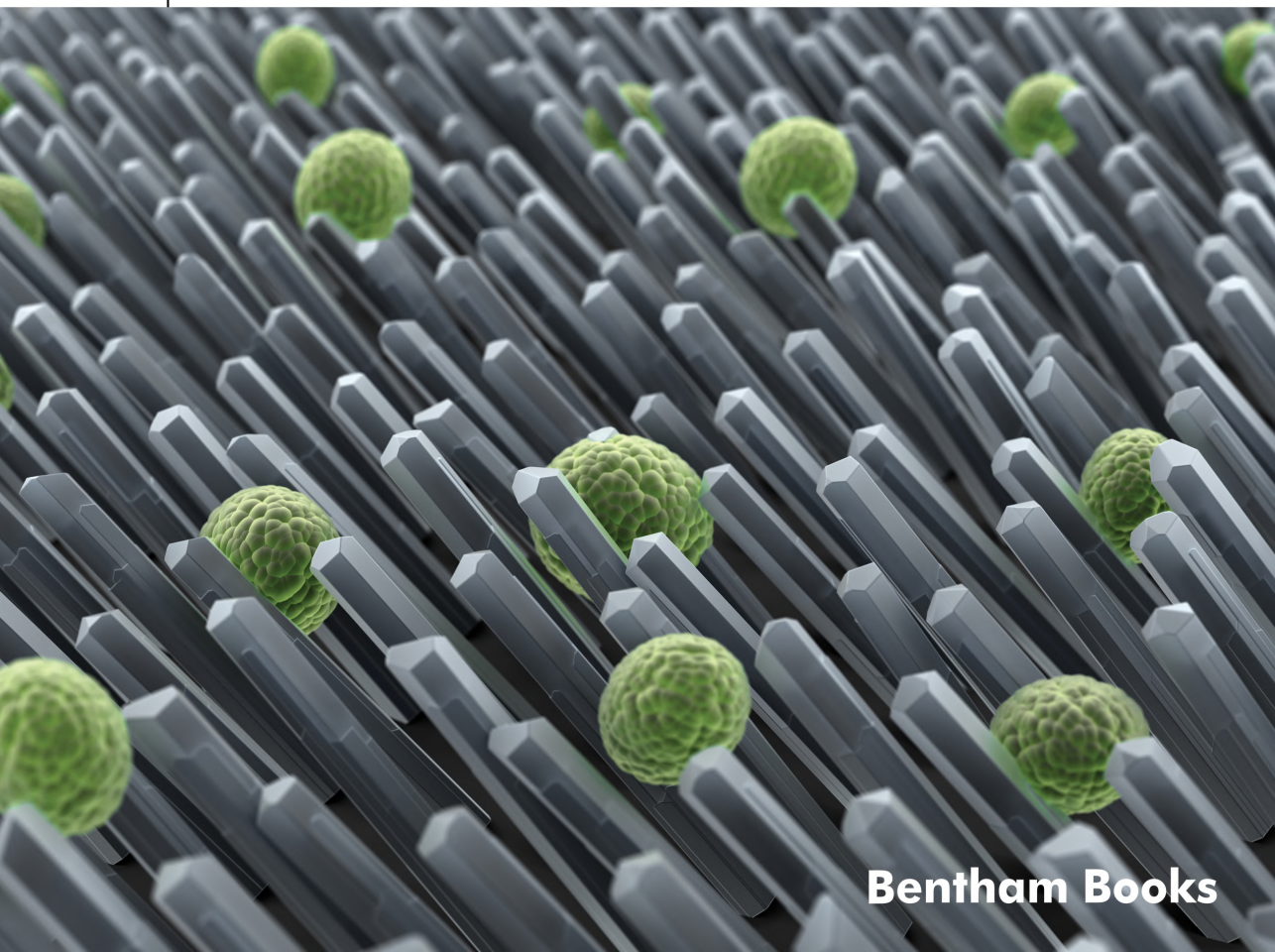
ELECTROCHEMICAL BIOSENSORS IN PRACTICE

MATERIAL AND METHODS

Seyed Morteza Naghib
Seyed Mahdi Katebi
Sadegh Ghorbanzade



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Electrochemical Biosensors in Practice

Electrochemical Biosensors in Practice: Materials and Methods

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Electrochemical Biosensors in Practice: Materials and Methods

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CONTENTS

PREFACE

Since Clark's first invention of biosensors in 1956, various enhancements have been made, and new detection methods have been proposed for their future development. The term “biosensor” refers to any analytical instrument that detects an analyte using a bioreceptor and a transducer in addition to a physicochemical detector. They exhibit a high degree of selectivity due to the interactions between the bioreceptors' structure and the analyte (biorecognition). Due to their unique interaction, biosensor signals cannot be tampered with by other substances. Numerous biorecognition molecules, including aptamers and antibodies as well as enzymes and nucleic acids, have been employed in the creation of biosensors because of new technology in electronics and microprocessors. Because of these changes, biosensors can now be put on a smaller surface.

Electrochemistry is a common technique of signal transduction in biosensors. It includes electrochemiluminescence, potentiometry, impedance spectroscopy, amperometry, conductometry and voltammetry. Recent advancements in nanotechnology and nanoscience have enabled biosensor researchers to conduct ground-breaking research into novel biomaterials and materials with superior physical, biocompatible, mechanical and electrical properties, paving the way for manufacturing of even more efficient electrodes. Innovative electrochemical biosensors are finding new applications as a consequence of this study. Nanostructured biomaterials are one of the most versatile forms of biomaterials since they may be utilized to produce electrodes with micrometer-sized surface areas. For instance, carbon nanotubes and quantum dots, which are used in biosensors, display hitherto unseen properties. As a result, biosensors have become a strong and interesting field thanks to the development of small electrodes that can detect even the smallest amounts of analytes in living systems.

As a result of these advancements, this book will present an overview of electrochemical biosensors, covering the many types and surface modification methods that are now available. The subjects explored in this book will pique the curiosity of a wide variety of readers. This category of nanomaterial-based systems includes carbon nanomaterials and biosensor signal monitoring devices. Electrochemical biosensors based on microbial cells, nucleic acids, aptamers, and enzymes, as well as receptor-based biosensors for metabolite detection and physiological process research, highlight how electrochemistry may be utilized for metabolite detection and physiological process research. If you are a student or a scientist, this book will help you. It includes contributions from well-known experts in the field of electrochemical transduction for biosensors.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

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CHAPTER 1

Introduction to Electrochemical Biosensors

Abstract: The book starts with the definition of biosensors and their classifications upon transduction, which is divided into five systems: Electrochemical, Optical, Thermal, Mass-bass, and Energy and bioreceptor components, which are divided into six types, including Enzymes, antibodies, Nucleic Acids, Aptamers, Cells, and Microbial. Afterward, it continues with electrochemical biosensor fundamental descriptions and then introduces all the electrochemical types like Voltammetric, Potentiometric, and Impedimetric. Finally, Chapter 1 concludes with a short discussion of the electrochemical biosensor market. This talk will focus on biological sectors, food production, and environmental protection and will finish with a look at the newly revealed numbers.

Keywords: Bioreceptor, Biosensor, Cell, Electrochemical, Transducer.

INTRODUCTION

Nowadays, the significance of monitoring and controlling various factors is growing, whether in the food business, clinical diagnosis, hygiene, environmental protection, drug development, or forensics. As a result, it is critical to have dependable analytical equipment accessible to conduct rapid and accurate tests. Using a correctly constructed biosensor is one approach to circumvent many drawbacks of traditional techniques [1]. A biosensor is a device that combines a biological sensing element with a transducer [2]. A biosensor is a chemical sensor that uses a broad and scientific description of the recognition characteristics of biological components in the sensitive layer [3].

According to the International Union of Pure and Applied Chemistry (IUPAC), a biosensor is a device that detects chemical compounds through specific biochemical processes mediated by whole cells, organelles, tissues, immunosystems, or single enzymes (McNaught and Wilkinson 1997) [1]. Apart from these meanings, the word “biosensor” has a variety of implications depending on the user's area of expertise:

● For instance, a biologist defines a biosensor as “a device that converts biological factors such as chemical concentrations, movement, or electric potentials into electrical signals.”

Seyed Morteza Naghib, Seyed Mahdi Katebi and Sadegh Ghorbanzade

● To the scientist, a more appropriate description would be "a device that detects chemical substances through particular biochemical processes mediated by individual entire cells, organelles, tissues, immunosystems, or enzymes."

● A physicist could characterize a biosensor as follows: "a device that sends data, records, and detects a physiological change or process" [4].

Nonetheless, we must understand what Biosensors are in order to apply these concepts. As a result, the majority of sensors are composed of three primary components (Fig. **1**) [2, 5]:

1) To begin, there must be a component that recognizes the analyte of interest selectively. Typically, this is accomplished *via* a binding event between the target and the detection component (like Bioreceptors) [5].

2) Second, to convert the biological binding event into a measurable indication, a transducing element is needed. This may result in the formation of electrochemically detectable species such as protons or H_2O_2 and a change in conductivity, mass, or optical properties such as refractive index (Like transducers) [5].

3) Thirdly, some mechanisms for measuring and detecting physical change must exist, for example, sensing a current of optical, mass, or electricity alteration and translating it to helpful information (like microprocessors) [5].

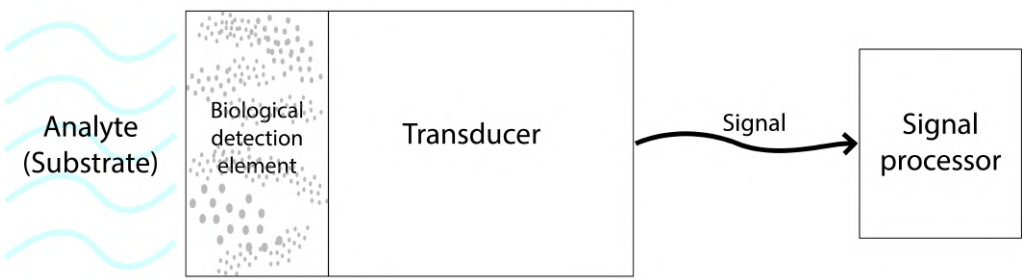

Fig. (1). A biosensor's schematic layout.

Basic Principle of Biosensor

A Bioreceptor is any biological or biomimetic substance, such as antibodies, enzymes, nucleic acids, viruses, bacteria, or tissues. A bioreceptor will bind precisely to a target analyte and trigger the generation of a voltage signal by a transducer [6]. The nose is one of the natural biosensors; the olfactory nerves serve as a bioreceptor, the nerve cell acts as a transducer, and the brain acts as a

microprocessor (Fig. **2**) [2]. A transducer converts an observable change (chemical or physical) into a measurable signal, most often an electrical signal with significance proportional to the concentration of a specific chemical or set of chemicals [2].

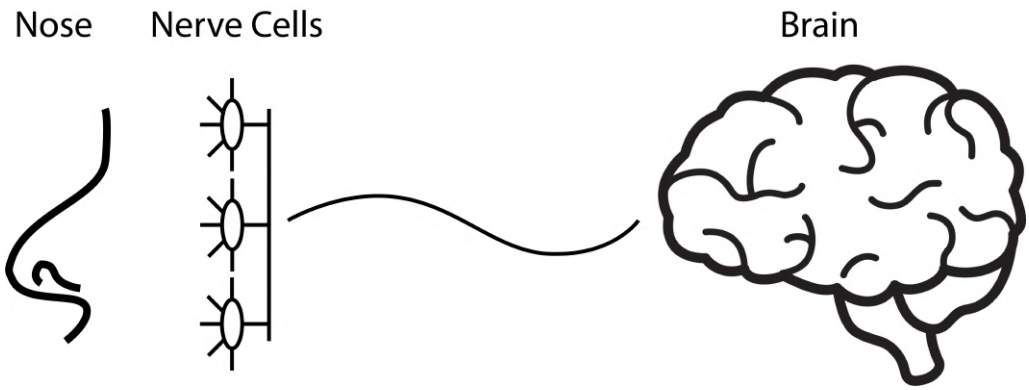

Fig. (2). Simple Biosensor in the human body is noise.

On the other hand, biosensors are classified in various ways, discussed in more depth in the following sections. However, the two most common types are (a) affinity-based and (b) catalytic biosensors [7].

Clark developed the first "biosensor" in 1956, and Clark and Lyons (enzyme electrodes) demonstrated it in 1962 by sandwiching soluble GOx (glucose oxidase) between the gas-permeable membrane and an outer dialysis layer of a voltammetric oxygen (O_2) electrode. The oxidation of glucose, mediated by glucose oxidase (GOD), is a chemical process.

$$G + O_2 + H_2O \xrightarrow{GOD} Gluconic\ acid + H_2O_2$$

At the electrode:

$$O_2 + 2e^- + 2H^+ = H_2O_2$$

Between the anode and cathode, which are platinum and silver, respectively, A - 0.7 V voltage is applied, sufficient to deplete the oxygen. The current flowing through the cell is determined, which is relative to the direction of the oxygen concentration [2, 4].

Later that year, in 1967, Updike and Hicks added another Oxygen electrode to compensate for O_2 fluctuations in the model. It was quickly recognized that

enzyme electrodes could be produced by connecting relevant enzymes to a suitable electrode network for various additional therapeutically critical analytes [4].

Another early biosensor was used to detect the presence of urea. Guilbault and Montalvo invented this biosensor (1969). The ammonia concentration is determined using an ammonium ion-elective electrode whose comparable voltage is evaluated near zero current. This voltage is proportionate to the ammonia concentration's logarithm and is directly related to the urea concentration [2].

$$CO\left(NH_2\right)_2 + H_2O \xrightarrow{urease} CO_2 + 2NH_3$$

By halting living microorganisms on an NH_3 gas-sensing electrode plane, Rechnitz created a bioselective electrode for arginine in 1977. This word was then abbreviated to “biosensor” and has endured a common abbreviation for each analytical instrument that amalgamates a biological identification system *via* a physicochemical transducer [4].

Throughout the late 1980s and early 1990s, efforts were made to promote the direct electrical connection between the electrode plane and the redox heart of GOx and the creation of minimally invasive subcutaneously implanted devices. During the 1980s, intense efforts concentrated on creating “second-generation” glucose biosensors based on mediators [4].

Third-generation biosensors will incorporate the biological element directly into the electrical device, for example, by embedding an enzyme inside a conducting polymer or semiconductor material (Foulds & Lowe 1985) [21].

McNeil & Bannister invented the first electrochemical Biosensor in 1986. One novel method, which may result in a simple voltammetric test strip, is electrochemically detecting a standard enzyme label, alkaline phosphatase [8].

One of the important aspects of biosensors is that regulating their physicochemical characteristics is a common barrier in developing all next-generation biosensors due to their interface's inadequate stability and repeatability [3].

Classification of Biosensors Based on Transducers

According to the preceding section, a transducer is an analytical instrument that generates an output amount proportional to the input quantity [1]. Biosensors might be classified based on their bio transducers or the biological specificity mechanism [9]. As a result, this section will concentrate on bio transducers.

Different biosensor categories will be discussed and shown, including thermal, energy, optical, mass-based, and electrochemical biosensors [9].

Electrochemical Biosensors

The fundamental concept behind this type of biosensors is that chemical interactions among the aim analyte generate the immobilized biomolecule or use electrons or ions, thus changing the solution's observable electrical characteristics, such as current or potential [1]. Electrochemical transduction biosensors use the biocatalytic response or a solution-based reporter's redox activity of an electroactive label linked to a probing instrument or an objective [7]. Several benefits include the fact that electrochemical biosensors are volume-independent (Even samples with minimal volumes can be quantified.) [9], affordable, portable, vulnerable, and compatible with current microfabrication methods [10].

Typically, the sensor substrate includes both working and reference electrodes, whereas electrochemical biosensors rely on enzyme catalytic processes [9].

Additionally, there are many monitoring methods available for electrochemically detecting a signal: the act of accumulating quantifiable charges potential (potentiometry) or density, measuring impedance (impedimetry), and altering the conductivity of measuring current changes (amperometry), or the average among unlike electrodes (conductometry). As a result, depending on their electrochemical sensing methods, they can be classified as potentiometric, impedimetric, conductometric, field-effect transistor, or voltammetric biosensors. The mobility and small electrochemical biosensors allow them to be utilized as a point-of-care apparatus by the patient at a medical clinic or a home [9].

Optical Biosensors

The output transducer signal is light [1]. Initially, the optical Biosensor was designed to monitor dissolved oxygen, carbon dioxide, and pH [11]. Examples include light scattering spectroscopy, surface plasmon resonance, internal reflection, luminescence, fluorescence, and absorption of optical transducers. For instance, a surface plasmon resonance (SPR) sensor was developed using gold nanoparticles as the platform to detect the presence of casein on the surfaces [12].

The benefits of optical biosensors are their fast detection speed, sensitivity, robustness, and capacity to detect numerous analytes [10]. Optical transduction occurs when the optical characteristics of the transducer surface change as a consequence of a biorecognition event. These changes include refraction, reflection, scattering, transmittance, emission, and absorption. Labeled or label-free biosensors can track these optical changes with or without a label

(fluorophore or chromophore) attached to a target or probe [7].

Optical biosensors of all kinds are covered, include fluorescence effects, Raman Spectroscopy, FT-IR spectroscopy, and SPR. Because electrical and mechanical biosensors have some limitations, optical biosensors are being explored to detect various biological components for diagnostic and analytical applications [7].

Mass-Base Biosensors

Mass-based biosensing uses a mass variation to identify analytes determined by a change in different kinds of sensors. Surface acoustic waves, a QCM (quartz crystal microbalance), or a piezoelectric sensor are the three primary kinds utilized in mass-based biosensing applications [9]. Mass-sensitive biosensors provide some benefits, including operating and monitoring in real-time in liquid, vacuum, and air conditions [10].

Thermal Biosensors

Thermal transducer biosensors are a unique analytical instrument used to measure the amount of heat produced during a biological process. In this formula, molar enthalpy is equal to the concentration/amount of the target analyte, and the total amount of heat produced or absorbed is proportional to the molar enthalpy and the target analyte concentration/amount. The thermal Biosensor is a compact calorimetric apparatus fitted with a high-sensitivity thermistor capable of detecting temperature changes between 0.0001 and 0.05°C. Additionally, it can detect concentrations of the desired analyte concentration as low as 10-5 molarity. By first measuring H (Enthalpy), the reaction's enthalpy at various temperatures, and thus collecting the basic thermodynamic data, G (Gibbs free energy) and S (Entropy) can be computed for a process. Thermal Biosensors are classified as Thermometric Sensors, Terahertz Effect Sensors, and Thermal Radiation Sensors [9].

Historically, thermometric biosensors have been primarily used to monitor clinical and industrial processes [10].

Energy Biosensors

The cellular mechanism is also often stated to store energy in the shapes of molecules [9].

Energy Biosensors are classified into two categories: Fluorescence Resonance Energy, Adenosine Triphosphate. Application of energy biosensors: Food molecules are formed when water and carbon dioxide are oxidized in the

mitochondria, one of the most important organelles in the cell. In glucose metabolism, the adenosine triphosphate (ATP) ratio to adenosine diphosphate (ADP) is a key component influencing the cellular energy metabolism structure, which finds changes in free energy required for ATP hydrolysis and driving force generation. So, a biosensor can detect ATP's disturbance in live cells *via* metabolic activity, specifically by measuring ATP and metabolic activity's common effect, ATP. An important part of detecting material energy is fluorescence sensors [9].

Classification of Biosensors Based on Bioreceptor

Bioreceptors, or biological recognition components, are required for highly specialized biosensor technologies. The primary difference between a biosensor and a standard sensor is its biological or bioreceptor recognition element. The bioreceptor is the sensor's method of recognition for the analyte of interest. A bioreceptor is a molecular species that identifies other molecules through a biological process. The sensor-surface adherence is their responsibility [13]. There are six types: enzymes, antibodies, nucleic acids, aptamers, microbes, and cells.

Enzyme

Leyland Clark developed one of the first biosensors by coating an oxygen electrode with a film containing a dialysis membrane and glucose oxidase. This might be used to determine blood glucose levels; the enzyme transformed glucose to hydrogen peroxide and gluconolactone while also using oxygen. The decrease in dissolved oxygen might be detected at the electrode, and with proper calibration, blood glucose levels can be estimated [5].

In biosensor applications, enzymes have been the most often employed bioreceptor molecules. Because of their unique ability to catalytic and bind action, enzymes are often employed as bioreceptors. A catalytic process amplifies the detection in biocatalytic recognition systems [13].

All enzymes, except for a tiny subset of catalytic ribonucleic acid molecules, are proteins [13]. They are amino acid-based proteins joined together through peptide bonds to create lengthy chains folded into spherical shapes. A biorecognition layer, including enzymes, metabolizes an analyte identified *via* the production of end products.Otherwise, an analyte competes with the enzyme in the biorecognition layer for the enzyme's substrate, reducing the production of enzymatic products that ultimately correspond with the analyte concentration [7].

The bioreceptors' mechanisms of action can include the following: (1) The process of turning the analyte into a detectable sensor product; (2) the measurement of an analyte that can block or activate an enzyme; or (3) the assessment of how the analyte modifies the enzyme's properties when it interacts with the analyte [13].

Enzymes are used in biosensors because they are naturally occurring proteins that catalyze a change in a particular substrate molecule to a product without being eaten in the process [13]. Enzymes are often utilized in the creation of biosensors as biomaterials. These biosensors are based on enzymes Table **1** [1].

Table 1. The categories of enzymes and their functions are utilized by biosensors to identify their competent substrates as analytes.

Enzyme class	Function
Oxidoreductases	Reactions of oxidation/reduction
Transferases	Transfer of chemical groups between molecules
Hydrolases	Cleavage by hydrolysis
Lyases	Other than oxidation or hydrolysis, cleavage of C—N, C—O, and C—C bonds
Isomerases	Rearrangement on an intramolecular level
Ligases	The fusion of two molecules

Enzymatic sensors are classified into substrate and inhibitor sensors based on their functionalities. Biosensors for substrates are used to determine the particular substrates of enzymatic processes. Inhibitor sensors are used to detect the presence of substances that interfere with an enzyme's function [12].

Enzymes are ideal candidates for biosensors due to their high selectivity; for example, glucose oxidase will only interact with glucose and will not interact with other sugars. Due to their high catalytic activity, enzymes exhibit fast substrate turnover, which is essential because they may become saturated or generate insufficient active species to identify. Nevertheless, they have certain drawbacks: an enzyme specific to the object of interest does not exist. Additionally, enzymes could be costly and difficult to extract in adequate amounts and might be unstable, quickly denaturing, and rendered ineffective. Some species may also poison them. Furthermore, detecting enzyme turnover can be difficult [5].

Antibody

In the 1950s, an antibody-based biosensor was unexpectedly linked to recognition, paving the door for the possibility of immuno-conclusion. From that moment forward, strenuous efforts have been undertaken to develop an

immunosensor made of antigen/immunizer as a clinical diagnostic bioreceptor device [14]. A counteracting agent is an immunoglobin (Ig) produced in the 'Y' configuration, which consists of two substantial chains (H), as well as two light chains (L). On the other hand, specific human antibodies form dimeric or pentameric complexes through disulfide bonds and an extra protein termed the joining or J-chain. Each chain has a constant and a variable component. The variable portion is unique to the antigen associated with the corresponding antigen, which is unique [14]. They exhibit remarkable selectivity in their binding to particular species (antigens) through a combination of hydrogen bonds and other noncovalent interactions. The binding occurs inside the protein molecule's cleft (Fig. **3**) [5, 13].

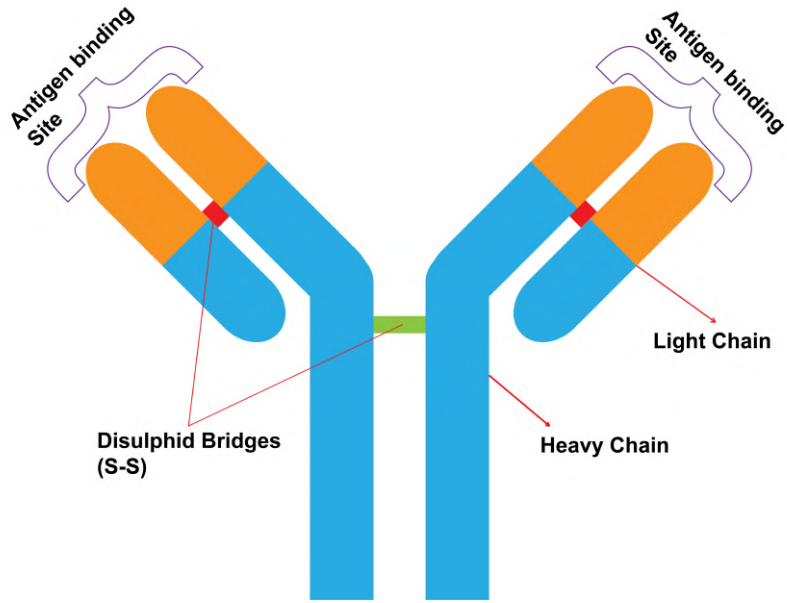

Fig. (3). A schematic representation of an antibody's Y-shape structure.

Thus, an immunosensor constructed from antigen as a bioreceptor takes advantage of the immune response's ability to associate with a contrasting antigen that is deeply specific, durable, and adaptive. A component of an immune response's selectivity for its antigen's coupling side is its amino acids [14]. Immunosensors use either an antibody or an antigen as a biorecognition layer to leverage the antigen-antibody interaction [7]. The majority of immunosensors are constructed using the solid-phase immunoassay principle, in which antigens and antibodies are immobilized on a stable substrate. As a result, contact between antigen and antibody occurs at the solid-liquid interface [10].

Protective proteins, such as immunoglobulins, are produced *via* an organism's immune system in reaction to the infiltration of foreign biological substances (antigens), which serve as the biochemical receptors in this circumstance [12].

After the antibody is produced, Adsorption may occur on a transducer to create a biosensor, as an example of it is given in Fig. (**4**) [5].

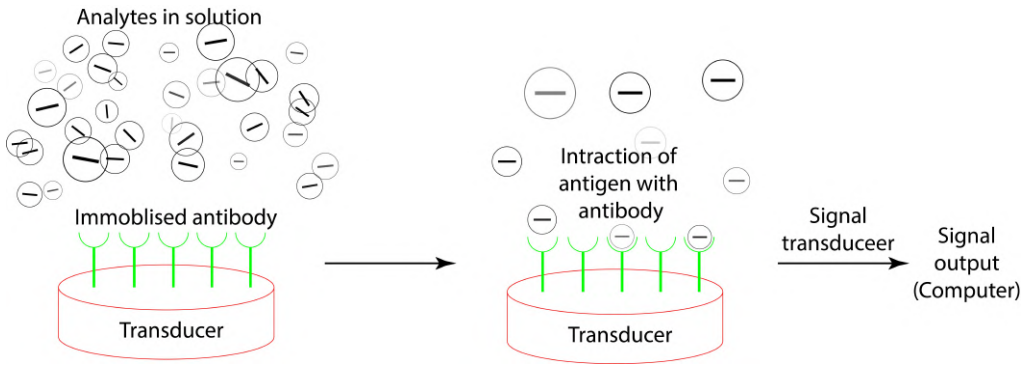

Fig. (4). An immunosensor based on antibodies is shown schematically.

Immunosensors operate in three styles: (a) sandwich mode, which includes the use of an antigen with two epitopes that binds both an immobilized antibody and a secondary antibody that has been tagged; (b) competitive mode, which includes a competition for binding between a labeled and an unlabeled antigen to the immobilized antibody's limited binding sites; and (c) straight mode, which entails the interaction of an unlabeled antigen with an unlabeled antibody [7].

An antigen-specific antibody binds to its specific antigen in a highly specific manner. This particular feature of antibodies is critical for their application in immunosensors because the antibody binding site can only accommodate the specific analyte of interest, the antigen [1]. Thus, immunosensors are utilized to identify the players in immunochemical interactions, particularly antibodies, antigens, and quantifying proteins [12, 13].

One significant advantage of antibodies is that they can be “grown” in laboratory animals by inoculating them with the desired antigen; the animal's innate defense mechanism is to produce antibodies to the antigen. One disadvantage is that when antibodies create a compound with their antigens, no easily quantifiable byproducts are found. These include redox-active species and electrons [5].

Aptamers

Aptamers are synthetic peptides or oligonucleotides that are intended to affix certain nucleic acids. They are often generated by SELEX (SELEX is the acronym for Systematic Evolution of Ligands by Exponential Enrichment, which is the method of choosing an aptamer), a predominantly random pool of genes, or a sequences library [15]. Aptamers, which are *in vitro* synthesized oligonucleotides *via* ligands, have evolved methodically *via* exponential growth (SELEX), act as a biomimetic biorecognition layer capable of detecting a wide variety of analytes, including organic dyes, nucleic acids, metal, drugs, ions cofactors, antibiotics, and amino acids [7].

Aptamers are a class of DNA/RNA -like oligonucleotides that attach to various objectives, counting cells, peptides, medicines, and proteins. When they bind their intended recipients, the aptamer undergoes conformational changes; for instance, it can wrap around molecules. These structural modifications are often visible, aptamers are excellent candidates for sensing applications [5].

