124] Mohanty, S.P. and Kougianos, E. Biosensors: a tutorial review, *IEEE Potentials*, 25(2):35-40, 2006.

[125] Mullett, W. M., Lai, E. P., and Yeung, J. M. Surface plasmon resonance-based immunoassays. *Methods*, 22(1):77-91, 2000.

[126] Strianese, M., Staiano, M., Ruggiero, G., Labella, T., Pellecchia, C. and D'Auria, S. Fluorescence-based biosensors. *Methods in Molecular Biology*, 875:193-216, 2012.

[127] Su, Y.L., Li, J.R., Jiang, L., and Cao, J. Biosensor signal amplification of vesicles functionalized with glycolipid for colorimetric detection of Escherichia coli. *Journal of Colloid and Interface Science*, 284(1):114–119, 2005.

[128] Pohanka, M. The Piezoelectric Biosensors: Principles and Applications, a Review. *International Journal of Electrochemical Science*,12:496 -506, 2017.

[129] Rakow, N. A., and Suslick, K. S. A colorimetric sensor array for odour visualization. *Nature*, 406(6797):710-713, 2000.

[130] Ansari, S. A., and Husain, Q. Potential applications of enzymes immobilized on/in nano materials: A review. *Biotechnology advances*, 30(3):512-523, 2012.

[131] Schnell, S., Chappell, M.J., Evans, N.D., and Roussel and M.R. The mechanism distinguishability problem in biochemical kinetics: The singleenzyme, singlesubstrate reaction as a case study. *Comptes Rendus Biologies*, 329:51- 61, 2006.

[132] Bankar, S. B., Bule, M. V., Singhal, R. S., and Ananthanarayan, L. Glucose oxidase—an overview. *Biotechnology advances*, 27(4):489-501, 2009.

[133] Koshland Jr, D. E. The key–lock theory and the induced fit theory. *Angewandte Chemie International Edition in English*, *33*(23-24):2375-2378, 1995.

[134] Manohar, B. and Divakar, S. An artificial neural network analysis of porcine pancreas lipase catalysed esterification of anthranilic acid with methanol. *Process Biochemistry*, 40:3372-3376, 2005.

[135] Mathewson, P. R. Enzymes: Practical guides for the food industry. *Enzymes, Eagan Press Handbook Series, American Chemical Society, St. Paul*, 1998.

[136] Burton, D.R. The structure of immunoglobulins and their interaction with complement. Activators and Inhibitors of Complement,7:17-36, 1993.

[137] Campbell, A.M. Monoclonal Antibody and Immunosensor Technology, 1st edition, 23, 1991.

[138] Mendes, A. C., Baran, E. T., Reis, R. L., and Azevedo, H. S. Self-assembly in nature: using the principles of nature to create complex nanobiomaterials. *Wiley interdisciplinary reviews: nanomedicine and nanobiotechnology*, *5*(6), 582-612, 2013.

[139] Friguet, B, Chaffotte, A.F, Djavadi-Ohaniance, L. and Goldberg M.E. Measurements of the true affinity constant in solution of antigen – antibody

complexes by enzyme-linked immunosorbent assay. *Journal of Immunological Methods*, 77:305-19, 1985.

[140] Mayer, G. Immunoglobulins-antigen-antibody reactions and selected tests., Ph.D thesis, Emertius Professor of Pathology, Microbiology and Immunology, University of South Carolina, 2017.

[141] Zhou, Y., Goenaga, A.L., Harms, B.D., Zou, H., Lou, J., Conrad, F., Adams, G.P., Schoeberl, B. Nielsen, U.B., Marks, J.D. Impact of intrinsic affinity on functional binding and biological activity of EGFR antibodies. *Molecular Cancer Therapeutics*,11(7):1467-1476, 2012.

[142] Hughes-Jones, N.C., Gardner, B., Telford, R. The effect of pH and ionic strength on the reaction between anti-D and erythrocytes. *Immunology*, 7:72-81, 1964.