Aptamers are categorized as follows (Fig. **5**) [13, 15]:

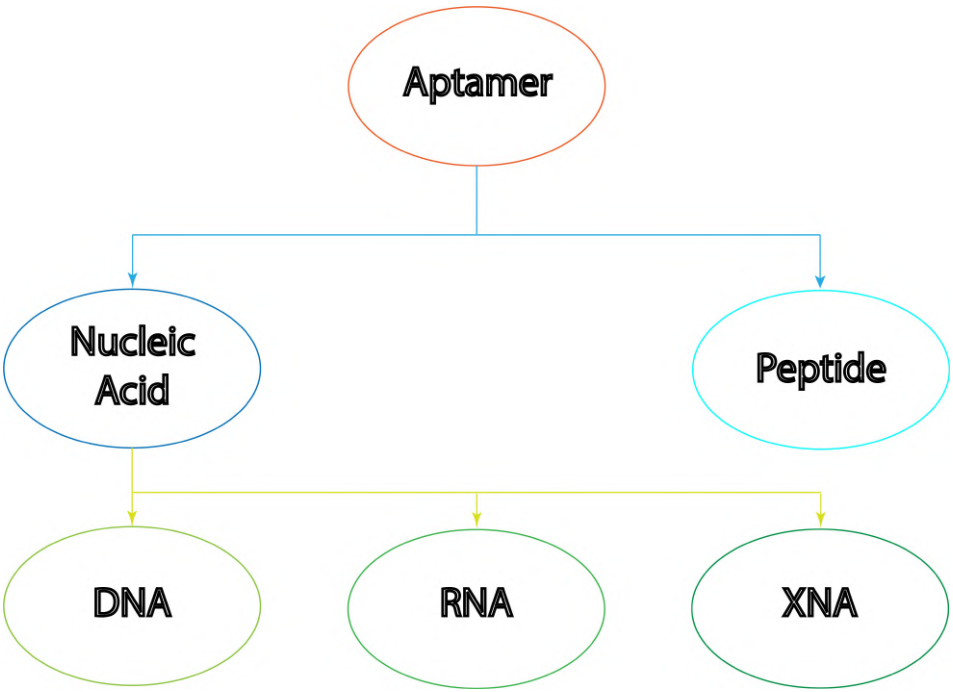

Fig. (5). Classification of aptamers.

• Nucleic acid aptamer

• Peptide aptamers

That nucleic acids can be into three classifications:

• DNA

• RNA

• XNA

Nucleic Acids

Nucleic acids are macromolecules accountable for transferring all living organisms' genetic information from generation to generation. They are made up of a base (pirimidic or purine), a sugar (deoxyribose or ribose), and phosphate [15]. These biosensors rely on hybridization between a nucleic acid probe sequence (bioreceptor) mounted on the surface and its matching target sequence [7].

The specific affinity binding process that occurs between two single-stranded DNA (ssDNA or RNA) chains to produce double-stranded DNA (dsDNA) is used in nucleic acid-based biosensors, which recognize biological entities *via* the use of nucleic acids (Fig. **6**) [13, 15].

DNA, which serves as a template for protein synthesis and can be seen as a molecular data storage device, is present in all living cells. RNA has many functions in alive organisms, serving as a messenger between the ribosomes and DNA that build proteins and as a gene expression manager. RNA and DNA are polymeric species with a sugar-phosphate backbone, and the thymine, guanine, cytosine, and nucleic acids adenine as side chains in DNA [5]. Biosensors based on nucleic acids (NA) include a NA as the biological recognition element (natural and biomimetic forms of oligo- and polynucleotides) [1].

DNA sensors typically consist of one oligonucleotide chain attached to an appropriate transducer, such as a QCM (quartz crystal microbalance), SPR (surface plasmon resonance) chip, or electrode exposed to a solution containing the desired oligonucleotide strand. The surface-bound oligonucleotide is chosen to complement the target oligonucleotide and the bound, and during the recognition event, sequence-specific hybridization occurs in solution strands [5].

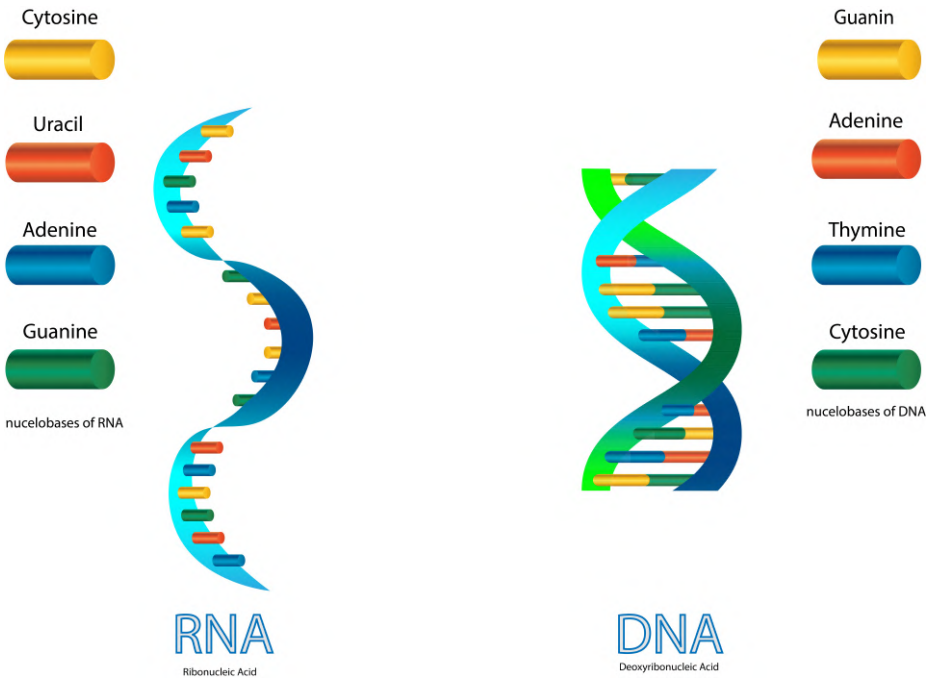

Fig. (6). Schematic representation of three essential nucleotide structural elements, DNA, RNA, and nucleotides.

Biosensors based on peptide nucleic acid, RNA, or DNA benefit from the formidable base-pair attraction between mutually beneficial portions of nucleotide strands that are aligned [1]. Electrochemical DNA biosensors that translate the identification of base pairs to a quantifiable electrical signal are promising possibilities for fast and low-cost genetic disease diagnostics and the detection of harmful biological organisms. Their clinical utility and adaptability with microfabrication technology make them attractive candidates [1].

DNA and RNA are both chains made up of conventional oligonucleotides [15].

Due to their cheap cost, specificity, and excellent stability, DNA-aptamer-based biosensors have been created as an alternative to antibodies. It can attach to particular bacteria, viruses, proteins, hormones, analytes, and even tiny molecules and ions with high specificity and affinity. Van der Waals forces and Hydrogen bonds are the primary kinds of bonds utilized in this binding [10].

XNA: Similar to nucleic acids, but modifications to the phosphate phosphodiester, pentose sugar, or nucleobase skeletons [15].

Peptides

Peptides are naturally occurring or synthesized polymers of amino acids that are constructed similarly to proteins. When binding to targets, many proteins exhibit high selectivity and specificity, so peptides with the proper amino acid sequence should exhibit the same properties. These recognition receptors may be generated with a specific sequence, or affinity to a certain target can be determined using a library of peptides [5].

Peptides have many benefits over proteins; they have a higher degree of conformational and chemical stability and are considerably less prone to denaturation. Additionally, they can be produced utilizing well-established solid-phase synthesis methods and readily replaced with labeling groups without impairing their action. However, one problem is that immobilizing them on a solid surface may result in structural changes that render them inactive [5].

Finally, Aptamers have several benefits over many other recognition elements, including antibodies and enzymes. They can be produced *in vitro* without the need for animal hosts and with a high degree of selectivity and specificity for virtually any object, from tiny molecules to entire cells, proteins, and peptides. Apta sensors will be more robust and adaptable to the circumstances encountered during sample acquisition. It is commercially manufactured in its pure state and often outperforms other biological compounds in terms of stability. One disadvantage is that, since aptamers form counterparts, no readily observable product, such as a redox-active species, is produced [5, 13].

Cells

The bioreceptor layer comprises live cells that detect useful details about biologically active analytes, both extra- and intracellular microenvironments changes, and extrinsic stimuli, such as medicines and receptor ligands, on cells (chemical stimulus), and potential for induced stimuli (electric stimulus). Numerous non-invasive methods can be used to monitor microenvironmental changes caused by such stimuli, including the concentration of ions or extracellular chemicals, the action potential, and the impedance change caused by cellular metabolism (Fig. **7**) [1, 7].

Cell-based sensors are a type of Biosensor that utilizes living cells as the biospecific sensing component. They depend on live cells' ability to detect extra- and intracellular microenvironment variables and physiological factors and to produce a reaction through the collaboration of jolt and cell [14].

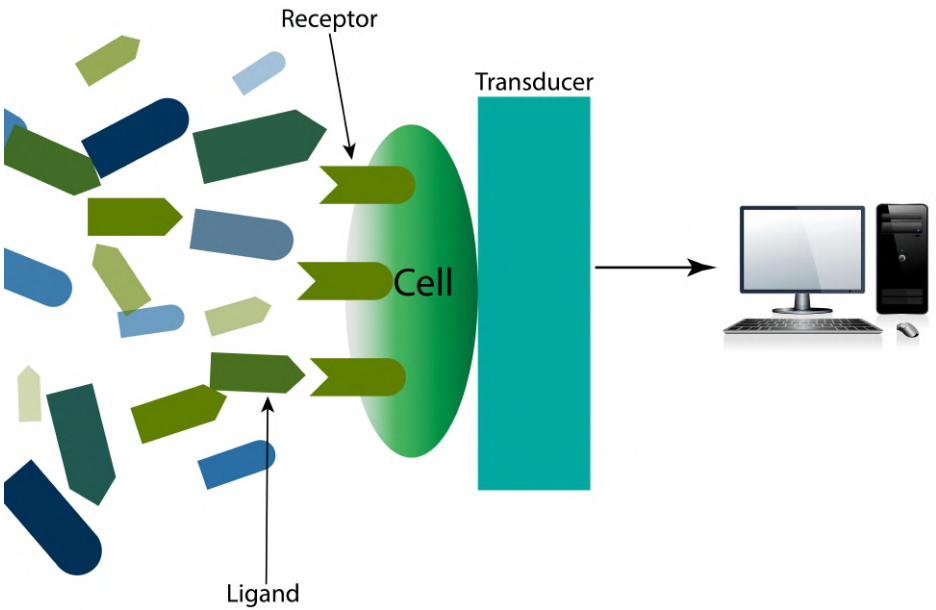

Fig. (7). Scheme of the cell-based Biosensor.

A microbial whole-cell strain of genetically modified Biosensors uses either prokaryotic or eukaryotic cells to rapidly and cost-effectively report chemical composition, toxicity, carcinogenicity, and mutagenicity [10]. Another significant benefit of utilizing this type of bioreceptors is the possibility of low detection limits due to signal amplification [1].

Microbial

A microbial biosensor is an analytical instrument that immobilizes microorganisms on a transducer to detect specific analytes. Fungus and bacteria may be employed as biosensors to discover particular chemicals or the "state" of the surrounding environment in general [13].

Microorganisms are used as biological components in biosensors that are metabolically active, often accompanied by carbon dioxide or oxygen consumption, and monitored electrochemically [13].

Electrochemical Biosensors

Electrochemical biosensors make use of the intrinsic charges of probes mounted on a transducer's surface. The sensing platform may then be immersed in a solution containing charged molecules, such as Ferro/ferricyanide ($[Fe(CN)_6]^{3-/4-}$)and a phosphate buffer (PB). This allows for studying the electrical

characteristics and interactions of various biological probe chemicals on the surface. Electrochemical analysis for the expansion of biosensors is usually performed using a three-electrode cell configuration Refer to Figs. (**8** and **9**), consisting of CE (a counter electrode), RE (a reference electrode), and WE (a working electrode), or a pseudo-reference electrode paired with the WE in a two-electrode configuration [16, 17].

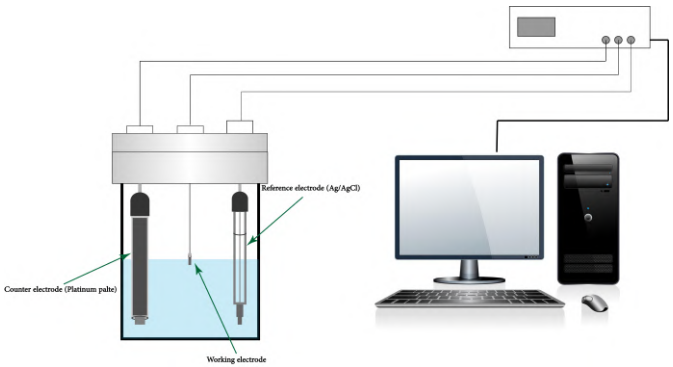

Fig. (8). Schematic of a three-electrode electrochemical cell.

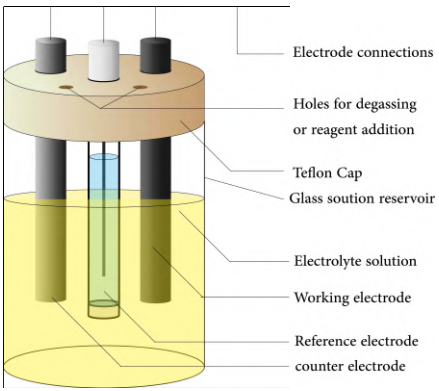

Fig. (9). The working electrode of a conventional three-electrode cell is gold-base, whereas the reference electrode can be made of SCE, Ag/AgCl, or $Hg/HgSO_4$.

Fundamentals and Terminology

Electricity is generally recognized as the most lucrative and widely used type of energy transmission in the modern era. It is mostly generated by power generators by transferring energy *via* different flammable substances and chemicals. This method of energy generation has two significant disadvantages: (I) thermodynamic rules require that thermal engines (*e.g.*, automobiles) have an efficiency considerably less than 100%, and (II) burning processes generate gaseous pollutants that contaminate the barley and surrounding ambiance. The conversion of energy through electrochemistry is according to the straight transmission of electrical energy with an excellent yield of about 90% when a

chemical reaction occurs spontaneously in a galvanic/ electrochemical cell, such as hydrogen's "cold combustion" in a fuel cell [18].

Alessandro Volta discovered electrochemical processes for the first time in 1793 when he showed that electricity could be generated by electrically connecting two different metals with wet paper in between. This was the world's first basic battery (Fig. **10**) [19].

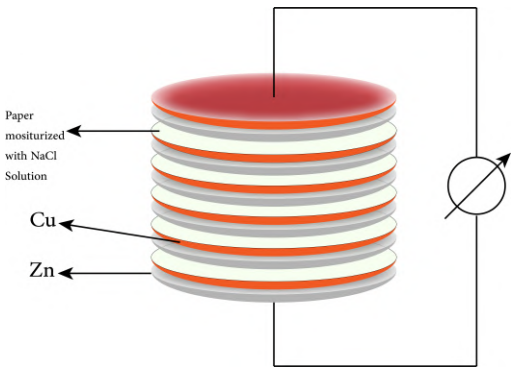

Fig. (10). Model and schematic of the first fundamental battery.

A few years later, the Volta battery was used to break down water into hydrogen and oxygen using electricity. This chemical landmark revealed that hydrogen and oxygen atoms had positive and negative electrical charges that act as bonding forces. In the year 1812, Berzelius, a Swedish physicist, suggested that each atom is electrified, hydrogen and metals are positive, whereas nonmetals are negative. While two electrodes immersed in a solution are supplied with electricity, it supplies energy for the attraction forces to be broken up and ions to form. The term ion is derived from the Greek meaning "traveler". Although the Lewis theory of bonding eventually supplanted Berzelius's notion of shared electrons, it marks a critical step forward in our knowledge of chemical conjunction [19].

Humphrey Davey demonstrated continued use of newly found electrochemical principles to draw fresh thoughts, which sodium hydroxide melt could be electrolyzed to create elemental sodium. Michael Faraday, the former assistant to Davey, created important advances to our knowledge of electrochemical establishing procedures by connecting the electrical charge's magnitude passing *via* a solution and how much material reacted or was created. These are referred to as Faraday's Laws of Electrolysis [19].

This new "perception" of the world prompted James Clerk Maxwell to postulate the possibility of an "electrical molecule," the electron's initial concept. However, the idea was not recognized until the late nineteenth century [19].

Sir William Grove is credited with inventing the first fuel cell in 1837 by immersing in sulfuric acid, and platinum electrodes. At the time, he noticed occurrences like the current density and three-phase boundary, which remain enigmatic and important to our knowledge of electrochemistry. Additionally, Grove accurately recognized the overpotential or loss in electrochemical cells by realizing that additional solitary fuel cells needed to be linked in a heap to provide the voltage required for water electrolysis [19].

The nineteenth-century history of electrochemistry would be incomplete without including these forerunners: Johann Ritter (1776–1810), inventor of the first dry cell battery in 1802 and discoverer of the connection between chemical and galvanism reactivity; John Frederic Daniell (1790–1845), who invented the first commercially successful telegraph power supply using electrodes of copper and zinc in a solution of copper sulfate; and Edmund Becquerel (1820–1891), who created the first Amorphous silicon solar cell [19].

Walther Nernst (1864–1941) was honored for his electromotive force theory in voltaic cells, Frederick Cottrell (1877–1948) for his contributions to the field of In electrochemical systems, diffusion effects exist, and Jaroslav Heyrovsk (1890–1967) for developing the mercury electrode and the polarogram [19].

Numerous notable scientists impacted the area of electrochemistry throughout the second half of the twentieth century, including Allen Bard (the University of Texas at Austin), John Bockris (Texas A&M), Ernest Yeager (Case Western University), and Rudolph Marcus (Caltech) [19].

Finally, The Nobel Prize in Chemistry was awarded to John B. Goodenough, M. Stanley Whittingham and Akira Yoshino for inventing lithium-ion batteries [19].

Electrochemistry is a science that exists at the interface of electricity and chemistry. It is concerned with the events and a method that happens due to chemical reactions generating electricity or electrical current induces chemical reactions. Electrochemical systems and methods are ubiquitous in contemporary science and technology, as well as in daily life. Electrochemistry is a fundamental area of science that encompasses everything from fuel cells and batteries to corrosion prevention, chlorine generation, and metal recovery. Electrochemistry is the science that examines processes in which ions pass across the contact between a solid (a metal electrode) and fluid or electrolyte on a microscopic level. These reactions are essentially thermodynamically and kinetically regulated by the potential dissimilarity between the solution and the electrode [19].

The essential divisions in electrochemistry are 1. equilibrium electrochemistry and 2. dynamic electrochemistry or electrochemical kinetics. The first case is

concerned with ionic equilibria and electrochemical cell thermodynamics; on the other hand, the latter incorporates a temporal dimension into the study of electrochemical processes. The focus of this study is on the fundamental principles of electrochemistry and the thermodynamics of electrochemical cells. Notably, very little education research has addressed issues like mass transfer or the kinetics of electrode operations so far [20].

Cell

An electrochemical cell is formed when two electrically conducting electrodes are submerged or come into touch with an electrolyte (Fig. **11**). The electrodes are electrical conductors, or semiconductors, in the sense that they conduct electrons. Due to the electrons' inability to carry charge in salts or solutions must be transmitted beyond electrodes through charged molecules or atoms called ions. Ions in the electrolyte interact with the electrode surfaces in heterogeneous ways, leading to electron transport to and in the conducting electrodes. Additionally, On electrodes, neutral atoms or molecules may react to create ions or to be converted into ions as a result of electron loss or gain [19].

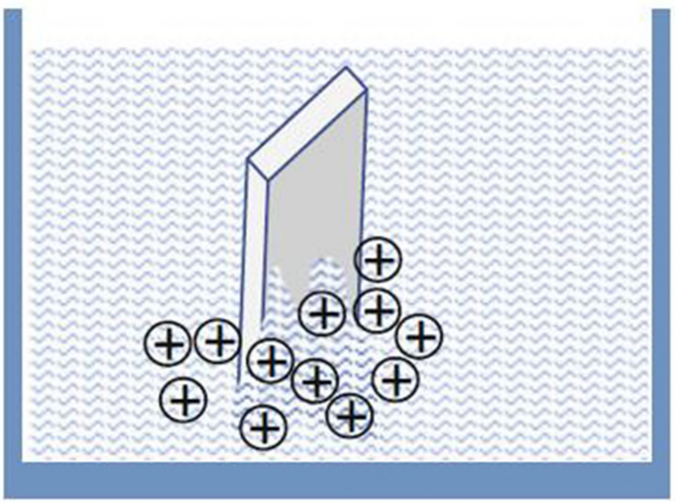

Fig. (11). Lithium ionization illustration in water.

Fig. (**12**) depicts an electrochemical cell. Two electrodes are shown submerged in the solution, while negative and positive ions conduct charge in the electrolyte. The electrons flow *via* the external circuit; they make their way into the electrode responsible for the reduction and exit the electrode responsible for oxidation. Three operating modes are denoted by the external circuit symbols (which cannot

be utilized concurrently): the voltage symbol denotes an electrolytic cell that affects the solution and produces chemicals, a galvanic cell that produces power and is as shown by the resistance of an electroanalytical cell that measures current and voltage [19].

Transfer of charge happens at the contact between the solid electrode and the fluid electrolyte, which is one of the distinctive features of electrochemical processes. Due to the complexity of this process and the interaction of chemical and electrical effects, electrochemistry is a fascinating interdisciplinary field of study. Among other things, it is required for electrolysis, batteries, solar cells, fuel cells, and corrosion [19].

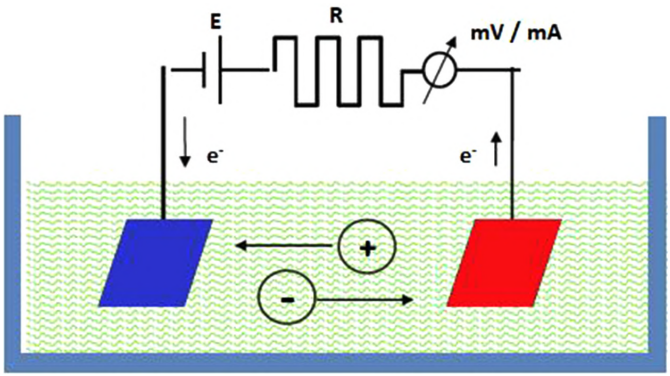

Fig. (12). Schematic of an electrochemical cell.

The Anode and Cathode

At the cathode, electrons from the external circuit enter the circuit and react to the reaction. The cathode experiences a reduction. In this experiment, oxygen gas is reduced in a fuel cell, and chlorine gas is consumed [19].

$$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$$

$$Cl_2 + 2e^- \rightarrow 2Cl^-$$

Electrons leaving the electrode are known as the anode. Electrons are the result of oxidation, which occurs on the anode. Chlorine gas is often produced in an anode reaction, whereas hydrogen oxidation may occur in a fuel cell:

$$2Cl^- \rightarrow Cl_2 + 2e^-$$

$$2H_2 \rightarrow 4H^+ + 4e^-$$

The most basic memory method is that the two words begin with the vowel, o/a, in the oxidation-anode connection. In contrast, Both begin with a consonant, r/c, in the reduction-cathode connection [19].

Electrolyte

Ions, which are charged particles, are responsible for the electrolyte's ionically conductive properties. This may be a liquid, or it may be a solid, a molten material, or a polymer [19].

Electrolyte solutions are usually watery (*i.e.*, acidic, basic, or salty) solutions of acids, bases, or salts. This complex dissociates into ions due to the process termed solvation. One good example is:

$$NaCl_{(S)} \rightarrow Na^{+}_{(aq)} + Cl^{-}_{(aq)}$$

When carbon dioxide or sulfur dioxide combines with water, hydronium (H_3O^+), carbonate, and sulfate ions from a solution comprising hydronium, carbonate, and sulfate ions—dissolving a salt without melting it in water yields the electrolyte. A salt melts and dissociates into ions at a particular temperature, which is its melting point. The salt Na_2CO_3 liquifies at 650 degrees Celsius and is utilized in molten carbonate fuel cells.

$$Na_2CO_3 \rightarrow 2Na^{+} + CO_3^{-2}$$

It may also be a solid electrolyte. Ceramic structures constructed with ions capable of flowing inside the structure due to concentration gradients and electrical fields are called electronic, ionic structures. ZrO_2 is a good example of a solid electrolyte since it is utilized in solid oxide fuel cells to manufacture oxygen sensors. At 800°C, zirconium oxide may conduct oxygen ions [19].

If the polymer's structure is changed to allow the flow of ions, it may also be considered an electrolyte. Polymer electrolyte membranes (also known as Fuel cells using a polymer electrolyte membrane or PEM fuel cells) are examples of polymer electrolytes. Teflon or Polytetrafluoroethylene (PTFE) is reformed with branching HSO_3^- groups in the fundamental polymer structure in order to enable the polymer electrolyte to conduct protons, commonly known as hydrogen ions (H^+) [19].

Electrolyte ionic conductivity is typically represented as S/cm per degree centigrade. As stated above, strong ionic conductivity is important in

electrochemical apparatuses, for example, electrolysis cells, fuel cells, and batteries. This allows processes to occur quicker and at lower voltage drops, resulting in increased power. In order to avoid a short or a leakage current, electrolytes must have a very low electronic conductivity [19].

They may also be organic-based liquid electrochemical systems. It must be assumed that the solvent does not consist of water to draw this conclusion. The electrolyte is created by dissolving specific salts in an organic solvent to achieve ionic conductivity. Electrochemical cells are often employed with organic solvents. These include tetrahydrofuran, diethyl ether, acetonitrile, formamide, glycol, ethanol, methanol, *etc*. Inorganic electrolytes and salts used as examples include NaCl, $LiBF_4$, and $HClO_4$ [19].

Electrodes

Various conducting electrodes may be distinguished into two distinct groups, depending on the substance from which they are formed. Carbon (graphite, diamond, graphene, *etc*.), metal oxides (MoO_2, MnO_2, CoO_2, *etc*.), liquid metals (Hg, amalgam), Solid metals (Pt, Au, Ag, *etc*.), and semiconductors have all been utilized as electrodes: semiconductors (ITO(indium-tin-oxide), Si, *etc*.), metal oxides (MoO_2, MnO_2, CoO_2, *etc*.), liquid metals (Hg, amalgam), and solid metals (Pt, Au, Ag, *etc*.) [19].

The electrode geometries are many and may include anything from a disc to a tube to a wiry mesh to a liquid to a microscopic to an ultra-micro electrode [19].

Micro or ultra-micro electrodes are used in voltammetry (*i.e.*, analytical applications). Measurements in weakly conducting fluids are possible with these electrodes since shallow currents are involved. These electrodes also have a low voltage drop and a little change in the electrode-solution interface [19].

RDEs (Rotating disc electrodes) are electrodes that utilize a moving circular disk to obtain concentrations and mass transport effects in electrochemical systems. By encapsulating a metal wire in a cylindrical polymer body (*e.g.*, Teflon), the spinning disk electrode is created. Special hydrodynamic conditions are generated in the water column by spinning the polymer cylinder electrode, allowing us to examine the flow of substance and ions starting with the bulk solution and ending with the electrode. The rotating disk-ring electrode is used to study electrode processes and associated intermediate species. Fig. (**13**) depict disk and disk-ring electrodes [19].

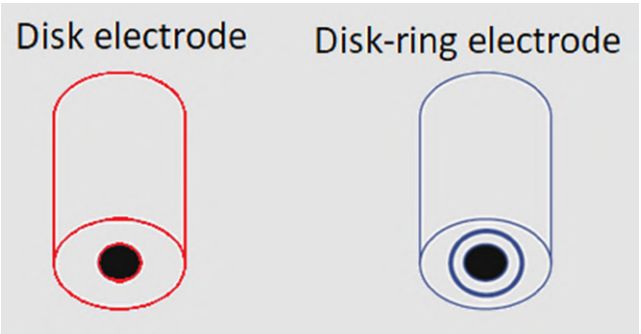

Fig. (13). Some preliminary schematic representations of disk and disk-ring electrodes. Single electrodes are shown, but no equipment for the rotator is included.

Another way to classify electrodes is according to their role in an electrochemical cell. The most basic electrochemical cell has only two electrodes, referred to as a two-electrode system (or embellishment). Typically, this arrangement is utilized in electrochemical industry processes and galvanic cells, such as fuel cells, batteries, and electrolysis. Typically, these cells' electrodes are referred to as cathode and anode or negative and positive [19].

When utilizing a potentiostat/galvanostat, a two-electrode arrangement allows for the most detailed study of the main response; the electrodes are the counter and working electrodes. The reaction occurring on the counter electrode serves a purpose just to complete the electrochemical cell in these cells [19].

The essential characteristics of electrode processes and in electroanalytical chemistry, a frequently utilized arrangement or configuration includes more electrodes than the standard two-electrode system. In electroanalytical studies and electrochemical characterization, the difficulty is determining the electrode's useful potential for both electrodes. Assume that the electrode's potential of interest (*i.e.*, the working electrode) is monitored about the counter electrode. It may not be correct in this instance since the counter electrode potential is not constant and can vary throughout the experiment or reaction. That is to say, although the voltage of a cell may be accurately measured, the individual potential values (on a defined scale) cannot [19].

As a result, a third electrode is added, which has a constant potential and is not vary by the response, *i.e.*, the current does not flow *via* it, however in the middle of the counter and working electrodes. Individual potentials are recorded as the electrochemical reaction among the counter, and working electrodes occur (using a potentiostat or a voltmeter). When the working electrode's potential is altered or regulated intentionally, such as when using a potentiostat, its potential is precisely

managed by monitoring its potential against adjusting accordingly and the reference electrode [19].

Reference or sense electrodes are the terms used to refer to these extra electrodes. Thus, electrochemical systems may consist of two, three, four, or five electrodes. Additionally to a cathode and anode, a reference electrode is used to accurately calculate and regulate the potential of one or both of the electrodes under consideration, *i.e.*, cathode and anode. When multiple reference electrodes are utilized, the extra electrodes are referred to as sensitivity electrodes, and their purpose is to precisely compute the potentials of the counter and working electrodes . Additionally, the sensitivity electrodes are located near the counter and working electrodes; they "sense" to reduce possible distortion caused by unequal electrolyte concentrations and shifting electrical fields. Fig. (**14**) schematically depicts a five-electrode cell arrangement [19].