[143] Atchley, W.A., Bhagavan, N.V., Masouredis, S.P. Influence of ionic strength on the reaction between anti-D and D-positive red cells. *The Journal of Immunology*, 93(5):701-12, 1964.

[144] Ahuja, T., Mir, I.A., Kumar, D. R. Biomolecular immobilization on conducting polymers for biosensing applications. *Biomaterials*, 28:791-805, 2007.

[145] Brena, B. M., and Batista-Viera, F. Immobilization of enzymes: a literature survey. *Immobilization of enzymes and cells*, 15-30, 2006.

[146] Mansur, H. S., Orefice, R. L., Vasconcelos, W. L., Lobato, Z. P., & Machado, L. J. C. Biomaterial with chemically engineered surface for protein immobilization. *Journal of Materials Science: Materials in Medicine*, 16(4):333-340, 2005.

[147] Nanduri, V., Sorokulova, I. B., Samoylov, A. M., Simonian, A. L., Petrenko, V. A., and Vodyanoy, V. Phage as a molecular recognition element in biosensors immobilized by physical adsorption. *Biosensors and Bioelectronics*, 22(6):986-992, 2007.

[148] Sirisha, V. L., Jain, A., and Jain, A. Enzyme immobilization: an overview on methods, support material, and applications of immobilized enzymes. *Advances in food and nutrition research*, 79:179-211, 2016.

[149] Alzari, P. M., Lascombe, M. B., and Poljak, R. J. Three-dimensional structure of antibodies. *Annual review of immunology*, 6(1):555-580, 1988.

[150] Scouten, W. H., Luong, J. H., and Brown, R. S. Enzyme or protein immobilization techniques for applications in biosensor design. *Trends in biotechnology*, 13(5):178-185, 1995.

[151] Dave, B. C., Dunn, B., Valentine, J. S., and Zink, J. I. Sol-gel encapsulation methods for biosensors. *Analytical chemistry*, 66(22):1120A-1127A, 1994.

[152] Monosik, R., Streansky, M., and Sturdík, E. Biosensors-classification, characterization and new trends. *Acta chimica slovaca*, 5(1):109-120, 2012.

[153] Jung, Y., J. Y. Jeong, J.Y., and Chung, B.H. Recent advances in immobilization methods of antibodies on solid supports. *Analyst*, 133:697-701, 2008.

[154] Wang, C., and Feng, B. Research progress on site-oriented and threedimensional immobilization of protein. *Molecular Biology*, 49:1, 2015.

[155] Welch, N.G., Scoble, J.A., Benjamin W. Muir, and Paul J. Pigram. Orientation and characterization of immobilized antibodies for improved immunoassays (Review). *Biointerphases*, 12(2):301-312, 2017.

[156] Migneault, I., Dartiguenave, C., Bertrand, M. J., and Waldron, K. C. Glutaraldehyde: behavior in aqueous solution, reaction with proteins, and application to enzyme crosslinking. *Biotechniques*, 37(5):790-802, 2004.

[157] Hossain, M. J., Al-Mamun, M., and Islam, M. R. Diabetes mellitus, the fastest growing global public health concern: Early detection should be focused. *Health Science Reports*, 7(3):e2004, 2024.

[158] Roglic, G. WHO Global report on diabetes: A summary. *International Journal of Noncommunicable Diseases*, 1(1):3-8, 2016.

[159] Stitt, A. W., Curtis, T. M., Chen, M., Medina, R. J., McKay, G. J., Jenkins, A., ... and Lois, N. The progress in understanding and treatment of diabetic retinopathy. *Progress in retinal and eye research*, 51:156-186, 2016.

[160] Calcutt, N. A., Cooper, M. E., Kern, T. S., and Schmidt, A. M. Therapies for hyperglycaemia-induced diabetic complications: from animal models to clinical trials. *Nature Reviews Drug Discovery*, 8(5):417-430, 2009.