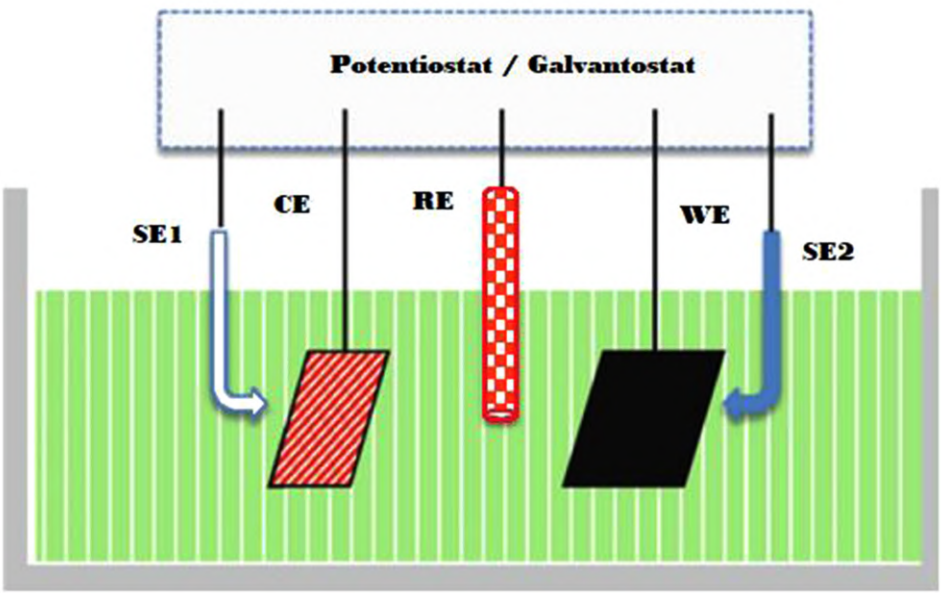

Fig. (14). Electrochemical cell with five electrodes.

One of the key technical aspects of next-generation bio/chemical microsystems is the use of electrochemical principles. Ionic solutions and electrodes are at the heart of electrochemistry. It looks a long way from the world of biology, where you are certain to find flesh and blood and bone. However, because of this, Galvani's research lab in Bologna, Italy, in 1791 was among the first to combine bioelectrochemistry with electrochemistry. Electrochemistry emerged historically out of electrochemistry [21].

Because Becker goes into considerably more detail in his book The Body Electric, we may safely conclude that electrochemical processes occur everywhere you look into biological systems. Nerves may be seen as the wiring that connects all the body's enzymes to the electrodes. There are membranes inside the body, as well as in electrochemical cells. However, it is exactly what occurs in electrolysis, in electrochemical reactors. As a result, we may turn to the brain, and what phenomena are the least understood and documented. Maxwell-Cade (1996) discovered that electrical oscillations not only alert the brain to mental activity but are also closely associated with consciousness. The electrochemical origin may be found in both of them [21].

Voltammetric (or amperometric), potentiometric, and impedimetric biosensors are the three primary classifications of electrochemical biosensors based on the transduction method employed and the type of signal recorded.

Voltammetric Biosensors

Voltammetric and amperometric methods are defined by applying a potential to a working (or indicator) electrode in comparison to a RE and the subsequent measurement of current [22]. The most widely used kind of Biosensor is the voltammetric Biosensor. Most biochemicals may now be detected and measured Voltammetrically through their electrooxidation or electror eduction mediated by an enzyme. Their hydrolysis/phosphorylation is catalyzed by an enzyme followed by electro reduction/electrooxidation, or their participation in a bio affinity process allowing electrooxidation/electro reduction (Fig. **15**) [1, 23].

Leyland and Clark invented the first commercial Biosensor for glucose monitoring. It was a voltammetric electrode [24].

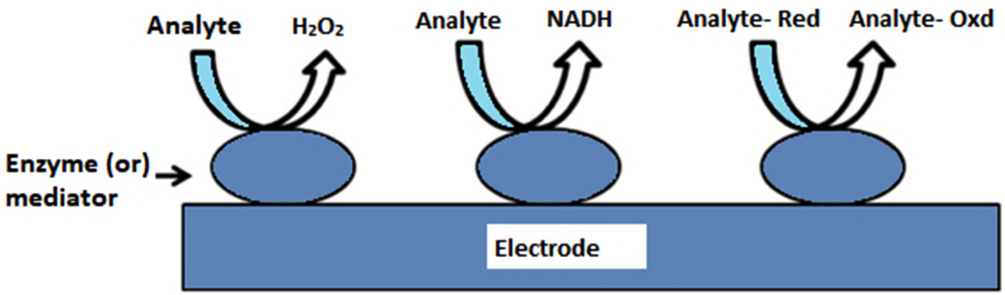

Fig. (15). Various types of voltammetric biosensors.

Voltammetry is the term used to describe the technique in which current is measured using a controlled variable voltage [17]. Voltammetric sensors examine

the impact of the detecting species' concentration on the current potential characteristics of a particular reaction's reduction or oxidation [23].

The flexibility of amperometric biosensors is further shown by their capacity to perform direct or indirect measurements. Direct amperometry takes advantage of the close connection between the redox reaction products and the measured current. On the other hand, indirect amperometry utilizes ordinary detectors to determine the analyte's metabolic substrate or product [23]. Voltammetric techniques include stripping voltammetry, polarography, AC voltammetry (alternating current voltammetry), SWV (square-wave voltammetry), DPV (differential pulse voltammetry), HV (hydrodynamic voltammetry), CV (cyclic voltammetry), and LSV (linear sweep voltammetry) [13, 17].

The word “amperometry” refers to the technique of tracking changes in current (as a result of electrochemical reductive or oxidation) throughout time, maintaining the cell's or electrodes' potential constant. This technique determines the current by reversing the potential equivalent to the desired value or keeping it at that value. In this respect, the measured maximum current value across a possible linear range is proportional to the analyte's bulk concentration in the solution [17]. The absence of a scanning potential distinguishes amperometry from voltammetry [22].

Voltammetry determines the potential difference between an electrode executing a task and a RE. The current flow between the CE and a WE is measured using a voltmeter attached to the latter (whose purpose is to shut the circuit). In an electrochemical cell, electrodes are inserted that hold a solution containing the desired analyte. Electroactive species initiate electron transfer processes across the WE, and the resultant current is commensurate to transferred electrons [15].

The Cottrell equation is a straightforward mathematical formula that relates the measured current I in the working electrode (of plane geometry) to various times (t) in terms of the analyte concentration (C) in the solution:

$$i(t) = \frac{nFAC^{\frac{1}{2}}}{\left(\pi^{\frac{1}{2}} t^{\frac{1}{2}}\right)} \quad \textbf{(1)}$$

Where n is the number of electrons transferred during the redox reaction, A denotes the electrode area, and F denotes the Faraday constant [15].

The Voltammetric Biosensor is on the basis of the rate of electron transmission at the WE's face [25]. The literature indicates that this mode of transduction has been extensively investigated in biosensors for protein detection, glucose, lactate, and sialic acid [23, 26].

Current is monitored at time intervals in fast-scan cyclic voltammetry due to the triangle pulsed potential waveform delivered to the UME (Ultramicroelectrode) [13].

Amperometric biosensors provide a high degree of selectivity because the potential generated by the oxidation or reduction process utilized to detect the analyte species is a unique characteristic of that species [17]. The primary benefits of these sensors are their rapid reaction times and dynamic ranges and their high sensitivity, suitability for mass manufacturing, and sensitivity [1, 27]. However, it does not allow the differentiation of various oxidizable species at the chosen working electrode potential [22].

Voltammetric techniques having a large linear dynamic range are well suited for measuring low-level values [17]. Due to the fact that rest intervals are required between scans, the temporal resolution is less acceptable than in amperometry. Additionally, capacitive currents are substantial and tend to obscure the faradic information unless they are sufficiently stable to be removed [22]. Voltammetry is the least susceptible to the noise of the electrochemical methods [26].

Potentiometric Biosensors

The accumulated charge potential at the indicator electrode is monitored. In comparison, the RE in potentiometric devices provides important information about the electrochemical process's ion activity (In conditions of zero or negligible flow currents between reference electrodes and the indicator) [17]. This transducer detects the difference in potential produced between two solutions separated by an ion-selective membrane with practically little current flow (Fig. **16**) [1, 9].

The general process of potentiometric signal production is based on the charge separation of two phases, which occurs due to the perm-selective transfer of analyte ions from the aqueous to the organic phase. Potentio metric biosensors are constructed using an ion-selective electrode (ISE) and ion-selective field-effect transistors (FETs) (ISFET). Ion-selective field-effect transistors (FETs) are FETs with an ion-sensitive surface [23].

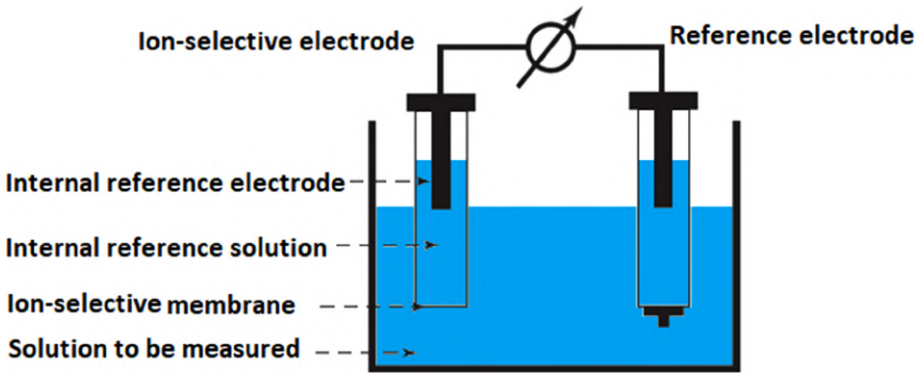

Fig. (16). Structure and working process of potentiometric biosensors.

The observed potential is ascribed to the quantity of the electroactive type present in the instance under these circumstances. The reduction potential is proportional to the analytes' concentrations using the Walther Nernst equation.

$$E = E^{\circ} - \left(\frac{RT}{nF}\right) \times \ln\left(\frac{\alpha \mathrm{Re}\, d}{\alpha Ox}\right) \quad \textbf{(2)}$$

Where Ox/Red means the activity proportion of the oxidant and reductant species, F denotes Faraday's constant, n denotes a number of electrons exchanged during the redox reaction, T denotes the cell's constant temperature in Kelvin degrees, R denotes the gas constant, and E^{o} denotes the standard-state potential, respectively [17]. This is true only when layer selectivity or the membrane is infinite or when the concentration of interfering ions is constant or sufficiently low. Except at the membrane-sample solution boundary, the potential changes at various phase boundaries are negligible or constant [28].

They have many benefits, including their simplicity, clarity of operation, and lack of chemical reaction with the sample; they are also well suited for measurements at low concentrations and in samples with small volumes [17, 27]. Additionally, they have a known capability for downsizing, which gives them an edge over conventional electrochemical transducers. In contrast to voltammetric methods, the signal is not reliant on the surface area of the electrode [23]. However, due to their low selectivity for ions, sensors potentiometric are not reliant on electrochemical reduction and oxidation processes [9].

Impedimetric Biosensors

Schulze and Lorenz developed electrochemical impedance spectroscopy in 1975 to determine the material's capability and resistance by disturbing the system amplitude with a sinusoidal AC functional wave [9]. Potentiometry is a technique for determining the difference in potential between a WE and a RE at near-zero current levels. While many potentiometric electrodes are available, the greatest often utilized are ISE (Ion Selective Electrodes). This kind of transducer is composed of membranes that have a high selectivity for particular ionic species produced or used during a particular biological activity [26].

These devices are based on their capacitance (C) and constituent's resistance (R) or impedance (Z). Inductance has a minimal impact on how a typical electrochemical system operates. Thus, impedance is represented in the following manner:

$$Z^2 = R^2 + \frac{1}{\left(2fC\right)^2} \quad \textbf{(3)}$$

Conductance is the inverse of resistance therefore, some scientists refer to such systems as conductometric. Impedance biosensors typically consist of three electrodes with an alternating voltage delivered at amplitudes ranging from a few to 100 mV [23].

The impedance spectra of this system are obtained by varying the frequency across a wide range. The resistive and capacitive impedance components are determined by measuring out-of-phase and in-phase responses to current. This technique is capable of carrying out high-frequency electron transfer as well as low-frequency mass transport [9].

The most significant phenomena that this approach may investigate are the impedance method, species diffusion, the double layer's capacitance, and the transmission of electrical charges for binding designation monitoring. For instance, the current is determined, and a known voltage is applied to the electrode during the procedure [9, 15].

This method is widely employed in microbial electrochemical analysis because of its compact size and rapid reaction; nevertheless, even when coupled with nanomaterials, it cannot reach the sensitivity of other techniques when used with low-conductance samples [26]. The primary drawback of impedance biosensors is the possibility of false-positive findings owing to electrolytes in the samples. In comparison to potentiometric and impedimetric biosensors, impedimetric

biosensors are used less often [23].

Finally, all cases are summarized in Table **2**.

Table 2. Environmental water monitoring: Classifications of electrochemical biosensors and their related benefits and drawbacks.

-	Voltammetric (Amperometric)	Potentiometric	Impedimetric
Enter	Controlled constant voltage or a succession of controlled voltages (*e.g.*, linear potential sweep, cyclic voltammetry, differential pulse voltammetry, *etc*.).	At the reference electrode, a specified voltage is applied. Presently, there is little to no presence.	A DC bias potential and a broad range of alternating current frequency scans (Faradaic impedance) or a complete range of alternating current frequency scans (non-Faradaic capacitance).
provide	Current variation with respect to time or current variation with respect to applied voltages.	The possibility of a distinction between the WE and the RE (a constant potential).	Complex impedance or capacitance is a term that refers to both.
how to maximize	The measured Faradaic current is proportional to the analyte's concentration.	The difference in potential between WE and RE is proportionately evident in the sample's ion activity. They are often used in conjunction with ion-selective electrodes (ISE).	Complex impedance measurements provide information on the charge transfer resistance (if a redox couple is present) and the capacitive characteristic of the electrochemical double layer around the WE.
How to enhance	To enhance the signal and increase the signal-to-noise ratio, use an electron mediator or conductive nano-architectures (*e.g.*, nanotubes) or interdigitated electrodes.	Utilizes carbon nanotubes in the sensor to enhance heterogeneous charge transfer and surface area	Immobilize nanoparticles to enhance the surface area and improve charge transmission. Utilize single frequency monitoring for capacitive sensors to create a simpler and quicker system.
Vantages	The system's simplicity (ease of integration and possible cost savings), rapid detection; low detection limit and high sensitivity; possibility for intercalator-based DNA detection utilizing PNA probes.	Appropriate for mass manufacturing.	The electrode may be label-free and is simple to produce.

(Table 2) cont.....

-	Voltammetric (Amperometric)	Potentiometric	Impedimetric
Inadequacy	Changes in pH caused by the addition of biological material may impair enzyme function.	The high need for the reference electrode (stable and accurate); the same disadvantages associated with the use of an enzyme as a label or a BRE; intrinsically sensitive to the pH value of the surrounding environment during measurements since the detecting technique is dependent on ion activity; restricted applicability owing to problems in using affinity-based detections.	The time required to conduct a single measurement is relatively long due to the time-consuming frequency scan process; miniaturization is more difficult due to the more complicated input and output control; and the label-free nature of the instrument, an adequate antifouling system and certain signal amplification modifications may be required.

Economic and Market for Biosensors

Biosensors have a wide variety of commercial uses in a wide variety of activities. Medical (at home or in hospitals) and process control in the food manufacturing sector is the most significant uses. Other uses include environmental protection and defense [9].

The Biomedical Sector

The majority of sensors presently enzyme sensors are used in medicine. Due to their measuring specificity, simplicity of installation, and commercial availability of the enzymes and their associated transducers. The glucose oxidase sensor has garnered the greatest interest because it can detect blood and urine glucose levels for diabetes diagnosis. This is also why this sensor has received so much research attention. One of two methods is being utilized to establish the blood glucose levels of individuals with diabetes. One of them employs an open-loop system in which insulin is administered to the patient according to a preset schedule without respect for a person's glucose levels. The following method employs a closed-loop system that automatically injects insulin or other chemicals in response to the glucose levels at any given moment. The enzyme electrode continuously monitors the glucose level in the blood the device could be thought of as a sham pancreas. Biosensors *in vivo* necessitate resolving biocompatibility issues, particularly those relating to platelet and fibrin deposition on the membrane that is enzymatic. Apart from the glucose electrode, biosensors may be used to measure medium biological metabolites. The creatinine and urea electrodes are used to monitor renal work; the cholesterol electrode is utilized to detect and prevent

arteriosclerosis; Neurotransmitters are measured using the acetylcholine electrode involved in chemical communication synapses; the Lactate electrode is used to assess the intensity of physical exertion. Microbial sensors have limited uses in medicine because of their incompatibility with biological fluids, often used as the medium in which bacteria grow. The development of the biomass deforms the biocatalytic matrix, resulting in cell leakage and pollution of the representative media. Additionally, since microbial sensors include many enzymes, they are insufficiently selective in numerous biological investigations. Due to the specificity of Immunological responses, the more significant potential for application in medicine is inside immunological sensors. These sensors assess medicines' concentration, such as the human chorionic gonadotropin hormone and theophylline for pregnancy diagnosis, cancer detection with alpha-fetoprotein, and the hepatitis B surface antigen. Though, these sensors are unable to be utilized *in vivo* since the enzymatic amplification required for sensor function needs the inclusion of a substrate. Additionally, the antigen-antibody complex synthesis is sluggish and needs many stages. The immunological sensor operates sequentially and is unable to provide continuous control [9, 29].

The Food Production Industry

Food manufacturing processes are getting more mechanized. There is an enormous need for biosensors to monitor the various manufacturing phases and the final product's quality control [29].

Due to biosensors' sensitivity to biological products are well suited for the purpose of determining the composition of substances, food additives, contaminants, and toxins. Meat freshness can be determined by measuring the glucose consumption of microorganisms on a glucose electrode. Similarly, inosine, hypoxanthine, and the inosine-5'-monophosphate sensor are used to determine the freshness of fish. Biosensors have a variety of applications in the food production sector, including determining lactate for wine and yogurt quality control and glucose and penicillin for fermentation monitoring. Additional instances contain the measurement of amino acids (for instance, lysine) to control the nutritional value of meals and the protein content; sucrose is used to manufacture jams, syrups, and ethanol to determine the alcohol content in alcoholic drinks. Similar to what was done with medicinal applications, enzyme sensors are most adapted to these needs [29].

The application of biosensors in fermentation processes continues to present many challenges. Biosensors, which are enzymatic reaction-based, are invasive; they release minute amounts of reaction products. To prevent this, In a derivative loop, the Biosensor is placed, and the sample has measured components that are not

recycled. Additionally, this device eliminates contamination concerns inside the fermenter. However, extraction results in the disappearance of the artistic medium, and the sample obtained is not necessarily the fermentation broth's representation as a whole. In other experiments, the Biosensor was placed in an autoclave without its Biocatalyst. After that, the enzyme is placed in a microchamber and retained throughout the Biosensor's process. It is possible to regenerate an enzyme if its activity is deemed insufficient [29].

As stated before, there is a rising need for biosensing technology in food sustainability, which addresses all five of the main issues. One of the difficulties in developing alternative energy sources is that our present dependence on fossil fuels has constrained their supply, resulting in pollution. Bioelectrochemical systems (BES) are emerging as a means of addressing the energy problem by developing sustainable waste remediation, resource recovery, chemical synthesis, and power sources. These one-of-a-kind devices may convert chemical energy to electrical energy in both directions utilizing microorganisms generated from organic wastes such as low-strength wastewaters and lignocellulosic biomass as catalysts. The systems can be configured to produce peroxide, caustic, hydrogen, electrical energy, and nutrients, as well as to recover metals and eliminate refractory substances. These systems have resulted in the development of novel ideas and designs for separators, electrodes, and catalysts (Fig. **17**) [29, 30].

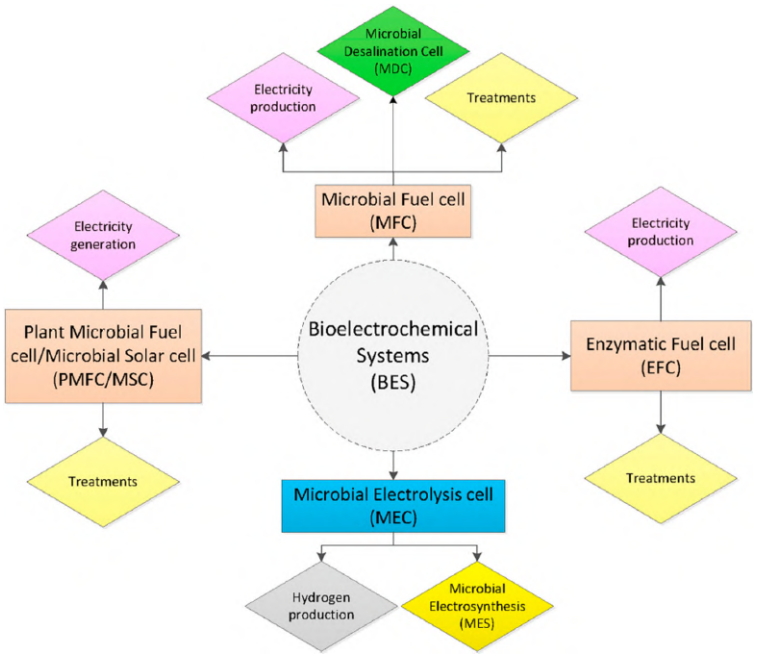

Fig. (17). A graphical depiction of the different kinds of bio-electrochemical systems (BESs).

Global land degradation is one of the most severe food production problems owing to increasing industrialization, pollution, urbanization, and unsustainable land use. Land degradation has expanded rapidly during the last several decades, impacting 12.2 billion hectares worldwide and 1.5 billion people. Alternatively, As an assuring technique, bioremediation for restoring damaged and contaminated land may have field constraints. Fortunately, innovative advances in biotechnology are paving the way for new approaches in sustainable land restoration, such as using highly specialized enzymes, creating microbial consortia, and using plants with microbial partners. The primary worries are that recovery of land activities contaminant must exist and be location-specific to accommodate adjacent areas' soil and social situation. Restoration actions should be linked to extra advantages, for instance, biomass crops, industrial bioproducts, carbon sequestration in soil, and biofuel (Fig. **18**) [30].

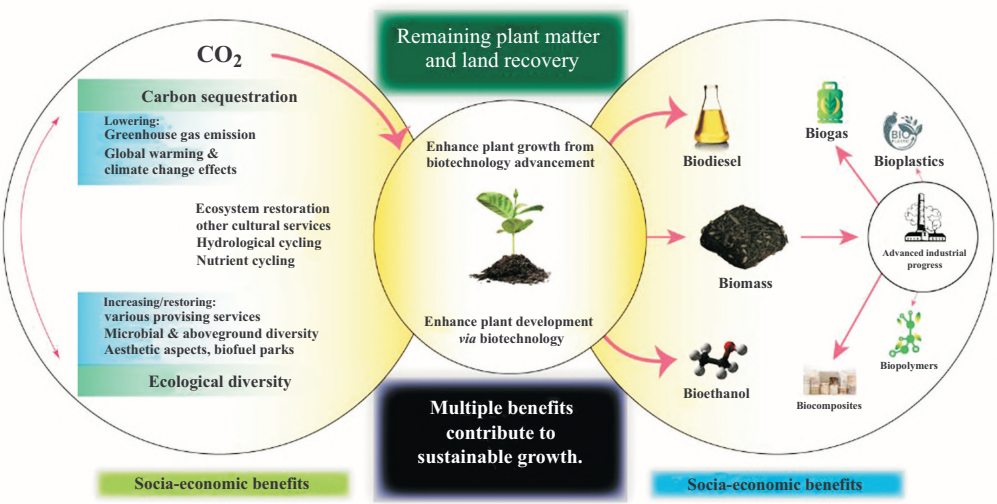

Fig. (18). Coupling bioremediation with the production of bioenergy and other value-added products in order to sustain a Bio-based economy.

Protection of the Environment

The situation of the environment in metropolitan zones is proportional to how air and water pollutants are controlled. Domestic and industrial waste contribute to water pollution, and biosensors may help quantify organic contamination and assess hazardous chemicals [29].

a) Determination of Organics:

Organic contamination is quantified using a well-established, standardized method that quantifies the biological oxygen requirement (BOD). The experiment

needs five days of incubation at 20 °C for the sample. A Microbial biosensor can perform the BOD measurement in less time (about 15 minutes). This sensor works by immobilizing entire On the oxygen-permeable membrane of a pO_2 electrode hydrogen-producing bacteria (Clostridium butyricum). After the model has been drowned in oxygen, it is exposed to the organic chemical. The bacteria's consumption of oxygen results in a decrease in the electrode signal. The BOD value is then calculated using a calibration curve [29].

b) Identification of Poisonousness:

Poisonousness is challenging to assess due to the diversity of chemicals that are harmful in varying degrees. Total toxicity is often determined using susceptible aquatic species, such as trout. However, It is challenging to automatically identify the demise of a single or several trout without being harmed by false alarms caused by unintentional death. Additional biological assays, such as the Microtox bacteria assay and the mobility of daphnia assay, rely on huge crowds of live organisms to increase their dependability. These biological examinations continue to be challenging to automatize, so researchers are investigating biosensors sensitive to overall toxicity. Toxic chemicals function by inhibiting enzymes, and the immobilization of aim enzymes on transducers enables ongoing monitoring of the Biocatalyst's activity. The Biosensor simulates the toxication of live creatures and determines the real degree of toxicity. On this premise, the Cholinesterase electrode was developed for the complete measurement of Organophosphates and Carbamates. Additionally, this electrode is sensitive to a variety of additional hazardous chemicals with meager discovery restrictions. This kind of Biosensor is based on enzymatic inhibition and possibly be integrated into an automated system for monitoring water's purity and, in particular, wastewater [29].

Markets and Application Fields

The combined yearly revenue of all biosensor providers in 2014 was U.S $ 11.5 billion U.S $, and is projected to reach at 28.78 billion U.S $ in 2021. This equates to a 12.2% annual growth rate. The following sections outline the various applications for odor sensors and their associated market quantities [31].

• Healthcare: The healthcare market encompasses the contribution of established hospitals, dentists, doctors, and another service suppliers, to ambulatory and stationary accomplishment. Germany's health care system generated €86.5 billion in revenue in 2019. In 2018, Germany had 48,346 healthcare-related businesses. Diagnostics is one example of a potential area of use. In comparison to healthy individuals, sick individuals emit various VOCs. As biomarkers, these volatile organic compounds (VOCs) can be detected in urine, breath, and other bodily fluids. Making a diagnosis simply based on a patient's odor needs exact diagnostic

apparatus. When it comes to illness diagnosis, sensors for smells show to be an effective diagnostic instrument. Non-invasive diagnostic techniques are in high demand within the healthcare industry. These sensing devices need to be capable of monitoring in real-time and portability and reasonably priced [31].

• Food Manufacturing: Food manufacturing consists of producers of food and feed and the beverage sector. The German food sector employs about 6000 firms with more than 20 workers. These businesses occupied greater than 500,000 individuals in 2018. The food business is one of Germany's biggest industries, with about €180 billion yearly revenue. The odor sensors used in this sector should be capable of rapidly detecting changes in product quality during manufacturing. Impurities and pathogens are detected during quality control. Additionally, the proper composition, aroma, and flavor of the generated meal can be determined [31].

• Farming: farming is an economic activity in which dirt, animals, Manpower, and knowledge are used to create agricultural goods that guarantee the human population's access to plant and animal sustenance. In 2018, Germany had a total of 266,600 active businesses. In 2018, they generated 38.3 billion € in revenue. In agriculture, smell sensors are used to assess the quality of products and resources. According to their smells or volatile organic compounds (VOCs) or discover pests and other harmful effects already operating in the sector [31].

• Cosmetics Industry: Cosmetics are defined as any substance that has a therapeutic function but is also utilized for cosmetic purposes. The major consumer product groups mostly determine the industry.137 German businesses in 2018 manufactured cosmetics, earning about €6.4 billion in sales. In the cosmetics business, sensors for odors are primarily used to monitor the quality of manufacturing products. In the manufacturing process, odor sensors can verify the correct composition of goods, allowing for the analysis and creation of more distinct odors [31].

• Safety Applications: These are various kinds of applications that are designed to identify potentially harmful chemicals. Smells provide critical environmental and activity-related data that are important for army and security requests. This involves the identification of explosives or potentially dangerous items. Nonetheless, a smell detector could also be used for crime prevention, such as airport security checks or drug detection. Germany's security sector is expected to earn €9.2 billion in sales in 2021 [31].

• Environmental Monitoring: Environmental monitoring analyzes air both outdoors and inside to identify problems with air quality resulting from hazardous VOCs. These problems may arise during the manufacture of furniture, for

example. Odor sensors are also useful for detecting hazardous and poisonous chemicals. In addition, industrial emissions and air quality may affect the quality of surface water and groundwater. Due to increasing environmental notice and contamination, technology environmental monitoring solutions are expanding. In 2018, environmental protection in the German sector generated revenue of €71 billion [31].

CONCLUSION

In conclusion, in a simple example to define a biosensor, we can name the sensory organs of the body, which is like a biosensor, such as a nose, and has three main components: biological elements, transducer, and signal processor, and accordingly will be divided into two types. One is based on transducers, and the other is based on biological elements. Subsequently, it is divided into three types based on transducers: electrochemical, optical, thermal, and mass. In addition, based on biological elements, it is divided into antibody, antigen, aptamer, cellular and microbial. This book studies more electrochemical biosensors based on the electrochemical transducer. This chapter serves as the foundation for the material covered in subsequent chapters. In fact, by understanding the fundamental concepts of electrochemical biosensors, the reader can enter into a discussion about sensor design.

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CHAPTER 2

Electrochemical Biosensors Design Steps

Abstract: Designing a biosensor is a complex engineering process requiring careful consideration. This chapter takes a brief look at the design-to-fabrication process of electrochemical biosensors and the evaluation of their performance. This review helps us to build a roadmap for designing reliable and valuable biosensors for various applications. The design roadmap consists of ten steps. The first section discusses the importance of these steps, then some of them will be discussed in detail. This chapter helps researchers to study the field of biosensors in a systematic and practical manner.

Keywords: Electrochemical biosensors, Immobilization method, Optimization method, Process design.