[161] Klatman, E. L., Jenkins, A. J., Ahmedani, M. Y., and Ogle, G. D. Blood glucose meters and test strips: global market and challenges to access in low-resource settings. *The lancet diabetes & Endocrinology*, 7(2):150-160, 2019.

[162] Deshmukh, M. A., Kang, B. C., and Ha, T. J. Non-enzymatic electrochemical glucose sensors based on polyaniline/reduced-graphene-oxide nanocomposites

functionalized with silver nanoparticles. *Journal of Materials Chemistry C*, 8(15):5112-5123, 2020.

[163] Newman, J. D., and Turner, A. P. Home blood glucose biosensors: a commercial perspective. *Biosensors and bioelectronics*, 20(12):2435-2453, 2005.

[164] Wang, J. Electrochemical glucose biosensors. *Chemical reviews*, 108(2):814-825, 2008.

[165] Kondepati, V. R., and Heise, H. M. Recent progress in analytical instrumentation for glycemic control in diabetic and critically ill patients. *Analytical and bioanalytical chemistry*, 388:545-563, 2007.

[166] Bruen, D., Delaney, C., Florea, L., and Diamond, D. Glucose sensing for diabetes monitoring: recent developments. *Sensors*, 17(8):1866, 2017.

[167] Chen, C. M., and Chang Yeh, M. The experiences of diabetics on self-monitoring of blood glucose: a qualitative metasynthesis. *Journal of Clinical Nursing*, 24(5-6):614-626, 2015.

[168] Vashist, S. K. Non-invasive glucose monitoring technology in diabetes management: A review. *Analytica chimica acta*, 750:16-27, 2012.

[169] Reddy, K. R. N., Salleh, B., Saad, B., Abbas, H. K., Abel, C. A., and Shier, W. T. An overview of mycotoxin contamination in foods and its implications for human health. *Toxin reviews*, 29(1):3-26, 2010.

[170] Robens, J. F., and Richard, J. L. Aflatoxins in animal and human health. *Reviews of Environmental Contamination and Toxicology: Continuation of Residue Reviews*, 69-94, 1992.

[171] Cano-Sancho, G., Ramos, A. J., Marín, S., and Sanchis, V. Presence and co-occurrence of aflatoxins, deoxynivalenol, fumonisins and zearalenone in gluten-free and ethnic foods. *Food Control*, 26(2):282-286, 2012.

[172] Nakazato, M., Morozumi, S., Saito, K., Fujinuma, K., Nishima, T., and Kasai, N. Production of aflatoxins and aflatoxicols by Aspergillus flavus and Aspergillus parasiticus and metabolism of aflatoxin B_1 by aflatoxin-non-producing Aspergillus flavus. *Eisei kagaku*, 37(2):107-116, 1991.

[173] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans., International Agency for Research on Cancer, World Health Organization, Some traditional herbal medicines, some mycotoxins, naphthalene and styrene., International Agency for Research on Cancer, 2002.

[174] Ismail, A., Naeem, I., Gong, Y. Y., Routledge, M. N., Akhtar, S., Riaz, M., ... and Ismail, Z. Early life exposure to dietary aflatoxins, health impact and control perspectives: A review. *Trends in Food Science & Technology*, 112:212-224, 2021.

[175] Frenich, A. G., Vidal, J. L. M., Romero-González, R., and del Mar Aguilera-Luiz, M. Simple and high-throughput method for the multimycotoxin analysis in cereals and related foods by ultra-high performance liquid chromatography/tandem mass spectrometry. *Food Chemistry*, 117(4):705-712, 2009.

[176] Li, P., Zhang, Q., Zhang, W., Zhang, J., Chen, X., Jiang, J., ... and Zhang, D. Development of a class-specific monoclonal antibody-based ELISA for aflatoxins in peanut. *Food Chemistry*, 115(1):313-317, 2009.