INTRODUCTION

A common sensing device based on transducing biological events to electrical signals is the electrochemical biosensor, which is one of the most widely used. An electrode is a critical component in this sort of sensor since it serves as a stable base for the immobilization of biomolecules as well as the flow of electrons. Numerous nanomaterials with large surface areas enable synergic effects by improving loading capacity and mass transport of reactants, resulting in improved analytical sensitivity due to the increased loading capacity and mass transport of reactants enabled by the large surface area of nanomaterials. Electrochemical biosensors have received a great deal of attention from the scientific and industrial communities because of the benefits they offer and the promises they show [1]. Electrochemical biosensors are utilized in clinical diagnostics, quality control in food processing, and environmental monitoring applications [1, 2]. Variety of applications causes biosensors to be an interdisciplinary field. Because of their interdisciplinary nature, electrochemical biosensors are studied in various fields, so a clear roadmap for designing biosensors is essential. This roadmap helps researchers follow a systematic approach to find reliable results. The diversity of expertise of researchers active in the biosensors field can cause some research aspects of biosensors research to receive less attention. In some cases, the engineered design process of biosensors becomes an incomplete, not fully designed process. For more clarification of the path, from biosensor design to its

Seyed Morteza Naghib, Seyed Mahdi Katebi and Sadegh Ghorbanzade

application, especially for young researchers, this chapter examines the progress of sensor design and details some of the steps, from design to test. The designing and testing of a biosensor are shown step by step in the chart below (Fig. **1**).

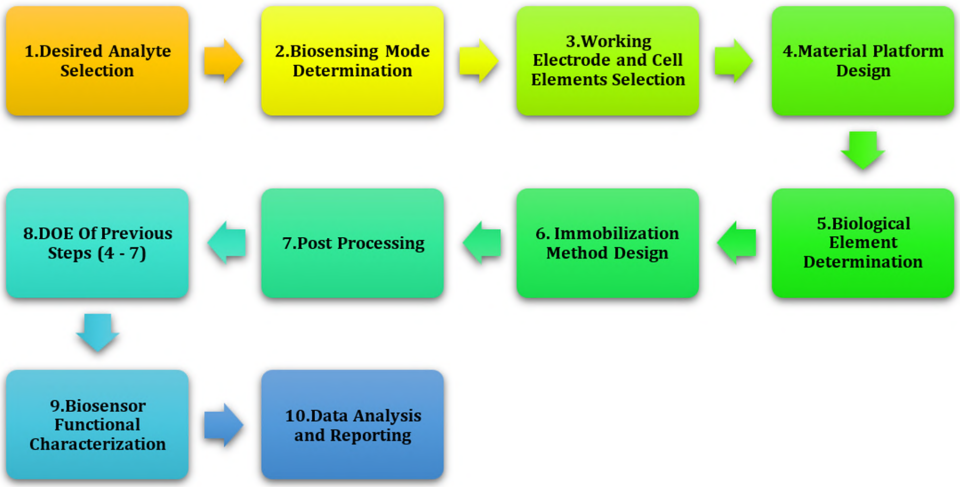

Fig. (1). Progress of biosensor design, fabrication and evaluation.

Electrochemical Biosensors Design Steps

Step 1: Desired Analyte Selection

The purpose of building biosensors is to measure a biomarker or analyte in an environment; therefore, to start the design process of a biosensor, the target analyte must be specified. Each analyte place restrictions on the design process due to environmental conditions and the appropriate concentration range. The analyte selection significantly affects the subsequent steps; for example, when the analyte is a cell with desired characteristics, the optimal biosensor mode would be EIS, or membranes cannot be used in the Post Process step.

Step 2: Biosensing Mode Determination

The biosensing mode will be determined in the second step, given the considered analyte, measurable concentration range, facilities, *etc.* Generally, the available modes for electrochemical biosensors are Amperometric, Voltammetric, Impedimetric, Capacitive, and Potentiometric. The biosensing mode can be determined according to the considered structure of the biosensor and the available facilities. In some cases, the analytics conditions restrict the available options and direct the designer to a specific option. For example, when working with cellular biosensors, the current and voltage of the working electrode drastically decrease due to the blockage of the surface electrode by cells;

therefore, the sensor process is practically disrupted. In this case, impedimetric or capacitive biosensing modes are recommended.

Step 3: Working Electrode and Cell Elements Selection

With determining the biosensing mode, the electrochemical cell structure would be determined, in general; yet there are parameters to be considered and selected which can be used for designing more optimal biosensors. For example, by selecting the Amperometric mode, the cell structure will form three electrodes. In this cell, the reference electrode can be selected among the available options given the required reactions in the following steps. Also, selecting the material and type of working electrode facilitates designing the material platform and fabricating the biosensor.

Step 4: Material Platform Design

In this step, according to the problem requirements and the potential of different materials, a material platform should be developed to achieve two critical goals. First, to provide a suitable condition for stabilizing biological elements to the electrode. Second, with its electrical, chemical, and biological properties, the platform can increase the detection quality; for example, it would increase sensitivity or be antifouling.

Step 5: Biological Element Determination

The core of a biosensor is its natural element. Since biological elements carry out the analyte detection and capture, their selection is critical in the biosensor design process. When selecting the target analyte, the biological elements suitable for analyte detection are very limited; however, it is important to consider the options available in this step. Besides selecting the natural element, the physicochemical and biological conditions of the biological element must be examined. These parameters are very effective in the biosensor performance quality.

Step 6: Immobilization Method Design

Once the material platform and the biological element have been selected, the appropriate method for connecting the two elements must be determined. How the natural element is stabilized on the surface affects its performance and biosensor quality. Generally, the biological elements can be stabilized and connected to the surface (electrode or material platform) in two approaches: physical and chemical. The choice of connection method depends on the physical and chemical characteristics of the material platform surface and the natural element.

Step 7: Post Processing

In this step, depending on the biosensor requirements, other processes may take place to improve the performance of the biosensors. For example, by adding a membrane to the external surface of the biosensor, the selectivity of the sensor can be enhanced, or by blocking the unconnected linkers, unrelated links are prevented during the biosensor test.

Step 8: DOE of Previous Steps

Since steps 4 to 7 are experimental processes, the parameters involved should be examined and optimized. Optimizing these parameters will maximize the output of the biosensor. For this purpose, the Designs of Experiment methods should be applied, determining the most optimal state for laboratory processes with the least amount of time and money.

Step 9: Biosensor Functional Characterization

After designing and optimizing the laboratory processes, the biosensor is ready for performance testing. The biosensor performance test (step 9) and optimization (step 8) form an iterative loop; therefore, some of the former parameters may require modification or revision based on the output quality of functional and performance tests. The functional parameters of the biosensor are defined according to the designed purposes and problem constraints. In other words, a parameter with a certain value may be proper in a particular condition but considered unacceptable in another. A wide range of performance parameters is defined for biosensors. These parameters are sensitivity, selectivity, response time, linear range, *etc.*

Step 10: Data Analysis and Reporting

After investigating the sensor performance, the data required for the analysis will be available. The data should be analyzed and reported in a proper format, which significantly contributes to the reliability and repeatability of the research. Proper statistical knowledge will be beneficial to a successful data analysis and appropriate test design.

Biosensing Mode Determination

Detection or Measurement Mode Based on Process

Biosensors are categorized based on the analytes or processes they monitor. It is important to distinguish between direct and indirect monitoring of analytes or biological activity.

Direct Monitoring of Analytes or Biological Processes that Produce or Consume Analytes

Direct analyte monitoring has been the primary use of biosensors. However, it should be noted that the same biosensor may also be used for direct monitoring of enzyme or live cell activities by detecting the synthesis or consumption of a particular chemical in a continuous or sequential manner.

Indirect Monitoring of Inhibitor or Activator of the Biochemical Receptor

It is also possible to use biosensors to detect inorganic chemicals as well as pesticides that inhibit the biosensor's biocatalysis. These devices, however, may be irreversible. On the other hand, immuno-sensors can typically only be returned to their original biological activity after chemical treatment, so they are not considered reagentless devices. Thus, their applications, especially when monitoring the environment, are often as warning systems that do not require the precise measurements of the analyte concentration [3, 4].

Detection or Measurement Mode Based on Electrochemical Transduction

The amperometric method measures the current generated by an electrochemical reaction. Low currents can be achieved by maintaining a constant potential on a Pt, Au, or C-based working electrode or using multiple electrode arrays with reference electrodes, which may also serve as the auxiliary electrode. The resultant current depends on the bulk concentration or rate of synthesis or consumption of the electroactive species within the biocatalytic layer. Steady-state currents are generally proportional to bulk analyte concentration due to the 1st order dependence of biocatalytic reaction rates [4, 5].

Potentiometric measurements are made when no significant current flows between an indicator and a reference electrode or between two electrodes separated by a perm-selective membrane. Ion-selective electrodes (ISE) utilize thin films, selective membranes, or electrochemical sensors to recognize ions of interest. There are a variety of potentiometric devices available, including pH electrodes as well as ion (F, I, CN, Na^+, K^+, Ca_{2+}, NH_{4+}) or gas-selective electrodes (CO_2, NH_3). The Nernst-Donnan equation states that the difference in potential between indicators and reference electrodes depends on the logarithm of ion activity or gas fugacity (or concentration). This is only true if (I) the membrane or layer selectivity is infinite or if the concentration of interfering ions is constant or low enough; and (ii) potential differences at various phase borders are either insignificant or constant, with the exception of the membrane/sample-solution boundary.

Analyte diffusion to the biocatalyst layer, reaction with the biocatalyst, and reaction product diffusion towards the detector and bulk solution are all factors to be considered when setting up a biocatalyst layer near a potentiometric detector. Potentiometric biocatalytic sensors exhibit steady-state or transient responses but not equilibrium responses. On the other hand, enzyme-labeled immunosensors are evaluated under steady-state analyte consumption conditions, while the Ab-Ag complexes are expected to approach equilibrium and reactions to be reversible or irreversible. It is highly dependent on the buffer capacity and ionic strength of the sample for ISE-based biosensors, such as pH electrodes, to respond [6, 7].

It has been found that Ion-sensitive field-effect transistors are a significant modification of ISFETs, which are usually used to detect ion concentrations. Typically, the gate of an ISFET is covered with an ion-selective membrane. As a result, these ISFETs can be referred to as enzyme (ENFET) or immunological (IMFET) field-effect transistors when combined with a biocatalytic or bio complexing layer. ENFET and IMFET-based devices' operating characteristics are closely linked to those of ISE-based biosensors [4, 8, 9].

Interdigitated microelectrodes and impedimetric or conductometric sensors can monitor enzyme activities, such as urease and many biological membrane receptors. In a differential measurement, a sensor with an enzyme is compared to an identical sensor without an enzyme because the parallel conductance of the sample solution reduces the measurement's sensitivity [4, 10].

Biological Element Determination

The three main components of a biosensor are the receptor, the sensor transducer, and the signal processing system. Biosensors can be categorized by their bioreceptor or transducer. Bioreceptors, or biological recognition components characterize biosensor technology. Biological recognition elements, or bioreceptors, are what distinguish biosensors from other sensors. Biological receptors are sensors' mechanisms for recognizing analytes. Bioreceptors are molecular species that recognize themselves through biological processes. The sensors are responsible for attaching analytes to the sensor's surface for measurement. Bioreceptors are categorized into five main types: enzymes, antibodies/antigens, nucleic acids/DNA, cellular structures/cells, and biomimetics. Sample components of biosensors contain a biosensitive layer containing bioreceptors or covalently bonded bioreceptors. Biosensors commonly use the following types of bioreceptors:

1. Interactions between antibodies and antigens

2. Interactions between nucleic acids

3. Enzymatic interactions

4. Cellular interactions (*i.e.*, microorganisms)

5. Biomimetic interactions (*i.e.*, synthetic bioreceptors)

There are two major types of bioreceptor utilized in biosensor applications: enzymes and antibodies. On molecular recognition, a biosensor's success is based. Through bonding interactions and molecular structure, a molecule may "recognize" another molecule. A wide variety of noncovalent interactions between molecules exists, including hydrogen bonding, metal coordination, hydrophobic forces, van der Waals forces, pep interactions, halogen bonds, electrostatics, and electromagnetics. Molecular recognition involves molecular complementarity between the host and guest. For example, this occurs between receptors and ligands in biological systems as well as between RNA and ribosomes in RNA and RNA-based systems [11 - 13].

Molecular recognition is a process where a host interacts with a guest in order to create a detectable alteration that can be read by a device. Vancomycin, an antibiotic that preferentially binds to peptides containing terminal D-alanyl-D-alanine in bacterial cells, is an important example of molecular recognition. Upon binding to these specific molecules, vancomycin is deadly to bacteria because it can no longer be utilized to build their cell walls. In biology, molecular recognition is essential for a variety of activities. Enzyme catalysis, multiprotein complex assembly, active and passive ion transport across membranes through ion pumps, ionophores, and Channels are some of these activities [11, 14].

Immobilization Method Design

Immobilized enzymes are described as "enzymes that are physically restricted or localized in a specified area of space while retaining their catalytic activity and may be utilized again and continuously." In certain instances, the use of immobilized catalysts has significantly enhanced both the technical and economic performance of industrial processes. Immobilization technology has advanced quickly in the last three or four decades, and it has increasingly become a matter of logical design; nevertheless, there is still room for improvement. New methods and greater knowledge of existing procedures will be required to extend the application of immobilized enzymes to additional practical processes. Irreversible and reversible immobilization are the two types of immobilization. In the next part, we go through these two approaches in depth [15].

Methods of Irreversible Immobilization

An irreversible immobilization can be described as the situation in which an enzyme, once connected to a support, cannot be removed without affecting either the enzyme or the support. Covalent coupling, entrapment or micro-encapsulation, and cross-linking are the most prevalent methods of irreversible enzyme immobilization.

The most common methods of protein immobilization are based on covalent bonds. These techniques have the benefit of not releasing the enzyme into the solution due to the stable nature of the bonds established between the enzyme and the matrix. However, the necessary amino acid residues for a catalytic activity must not be implicated in the covalent attachment to the support in order to obtain large levels of bound activity; this criterion may be difficult to meet in certain instances. When there is a stringent need for the lack of enzyme in the product, covalent immobilization techniques are used. Depending on the functional groups accessible on the matrix, a broad range of reactions have been created. In general, there are two types of coupling methods: (1) matrix activation by adding a reactive function to a polymer, and (2) alteration of the polymer backbone to create an activated group. The activation procedures are often intended to produce electrophilic groups on the support, which react with the strong nucleophiles on the proteins during the coupling phase. The fundamental factors that govern the course of covalent coupling to matrices are similar to those that govern protein chemical alteration [15, 16].

The amino acids lysine (amino group), cysteine (thiol group), aspartic and glutamic acids are the most often utilized side chains in processes (carboxylic group). There are many commercially available immobilization supports; the optimum option in each instance necessitates the evaluation of certain important catalytic characteristics as well as the desired application. However, it is common to have to test a few different approaches before settling on one that works best for the situation. The most frequent covalent processes produce enzymes connected to the support through amide, ether, thiol-ether, or carbamate bonds. As a result, the enzyme is firmly attached to the matrix and, in many instances, stabilized. However, due to the covalent nature of the connection, the matrix, along with the enzyme, must be destroyed after the enzymatic activity has degraded. The expense, in terms of usually poor yields of immobilized activity and the nonreversible nature of this binding, is partly compensated by the advantage of establishing a leak-proof binding between enzyme and matrix as a consequence of these processes. One approach to circumvent this issue is to use enzymes that are covalently linked to solid supports through disulfide bonds [17].

Methods of Reversible Immobilization

Because of the type of binding between enzymes and supports, enzymes that have been immobilized can be removed from support under mild conditions. In the case of enzyme immobilization, the use of reversible techniques is appealing mainly because new enzymes can be added to the support as the aged enzyme degrades. Indeed, the cost of the support is often a major component of the total cost of an immobilized catalyst. The ability to immobilize labile enzymes and use them in bioanalytical systems requires reversible immobilization of enzymes [16].

Adsorption

Immobilization by nonspecific adsorption is the simplest of the techniques, and it relies mostly on physical or ionic adsorption to achieve results.

Physical Adsorption or Nonspecific Adsorption

Physical adsorption uses hydrogen bonds, van der Waals forces, and hydrophobic interactions for enzyme attachment, while ionic bonding uses salts. Due to the nature of the forces causing noncovalent immobilization, the process can be reversed by adjusting the circumstances affecting the strength of the contact. Adsorption immobilization is a gentle, simple procedure that maintains the enzyme's catalytic activity. As a result, such techniques are cost-effective, although they may have issues such as enzyme leakage from the matrix if the connections are weak [18].

Using Ionic Bindings

The principles of chromatography may be utilized to immobilize enzymes reversibly. Ion exchangers were one of the earliest chromatographic applications in reversible enzyme immobilization. Generally, it is challenging to establish circumstances in which the enzyme stays firmly attached and completely active. Recently, immobilized polymeric-ionic ligands have been used to modulate protein-matrix interactions and improve derivative characteristics. Many patents have been filed on polyethylenimine binding enzymes and entire cells. However, using a highly charged support when the substrates or products are charged may cause difficulties; partitioning or diffusion can alter the kinetics. Such characteristics as pH optimum or stability may vary. Depending on the application, it may be beneficial to move an enzyme's optimum conditions toward more alkaline or acidic settings [15, 18, 19].

Hydrophobic Binding

The utilization of hydrophobic interactions is another option. It is an entropically

driven interaction that occurs in this technique rather than creating chemical bonds. For more than three decades, hydrophobic adsorption has been employed as a chromatographic concept. pH, salt content, and temperature are all well-known experimental variables. Both the hydrophobicity of the adsorbent and the hydrophobicity of the protein have a role in the strength of the interaction. The degree of support substitution and the size of the hydrophobic ligand molecule may both influence the adsorbent's hydrophobicity. It has been shown that b-amylase and amyl glucosidase may be immobilized on hexyl-agarose carriers in a reversible manner. There have been many additional reports of strong reversible binding to hydrophobic adsorbents [20].

Entrapment:

The entrapment technique involves occluding an enzyme inside a polymeric network that enables substrate and products to flow through while keeping the enzyme in place. Entrapment of enzyme immobilization has the benefit of being quick, inexpensive, and require moderate reaction conditions. Enzyme entrapment may be accomplished in a variety of ways, including gel or fiber entrapment and microencapsulation. Microbial contaminants, proteins, and enzymes in the microenvironment are all protected by the support matrix. Mass transfer restrictions *via* membranes or gels limit the practical application of these techniques [17, 20].

Microencapsulation

A chemical may be microencapsulated within a microsphere/microcapsule, with an average diameter of 1 mm to several hundred micrometers. A range of polymeric and nonpolymeric materials like poly (ethylene glycol), poly(methacrylate), poly(styrene), cellulose, poly(lactide), poly(lactide-c--glycolide), gelatin-acacia, *etc.* Depending on the ultimate usage of the encapsulated goods, these microcapsules employ various release methods to release their contents. Encapsulation is used to protect the local surroundings, separate incompatible components, convert liquids to solids, improve stability, conceal smells, taste, and activity of encapsulated chemicals, and isolate incompatible components [17, 21].

Metal Binding

Coupling with nucleophilic groups on the matrix binds transition metal salts or hydroxides placed on the surface of organic materials. The technique is known as “metal bond immobilization,” and it has mostly been employed with titanium and zirconium salts. Heating or neutralization causes the metal salt or hydroxide to precipitate onto the support. Due to steric constraints, the matrix is unable to

occupy all of the metal's coordination sites; as a result, some places are left open for interaction with enzyme groups. The technique is straightforward, and the immobilized specific activity achieved with enzymes in this manner has been impressive. The operational stabilities obtained, on the other hand, are extremely varied, and the outcomes are difficult to repeat. The presence of nonuniform adsorption sites and substantial metal ion leakage from the support are most likely to blame for the lack of repeatability [15, 22].

Disulfide Bond Formation

These techniques are unusual in that, despite the formation of a stable covalent link between the matrix and the enzyme, it may be disrupted under moderate circumstances by reacting with an appropriate chemical such as dithiothreitol. Furthermore, since the reactivity of the thiol groups may be controlled by changing the pH, the activity yield of disulfide bond formation techniques is typically high if a suitable thiol-reactive adsorbent with high affinity is employed [17].

Post Processing

Inner and Outer Membranes

A biosensor may have one or more internal or external layers as well as the responding layer or membrane, particularly if it is intended for biological or clinical use. These membranes provide three critical functions:

Semipermeable Barrier

The outer membrane prevents biomolecules, such as proteins or cells from biological material, entering and disturbing the reaction layer. In addition, it reduces the number of reactive layer components that leak into test samples. In implanted glucose sensors, since the glucose oxidase is not of human origin, the outer membrane plays an essential role in preventing immunological reactions. Furthermore, a well-chosen membrane may possess perm selective characteristics that are beneficial to the biosensor. It can potentially reduce the impact of any interfering species identified by the transducer. The majority of *in vivo* and *ex vivo* glucose biosensors include a negatively charged inner membrane of cellulose acetate that reduces the interference of ascorbate or urate, which are electrochemically detected alongside naturally occurring hydrogen peroxide [4, 23].

Diffusional Barrier

Most enzymes follow a type of Michaelis-Menten kinetics, causing their reaction rates to be non-linear. However, if the sensor response is influenced by substrate diffusion through the membrane rather than enzyme kinetics, linear dynamic ranges may be enormous. As a result of this regulation, a thin outer membrane is placed over a highly active enzyme layer. As an added benefit, such a diffusional barrier ensures that sensor response is independent of the quantity of active enzyme present, and increases [23, 24].

Biocompatible and Biostable Surfaces

When biosensors come into direct touch with biological parts or fluids, such as when they are implanted *in vivo* or, more broadly, in physiologically active matrices such as cell cultures, they undergo two sets of modifications:

● Biosensors are capable of triggering different kinds of reactions in a host biological sample, including toxicity, mutagenicity, carcinogenicity, thrombogenicity, and immunogenicity.

● An element or structure in a sample may modify the operation of the sensor: Fouling of the exterior layer or the inner detector, blockage of the biorecognition process, and increased transport of substrates and co-substrates towards the biosensing region are all factors contributing to the change in performance.

There are only two kinds of coating materials that need to be used in biological devices. The first type is molecules that interact with receptors in the body, such as molecule-based systems or transducers that need to be in direct contact with the target organ for a stable response. The second type is an outer layer, and without it, a device's response will degrade after implantation. In the case of sensor diameter, depending on the membrane material you are using, you may either use pre-cast membranes, such as collagen, polycarbonate, or cellulose acetate, or dip- or spin-coat the membrane, such as with cellulose acetate, Nafion, or polyurethane. The electropolymerization process is used to create micro-sized biosensors. In order for a biosensor to be considered biocompatible, it must either not adversely affect the normal function of the host medium or its own functioning should not be negatively affected by the host medium [25].

Biosensor Functional Characterization and Reporting

Because operating parameters may reveal the nature of the rate-limiting processes (transport or reaction), characterizing a biosensor's response has the same importance as characterizing any sensor based on chemical recognition. We'll go

over some of the most important performance requirements and how they relate to the characteristics of the receptor and transducer portions of electrochemical biosensors in the below sections of the chapter. Enzyme-based biosensors are the focus of much of the discussion below. An important consideration in the design of immunosensors is the surface's capture capacity or the amount of biologically active elements present on the surface. A simple way to evaluate this metric is by calculating the specific activity, which is the ratio of the number of active molecules to that of immobilized molecules. Because the immobilization method (molecular orientation, attachment sites) changes, this value fluctuates widely, rarely reaching 1. In microfluidic applications, when the surface area is reduced, this capture readiness becomes even more critical. Whether the surface can be renewed without substantial loss of function is another key problem for immunosensors.

Calibration Characteristics

Sensor calibration is usually done by adding standard analyte solutions and graphing steady-state responses against the analyte concentration or its logarithm, potentially adjusted for a blank (commonly termed background) signal. For sequential samples, transient reactions are essential, but for continuous monitoring, they are less so.

Working and Linear Range

The linear range of an analyte concentration is defined as the range of values in which the sensor output varies linearly. To Prepare the linear calibration curve for the specific concentrations of the analyte is by graphing the different concentrations of the analyte against each other, along with the current responses acquired for each concentration. The linear range of electrochemical biosensors is always limited. This limit is directly linked to the biocatalytic or affinity characteristics of the biochemical or biological receptor, but it may be substantially increased in the case of enzyme-based biosensors by employing an exterior diffusion barrier to the substrate. The obvious trade-off for such an increase in the linear range is a reduction in sensor sensitivity. Within the reaction layer, the local substrate concentration may be at least two-fold lower than in the bulk solution [4].

Detection and Quantitative Determination Limits

The lowest amount of a material that can be discriminated by the sensor output signal from the absence of that solute (control) is the detection limit or lower limit of detection.

Sensitivity

The lowest magnitude of the input signal needed to generate output with a given signal-to-noise ratio is described as sensitivity. It is determined by the measurement's standard deviation. In general, it is computed by graphing the measured current on the Y-axis and the concentrations of the specific analyte on the X-axis on the slope of the calibration curve. The greater the slope value, the greater the sensitivity of the biosensor [4, 26].

Selectivity

In chemical analysis, selectivity is defined as the ratio of signal output with the analyte alone to signal output with the interfering substance alone, where both substances are present at the same concentration of the analyte. By monitoring the biosensor response to interfering substances being added at their anticipated concentration into a measuring cell that already contains the normal analyte concentration, it is possible to get insight into this phenomenon. It is based on the concentration range of the analyte as well as the selection of the biological receptor and transducer. The majority of biological recognition elements (enzymes) are selective; nevertheless, others are nonselective in nature. The measurement of analyte in a biological medium is complicated by the presence of many coexisting substrates, each of which may interfere with the measurement unless suitable membranes are used to limit their effect [27].

Two techniques for determining biosensor selectivity are suggested based on the measurement goal. The first involves comparing the biosensor response to interfering substances to the analyte calibration curve under similar operating circumstances. Selectivity is the ratio of the analyte signal to the interfering substance signal at the same concentration. In the second process, interfering chemicals are introduced to the measuring cell, which already has an analyte concentration in the anticipated range. The second approach, more readily measured than the first, is specific to each application and has more limited relevance. The selectivity may be influenced by the analyte concentration range [4].

Reliability

The selectivity and repeatability of biosensors for specific samples determine their reliability. It must be determined in real-world operational circumstances, including the presence of potentially interfering chemicals. The biosensor response should be directly linked to the target concentration and should not fluctuate with variations in the concentrations of interfering chemicals within the

sample matrix in order to be trustworthy for an analyst. As a result, for each kind of biosensor and sample matrix, the acceptable interference that should be considered and how its impact should be measured should be stated explicitly. This reliability evaluation is required for each application's accuracy assessment [11, 13].

Steady-state and Transient Response Times

For each new analyte that is added to the measuring cell, the steady-state response time is readily calculated. It takes time to achieve a steady state for 90% of the reaction. Response time varies depending on the speed of the output signal's first derivative, which rises to its maximum value after the analyte is added. This is important to consider since reaction times depend on how quickly the analyte, co-substrate, and product move through various layers or membranes. When everything is all said and done, it is critical that the thickness and permeability of these layers be considered. The activity of the molecular recognition system also influences both response times. The longer this reaction time, the greater this activity must be. Finally, sample mixing time must be included when calculating batch-measuring cell measurements. The steady-state and transient reaction durations are specified as in batch if the analyte concentration is changed stepwise; otherwise, only transient responses are provided if analyte pulses are injected into the circulating fluid. The operational response time of sensors that are implanted in the body or are positioned in or around industrial reactors takes into account the rates of analyte and co-substrate transit to the sensor location [4, 26, 28].

Sample Throughput and Recovery Time

In the case of sequential measurements using biosensors, whether, in batch or flow-through configurations, the sample throughput is a measure of the number of different batches processed in a given amount of time. This metric takes into consideration not only the steady-state or transient reaction durations but also the recovery time, which is the amount of time required for the output signal to return to its baseline. Depending on the sample components, analyte concentration, or sensor history, both response times, as well as sample throughput, may be affected: these dependencies should be evaluated and measured [4].

Reproducibility

The term “reproducibility” refers to the variation in a set of observations or findings over time. It is usually calculated for analyte concentrations that are within the acceptable range. It is studied by utilizing the same method to build

four distinct biosensors and evaluate their electrochemical reactions to the analyte's oxidation/reduction [4, 29, 30].

Stability

The practical stability of a biosensor response depends on the sensor shape, preparation technique, receptor and transducer used. A substrate exterior, inner diffusion, or biochemical sensing reaction is also a significant limiting element. Finally, it varies greatly based on operating circumstances. We suggest considering analyte concentration, biosensor contact time with the test solution, temperature, pH, buffer composition, organic solvent presence, and sample matrix composition when determining operational stability. Although certain biosensors may be used for over a year in the laboratory, their useful lifespan in industrial processes or biological tissue is unclear or restricted to days or weeks. Storage conditions (dry or wet), atmospheric composition (air or nitrogen), pH, buffer composition and additives are important factors for assessing storage stability.

A biosensor's laboratory bench stability can be determined easily, but determining its behavior in industrial reactors over many days is a considerably more complicated and challenging task. In both cases, *i.e.*, bench or industrial, it is necessary to specify whether the lifetime is a storage (shelf) or operational (use) lifetime, the storage and operating conditions, and the substrate concentrations compared to the apparent Michaelis-Menten constant KM [4, 13].

Lifetime

Lifetime should be defined as the amount of storage or operating time required to cause a 50% reduction in sensitivity within a linear range of concentrations. The storage lifetime of biosensors should be determined by comparing their sensitivity after being stored for a variety of times under the same conditions. The stability of a biosensor may also be measured as the drift when the sensitivity is tracked in either storage or operating conditions. In particular, the drift calculation is helpful for biosensors that have been tested very slowly or in a short period of time. Lastly, the method by which the lifetime will be determined should be described, such as using starting sensitivity, the linear range of concentrations in the calibration curve, or precision and repeatability [13].

CONCLUSION

The scientific and industrial sectors are interested in electrochemical biosensors because of their benefits and potential. Since electrochemical biosensors are multidisciplinary, they are investigated widely. Various levels of experience among researchers in this field mean that some aspects of biosensor research may

be overlooked. In certain circumstances, the engineering design of biosensors is imperfect. As a novice researcher, this chapter discussed some of the processes involved in the design and testing of sensors. There are ten steps in the design of biosensors. In the beginning, we mentioned the importance of these steps; after that, some of them were discussed in more detail. This roadmap may be a good starting point for you if you are interested in discovering how you think as a biosensor researcher.

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CHAPTER 3

Material and Biomaterial for Biosensing Platform

Abstract: The fourth chapter focuses on essential materials for biosensing platform research, including graphene, carbon nanotubes, conductive polymer, and other advanced materials. This chapter describes the function of each biosensing platform and the most recent advances in the synthesis and application of advanced materials. After three sections on the subject's fundamentals, this and the following two chapters present experimental and research-relevant material. For this purpose, carbon-based materials will be examined first, including the following categories: fluorines, carbon nanotubes, graphene, nanodiamonds of carbons, carbon nanohorns, carbon dots, and carbon nanofibers. This section examines the research on these materials and the types of conductive polymers utilized in electrochemical biosensors. Several polymers and their functional techniques, including MNPPFs, MIP/SIPs, and dendrimers, are examined in the following sections. The nanoparticles, such as Au, Pt, Ag, Pd, Ni, Cu, Fe_2O_3, TiO_2, ZnO, zeolites and other aluminosilicates, inorganic quantum dots, doped inorganic NMs, nanowires, Carbon black, and calixarenes, are then investigated. Then, biological materials are examined, including enzymatic nanocomposites, nucleic acid nanocomposites, immunoassay-based nanocomposites, aptamers, and biopolymeric nanocomposites. Finally, sandwich- or composite-based biosensor materials are discussed.

Keywords: Carbone, Electrode materials, Recognition element, Polymers.

INTRODUCTION

Carbon materials are commonly employed in the construction of electroanalytical chemistry electrodes because of their relative chemical inertness in the majority of electrolyte solutions, low background current, cheap cost, and large potential windows in aqueous media. Carbon materials are accessible in various microstructures, including carbon dots, graphene, Carbon nanotubes (CNTs), graphite, carbon fiber, glassy carbon (GC), amorphous powders, and diamond, and dimensional carbon nanomaterials are generating significant interest due to their unique features. Graphene, a two-dimensional densely packed honeycomb lattice, and one-atom-thick, has been the subject of much research in recent years because of its unusual electrical, mechanical, and thermal characteristics since its discovery in 2004 [1].

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Numerous biomolecules such as DNA, antigens (antibodies), or enzymes are combined with natural polymeric nanomaterials to form a range of nanosystems. The self-organization of biomolecules with different shapes, such as nanotubes, nanorods, and nanoparticles is a constant source of inspiration for scientists working in biological and biomedical applications. Substances like proteins, RNA, and DNA serve as the starting point for the development of extraordinary nanomaterials. For example, self-assembled peptides are biocompatible building blocks with a wide range of chemical endurance, flexibility, and properties. Numerous peptides organize to form a variety of nanostructures under moderate circumstances, including nanoparticles, nanotubes, and nanofibers. Generally, amyloid-type nanofibrils derived from a natural fibrous protein are used to change the surface of the Au electrode. Peptide biomaterials are very selective and effective ligands for a wide variety of metal ions, with the capacity to complex the appropriate metal ions. Bionanomaterials have become a catch-all word for nanocomposites formed by combining DNA, antigens (antibodies), enzymes and biopolymers with inorganic species over the past several years [2].

Carbone-Based Materials

Fullerenes

R. F. Smalley, R. F. Curl, and H. W. Kroto discovered fullerene, an allotropic modification of Carbon, in 1985 [3]. It was the first nanomaterial to be separated successfully. Fullerenes are characterized by forming certain atomic Cn clusters ($n > 20$) of carbon atoms on a spherical surface. In fullerenes, the carbon atoms establish covalent connections with one another during sp2 hybridization. They are often seen on the sphere's surface near the vertices of hexagons and pentagons. C60 is a well-studied and researched fullerene. It consists of symmetric spherical molecules made up of 60 carbon atoms located at the vertices of 60 carbon, or 12 pentagons and 20 hexagons atoms arranged in 20-six member rings and 12-five member rings [4].

Due to the unusual dimensions and electrical structures of C_{60}, an electron acceptor, it exhibits a variety of electrochemical characteristics. Under severe circumstances (-10 °C temperature, acetonitrile-toluene mixture, and vacuum), C_{60} undergoes 6 different one-electron reversible redox reactions. As with other CNs, C_{60} and its derivatives may be changed or immobilized in a variety of ways, enabling the construction of electrochemical biosensors based on C_{60}.the first amperometric biosensor mediated by C_{60} (Chaniotakis, 2000). C_{60} was constructed on a porous carbon electrode in this glucose biosensor and served as a mediator for electron transport [5].

Han *et al.* [6] have developed a bifunctional C_{60}-based nanomaterial that may be used as a novel kind of redox nanoprobe and nanocarrier to mark detecting antibodies in an electrochemical immunosensor for erythropoietin (Fig. **1**) [5].

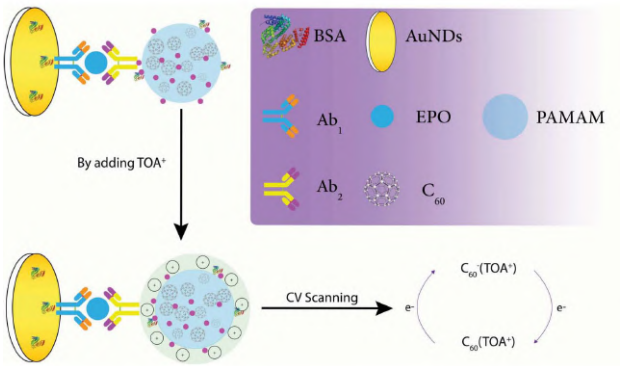

Fig. (1). An electro-immunochemical assay based on C_{60} is shown schematically.

Fullerenes are a zero-dimensional graphitic carbon shape that may be seen as an uneven graphene sheet coiled into a spherical by subjoining pentagons to its structure. They are available in a variety of shapes and sizes, spanning between 30 and 300 carbon atoms. They may be produced by sputtering, electron beam ablation, or arc discharge. Fullerenes are also found in combustion soot and may be produced using graphitic electrodes. Initially, Fullerenes were produced by evaporating Graphite electrodes in a Helium environment. Nonetheless, fullerenes' practical use is constrained by their poor yields associated and high synthesis cost with the techniques presently available for their manufacture [4].

Carbone Nanotube

CNTs (Carbon nanotubes), one of the allotropic modifications of Carbon, were figured out in 1991 by a Japanese scientist S. Ijima [7]. Through sp2 hybridization, each carbon atom with three electrons creates trigonally coordinated s bonds with 3 other carbon atoms in CNTs. CNT is a single sheet of graphene that has been smoothly rolled into the shape of a hollow tube. Carbon nanotubes are characterized by rolled graphene sheets piled in tubular/cylindrical formations with a diameter of numerous nanometers. CNTs may be customized in terms of chirality vectors (the symmetry of the nulled Graphite sheet), diameter, layer count, and length. CNTs perhaps classified into two main categories based on their structure: SWCNTs (single-walled carbon nanotubes) and MWCNTs (multi-walled carbon nanotubes) [4].

Utilization of Carbon Nanotubes as Nanoprobes

Oxygen-containing groups and the critical roles of defect sites in CNTs' electrochemical properties have been extensively investigated, providing insight into the electrocatalytic process of CNT-related nanomaterials. Numerous electrochemical biosensors have been created to date entirely and directly based on the electrocatalytic activity of carbon nanotubes; therefore, they may be called nanoprobes for electrochemical biosensors [5].

Utilization of Carbon Nanotubes as Nanocarriers

Owing to the benefit of carbon nanotubes (*e.g.*, excellent conductivity, a flexible sidewall, good biocompatibility, and a large specific surface area), coating other materials upon the surface of carbon nanotubes may result in the formation of novel nanomaterials or nanostructures with enhanced properties. Additionally, many techniques have been used to accomplish the immobilization procedure. As a result, carbon nanotube composites serve as critical scaffolds for electrochemical biosensors. MNs and DNA are the most productive materials utilized with peptides, antibodies, redox enzymes, CNTs, and other proteins [5].

CNTs may mediate between the active sites of redox enzymes and the electrode, thus boosting the enzyme's DET (Direct electron transfer). Gooding demonstrated the electrical communication paradigm [8] and Willner's group constructed a conventional biosensor (Fig. **2**) [5, 9].

Detection by Carbon Nanotubes as Electrochemical Biosensors

Detection of dopamine using carbon nanotubes as electrochemical biosensors. - The first documented use of a carbon nanotube electrochemical biosensor was in 1996 when it was used to detect dopamine [10]. Glucose biosensors based on carbon nanotubes. - A polyethyleneimine (PEI)-functionalized carbon nanotube (CNT)-based electrochemical phage-based biosensor has been reported to recognize *E. coli*.- The detection of trichloroacetic acid and nitrite (NO^{2-}) using a DNA hybridization biosensor based on SWCNT and ssDNA has been described. The GCE modified with mesoporous silica MCM41 nanoparticles functionalized with the hemoglobin (Hb) hybrid conjugate and Nafion-coated MWCNTs were used as a biosensor for the recognition of trichloroacetic acid (TCA) and nitrite (NO^{2-}), presenting increased sensitivity and selectivity, that is ascribed to Hb's brilliant biocatalytic activity the nanoparticles' and Hb's unusual biocatalytic activity.- Amperometric electrochemical biosensors-detection of cancer cells-have been used to determine the blood cholesterol level. A CNT coated with

polydopamine and functionalized with folic acid was utilized to detect HL60 and HeLa cancer cells. Hybridization detection.- The cellular prion protein was identified using a fluorescent-label aptamer, which requires non-covalent alteration of the Multi-wall carbon nanotubes to quench the fluorescence-prostat--special antigen detection. A very precise electrochemical biosensor based on carbon nanotube bioconjugates and the Single-wall carbon nanotubes jungle platform has been announced to detect secondary antibody ratio/the horseradish peroxidase (HRP) of PSA (prostate-specific antigen PSA). Detection of the cholesterol level in the blood.- The electrochemical biosensors based on carbon nanotubes were synthesized utilizing dehydrogenase enzymes like alcohol dehydrogenase, phosphatase, D-fructose dehydrogenase, and GDH (Glutamate Dehydrogenase) -protein detection.- CVD (Chemical vapor deposition)-fabricated carbon nanotube fibers were utilized as a detecting electrode to sense the glucose presence -enzymatic biosensors foundation on carbon nanotubes [4].

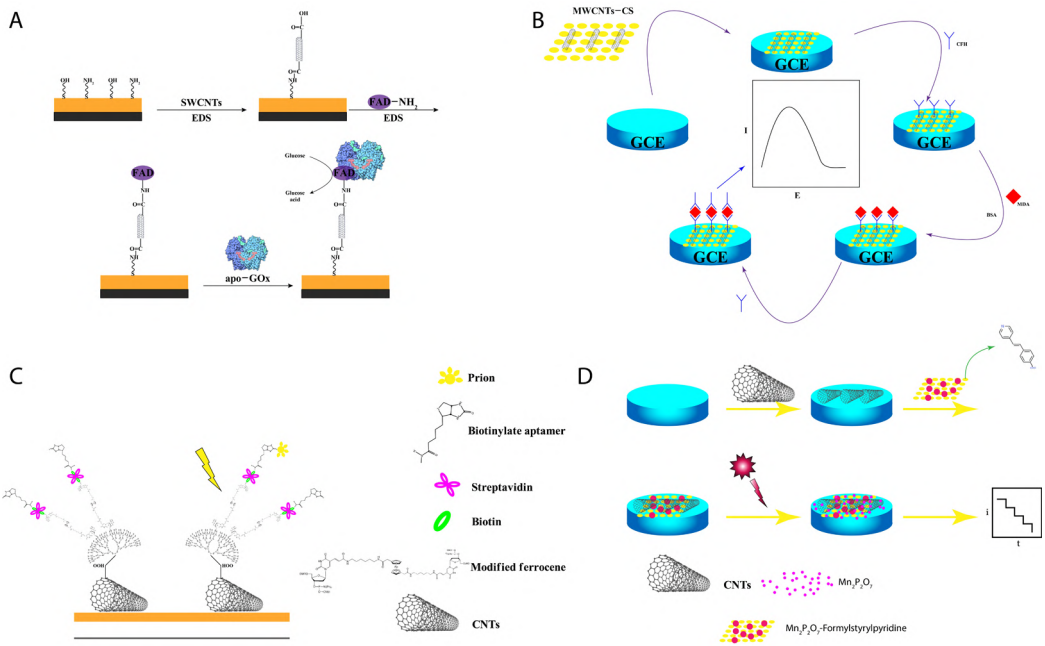

Fig. (2). Electrochemical tests using carbon nanotubes as nanocarriers. (A) Assembling the SWCNT-based glucose oxidase electrode with an electrical contact. (B) Schematic of the label-free biosensor constructed using MWCNTs. (C) Schematic illustration of a biosensor composed of an aptamer- streptavidin-biotin-ferrocene and MWCNT-polyamidoamine dendrimer. (D) Schematic representation of a new MWCNT-based superoxide anion biosensor.

Synthesis

LA (Laser ablation), CVD, and Carbon arc discharge methods were used to

manufacture the carbon nanotubes. The carbon arc discharge method was first utilized to manufacture MWCNTs or SWCNTs with more control over the nanotube size and a high yield. The CVD technique produced CNTs with lower yields and smaller diameters but of higher quality. Laser ablation results in a considerably smaller diameter and a reduced yield but with a lot higher quality. Semiconducting and metallic carbon nanotubes may be produced through selective functionalization, selective destruction *via* separation or electrical heating using density gradient ultracentrifugation. Using transition metal nanoparticle catalysts, CVD was utilized to generate high-quality MWCNTs and SWCNTs in a vertically aligned array. They have been produced in large quantities using CVD and arc discharge techniques (Mo-Co Catalysts). CVD requires less operating and equipment at relatively low temperatures and pressures, making it more suitable for large-scale carbon nanotubes manufacturing rather than the other two techniques [4].

Vertically aligned arrays of carbon nanotubes have been synthesized on metallic and quasi-crystalline substrates. The production of carbon nanotubes in the presence of other hydrocarbons has been reported using pyrolyzing metal carbonyls. The transition metals contained in graphite electrodes have resulted in increased product production and repeatability of carbon nanotubes. Transition metal catalysts and the chemical vapor deposition technique have been investigated to obtain high-quality carbon nanotubes in vertically aligned arrays [4].

Graphene

It is a two-dimensional allotropic shape of C composed of a single carbon atom layer. In sp2 hybridization, the C atoms form a hexagonal crystal lattice connected by sand p bonds, with an interatomic spacing of 0.142 nm between the C hexagons. P. R. Wallace, a Canadian theoretical physicist, discovered graphene for the first time in 1947. In comparison, the samples were examined by K. Novoselov, a Russian-British scientist, and A. Geim, a Dutch-British physicist [4].

Graphene-related Materials May be Utilized as Electrochemical Nanoprobes for Electrochemical Biosensors

Graphene-related materials may be utilized as electrochemical nanoprobes for electrochemical biosensors in two ways. On the surface of GO, there are many oxygen-containing groups. Electrochemical reduction of Graphene Oxide yields well-defined signals from oxygen-containing compounds. Thus, GO may be directly applied to electrochemical biosensors as a label. In an amazing demonstration, TBA (thrombin aptamer) was physically fixed on the electrode

surface and then successively attached to GO and thrombin. The electrochemical signal of GO degradation was used to determine the concentration of thrombin (Fig. **3**). GR and its derivatives have shown inherent electrocatalytic activity in the past [5].

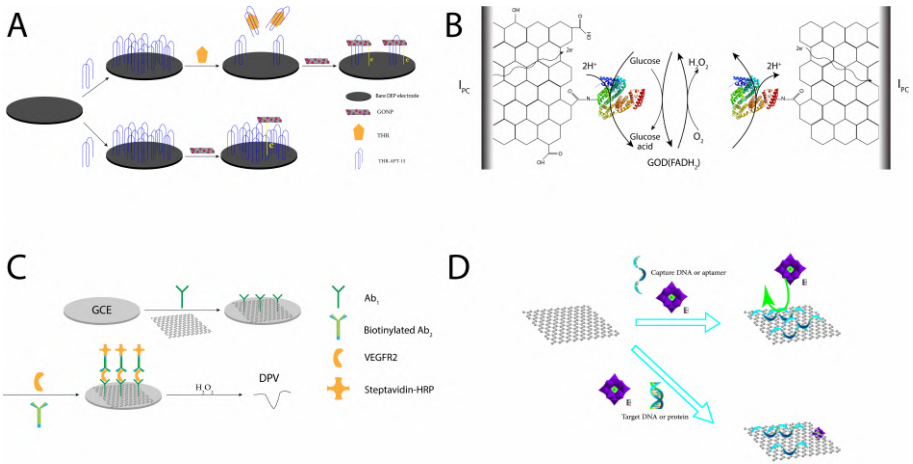

Fig. (3). Electrochemical tests based on graphene utilizing graphene, a nanocarrier or a nanoprobe. (A) picture demonstrating the use of Graphene oxide nanoplatelets as intrinsically electroactive markers for thrombin monitoring. (B) Schematic of the DET of the ERCGRGOx/GCE and oxygen and glucose electrocatalysis. (C) graphic illustration of the VEGFR2 electrochemical biosensing platform based on GR. (D) graphic representation of the label-free electrochemical biosensing device based on GO for DNA and protein analysis.

Utilization of GR as a nanocarrier: In addition to its electrochemical capabilities, GR has the simplicity of modification, high conductivity, and a huge specific surface area. Using GR as a nanocarrier, electrochemical biosensors may be built with redox probes, recognition components, or target molecules, resulting in enhanced analytical performance [5].

Detection by Carbon Nanotubes as Electrochemical Biosensors

Graphene as Electrochemical Biosensors: Glucose detection. - In 2009, the first graphene-based electrochemical biosensor based on graphene for glucose detection was announced detection of DNA. The electrochemical biosensor used to detect DNA has garnered researchers' interest owing to its excellent selectivity, high sensitivity, and cheap cost for identifying altered genes or DNA sequences associated with various human illnesses other biomolecule detection.- Graphene-based sensors for improved Dopamine detection electrochemically have been extensively studied and published [4]. Graphene for EIS (Electrochemical Impedance Spectroscopy) based DNA analysis.- Several methods have been used to conduct impedimetric DNA sequences on various nanostructured materials

with varying characteristics. With physical adsorption or chemical binding, hairpin or linear-shaped DNA probes have been fixed on graphene surfaces. In the first instance, the platform is stable for geno sensing due to the p–p stacking interactions among the hexagonal graphene cells and the aromatic ring of nucleobases. After modification with various compounds for chemical binding, DNA is covalently bonded to carboxylic group functionalized graphene platforms or surfaces with a great density of functional groups. In both instances, however, the DNA sequences are changed by particular linkers (*i.e.*, amino moieties) capable of forming covalent connections with the Gr surfaces. Graphene for immunoassays using electrochemical immobilization.- Based on the sensing method, Gr platforms for immunoassays may be divided into two main groups: aptasensors and immunosensors. Immunosensors are affinity biosensors based on antibodies. The recognition of the target analyte, in this instance, an antigen, occurs as a consequence of the antigen attaching specifically to a specific area of an antibody immobilized on the electrode surface. Aptasensors detect a target molecule by attaching it selectively to the immobilized aptamer on the electrode surface. Aptamers are sequences of peptides or oligonucleotides which bind precisely to a certain target. They are regarded as assuring for antibody development owing to their ease of manufacture, the potential for decreased well-understood, and cross-reactivity binding chemistry [11].

Synthesis

Novaselov and Geim separated graphene for the first time in 2004 at the University of Manchester by peeling off individual graphite layers using scotch tape [12]. This technique yields defect-free graphene. However, the sample's tiny size is suitable only for laboratory study and not for commercial uses. Graphite has been observed to peel in solvents such as the surfactant sodium dodecylbenzene sulphonate solution and N-methyl pyrrolidone. Due to its low manufacturing cost and simplified processing procedures, this method has been extensively utilized to synthesize graphene on a large scale. The multilayer graphene was synthesized through microwave-assisted exfoliation, liquid-phase exfoliation, epitaxial growth, CVD peeling off from graphite, and other oxidation methods. Additionally, graphene may be produced by reducing graphene oxide. However, the produced material has many flaws, while graphene generated from graphite has a low defect density [4].

CVD was utilized to fabricate graphene-modified electrodes and devices for electrochemical sensors. Graphene has also been synthesized on various transition metal substrates, including Pt, Po, Ni, and Cu. As a result of Carbon's poor stability in Cu, the Chemical vapor deposition development of Gr on Cu results in

a highly crystalline Gr layer. Epitaxial Gr may be produced by graphitizing doped Silicon Carbide single-crystal wafers and undoped SiC crystals at elevated temperatures [4].

Nanodiamonds of Carbon

Carbon Nanodiamonds (CNDs) are another allotrope of Carbon. They are NP with the crystal structure of the diamond and show many of the same characteristics as the diamond. The CND's are composed of a crystalline Diamond core restricted by an amorphous Graphite shell like an anion. They have a high capacity, a big surface area, and a small size for chemical attachment to biological molecules. They are very hard, with high thermal conductivity, a low refractive index, a low coefficient of friction, and excellent insulating properties. They are very non-toxic [4].

Zhao and his colleague [13] created a new glucose biosensor by covalently attaching Glucose to CNDs. This research showed that electrochemical pretreatment of CNDs might improve their performance. Gopalan *et al.* developed an H_2O_2 biosensor by electrochemically probing CND-based sponges with entrapped HRP (horseradish peroxidase). Lee and the company deposited Br-doped nanodiamonds on the surface of carbon nanotubes (Fig. **4**) [15].

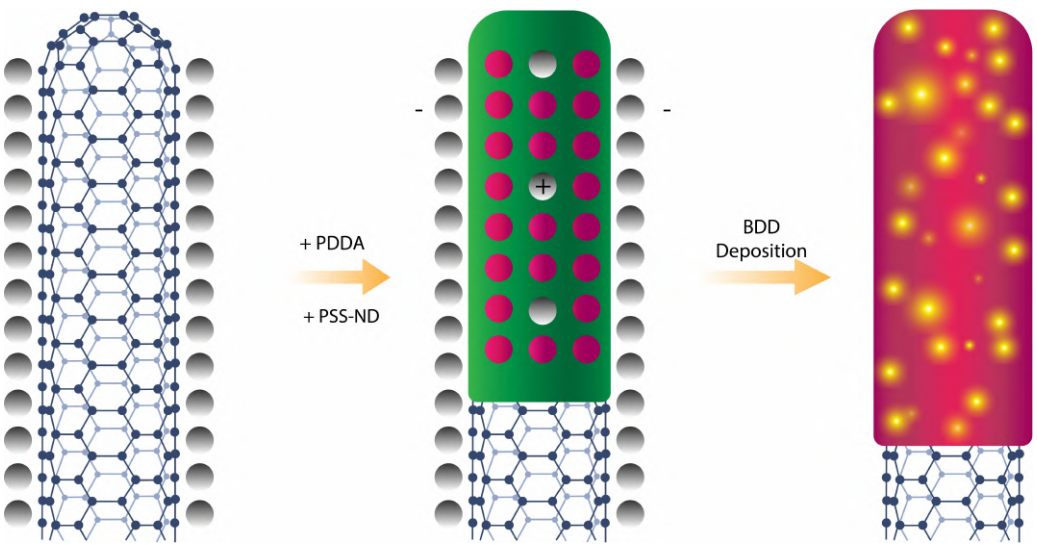

Fig. (4). 3D-Walled BDD/CNT shell-core nanowires with improved electrochemical performance are shown schematically.

Synthesis

CNDs are produced intentionally *via* the explosion of explosives. Due to the fact

that diamond displays fluorescence as a result of the existence of a complex defect (V-N) comprising a vacancy and nitrogen, fluorescent Carbone-based nanodots may be produced by doping N vacancies through free space annealing and electron irradiation. Scientific studies have discovered fluorescent CNDs made up of about Silicon vacancies and 400 Carbon atoms that may be used in sensing applications [4].

Carbon Nanohorns

CNHs (Carbon nanohorns) are a form of C with a length of 40–50 nm and a diameter of 2–5 nm. They are more advantageous than carbon nanotubes because they can be produced on a greater scale without the need for metal catalysts. They may be made by Joule heating, laser ablation of pure graphite, or arc-discharging carbon rods. The CNHs have high porosity and a large surface area, which may be used to their advantage in biosensing. Electrochemical Biosensor Using Carbon Nanohorns: A sensitive impedimetric immunosensor for alpha-fetoprotein detection has been created and published. It utilizes the electrocatalytic activity of Carbon nanohorns in conjunction with an enzymatic biocatalytic precipitation method [4].

Carbon Dots

CDs (Carbon Dots) are 0-dimensional CNMs (Carbon nanomaterials) made of C atoms with a diameter of less than 10nm. As shown by Quantum Dots, these materials display important electrical and optical characteristics. They do, however, exhibit minimal toxicity, stability, and biocompatibility, which creates them ideal for utilization as electrochemical biosensors. The CDs were created *via* the ablation of Carbon atoms using a laser. CDs have been manufactured using various techniques, including pyrolysis, hydrothermal synthesis, electrochemical approaches, and microwave synthesis. Additionally, they may be produced using the soot from a candle flame [4].

CDs as nanoprobes: Within the last several years, the electrocatalytic attributes of Carbon dots have been progressively identified, allowing for their application as nanoprobes in electrochemical biosensors. Due to the inherent catalytic activity of GO129 and the fact that GQDs are tiny bits of Graphene Oxide, Zhang and his colleague [14] tried to construct Graphene Quantum Dots on an Au electrode surface. They utilized the sensing application of this Au / Graphene Quantum Dots electrode (Fig. **5**) [5].

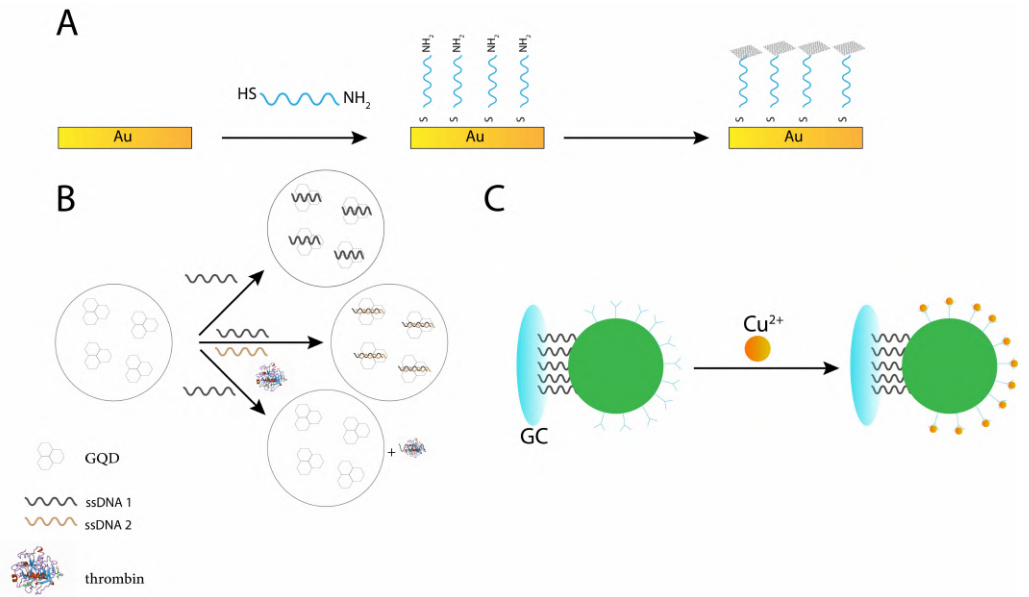

Fig. (5). Electrochemical tests using compact discs as nanocarriers or nanoprobes. (**A**) Illustration of the Graphene Quantum Dot /Au electrode manufacturing method. (**B**) Schematic representation of the GOD-modified PGE used as a platform for the development of various electrochemical biosensors. (**C**) An electrochemical technique for measuring Cu^{2+} is shown.

Utilization of compact discs as nanocarriers: Due to their unique characteristics, including biocompatibility, simplicity of modification, and a great surface-t--volume ratio, CDs may be used like new nanocarriers in electrochemical biosensors. Zhao and the company [15] described the development of the first electrochemical biosensors based on the robust interaction of Graphene Quantum Dots with ssDNA (Fig. **5**) [5].

Carbon Nanofibers

CNFs (Carbon Nanofibers) are cylindrical wire-shaped nanostructures composed of Gr sheets stacked in a herringbone pattern, platelet-like or ribbon-like. CNFs range in length from micrometers to tens of meters, while their diameters range from ten to five hundred nanometers. Their mechanical and electrical characteristics are identical to those of CNTs. CNFs feature more edge sites on their outside walls than CNTs due to the stacking of graphene sheets with various forms in different configurations. Due to the existence of edge sites, electrons may be transferred among the detector substrate and electroactive type in the solution. The CNFs have critical properties for electrochemical sensing applications, including ease of manufacturing, biocompatibility, a wide surface area, and high electrical conductivity. Additionally, CNFs may be readily

functionalized to accommodate a specific detecting method [4].

Carbon Nanofibres -modified electrodes for explicit recognition of tiny molecules: Marken and colleagues initially investigated the voltammetric behavior of CNF composite electrodes. Huang and colleagues studied and investigated nanocomposite electrodes composed of paraffin wax and carbon nanofibers as potential substrates for stripping operations and metal deposition. Additionally, they investigated the electrochemical characteristics of carbon nanotube–polystyrene composite electrodes and carbon nanotube–ceramic composite electrodes. Stevenson and Maldonado directly produced CNF electrodes by pyrolyzing FePcs (iron tetrasulfophthalocyanine) on Ni substrates. They examined the constancy of carbon nanotube electrodes and the electrochemical properties used as catalysts for oxygen reduction. They discovered that disorders in nitrogen functionalities, exposed edge plane defects, and graphite fibers all have a major role in affecting reactive intermediate adsorption and improving electrocatalysis for O_2 reduction. In comparison to nondoped CNF electrodes, N-doped (nitrogen-doped) CNF electrodes showed considerable catalytic activity for O_2 reduction between neutral and basic pH [16].

Detection by Carbon Nanofibers as Electrochemical Biosensors

Electrochemical Biosensors Based on Carbon Nanofibres: Glucose Detection.- A critical evaluation of biosensors based on CNFs has been published. The presence of Glucose was detected using Ni nanoparticle-loaded CNFs produced by electrospinning. DNA hybridization/protein detection.- AC Voltammetry has been used to recognize DNA hybridization using CNF arrays that are vertically oriented and are produced on silicon wafers [4].