[177] Li, P., Zhang, Q., and Zhang, W. Immunoassays for aflatoxins. *TrAC trends in analytical chemistry*, 28(9):1115-1126, 2009.

[178] Jallow, A., Xie, H., Tang, X., Qi, Z., and Li, P. Worldwide aflatoxin contamination of agricultural products and foods: From occurrence to control. *Comprehensive reviews in food science and food safety*, 20(3):2332-2381, 2021.

[179] Xue, Z., Zhang, Y., Yu, W., Zhang, J., Wang, J., Wan, F., ... and Kou, X. Recent advances in aflatoxin B1 detection based on nanotechnology and nanomaterials-A review. *Analytica chimica acta*, 1069:1-27, 2019.

[180] Khan, M. Z. H., M. S. Ahommed, and M. Daizy. Detection of xanthine in food samples with an electrochemical biosensor based on PEDOT: PSS and functionalized gold nanoparticles. *RSC advances* 10:36147-36154, 2020.

[181] Phongphut, A., Sriprachuabwong, C., Wisitsoraat, A., Tuantranont, A., Prichanont, S., and Sritongkham, P. A disposable amperometric biosensor based on inkjet-printed Au/PEDOT-PSS nanocomposite for triglyceride determination. *Sensors and Actuators B: Chemical*, 178:501-507, 2013.

[182] Manivannan, K., Sivakumar, M., Cheng, C. C., Lu, C. H., and Chen, J. K. An effective electrochemical detection of chlorogenic acid in real samples: Flower-like ZnO surface covered on PEDOT:PSS composites modified glassy carbon electrode. *Sensors and Actuators B: Chemical*, 301:127002, 2019.

[183] Kergoat, L., Piro, B., Simon, D. T., Pham, M. C., Noel, V., and Berggren, M. Detection of glutamate and acetylcholine with organic electrochemical transistors based on conducting polymer/platinum nanoparticle composites. *Advanced Materials*, 26(32):5658-5664, 2014.

[184] Liu, D., Rahman, M. M., Ge, C., Kim, J., and Lee, J. J. Highly stable and conductive PEDOT: PSS/graphene nanocomposites for biosensor applications in aqueous medium. *New Journal of Chemistry*, 41(24):15458-15465, 2017.

[185] Wong, A., Santos, A. M., and Fatibello-Filho, O. Determination of piroxicam and nimesulide using an electrochemical sensor based on reduced graphene oxide and PEDOT: PSS. *Journal of Electroanalytical Chemistry*, 799:547-555, 2017.

[186] Kanakamedala, S. K., Alshakhouri, H. T., Agarwal, M., and DeCoster, M. A. A simple polymer based electrochemical transistor for micromolar glucose sensing. *Sensors and Actuators B: Chemical*, 157(1):92-97, 2011.

[187] Senel, M. Simple method for preparing glucose biosensor based on in-situ polypyrrole cross-linked chitosan/glucose oxidase/gold bionanocomposite film. *Materials Science and Engineering: C*, 48:287-293, 2015.

[188] Zahed, M. A., Barman, S. C., Das, P. S., Sharifuzzaman, M., Yoon, H. S., Yoon, S. H., and Park, J. Y. Highly flexible and conductive poly (3, 4-ethylene dioxythiophene)-poly (styrene sulfonate) anchored 3-dimensional porous graphene network-based electrochemical biosensor for glucose and pH detection in human perspiration. *Biosensors and Bioelectronics*, *160*:112220, 2020.

[189] Rohaizad, N., Mayorga-Martinez, C. C., Sofer, Z., and Pumera, M. 1T-phase transition metal dichalcogenides (MoS_2, $MoSe_2$, WS_2, and WSe_2) with fast heterogeneous electron transfer: application on second-generation enzyme-based biosensor. *ACS applied materials & interfaces*, 9(46):40697-40706, 2017.