Synthesis

CNFs may be produced by arc discharge or laser ablation. CNFs have also been prepared *via* electrospinning, PECVD (plasma-enhanced chemical vapor deposition), and thermal CVD (chemical vapor deposition). The thermal chemical vapor deposition technique thermally decomposes a molecule comprising hydrogen and Carbon by using a metal catalyst at a constant temperature [4].

Polymers and Conductive Polymers

CPs (Conducting polymers) have been widely utilized as transducers in electrochemical biosensors for signal detection and amplification. As bio transducers, both inherently conducting polymer-nanocomposite and conducting polymer materials have been utilized. Metal nanoparticles and CNTs are two of maximum frequently used filler materials for functionalizing electrodes in

electrochemical sensors *via* polymer PANI (Polyaniline), Ppy (Polypyrrole), and PT (Polythiophene) are three of the most often utilized ICPs for creating electrochemical biosensors. Conducting polymers are excellent options for electrochemical sensing applications due to their ease of manufacturing, scalability, and low cost, as well as material characteristics such as high chemical specificities, tunable transport properties, and surface area. Thus, conjugated CPs act as a bridge between the final electrode and the biorecognition layer. Conjugated CPs have an alternating polymer chain of double and single bonds, resulting in delocalized electrons generating charge carriers. Redox mediators are distributed in the polymer matrix and added as chemically or dopants conjugated to encourage the selectivity and sensitivity of the biosensors. Additionally, the conjugated backbone of CPs enables the modification of their characteristics *via* immobilization (or attachment) to types of chemical moieties. For instance, (Fig. **6**) depicts PPy nanowires functionalized with avidin that are utilized to detect biotin-conjugated DNA molecules. Electrodeposition on polymerized films is a frequently used method for immobilizing macromolecules. This method enables the creation of highly repeatable ultrathin CP coating layers [17].

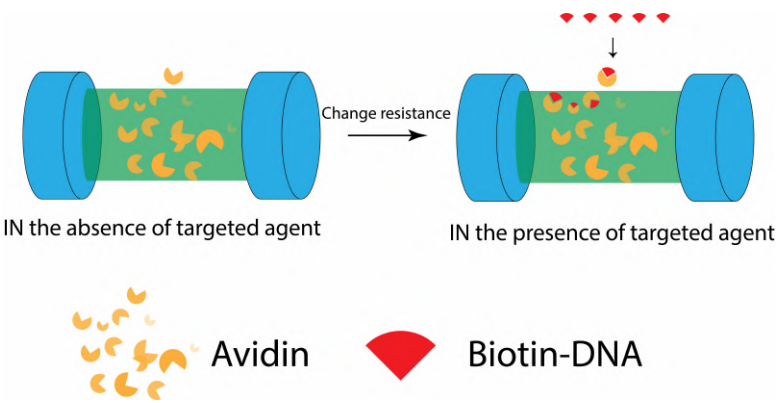

Fig. (6). The fabrication of a polymer (polypyrrole) nanowire on pre-patterned electrodes was shown, as well as its application to biosensing.

Conducting polymers (CPs) (alternatively referred to as intrinsically conducting polymers (ICPs) or electrically conducting polymers (ECPs)) are organic compounds that are also conductivity electrical. Typical organic polymers exhibit exceptional electrical conductivity, a high affinity for electrons, and redox activity. (Fig. **7**) illustrates the architecture of the principal conducting polymers poly(acetylene), poly(3,4-ethylenedioxythiophene) (PEDOT), poly(thiophene), poly(pphenylene vinylene) (PPV), poly(pyrrole), and poly(aniline) [18].

The factor of recognition is immobilized on the Conductive Polymer electrode in a Conductive Polymer-based electrochemical biosensor. Enzymes, antibodies,

aptamers, and oligonucleotides (ONs) are all examples of common recognition elements. Fig. (**8**) illustrates the designs and components of CP-based biosensors. The next sections describe measuring methods, target molecule types, and immobilization methodologies in depth [19].

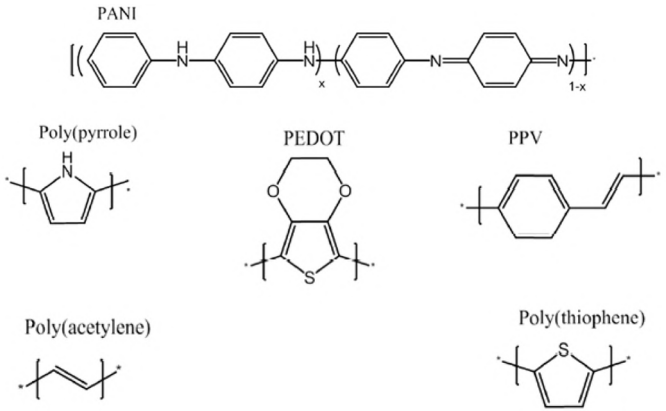

Fig. (7). poly(acetylene), poly(3,4-ethylenedioxythiophene) (PEDOT), poly(thiophene) (PTh), poly(p-phenylene vinylene) (PPV), poly(pyrrole) (PPy), and Poly(aniline) have different chemical structures (PANI).

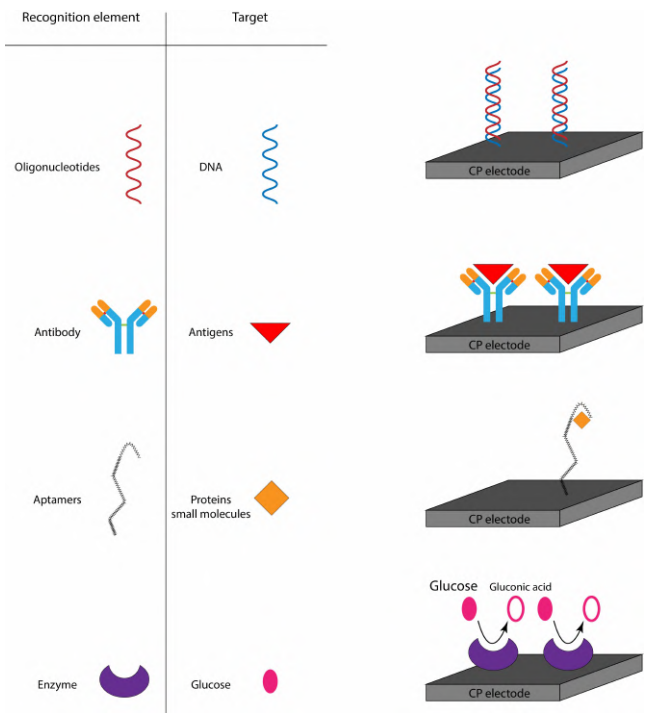

Fig. (8). CP-based biosensors: recognition components, target molecule kinds, and overall transducer architecture.

The development of biomedical electrochemical sensor systems depends on the biotic/abiotic interface's manufacturing characteristics. It has been shown that electrochemical biosensors and sensors or biomedical devices are made up of an electrical, mechanical, or physical transducer connected to an element of bio-recognition. Enhancing the sensing performance of polymer-based sensors is as simple as adjusting the following parameters: (i) great specificity – great recognition of bio-analytes; (ii) increased electrochemical signal transduction – rapid kinetics of analytes/carriers; (iii) permanence – covalent binding of the biomolecule; and (iv) biofunctionalization – this varies according to the exposed nature and surface of the biomolecule. Polymeric nanostructures based on molecularly imprinted polymers, CPs (conducting polymers), and dendrimers have been utilized to detect metabolites, antigens, proteins, enzymes, and DNA due to their excellent selectivity, hysteresis, linearity, and sensitivity [19].

Identification Element Immobilization Techniques

Immobilization of identification components is a critical phase in the construction of reliable and repeatable sensors. The appropriate immobilization method needs an in-depth understanding of the recognition probe's characteristics. In an ideal world, the immobilization procedure would be quick and simple while not impairing the recognition probe's functioning. Immobilizing recognition components on or inside CPs sensing films is often accomplished *via* affinity interactions, physical adsorption, covalent attachment, and electrochemical entrapment [18].

Well-defined Van der Waals forces and hydrophobic forces are examples of these interactions (Fig. **9A**). The bio probe's net charge, solvent type, pH, and temperature all serve as control factors for effective adsorption. Dicks and his colleague pioneered the utilization of adsorption-based CP biosensors for the immobilization of GOD on PPy, which was expanded to include additional enzymes and ONs. The primary benefit of physical adsorption is that it does not need the monomers to be functionalized. Umana & Waller [20] and Foulds & Lowe [21] pioneered the electrochemical entrapment method for incorporating GOD into Polypyrrole films through electropolymerization of pyrrole in the attendance of the enzyme. As a consequence, glucose oxidase and a matrix of CP were formed (Fig. **9B**). Similar techniques have been used to immobilize ONs, antibodies, other enzymes, and even cells inside sensor films in the years afterward. Covalent bonding of recognition probes to Conductive Polymer electrodes is often accomplished *via* the use of NHS/EDC (N-hydroxysuccinimide/1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) chemistry to link – COOH (carboxylic acid) to $-NH^2$ (amine) groups on the probe and CP, respectively (Fig. **9C**). The mechanism of action is as follows: 1)

electrodeposition of biotinylated monomers onto electrodes; 2) formation of avidin-biotinylated polymer bridges and incorporation of avidin, and 3) Biotinylated recognition probes are anchored onto avidin-biotinylated polymer composites (Fig. **9D**). The greatest non-covalent binding constant known (K_d = 10^{-15} M) creates a link between avidin and biotin. Nevertheless, it is resistant to the majority of organic solvents and very stable across a broad temperature and pH range. Aptamers, peptides, enzymes, ONs antibodies, and affinity interactions have been effectively mounted onto conductive polymer electrodes [18].

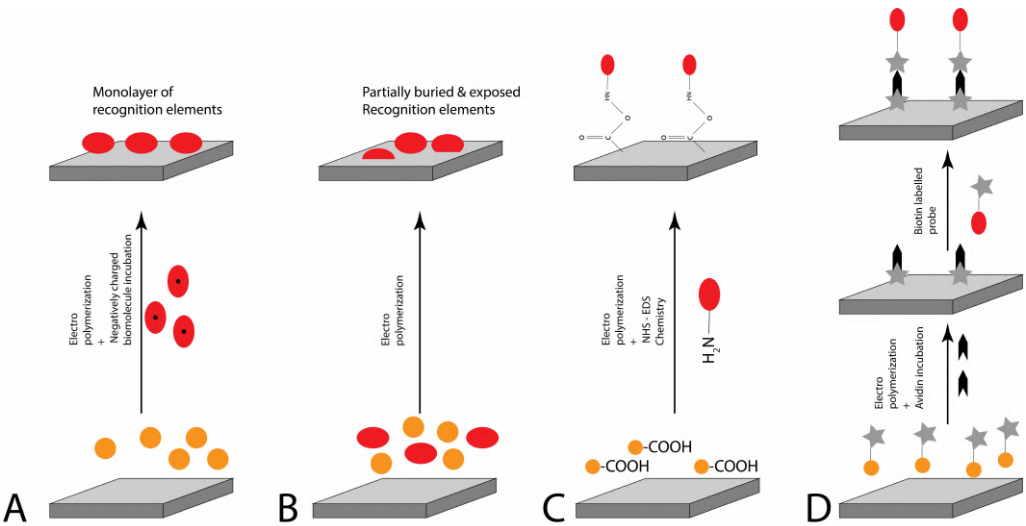

Fig. (9). Immobilization methods for bio probes: A) Adsorption on a physical level, B) entrapment electrochemical, C) covalent bonding, and D) Affinity interactions between avidin and biotin; the star denotes biotin, the red dot denotes a probe (or a recognition element, such as an enzyme, an ON, or an antibody), M-COOH denotes a carboxylic acid-functionalized monomer, and - M denotes a monomer.

Assortments of MNPPF for Electrochemical Biosensors and Transducers

In other words, MNPPF changed the electrode to serve as a better electron acceptor during the enzymatic oxidation of enzymes, resulting in an increase in the material's oxidation current throughout the analysis. MNPPF selectivity of electrochemical biosensors has been suggested as follows: in the attendance of oxygen conduct, target analytes, and an enzyme, a reaction in whom a by-product is produced, and the by-electro-oxidation product's present is measured following the utilize of an MNPPF modified electrode. The electrochemical biosensor's selectivity is critical because MNPPF is well Appropriate for target analytes. However, it is not fully developed at the moment [22].

Provision of Molecular Imprinted Polymers/Surface Imprinted Polymers for Electrochemical Biosensor

Numerous techniques have been used to synthesize SIPs/MIPs on electrodes in order to create electrochemical biosensors. They are generally synthesized in three phases: (i) Mounting of the template and functional monomer, (ii) polymerization of the monomer-template complex using initiators, porogens, cross-linkers, and under electrical-/thermal/photo conditions, and (iii) pattern takeaway to divulge highly specific binding microcavities. Nowadays, double and multi-template techniques have been developed, allowing Surface Imprinted Polymers/Molecular Imprinted Polymers -based biosensors to recognize an increased number of target analytes in a single complicated sample. Several techniques for the production of SIPs/MIPs include the following: 1. Deposition or Spin Coating on Electrodes 2. Self-Assembled Monolayer Assembly 3. Electropolymerization or Polymerization Induced by UV Light (Fig. **10**) 4. Imprinting through Micro-Contact (Fig. **11**) [23]

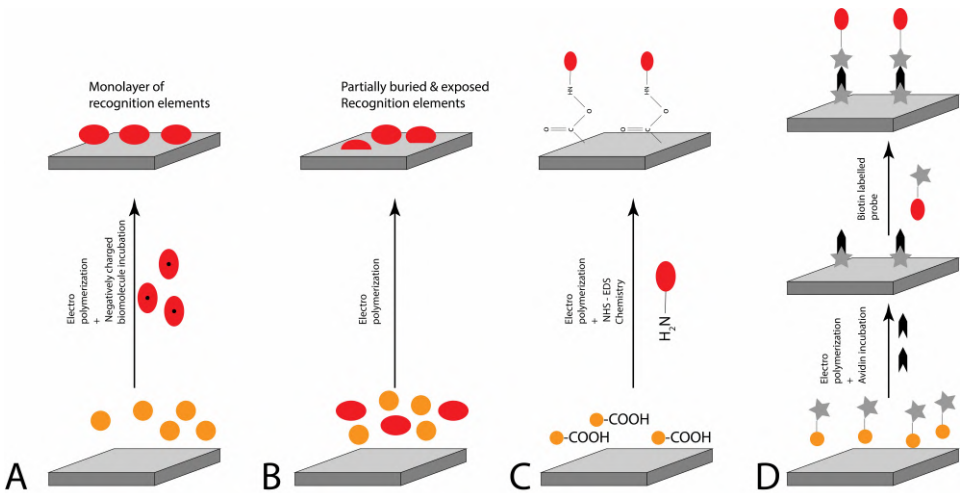

Fig. (10). (A) Schematic preparation methods for matching cAbs, and artificial capture antibodies (cAbs) height profiles. (B) Diagram preparation procedures for GO doped Surface Imprinted Polymers exposed to Ultraviolet light.

Several applications of MIPs/SIPs in clinical assays include the following: 1. Detection of Bacterial Infectious Diseases 2. Detection of Virus-Induced Infectious Diseases [23].

Dendrimers

Due to the unique structural characteristics of dendrimers, such as biocompatibility, well-ordered composition, truthfulness, and structural consistency, dendrimers-based electrochemical biosensor/sensor systems have

been widely used for detecting different bio-analytes. A notable forward movement has been achieved in recent years toward the development and use of electrochemical sensing electrodes based on dendrimers. Numerous biosensors have been developed based on the direct electrochemistry of enzymes and hemeproteins. Active biomolecules may be readily constrained on dendrimers without sacrificing their biological activity, allowing for the creation of efficient conducting surfaces useful in a variety of applications. Due to the abundance of amine groups in dendrimers, they easily interact with a large number of bioreceptors, substantially increasing the detection limit and sensitivity of the target bio-analytes [19].

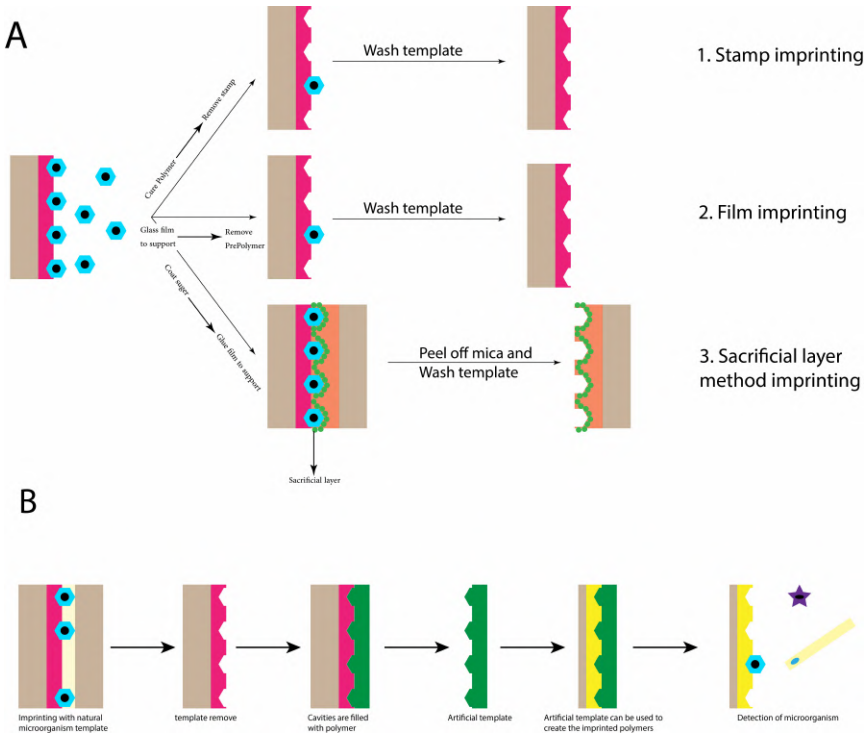

Fig. (11). Procedures for creating schematics for three distinct kinds of micro-contact imprinting (A) and indirect micro-contact imprinting (B).

Recently, Liu *et al.* developed a biosensor platform for *in vivo* electrochemical detection of dopamine (DA) utilizing a cell membrane-mimic PC polymer film-enabled microelectrode [24]. Some recent work by Conducting polymers includes developing an electrochemical neurotransmitter sensor based on Gold Nano Particles patterned on polyaniline nanowires (PANI) in uric acid and ascorbic acid Devaki *et al.* [25]. Miodek and the company created a DNA sensor for Mycobacterium TB utilizing a nanocomposite composed of redox poly-

(amidoamine) dendrimers (PAMAM) (MWCNTs-PPy-PAMAM) and MWCNTs coated with polypyrrole (PPy) [26]. A similar PAMAM-G4-based sensor was developed to detect paracetamol in human serum samples and commercial tablets with high sensitivity [19].

Electrochemical Biosensing Based on MNPPFs

1. MNPPF for mineral detection in biofluids;

2. MNPPF sensor for DNA and neurotransmitters;

3. MNPPF for the detection of particular amino acids and proteins: Identifying and quantifying specific proteins and amino acids is critical in therapeutic and medical research. MNPPF is utilized for protein and amino acid sensing because of its sensitivity to a variety of analytes. The current focus is on the development of protein and MNPPF-based amino acid sensors. Mercury electrodes pioneered label-free electrochemistry of proteins. Proteins and peptides containing cysteine/cysteine exhibited distinct electrochemical signals on mercury electrodes as a result of the development of the S–Hg bond [27, 28].

4. MNPPF sensor for urea

5. MNPPF sensor for cholesterol: MNPPF has recently become a desired material for cholesterol sensors due to its biocompatibility and increased surface area (nano-size materials), which significantly improves the loading enzyme per unit support volume and the immobilized enzyme's catalytic effectiveness. Numerous patentsandpapersonMNPPF have been published for cholesterol sensors [29, 30].

6. MNPPF sensor for creatinine;

7. MNPPF for glucose sensor: Monitoring and managing blood glucose levels using trustworthy sensors or home monitoring kits is one of the proactive steps required to manage diabetes mellitus. Electrochemical biosensors used in this application must be simple, dependable, and inexpensive [22, 31].

Nanoparticles

Numerous sensors have been manufactured using a variety of materials, including semiconductors, ceramics, metals, organic compounds, and metamaterials. Recent decades have seen a surge in interest in the creation of nanomaterials. Numerous nanomaterials have been suggested for this purpose, including nanoparticles, nanowires, and nanotubes of various sizes and forms [32].

Gold Nanoparticles

Gold Nano Particles have found many uses in electron microscopy as nanotechnology, electronics, biology, and contrast agents owing to their distinctive electrical and optical characteristics. Additionally, AuNPs have been extensively utilized as immobilizers and electrode modifiers in Glucose biosensors due to their electrocatalytic activity. The most stable MNPs are AuNPs. Additionally, AuNPs may bind to a variety of biological molecules (for example, nucleic acids, superantigens, and antibodies), which is advantageous for the fabrication of biosensors. Additionally, AuNPs exhibit the characteristics of other nanomaterials (for example, a huge surface-to-volume ratio that is essential for increasing the loading capacity of Glucose Oxide) [33].

A surface modification must offer a sufficient substrate for the Glucose Oxide enzyme attachment whenever retaining the electrode's original characteristics. When Gold Nano Particles are coated on the surface of an electrode, their affinity for Glucose Oxide is enhanced significantly. This property is presently being used to increase the number of enzymes immobilized in a variety of biosensors [33].

Incorporating polymers (for example, mesoporous silica and chitosan) and films or composites into Gold Nano Particle-based glucose biosensors has been shown to improve the surface's constancy, reduce enzyme leakage, and protect it from very harsh circumstances. Additionally, other modifiers such as AuNPs, a self-assembling double-layer 2-D network of MPTMS ((3-Mercaptopropyl)-trimethoxysilane), a composite of conductive PANI nanofibers and AuNPs, and a novel multilayer Gold NanoParticle/Multi-Wall Carbon Nanotube/Glucose Oxide membrane have been utilized to fabricate enzyme-based glucose sensors. German *et al.* have investigated and assessed several glucose biosensors based on glucose oxide adsorbed on bare carbon rod electrodes modified with Gold Nano Particles. They increased the linear detection range of biosensors by enzymatic Polymerization of pyrrole. In their research, LOD (The limit of detection) for Glucose was 0.07 mmol/L. AuNPs enhanced the calibration stability and sensitivity of the sensors and reduced the reaction time to the analyte glucose in the majority of reported investigations. In recent research, Gold NanoParticles were produced *in situ* on an eggshell membrane glued with Glucose Oxide on the surface of an O_2 electrode [33].

The nanoparticles provide an ideal milieu for the immobilization of enzymes. Immobilization of enzymes onto AuNPs substantially enhances their activity [34].

The researchers suggested utilizing laccase immobilized on an Au electrode modified with electrochemically generated Gold Nano Particles as a biosensor for determining the organophosphorus pesticide formetanate. We created a

conductometric biosensor for the detection of H_2O_2 utilizing HRP immobilized in a chitosan film without and with 11 nm Gold Nano Particles. A potentiometric biosensor was suggested to detect the herbicide glyphosate, which was based on urease immobilized with Gold Nano Particles 2.54 nm in diameter. The biosensor, which was recently developed for the purpose of measuring the activity of protein kinase A, was on the basis of AuNPs, antibodies (IgG), and horseradish peroxidase. A biosensor was suggested, especially for the colorimetric detection of AChE inhibitors [34].

Electrochemical Enzyme Biosensors Based on Gold Nanoparticles

Enzyme electrodes have received a great deal of attention in the field of biosensors using gold nanoparticles (For example, for the detection of HRP, GOx, *etc.*) [35]. Among the many methods, an effective and simple method is to directly deposit NPs onto the electrode surface [35].

Electrochemical Immunosensors Based on Gold Nanoparticles

Au NPs are critical in boosting the electrochemical signal-transducing response that occurs when antigens attach to antibody-immobilized surfaces and in stabilizing the number of immobilized immunoreagents (Like detection hIgG, α -Fetoprotein, α-1-Fetoprotein (AFP), Diphtheria antigen, Diphthero toxin, HbsAg (Hepatitis B surface antigen), *etc.* analyte) [35].

Electrochemical DNA Biosensors Based on Gold Nanoparticles

Due to their inherent benefits of fast response, sensitivity, and cheap cost, electrochemical DNA biosensors are advantageous analytical instruments for sequence-specific DNA detection and diagnostics. As with other electrochemical biosensors, gold nanoparticles may be used to immobilize DNA as appropriate labels and on electrode, surfaces to enhance hybridization event detection (Such as detection: Single-nucleotide polymorphisms (SNPs), cDNA, Sequence-specific PAT gene fragment, Norepinephrine in the presence of ascorbic acid, Nogalamycin, mithramycin, netropsin, 16-mer oligo-nucleotide, 55-mer oligo-nucleotide, ssDNA, *etc.*) [35].

Additional Biosensors using Gold Nanoparticles

The functional groups in hemoglobin (Hb) are easily reduced or oxidized by chemical redox agents. However, since the electron transfer process is protracted, it does not easily tolerate simple redox reactions at bare electrodes. Additionally, an amperometric sensor for H_2O_2 was created using a Nafion layer to immobilize

colloidal Au NPs and myoglobin on a GCE (Glassy Carbon Electrode). The electron transport of cytochrome P450scc (side-chain cleavage enzyme) was investigated using rhodium–graphite electrodes enhanced with Au NPs(Gold nanoparticles can also be used to detect Leukemia K562 cells, Living pancreatic Adenocarcinoma cells, Cyt c, Mb, Hb, *etc.* proteins) [35].

Platinum Nanoparticles

The antioxidant properties of PtNPs (Platinum Nanoparticles) have led to their use in various fields like medical applications, catalysis, and nanotechnology. The incorporation of PtNPs into glucose biosensors has the potential to substantially enhance the catalytic activity when Glucose is detected using hydrogen peroxide. PtNPs have been coupled with ordered mesoporous carbon nanocomposite, polypyrrole, and CNT-modified titania-NT (Nanotube) arrays mixed with iron-oxide/MWCNT/chitosan magnetic composites and GOx to make better the sensitivity of glucose biosensors, according to the research [33].

PtNPs have also been used in the fabrication of a chitosan glucose/functional graphene sheets/PtNP/GOx biosensor, according to the researchers. In another study, cationic dendrimer-encapsulated Platinum Nanoparticles and GOx were adsorbed onto the surface of negatively charged Multi-Wall Carbon Nanotubes in the presence of a negatively charged MWCNT. This amperometric glucose biosensor has a low LOD (limit of detection) for Glucose of 2.5 M. In this paper, we present an amperometric biosensor made of GOx that was co-immobilized with a PtNP/SnS_2 composite; The biosensor measured Glucose in two linear ranges (1.0–12 mM and 0.1–1.0 mM), and the limit of detection was 2.5 mM. However, platinum nanoparticles (PtNPs) are primarily used for amperometry; platinum nanoparticles were incorporated into a potentiometric biosensor for the detection of sulfites that was based on So_x (sulfite oxidase) that was immobilized in a PPy film containing platinum nanoparticles (Fig. **12**) [33].

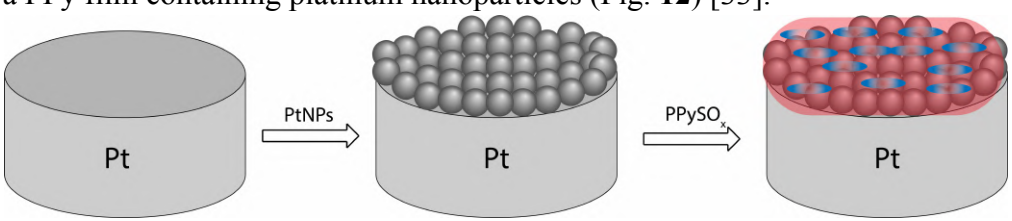

Fig. (12). The potentiometric sulfite biosensor is prepared by modifying the WE surface with PtNPs and then immobilizing SO_x (sulfite oxidase) in a PPy film.

Silver Nanoparticles

The advantages of utilizing AgNPs (silver nanoparticles) in biosensors are comparable to those using other metal NPs, including increased signal

amplification and matrix conductivity [33].

The urea determination amperometric biosensor was created using urease immobilization in a composite matrix comprising AgNPs, PVAc (Polyvinyl acetate), and Polyaniline stabilized in PVOH (Polyvinyl alcohol). Using lactate oxidase and AgNPs, an amperometric biosensor with a flexible electrode was created. The biosensor was designed to determine lactate levels in sweat. The linear detection range was between 1 and 25 mM, which corresponded to the specific lactate content in human sweat [33].

Palladium Nanoparticles

Due to their conductivity, catalytic activity, and cheaper cost than AgNPs or PtNPs, palladium nanoparticles (PdNPs) have gained increasing interest. Furthermore, PdNPs are capable of catalyzing H_2O_2 reactions (but maybe at a slower rate than platinum nanoparticles) [33].

Recently, it was shown that a glassy C electrode modified with 9 nm palladium nanoparticles efficiently lowers H_2O_2 at -0.12 V, which may aid in the creation of amperometric biosensors based on oxidases. Pd nanoparticles are often utilized in conjunction with other NMs. For instance, in reference [60], the glucose biosensor was constructed using MWCNTs (multi-walled carbon nanotubes), PdNPs, and GOx. Additionally, PdNPs increased the stability of biosensors. The biosensor was utilized to control the glucose content of honey and blood serum. PdNPs were combined with graphene and chitosan in a comparable biosensor [33].

Nickel Nanoparticles

NiO (Nickel oxide) nanostructured materials have been widely used in electrochemical sensors and supercapacitors, most notably in non-enzymatic glucose sensing, due to their superior electrocatalytic and low-cost characteristics. Due to the catalytic effect of the Ni^{3+}/Ni^{2+} formation on the electrode surface in an alkaline medium, Nickel-based nanomaterials exhibit catalytic oxidation activity for GOD, and most of the Nickel-based non-enzymatic sensors are produced for glucose detection [36].