[190] Jeong, J. M., Yang, M., Kim, D. S., Lee, T. J., and Choi, B. G. High performance electrochemical glucose sensor based on three-dimensional MoS_2/graphene aerogel. *Journal of colloid and interface science*, 506:379-385, 2017.

[191] Miao, Z., Wang, P., Zhong, A., Yang, M., Xu, Q., Hao, S., and Hu, X. Development of a glucose biosensor based on electrodeposited gold

nanoparticles–polyvinylpyrrolidone–polyaniline nanocomposites. *Journal of Electroanalytical Chemistry*, 756:153-160, 2015.

[192] Shi, L., Wang, Z., Yang, G., Chen, X., Gou, G., and Liu, W. Electrochemical immunosensor for Aflatoxin B_1 based on polyaniline/graphene nanohybrids decorated with Au nanoparticle. *Electrochemistry*, 85(7):384-390, 2017.

[193] Spinella, K. Development of a QCM (quartz crystal microbalance) biosensor to the detection of aflatoxin B1. *Open journal of applied biosensor*, 2(04):112, 2013.

[194] Jin, X., Jin, X., Liu, X., Chen, L., Jiang, J., Shen, G., and Yu, R. Biocatalyzed deposition amplification for detection of aflatoxin B1 based on quartz crystal microbalance. *Analytica chimica acta*, 645(1-2):92-97, 2009.

[195] Daly, S. J., Keating, G. J., Dillon, P. P., Manning, B. M., O'Kennedy, R., Lee, H. A., and Morgan, M. R. Development of surface plasmon resonance-based immunoassay for aflatoxin B1. *Journal of agricultural and food chemistry*, 48(11):5097-5104, 2000.

[196] Shahi, S. C., Dadmehr, M., Korouzhdehi, B., and Tavassoli, A. A novel colorimetric biosensor for sensitive detection of aflatoxin mediated by bacterial enzymatic reaction in saffron samples. *Nanotechnology*, 32(50):505503, 2021.

[197] Setlem, S. K., Mondal, B., and Ramlal, S. A fluorescent aptasensor for the detection of Aflatoxin B_1 by graphene oxide mediated quenching and release of fluorescence. *Journal of Microbiological Methods*, 193:106414, 2022.

[198] Xu, L., Zhang, H., Yan, X., Peng, H., Wang, Z., Zhang, Q., ... and Le, X. C. Binding-induced DNA dissociation assay for small molecules: sensing aflatoxin B_1. *ACS sensors*, 3(12):2590-2596, 2018.

[199] Jahangiri–Dehaghani, F., Zare, H. R., and Shekari, Z. Simultaneous measurement of ochratoxin A and aflatoxin B1 using a duplexed-electrochemical aptasensor based on carbon nanodots decorated with gold nanoparticles and two redox probes hemin@ HKUST-1 and ferrocene@ HKUST-1. *Talanta*, 266:124947, 2024.

[200] Jahangiri–Dehaghani, F., Zare, H. R., Shekari, Z., & Benvidi, A. Development of an electrochemical aptasensor based on Au nanoparticles decorated on metal–organic framework nanosheets and p-biphenol electroactive label for the

measurement of aflatoxin B_1 in a rice flour sample. *Analytical and Bioanalytical Chemistry*, 414:1973-1985, 2022.

[201] Dadmehr, M., Shahi, S. C., Malekkiani, M., Korouzhdehi, B., and Tavassoli, A. A stem-loop like aptasensor for sensitive detection of aflatoxin based on graphene oxide/AuNPs nanocomposite platform. *Food chemistry*, 402:134212, 2023.

[202] Adanyi, N., Levkovets, I. A., Rodriguez-Gil, S., Ronald, A., Varadi, M., and Szendro, I. Development of immunosensor based on OWLS technique for determining Aflatoxin B_1 and Ochratoxin A. *Biosensors and Bioelectronics*, 22(6):797-802, 2007.