Hydroxide and NiO are of considerable interest in biosensing development owing to their strong electrocatalytic action, chemical stability, non-toxicity, cost-effectiveness, and biocompatibility. Due to the unique characteristics of Nickel oxide nanostructures, they may be used to immobilize all kinds of molecules and biomolecules in order to fabricate sensors and biosensors [36].

Copper Nanoparticles

CuNPs (Copper nanoparticles) improve response current and are biocompatible and cheap. GR–CuNPs hybrids were utilized to detect analytes, particularly Glucose, in a non-enzymatic manner. There is just one study that examines the CuNPs–GR combination as a platform for enzyme immobilization. Huang *et al.* [37]. Developed an enzymatic biosensor for detecting Glucose. They disseminated the Graphene nanocomposite in Nafion before dropping it onto the GCE (Glassy carbon electrode) surface. By cyclic voltammetry, Copper nanoparticles were electrodeposited onto a modified electrode in a solution comprising NaCl and $CuCl_2$. Glucose oxide was immobilized on the surface of the modified electrode by electrostatic adsorption after CuNPs electrodeposition [36].

Iron(III) Oxide Nanoparticles

Fe_3O_4 (Iron oxide) nanoparticles accelerate the oxidation of H_2O_2, consequently increasing the responsiveness of O_2-based biosensors. However, iron oxide nanoparticles have lesser catalytic activity than PtNPs and PdNPs, the chemicals used to synthesize Fe_3O_4 NPs are much less expensive. Fe_3O_4 nanoparticles were recently utilized in the choline oxidase bienzyme/acetylcholine esterase system and developed biosensors based on GOx. Another study used urease to build a urea biosensor by trapping it in chitosan–Fe_3O_4 nanocomposite [34].

Titanium Dioxide Nanoparticles

TiO_2 (Titanium oxide) has a high active surface area due to its extensive nanopores and interior cavities. This, along with the charge transfer, non-toxicity, and chemical stability capabilities of Titanium nanotubes, makes them an attractive material for enzyme immobilization in electrochemical biosensors. Titanium oxide sheets are also used as components of perspective sensors [34].

For instance, GOx was immobilized using a composite material composed of titanium nanotubes and Polyaniline [34].

Zinc Oxide Nanoparticles

Analytes such as dopamine, DNA, cholesterol, uric acid, and Glucose can be detected using electrochemical biosensors based on zinc oxide (ZnO), which are highly effective. Bioreceptor: The bioreceptor is the biological detection component immobilized on the active site (Zinc Oxide) base and has a specific binding affinity for the analyte sample that has been determined. Among the common diagnosis components employed in the device are antigens, proteins, DNA, and enzymes, to name a few examples [38].

When it comes to detecting bio-element targets, electrochemical biosensors based on Zinc Oxide nanostructures have shown considerable promise, and they have also demonstrated excellent results during sensing experiments. Using ZnO nanoparticles as a model, Gunjan *et al.* classified them into two categories that are critical in biosensor applications: (1) a great surface-area-to-volume ratio and (2) size [39]. It has been demonstrated that ZnO nanoparticles can be used to address a variety of issues associated with electrochemical biosensors. Researchers Xing and colleagues discovered that employing an enzyme as a bioreceptor created a barrier between the enzyme layer and the electrode material used in conductive electrodes. The enzyme's active site was encased within the protein shell, protecting it from the environment [30]. As illustrated in Fig. (**13**), Zinc Oxide nanoparticles were used as sandwiches amid the electrode and the enzyme (glucose oxide) to improve the efficiency of the reaction. In part, this was due to the small size of the ZnO nanoparticles, which resulted in improved functionalization of the enzyme, which in turn led to improved efficiency of the biosensor system owing to the enzyme's effective absorption onto the surface of Zinc Oxide nanoparticles. ZnO nanoparticles also serve as a conductor between the enzyme and its electrode, thereby improving the electrical conductivity of the enzyme electrode as a result of their presence. In a separate study, Fidal *et al.* [40] discovered a similar tendency. Because of the small grain size of ZnO nanoparticles, they likely have a greater surface area. Thus, these nanostructures provided many sites for enzyme immobilization activities on their surfaces while simultaneously lowering barriers to mass transfer during analyte-enzyme interactions [38].

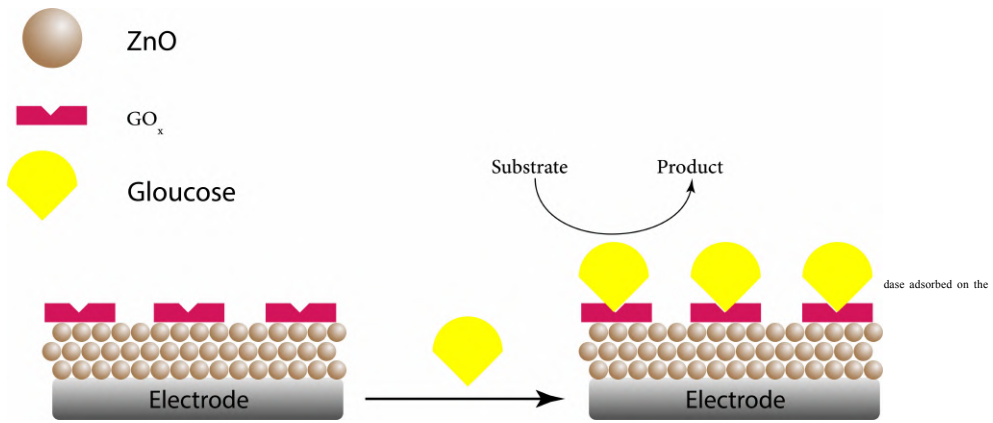

Fig. (13). ZnO nanoparticles are shown between the electrodes with glucose oxidase adsorbed on the nanoparticle surface.

Zeolites and other Aluminosilicates with Nanoscale Structure

Numerous nanosized aluminosilicates exist, including mesoporous silicon spheres and zeolites. Zeolites are further classified into Beta, A, Y, LTA, silica, and other varieties [34].

This substantially increases the surface area of the crystals available for enzyme absorption. Additionally, various zeolites with embedded metal ions may be utilized as charge carriers in electrochemical biosensors [34].

Various kinds of zeolites have been combined with conductometric, potentiometric, and amperometric biosensors, as well as glutamate oxidase, creatinine deiminase, butyrylcholinesterase, acetylcholinesterase, urease, and enzymes GOx, among others. Zeolites in biosensors facilitate immobilization and eliminate the need for hazardous reagents such as glutaraldehyde. Additionally, it was shown that the biosensor preparation was more reproducible. Several studies have shown a 20–30% improvement in the sensitivity of zeolite-based biosensors [34].

Inorganic Quantum Dots

Tyrosinase was immobilized in a chitosan and CdS (quantum dots) composite matrix in an amperometric biosensor for detecting phenolic chemicals. An amperometric biosensor for the determination of cholesterol and its esters was suggested. It was based on a bi-enzyme system of ChOx/Ch esterase immobilized in a composite matrix of chitosan and CdS (quantum dots) [34].

Doped Inorganic NMS

Doping may be used to adjust the characteristics of NMs; doping is a versatile method of modifying NMs without significantly altering their morphology, resulting in changes in catalytic, optical, electrical, and other properties. For instance, the newly reported thiourea sensor is based on manganese oxide nanoparticles with CoO codoped. The LOD values for acetone and ethanol sensors based on ternary doped metal oxides are very low, 0.05 nM and 0.127 nM, respectively. Melamine sensors based on Cd-doped Sb oxide nanostructures also exhibited an extremely low detection limit (14 pM). The titanium dioxide nanoparticles and nitrogen-doped carbon sheets were used to fabricate an ultrasensitive photoelectrochemical glucose biosensor. We constructed uric acid biosensors with a broad linear range (0.05–1.0 mM) using nitrogen-doped zinc oxide thin films [34].

Nanostructured Carbon Black

Dang *et al.* [41] and Zhang reported on electrochemical sensors [42, 43]. In these instances, acetylene black was used to label CB for voltammetric sensing of tetracycline, colchicine, and rutin. The source and/or type of CB may have a straight effect on the electroanalytic performance of the electrochemical (bio)sensor developed. Currently, CB is used for sensing applications to determine pharmaceuticals, environmental pollutants, food additives, biomolecules, and nicotine electrochemically. Xiao-He *et al.* [44] explored the use of CB as carbon support for the immobilization of glucose oxidase [45].

Calixarenes

Calixarenes are chemical compounds that have the form of a cup and are composed of multiple cyclic phenolic oligomers. Calixarene molecules come in a variety of forms and are often employed as selective binding agents in chromatography columns and sensors. Nevertheless, derivatives of calixarenes and calixarenes are sometimes employed as immobilization matrices for enzymes [34].

Gokoglan *et al.* [46] successfully immobilized GOx on an AuNPs/calixarene-modified electrode. CP poly(2-(2- octyldodecyl)-4,7-di(selenoph-2-yl)-2H-benzo[d] [1 - 3]triazole) was coated over the electrode to enhance conductance, followed by drop-casting of calixarene/AuNP solution, where calixarene established thiol linkages with AuNPs. Finally, NHS (N-(3-dimethy-laminopropyl)-N-ethylcarbodiimide hydrochloride (EDC)/N-hydroxysuccinimide) crosslinking agents were utilized to covalently bind GOx to calixarene. The biosensor was effectively utilized to diagnose the glucose content in drinks at a WP of -0.7 V *vs.* Silver wire. Calixarenes have been utilized to create a variety of biosensors for determining the glucose content of drinks; calixarenes may also be useful in conductometric enzyme biosensors for ammonia recognition [34].

Biological Elements and Biomaterials

The fundamental building blocks of bionanomaterial architectures are biomaterials and bionanocomposite materials [2].

(i) Owing to the presence of several binding sites and the presence of diverse binding domains, bionanocomposite materials enable the multi-directional development of nanostructures.

(ii) Modified biomaterials with particular anchoring groups facilitate their association with nanostructures/biomaterial surfaces, allowing for precise control

over the direction of nanostructure intensification.

(iii) Enzymes may serve as catalytic instruments in the organization of biomaterials, thus delivering nanostructures.

(iv) Bionanomaterials provide complementary and selective recognition interactions between hormone receptors, DNA and nucleic acid, and antibody and antigen.

Enzymatic Nanocomposites

Enzymes have unusual electrical activity under physiological circumstances and are readily detectable in a variety of systems. However, enzymes may be integrated into matrices or networks to create sophisticated nanoreactors. Numerous difficult studies have been conducted to stretch insoluble immobilized enzymes onto a variety of electrode substrates for selective electrochemical sensing. When compared to their soluble counterparts, the enzyme-modified or immobilized electrodes possessed several advantages, including (i) the competent direct of enzymatic activity, (ii) the repeatability and durability of electroanalytical platforms for a variety of applications, and (iii) the re-applicability of bio-derived catalysts reduce production costs through process control and proficient recycling [2].

A high-performance biosensor device based on a metallic–enzyme–polymer nanoparticle composite screen was shown to detect Glucose with excellent sensitivity. DA (Dopamine) is used as a monomer and reductant; galactose oxidase is used as metal ions, and an enzyme such as Au^{3+} or Pt^{4+} is used to initiate the Polymerization of DA in this biosensor platform. The enzyme-based electrodes as-built demonstrated significantly improved amperometric biosensing capability, with a sensitivity of 129 A cm^2 mM1 for Glucose and good durability. Gough *et al.* invented the glucose sensor in the 1980s using a two-dimensional enzymatic electrode design with an enzyme immobilized on the membrane [47]. O_2 was diffused into the membrane in 2 ways in this sensor arrangement, while Glucose was diffused in just one direction. Salimi and colleagues developed a biosensor system for lysozyme detection based on IDA–Cu complex (IDA–Cu ion) adsorbed on modified Au nanoparticles GC electrodes (Lys) [48]. The IDA–Cu complex is used as a structured recognition element in this platform to capture Lysine. The Gold NPs were used to immobilize the iminodiacetic acid–copper ion complex, and their interaction with Lys resulted in an improvement in the electrochemical signal (Fig. (**14**) [2].

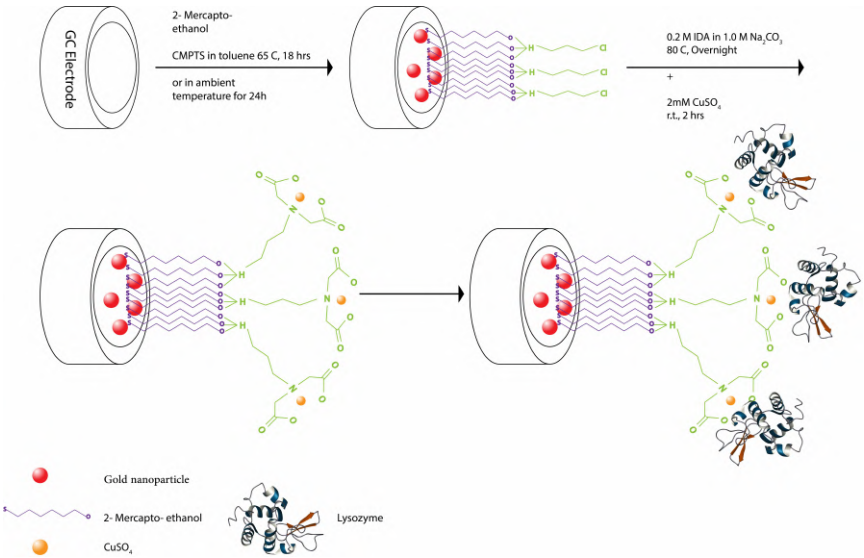

Fig. (14). The following diagram illustrates the different processes involved in the production of the Lys sensor.

Lin and colleagues created a biosensing technology for glucose detection based on a GOx-graphene-chitosan nanocomposite [49]. The immobilized enzyme maintained the bioactivity of the enzyme electrodes, showing a surface-confined, reversible $2e^-$ and $2H^+$ transfer process with a long lifetime. The linearity range of this GOxgraphene-chitosan nanocomposite film-based biosensor platform was 80 M to 12 mM glucose (lowest sensing limit of 20 M; sensitivity of 37.93 A mM^{-1} cm^{-2}). Bradar and colleagues produced rGO sheets coupled with tyrosinase and a bisphenol A (BPA) biosensor generated from chitosan polymer nanocomposite. The high sensitivity of the rGO-chitosan nanocomposite was achieved *via* the use of its increased electrochemical activity, large surface area, well-built structure, and electronic conductivity. The biosensor system was extremely sensitive to Bisphenol A (LOD: 0.74 nM) and demonstrated an improved sensitivity of 83.3 A nM1 cm^2 with a more extensive linear range of 0.01 - 50 M [2].

For glucose measurement, an electrochemical biosensor system was created by entrapping both HRP and GOx in the mesopores of SBA-15. The mesoporous SBA-15 materials enhanced the electron transfer mechanism between the substrate and HRP. Both GOx and HRP were immobilized and kept their catalytic activity inside the bienzyme-entrapped SBA-15 film. In the presence of Glucose, the enzymatic reaction of the GOx-glucose-dissolved O_2 system produces H_2O_2 in the bienzyme-entrapped mesopores. Ju and colleagues developed an electrochemical biosensor platform for detecting phenol using peroxidase-

tyrosinase immobilized on mesoporous silica [50]. The established phenol biosensor demonstrated a quick reaction time and a very high sensitivity of 14 A M1 cm^2. Lately, a lactate sensor based on lactate oxidase (limit of detection) and Graphene Oxide nanosheets/enzymes has been created. It demonstrated a dynamic range of 1 – 100 mM in human sweat and synthetic with a LOD 1.0 mM. It is an attempt to create biosensor platforms by fabricating integrated and structured structures, including enzymes. To accomplish this organization of the enzymes, rational approaches such as layer-by-layer (LbL), Langmuir–Blodgett (LB) techniques, and self-assembled monolayer (SAM) assembly were used, which are commonly used to produce a stable modified electrode. Zhang *et al.* created an electrochemical sensor for ionic lactic acid, Glucose, and strength in sweat using polymethylene blue-MWCNTs-lactic dehydrogenase/Au and multi-walled carbon nanotubes-glucose dehydrogenase/Au [51]. Another research used 5-hydroxyindole-3-acetic acid (5-HIAA) as a substrate for carcinoid tumor producers. The modified electrode was sensitized with 5-HIAA using the DPV method. This biosensor platform demonstrated a detecting limit of 1.4 M for 5-HIAA (linear response throughout the concentration range 1-50 M & sensitivity 0.72 0.01 nA M1) [2].

Nucleic Acid Nanocomposites

Nucleic acid is a polymeric biological molecule that includes DNA and RNA. It is made up of monomers called nucleotides. Due to their high stability, DNAzymes and DNA aptamers have been extensively studied for the purpose of building robust recognition and catalytic units for biosensor systems. According to the electrochemical signal, DNAzymes are regarded as a new biosensor for a variety of cofactors, including Pb^{2+}, UO^{2+}, Cu^{2+}, Zn^{2+}, and histidine [2].

Palecˇek first demonstrated electrochemical detection of DNA in the 1950s utilizing types of biocomposite electrode materials. *via* nanomaterials' great load capacity, they are excellent nanocarriers for catalytic G-quadruplex-DNAzymes used to produce a signal amplification tag. Vallée-Bélisle and colleagues developed a selective electrochemical protein sensing in whole blood utilizing DNA macromolecules through a Steric Hindrance Hybridization Assay (eSHHA) selective [52]. According to Shao and colleagues, the DNA-Gold bioconjugates act as bio-bar codes, enhancing the electrochemical signals used to detect Pb^{2+} [53]. The electrochemical DNAzyme biosensor developed demonstrated the ability to detect Pb^{2+} through catalytic reactions of a DNAzyme to Pb^{2+}. Yang and colleagues developed a biosensor system utilizing DNAzyme-functionalized DNA in a subsequent step [54]. Au nanoparticles/$Ru(NH_3)_6^{3+}$ created a signal with a LOD 0.028 nM on the electrochemical biosensor Pb^{2+}. He and colleagues developed an electrochemical biosensor for determining the nanomolar

concentration of kanamycin A using a signaling-probe displacement electrochemical aptamer [55]. HRP-DNAzyme (The hemin/G-quadruplex horseradish peroxidase-mimicking DNAzyme) was used to detect and quantify hydrogen peroxide. Ai *et al.* developed an electrochemical biosensor platform for detecting DNA methylation by activating G-quadruplex-DNAzymes with DpnI digestion [2, 55].

Ai and colleagues developed an electrochemical technique for screening inhibitors based on DpnI digestion and determining DNA MTase (methyltransferase) activity, which activates G-quadruplexDNAzymes [55]. With a LOD of 0.96 U/mL, this biosensor can readily monitor changes in DNA methylation levels with excellent selectivity. Willner and colleagues developed an electrochemical biosensor system based on the use of Platinum NPs/aptamers to enhance the electrochemical signal directed toward proteins [56]. Chen and colleagues recently showed the development of an impedimetric aptasensor based on an aptamer (ABA)-ATP (adenosine triphosphate) combination with Gold NPs [57]. Recently, He and colleagues developed an electrochemical biosensor based on AuNPs DNA and aptamer for Mycobacterium TB strain H37Rv [2, 58].

Immunoassay Based Nanocomposites

Immunoassays gained popularity in the 1970s for biological applications owing to their outstanding low detection limits and excellent selectivity for complicated sample analysis. Immunoassays and immunosensors may be performed using very simple methods and equipment. The original development of immunosensors utilizing electrochemical techniques was motivated by their rapid reaction time and great sensitivity. Multiple early radioimmunoassays for biological applications have been described, including the disease-associated proteins and the determination of hormones [2].

Valipour and colleagues have developed an electrochemical immunoassay for ultrasensitive chorionic gonadotropin recognition (hCG) [59]. A covalent attachment of Pt nanoparticles onto OMIMBF4 as ionic liquid (IL) nanocomposites, chitosan, and graphene was used to detect and determine hCG. Shen and colleagues have developed an ECL (electrochemiluminescent) biosensor platform for alpha-fetoprotein using CdTe quantum dots (QDs) nanocomposites (AFP) [60]. Li *et al.* described the development of a magneto-controlled microfluidic system for the direct electrochemical detection of squamous cell carcinoma antigen (SCC-Ag) in SCC-Ab (serum using anti-SCC antibody) functionalized magnetic mesoporous Au nanoparticles/thionine/NiCo2O4 composites [61]. Knopp and colleagues established a proof-of-concept for the immunoassay technique for Immunoglobulin G1 utilizing Au nanoparticles

tagged detecting antibodies, which exhibited a detection limit of 0.1 fg mL^{-1} for Immunoglobulin G1 [62]. Delequaire and colleagues reported on the utilization of metal nanoparticles as labels in another immunoassay [63]. After the antibodies captured proteins, the Ab_2-nanoparticles were attached, showing a LOD of 3 pM for Immunoglobulin G. Tang and colleagues developed a split-type PEC (photoelectrochemical) immunosensor for low-abundance PSA (prostate-specific antigen) by combining self-illumination with a DMM (digital multimeter) output [2, 64].

Aptasensor

The way this research was done shown in Fig. (**15**) was based on the idea that aptamers could act as chelating agents for analytes and undergo conformational changes, which would change the electrochemical properties of the aptamers with the redox label. Because of the T-Hg-T interaction, the conformation anti-Hg aptamer transforms into a hairpin shape that can accommodate multiple Hg^{2+} ions. In comparison, the anti-Pb aptamer produces a G-quadruplex, which accommodates the Pb^{2+} ion through G-Pb-G contact. In both instances, the redox labels approach the electrode surface, enhancing electron transport. Increases in the concentration of the target analyte may result in a rise in the concentration of coiled aptamers on the surface, which increases the electrochemical current [65].

Biopolymeric Nanocomposites

Due to their remarkable characteristics, biopolymers have gained considerable attention in fields ranging from fundamental research to biomedical technology. Chitin and chitosan-based biomaterials, in particular, encapsulate practical issues in a variety of forms, including thin films, gels, and solutions. The biosensor platforms combine efficient nucleic acids, proteins, and other biomolecules onto the electrochemical transducer's surface [2].

Another effective method for measuring cholinesterase inhibitory activity is to use an electrochemical flow cell for Choline dehydrogenase at the Choline dehydrogenase-altered chitin films placed on a Platinum electrode. Chitin, a biopolymer, may be used to embed protein-based biological detection components in enzymes. In the last few years, we have seen the use of flat, homogeneous, and thin chitin-based films as ideal biosensor substrates. Another intriguing sensor for humidity was created utilizing chitinpolyaniline blends based on a conductometric technique. Under gaseous conditions, the as-fabricated thin-film of mixes deformed their internal electrical resistance against the water vapor content. Additionally, Jayakumar and colleagues demonstrated the multifunctionalization of biodegradable CNGs (chitin nanogels) with MPA (mercaptopropionic acid)-capped CdTe-QDs (QD-CNGs) for biosensing studies and *in vitro* cellular

localization utilizing a controlled regeneration approach [66]. This nanocomposite may be beneficial in the delivery of drugs, imaging, and biosensing [2].

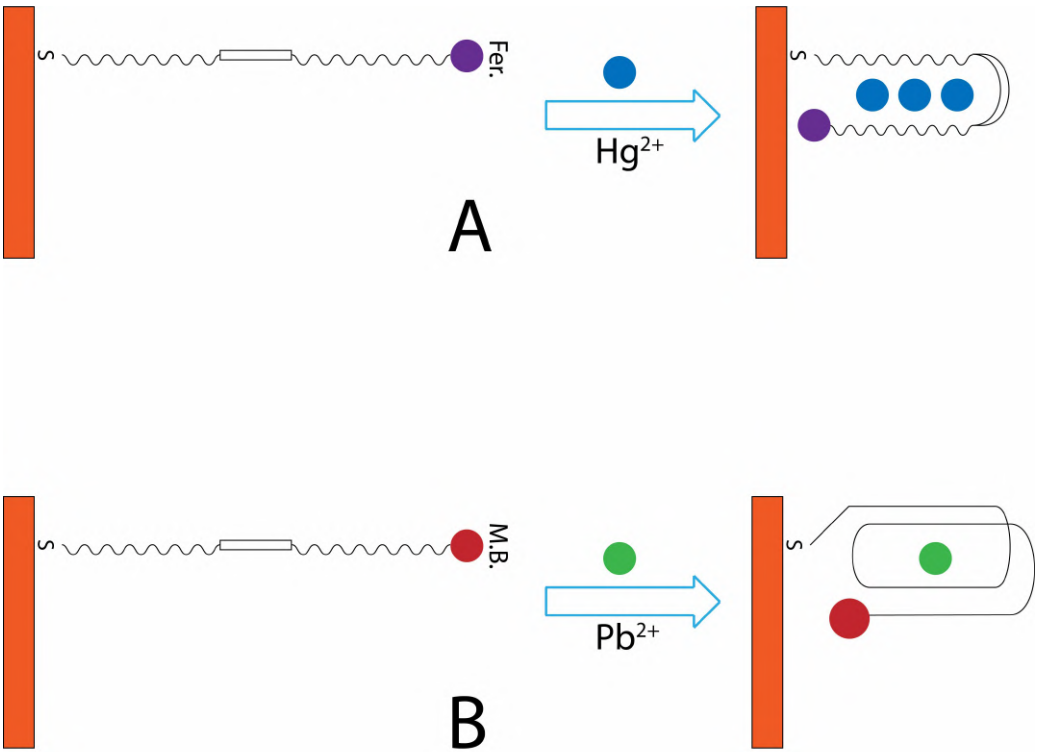

Fig. (15). Electrochemical detection of the heavy metal mercuric ion (A) and Lead ion (B) utilizing redox-labeled aptamers is shown schematically.

Bharathi and colleagues have developed a flexible amperometric glucose biosensor by directly immobilizing GOx (glucose oxidase) onto a chitosan-supported C nanocomposite [67]. Yaropolov and colleagues described a biosensor platform for DA recognition based on cellulose-derived tri-enzyme [68]. Mao and colleagues examined the electrochemical properties of MWNTs that have been non-covalently functionalized with cellulose derivatives such as methyl cellulose and hydroxyethyl cellulose [69]. Kerman *et al.* showed DNA/chitosan as a biosensor platform for probe immobilization *via* ionic interactions [70]. The surface of the chitosan-based DNA sensor may be maintained after the hybridization phase using urea and Ca^{2+} or Mg^{2+} salts. The chitosan-based immunosensing method uses the electrochemical property of antigen molecules interacting precisely with supplementary antibodies [2].

Composite and Sandwich Platforms

Sandwich Platform

Electrochemistry is the study of chemical reactions occurring in solution at the interface between an ionic conductor (the electrolyte) and an electron conductor (a semiconductor or a metal) that includes electron transfer in the middle of the electrolyte and the electrode or solution types. Electrochemical detection techniques have been widely used in immuno sensings and immuno assays because of their compatibility with modern micromachining technologies, low power consumption, cheap cost, and high sensitivity [71].

The test is designed in accordance with the current reports and employs a sandwich/competitive immunoassay template. The detectable signals diminish with and show an increasing signal-off tendency and analyte concentrations in the competitive-type immunoassay format. A high background signal is required in this situation. By contrast, sandwich immunoassays operate in a signal-enhancement (that is, mean signal-on) mode, resulting in a higher concentration of the target analyte. Consequently, the sandwich immunoassay is preferred for multivalent antigens, including several epitopes, particularly for low-concentration analytes. However, as shown in Fig. (**16**), the number of nanoparticles or pure enzymes used as labels was smaller than the number of enzyme labels coupled with nanoparticles in the last decade. According to signal-generating principles, electrochemical immunosensors and sandwich-type immunoassays are primarily categorized as amperometric, potentiometric, impedimetric, and capacity metrics [71].

Recently, much emphasis has been paid to signal amplification utilizing bio nanoparticle or multienzyme labels, and exploiting DNA as an amplified signal reporter in sandwich immunoassays. The recognition signal is typically derived from the labeled enzyme coupled with secondary antibodies for traditional sandwich-type enzyme immunoassays. Recently, the Tang group developed multiple sandwich amperometric immunoassays based on nanoparticles to detect biomarkers on functionalized magnetic immuno sensing probes using various nano labels, including nanogold/graphene nanosheets and enzyme-coated nanometer-sized enzyme-doped silica beads [61, 72]. Tang and the companies described an enzyme-free sandwich electrochemical immunoassay for the ultrasensitive recognition of alpha-fetoprotein utilizing gold nanoparticles enriched in carbon nanotubes as nanocatalysts/nano labels [73]. According to the HCR concept, a similar group constructed a sandwich-type electrochemical immunosensor for sensitive detection of Immunoglobulin G1 using G4 -based DNAzyme concatamers/hemin as bio labels and electrocatalysts [71]. Zhang *et al.*

demonstrated the feasibility of a unique and potent immuno-HCR assay for the measurement of huge target analytes, such as proteins, by linking the amplification capabilities of the HCR with the sensitive electrochemical signal of ferrocene molecules attached to hairpin probes (Fig. **17**) [62].

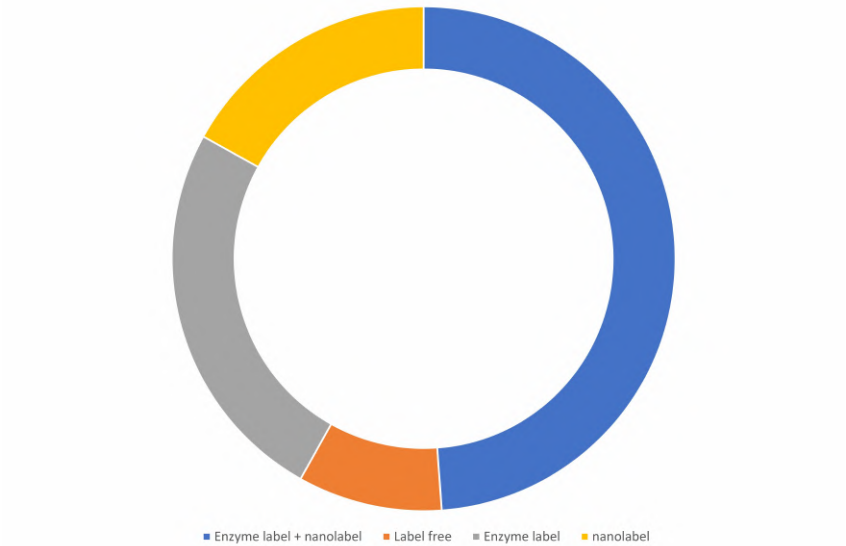

Fig. (16). From 2002 to 2011, the proportion of available papers relating to the various labels used in sandwich-type electrochemical immunoassays and immunosensors.

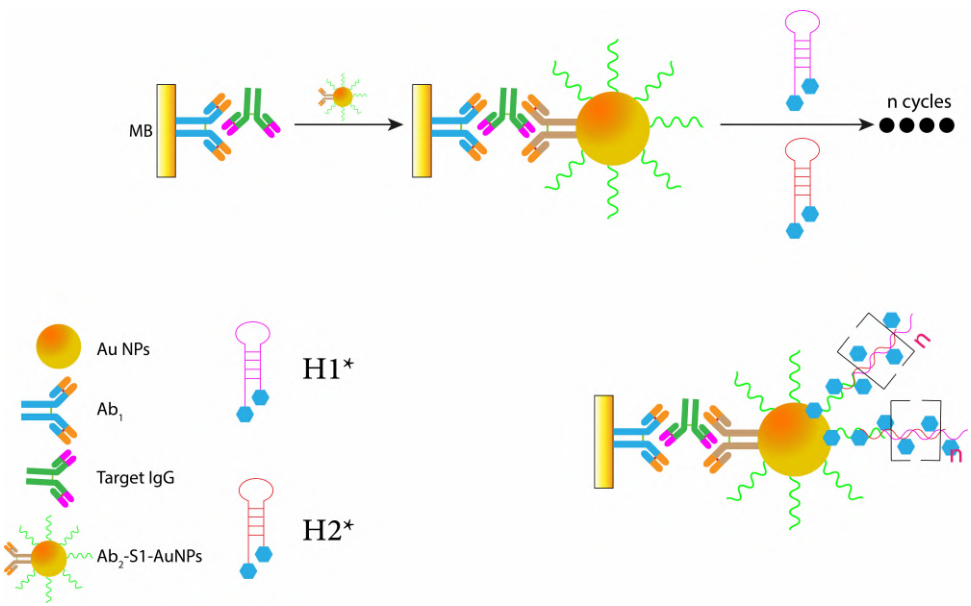

Fig. (17). The immuno-HCR assay method.

The potentiometric immunoassay was conducted in a microtiter plate using CdSe quantum dots labeled secondary antibodies and a sandwich immunoassay technique. Zhang and colleagues created a sandwich-type impedance immunosensor for the recognition of human Immunoglobulin G by amplification of the signal using nano Au-labeled secondary Immunoglobulins on a primary antibody-modified electrode with great resistance [73]. The CNP-labeled HRP-anti-AFP conjugates on the transducer were raised in proportion to the rise in AFP in the sample using a sandwich immunoassay technique. The immuno sensor's conductivity was reduced in the H_2O_2–KI system at a Limit detection of 0.05 ng mL^{-1} AFP [71].

Composite

Meek and Gupta, since 2018, produced 'hybrid' nanocomposites for ascorbic acid (AA) detection using metal NPs–grafted functionalized nanostructured polyaniline (PANi) and graphene nanosheets [74, 75].

Zhang and Li *et al.*, in 2018, investigated a novel dopamine biosensor-based CFMEs (on C fiber microelectrodes) modified with Cu^{+} sulfide functionalized rGO nanocomposites (Cu^{2+}S/reduction graphene oxide) for *in vivo* monitoring of neurotransmitters released by the Drosophila brain and the sensitive recognition of dopamine [76]. Sukrobov *et al.*, in 2018, reported the fabrication of electrochemical biosensors for sensitive non-enzymatic glucose recognition using a composite of nickel nanoparticles (NiNPs) coated on Nafion-Gr composite film [77]. Hang *et al.* 2018, developed a non-enzymatic sensor for H_2O_2 detection using an rGO-persimmon tannin-platinum nanocomposite (rGO-PT-Pt) [78]. Hatmluyi *et al.* in 2018, developed an electrochemical DNA biosensor for the detection of Rituxan (RTX) at low concentrations using a reduced GO nanocomposite/PAMAM dendrimer modified pencil Gr electrode (PGE/rGO/PAMAM) [74, 79].

Conductive polymers, nanocomposites and conductive polymers have recently been identified as promising materials for the creation of electrochemical DNA sensors. Electrochemical sensors for DNA recognition are being developed for various purposes, including forensic applications, rapid recognition of biological warfare chemicals, gene analysis, and DNA diagnostics [80].

The authors describe the development of an electrochemical DNA sensor based on a functional capture probe and PANI-AuNPs-Cts-GS (the polyaniline-gold nanoparticles-chitosan-graphene sheet) for detecting the ABL/BCR fusion gene in CML (chronic myelogenous leukemia). A DNA electrochemical biosensor was created using AuNPs, and PANI-nf (polyaniline nanofibers) was chemically synthesized onto them to form a nanocomposite material (PANI nf-AuNP). A

label-free DNA sensor based on PPy-PANI Au (polypyrrole-polyaniline-gold) nanocomposite films has been reported for electrochemical discrimination and detection of supplementary, non-complementary, double- and single-base incompatible target DNA hybridized surfaces. The dynamic detection range was $1*10^{-6}$ to $1*10^{-13}$ M by a LOD of $1.0*10^{-13}$ M. A DNA biosensor based on a PANI-Fe_3O_4-CNT nanocomposite was developed to detect DNA probes associated with *Neisseria gonorrhoeae* [80].

AuNp (Gold nanoparticles) is one of the most frequently utilized metallic nanomaterials in sensor applications. Besides their improved electrical characteristics and increased surface area, Gold nanoparticles have a biocompatible structure that is critical for biosensor usage [81].

The gold nanoparticle was often utilized as a modifier for other metallic-based Metal-organic framework compounds in sensor and biosensor applications. Wang and colleague [82] produced Gold nanoparticle-modified Copper-Metal-organic frameworks and utilized them to detect microRNA with high sensitivity (miRNA) [81].

Artificial enzymes have spread like wildfire in the scientific community due to their ease of production and purification, and stability. Additionally, they are reusable and do not need regular preparation. MOFs (Metal-Organic-Frameworks) have artificial enzyme characteristics, which make them very valuable for sensing and biosensor applications. For instance, iron(Fe)-based Metal-organic frameworks have been shown to have peroxidase-like enzymatic activity and are utilized in colorimetric recognition of thiol, AA, thrombin, Glucose, and H_2O_2 [81].

PANI has developed into one of the most assuring CP compounds in types of applications. Numerous devices for various purposes have been manufactured using polymer composites, including supercapacitors, sensors, and batteries. Electrochemical applications have shown the greatest promise for these composites. Specifically, the method of manufacturing sensors based on Polyaniline composites and Polyaniline has become well-known in a number of sensor investigations. The sensors exhibit high selectivity and sensitivity in identifying target species [83].

Additionally, Xue and colleagues created a phenol electrochemical sensor using polyphenol oxidase immobilized in a PNAI–PAN composite matrix. The resulting biosensor exhibited great stability and sensitivity [84]. Recently, an impedimetric immunosensor for detecting 2,4-D; acetic acid was developed using a PANABA-MWCNT (multi-walled carbon nanotube) nanocomposite functionalized with AuNP. Recently, it was revealed that a combination of PANI hydrogel

heterostructures and PtNPs (Pt nanoparticles) could concurrently detect Glucose. This sensor was shown a LOD of 0.0007mM and excellent sensitivity. Another PANI/PtNP hydrogel-based electrode was used to detect human metabolites, and the electrochemical sensor test demonstrated good performance. A polydopamine hybrid/polyaniline/gold nanoparticle electrochemical sensor was described for AA's selective and sensitive recognition (ascorbic acid). The sensor demonstrated a low detection limit of 0.000399 mM [83].

PoT is a very promising CP that is synthesized chemically or electrochemically from o-toluidine. Numerous research has been conducted to investigate polymer-lined electrodes. As sensing electrode materials, composites and PoT with metallic oxideMWCNTs and metal, enzymes, graphene oxide, graphene, and NPs have been described. The PoTAu/Cu nanocomposites demonstrated excellent biosensitivity and glucose selectivity [83].

Pan *et al.* created a sensor for the recognition of 4-nonyl-phenol that used a MIP/NGNRs-IL/GCE (molecularly imprinted poly(o-phenylenediamine-c--o-toluidine)-nitrogen-doped Gr nanoribbons-ionic liquid composite film) (Nanoparticles) [85]. This electrochemical sensor demonstrated superior performance, a LOD of 3.4 A/M, and high sensitivity. Change *et al.* created many more hydrogen peroxide sensors [86]. In 2013, graphene was integrated with poly(toluidine blue O) sheets and gold nanoparticles to create an electrochemical biosensor for determining H_2O_2. The modified electrode demonstrated extraordinary selectivity and stability for H_2O_2 [83].

O-anisidine polymer is one of the most advantageous conductive polymers, and its unmatched characteristics enable its usage in a wide variety of applications. Thin-film transistors, semiconductors, electrochromic devices, and Memory devices have all been manufactured using orthoanisidine-based CP composites. Regardless of these uses, polyorthoanisidine-based composite materials have been used to fabricate a variety of sensors, comprising those for the detection of Glucose, gases, neurotransmitters, and a variety of other analytes. Numerous combinations of compounds containing orthoanisidine-based polymers were investigated for their potential application as glucose biosensors. Electrochemically produced poly(o-anisidine) films doped with para toluene sulfonate anions and perchlorate, for example [83].

Modifying biosensors mainly composed of PPy composites and graphene has emerged as a viable electrochemical analysis method for achieving particular analytical objectives through the use of a variety of approaches. Recently, graphene was used with overoxidized PPy composites to identify guanine [83].

CONCLUSION

Much research has been done on different materials, with different advantages and disadvantages mentioned in this chapter. According to their field of expertise, any researcher can search on any of these cases; some of their advantages are mentioned in this section:

The utilization of carbon-based material in biosensors causes increases: in selectivity, sensitivity, cheap cost, thermal conductivity, insulating properties, porosity, surface area, stability, biocompatibility, and electrical conductivity; and decreased refractive index, coefficient of friction, and toxicity.

The utilization of polymers and conductive polymers in biosensors causes increases: in manufacturing, scalability, low cost, chemical specificities, transport properties, surface area, electrochemical signal transduction, durability, redox activity, an affinity for electrons, and biofunctionalization.

Nanoparticles' utilization in biosensors causes increases: in the surface's stability, protect biosensors from harsh circumstances, signal amplification, matrix conductivity, and superior electrocatalytic; and decreases enzyme leakage, cost and LOD.

Moreover, various materials can give an example of biosensors like biomaterial, composite and sandwich platforms, *etc.*, and of course, they have many advantages.

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CHAPTER 4

Basic Practical Principles for Studying Electrochemical Biosensors

Abstract: Due to the diversity of engineering disciplines involved in electrochemical biosensor studies, it is essential to be familiar with some topics, including experimental design, electrochemical laboratory tools, primary biology literature, and biological elements, to understand this area perfectly. The purpose of this chapter is to provide a quick review of these topics. In the section on the design of experiments (DOE), we discuss the principles of DOE, different approaches, guidelines for designing, and the DOE process. This section helps researchers to conduct studies systematically. After that, electrochemical instrumentation will be discussed. Potentio stat structure and function, elements of electrochemical cells, and experiments with two, three, and four electrodes are the topics that will be addressed. The final section of this chapter will introduce some basic biological concepts and elements.

Keywords: Biological element, Biosensor, DOE, Instrumentation, Practical Principles.

INTRODUCTION

For conducting a repeatable and reliable study, researchers must use systematic methods. In addition, knowledge and experience about the practical aspect of experiments are essential because the results will be reliable if a systematic study is designed and conducted [1]. Detailed studies usually consider multiple factors, and these factors have multiple levels. To determine the effect of each factor and level, dozens of experiments must be conducted. Many experiments will be time-consuming and costly. There are two general ways to reduce the cost of experiments [2]. As the first way, the use of DOE can reduce the number of experiments with the minimum effect on the study results logically.

As the second way, knowledge about instrumentation and the practical side of study will help increase the quality of experiments and prevent unnecessary repeats.

Seyed Morteza Naghib, Seyed Mahdi Katebi and Sadegh Ghorbanzade

Design of Experiments

Fundamentals

A study or experiment is a systematic process used to find an unknown effect, to test a hypothesis, or confirm a known result, all of which are conducted under controlled conditions. When studying a process (output), experiments are frequently used to determine which process inputs have a significant effect on the output and to establish what level these inputs should be at to achieve that outcome. To obtain this information, experiments may be constructed in a variety of ways. DOE, Designed Experiments, and Experimental Design are interchangeable terms for the Design of Experiments. To decrease design costs, experimental design can be used at the point of maximum leverage by speeding up the design process, minimizing late engineering design modifications, and reducing product material and labor complexity. The use of designed experiments is also a useful method for reducing manufacturing costs by reducing rework, waste, and inspection requirements [3].

Companies and laboratories often conduct experiments, so engineers (and scientists) use statistics to evaluate their results. Many engineers think that all are exposed to statistics during their undergraduate studies, which causes problems when tools are needed in the field. Either they do not know what kind of experimental strategy is needed for their problem and pick something inappropriate, or they pick the correct strategy but apply it incorrectly, or they pick the wrong strategy and it does not matter whether they use it correctly or not [4].

OFAT (one-factor-at-a-time) is a dated approach that is still extensively used by businesses and is often taught in colleges. It involves changing one variable at a time while keeping the others fixed. For complicated issues with numerous variables, DoE may be both fast and cost-effective because of its use of experimentation. More and more DoE case studies show the benefits and possibilities of the technology [5].

R.A. Fisher created the ANOVA (Analysis of Variance) method and the statistical approach to the Design of Experiments in 1920. In the years afterward, many people have worked to improve and advance this method. Techniques referred to as “Classical” in this chapter have all been based on Fisher's work. Engineers Shainin and Taguchi, on the other hand, have had a particularly large impact thanks to the introduction of two novel DoE methods they developed. As quality improvement methods, these new approaches go beyond the traditional Design of Experiments [6].

DoE (Classical, Shainin, and Taguchi) is better than OFAT in all three methods. There are proponents and opponents of each of the aforementioned strategies, and the discussion may become very intense at times. As long as the user is aware of the limits of the method, it may be helpful [4].

Approaches to Design of Experiments

For many years, the one-factor-at-a-time (OFAT) approach has been used. Ronald Fisher developed factorial experimental techniques based on factorial designs in the early 1920s, rendering earlier testing tactics obsolete. When testing is low-cost or when the number of variables under investigation is limited, these designs are very helpful (less than five).

Fractional factorial designs were developed in the 1930s and 1940s to address the large number of trials required by full factorial designs. A carefully chosen subset of the full factorial experimental design is used in this experiment. In exchange for ignoring certain high-order interactions, they provide a low-cost way of testing multiple variables in a single experiment. Due to high-order interactions often being negligible and difficult to understand in any case, there is little risk associated with this strategy [4].

The second stage of the traditional DoE approach began in the 1950s when Box and Wilson developed the subsequently known Response Surface Methodology. Due to their technique, DoE was used by the chemical industry and then by other industries. A few tests can be conducted andevaluated based on the results; new experiments can be designed. The researchers highlighted two advantages of industrial trials over agricultural studies: (a) Immediacy: Results can be acquired faster than in agricultural experiments, which might take a year; and (b) Sequentially: the experimenter can carry out a few tests, evaluate them, and then design new experiments based on past experiences. Central Composite Designs (CCDs) and Box-Behnken Designs (BBDs) were developed during this period [7].

In the 1980s, the Taguchi-Shainin method first appeared in the United States as a simple and efficient method of testing, ushering in the third era of classical testing. Statisticians and academics eventually recognized Taguchi and Shainin's engineering concepts. This resulted in beneficial improvements, such as incorporating numerous concepts from new methods (for example, variance reduction became an important study field within the classical design), and emphasizing the necessity of developing procedures and standards to facilitate implementation [8].

It was during an earlier era, when the democratization of statistics, along with Six

Sigma thinking spreading across all industries [9], that Design of Experiments became widely used across all types of industries. Furthermore, a growing interest in literature has been recommended for the Design of Experiments. Furthermore, software programs have simplified the creation of graphs and calculus, making DoE even more accessible [10].

Components of Experimental Design

A planned experiment examines three elements of the process: Factors, often known as process inputs. Controllable and uncontrolled variables are the two types of factors. A simple procedure like making a cake has two variables that can be controlled: its ingredients and the oven. We will refer to the controllable variables as factors throughout the text. The list of components for this example has been simplified; there may be many more elements that have a substantial impact on the outcome (oil, water, flavoring, *etc.*). Other variables can also exist, such as mixing techniques or instruments, mixing order, or even the individuals involved. Noise components are uncontrolled factors that cause variability in normal operating conditions, but we can control them during the experiment by utilizing blocking and randomization. The Fishbone Chart may be used to classify potential variables (Cause & Effect Diagram)

The settings or levels of each factor in the study, the temperature of the oven and the amount of sugar, flour, and eggs used for the testing are examples.

The experiment's response, or output, in the instance of baking, the cakes's flavor, consistency, and look of the cake are all quantifiable outcomes that may be affected by the variables and their amounts. Experimenters often choose not to optimize the procedure for one answer at the cost of another. Therefore, key outcomes are evaluated and studied in order to determine the components and the settings that would produce the best outcomes for the critical-to-quality features - both measurable variables and assessable qualities.

Purpose of Experimentation

Designed experiments may be used to improve processes and products in a variety of ways, including:

Contrasting Alternatives. In the case of cake making, we might compare the results of two different types of flour. Even if the price difference between wheat sellers turned out to be marginal, we might choose the lowest-cost vendor. When flour is important, we will choose the highest-quality flour. By conducting an experiment(s), we should be able to make an informed decision that takes into account both cost and quality.

Analyzing the factors that affect an output (response) - identifying those that are significant and those that are not. The question may arise, "What are the important variables besides flour, eggs, sugar, and baking powder?"

Achieving the Best Process Output (Response). "What are the required components, and what are the amounts of those variables, to create Mom's chocolate cake's identical flavor and consistency?"

Lowering Variability. "Can the recipe be altered so that it is more likely to provide the same results every time?"

Reducing, increasing, or focusing an output (Response). "How can I make the cake as moist as possible without it dissolving?"

Enhancing a process or product "Robustness" refers to the ability to function in a variety of circumstances. "Can the elements and their amounts (recipe) be changed so that the cake comes out roughly the same regardless of the kind of oven used?"

Optimization of several Critical Quality Characteristics (CTQCs) while balancing tradeoffs. "Using the simplest recipe (fewest ingredients) and the shortest baking time, how can you create the best-tasting cake?"

Guidelines for Designing Experiments

To solve the issues mentioned above, the design of an experiment must include the following:

- Testing the factors.
- Levels of each factor.
- Experimental runs are structured and laid out in a certain manner.

Experiments should be designed to collect as much information as possible be cost-effective, and be repeatable. Both competent measuring equipment and a steady process are required for trustworthy experiment findings. The experiment findings will be muddled if the measuring system adds significant inaccuracy.

When planning an experiment, keep the following four possible pitfalls in mind:

Other types of mistakes, such as unexplained variance, may muddle the findings in addition to measurement error. It is important to note that the terms "error" and "mistakes" are not interchangeable. Error is defined as any inexplicable

fluctuation that occurs inside an experiment run or between experiment runs that is related to level settings altering. Experiments that are well planned may detect and quantify error causes.

In normal operating conditions, noise variables are factors that are uncontrollable and cause fluctuation. This way, variables such as different machines, multiple shifts, raw materials, humidity, and so on can be accounted for in the experiment, so their fluctuation is not categorized as unexplained or experiment error. The capacity to select variables and settings that limit the impact of uncontrolled factors is a major strength of designed experiments.

Correlation is often mistaken for causality. Two variables that change in tandem may be strongly linked without one influencing the other they may both be driven by a third source. A misleading result may be formed if the data is examined without an understanding of the process.

Prior to carrying out the experiment, it is vital to take into account the combined effects or interactions of variables. Imagine an experiment in plant growth involving fertilizer and water. More water has been shown to promote growth, however, there is a point at which it causes root rot and has a negative effect. Additionally, more fertilizer has a positive effect until the roots are burned. In addition to the direct impacts, there may be additional interaction effects that would neutralize fertilizer's benefits by washing it away. A factor may generate non-additive non-linear effects, but these can only be investigated with more complicated experiments with more than two levels. There are two levels of linearity (two points create a straight line), three levels of quadraticity (three points create a curve), four levels of cubicity, and so on [11].

Experiment Design Process

The process of designing an experiment is shown in the flow chart below (Fig. **1**)

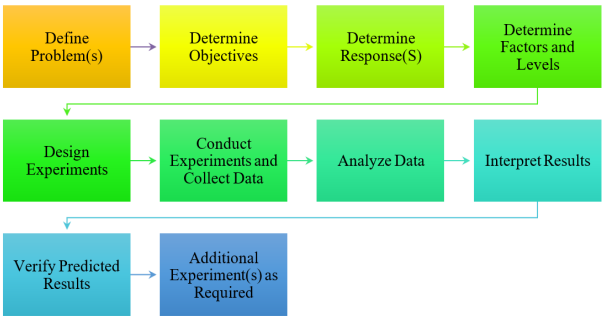

Fig. (1). Designing an experiment is a complex process that involves several steps.

Instrumentation

Electrochemical Cell

Electrochemical sensor investigations use an electrochemical cell. Electrodes are critical to the operation of electrochemical biosensors and electrochemical cells. In analytical chemistry, an electrochemical analyzer is a device that measures the current and potential inside an electrochemical cell [12].

A counter electrode (CE), a reference electrode (RE), and a working electrode (WE) comprise the electrochemical cell in which the experiments are conducted [12].

The traditional Electrochemical cell is a glass cell with a single compartment that must be thoroughly washed before each test. The kind of cell that is employed is determined by the quantity and kind of material, the method, and the analytical data that will be acquired. Fig. (**2**) depicts a typical electrochemical cell [12].

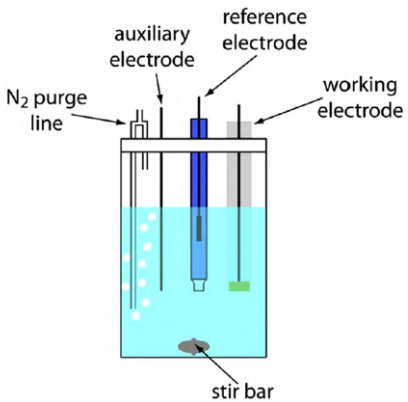

Fig. (2). Conventional three-electrochemical cell schematic depiction.

Working Electrode

The Working Electrode is an electrode on which the desired response occurs [12]. In a three-electrode electrochemical system, the WE is called anodic or cathodic based on whether the reaction occurring on the WE is an oxidation or a reduction. The WE material has a noticeable impact on the volumetric measurement function. Due to the fact that the desired reaction (oxidation or reduction) occurs upon the WE, It ought to have a good signal-to-noise ratio and a repeatable response. Thus, its selection is mainly determined by two factors: the background current in the measuring area and the redox behavior of the target analyte. Additionally, toxicity, availability, cost, mechanical characteristics, repeatability of the surface, electrical conductivity, and the potential window must be

considered. Numerous electrode types are available, including carbon paste electrodes, indium tin oxide-coated glass electrodes, silver electrodes, screen-printed electrodes, mercury electrodes, gold electrodes, platinum electrodes, and glassy carbon electrodes. The most often used WE materials for voltammetry are glassy carbon, mercury, gold, and platinum. For more specialized purposes, materials such as semiconductors and other metals are also utilized [13].

Reference Electrode

At the electrochemical cell, the RE maintains a constant potential (Fritz and Gyorgy, 2013). Because current passes with an electrode, it is possible to change its potential. In three-electrode setups, these effects are reduced for the reference electrode by considering two variables. To begin, its impedance ought to be modest since potentiostats are less tolerant of REs with a high impedance. A high impedance RE demonstrates the potentiostats' reaction (gradual ascension time) and exposes the system to environmental fuss (particularly power line fuss). Second, the reference electrode ought to be nonpolarizable because the passage of very tiny currents has no effect on the potential [14, 15].

Reference electrodes should be built with long-lasting half-cell components and maintain well-defined activity levels as temperature changes. They should have constant, repeatable electrode potentials. The most often used reference electrode is the SHE (standard hydrogen electrode), which is inert solid-like platinum. Submerged in a solution containing unit activity hydrogen ions and adsorption of hydrogen gas. The SHE half-cell response is provided by

$$2H^{+}(aq) + 2e^{-} \rightarrow H_2(g)$$

Plus, a zero half-cell potential value. $E^0 = +0.241$ V is the half-cell potential of the SCE (saturated calomel electrode). The Ag/AgCl electrode has a half-cell potential of $E^0 = +0.194$ V. The most often used RE for aqueous solutions is the silver/silver chloride electrode (Ag/AgCl), plus the potential controlled by the reaction [15].

$$AgCl(s) + e^{-} \rightarrow Ag(s) + Cl^{-}$$

Counter Electrode

The Counter Electrode (alternatively referred to as an auxiliary electrode) is used to shut the electrochemical cell's current circuit. It is often composed of an inert substance (for example, glassy carbon, graphite, gold, or platinum) and is not involved in the electrochemical process [16]. Due to the current flows between the

CE and the WE, the CE's total surface area (source/sink of electrons) must be greater than the WE's to avoid becoming a limiting factor in the electrochemical process's kinetics.

The other variables may be monitored when an investigated potential or current is placed between the working and counter electrodes in a two-electrode system. When anode, the counter electrode acts as a cathode, and the working electrode is used as an anode and contrariwise. The working electrode has a much lower surface area than the CE. The counter electrode's potential is not monitored in relation to the RE; instead is changed to bring the response back into equilibrium happening at the WE [15].

Other Electrochemical Cells

Above all, one of the electrochemical cells that make this kind of electrochemical system an ideal option for biosensing is the ability to create portable devices cheaply. The electrochemical biosensor depicted in Fig. (**3**) is among the most widely used electrochemical biosensors [17]. It utilizes SPEs (screen-printed electrodes) (Or Lab-On-Chip) decorated with mini electrodes (reference, counter, and working) forms that provide mass production, high operating speeds, and ease of low manufacturing costs. By offering surface modification and the capacity to interface to portable equipment, these flexible SPEs of reduced size have been developed for accurate on-site detection of target analytes. Finally, SPEs have surpassed traditional solid electrodes in terms of performance since they eliminate memory effects and time-consuming cleaning procedures. Combining these benefits enables SPEs to be used in various applications, including DNA and aptasensors, enzyme-based biosensors, and electrochemical immunosensors [17].

Microfluidic cells are another kind of electrochemical cell. A microfabricated electrochemical cell comprised of gold microelectrodes and was fabricated as a sensitive and compact alternative to the traditional electrochemical cell. A microfluidic electrochemical cell equipped with microelectrodes may significantly simplify online/in-line detection, electrode cleaning, and sample handling while reducing interferences; we may increase sensitivity [18]. Three distinct materials were optimized as electrodes in a microfluidic electrochemical cell: graphite-epoxy composite for the auxiliary electrode, silver epoxy composite for the pseudo-reference electrode, and gold film or graphite-epoxy composite for the working electrode. Microelectrodes behave differently than larger electrodes in that nonlinear diffusion is the main route of transport. This differential in mass transfer from the bulk solution to the electrode has many significant consequences, making microelectrodes very appealing in a wide variety of applications of electroanalytical chemistry. These benefits include a higher signal-

to-noise ratio, a faster creation of steady-state signals, a shorter time constant, and a smaller ohmic potential drop. Cyclic voltammetry was used to characterize the microfluidic cell's performance. Fig. (**4**) illustrates a microfluidic electrochemical cell [15].

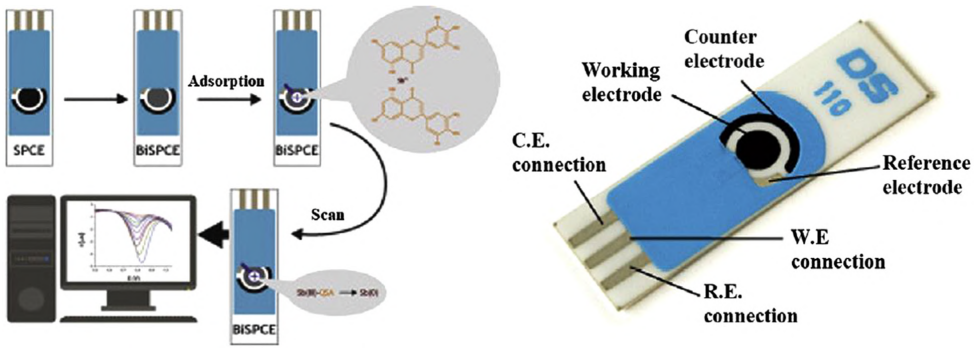

Fig. (3). A pixelated structure of screen-printed electrodes (Lab-On-Chip).

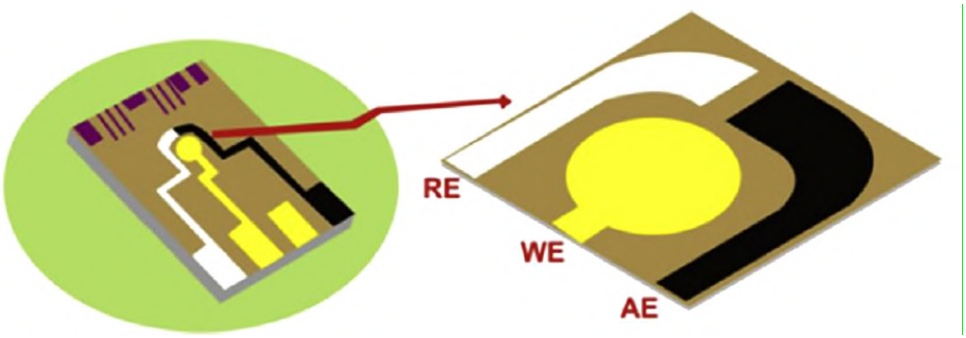

Fig. (4). The electrochemical cell on a microfluidic platform.

Principles of Potentiostats

A potentiostat regulates the difference between the voltages across a Working Electrode and a Reference Electrode. This is achieved by an Auxiliary (counter) electrode, which infuses current into the cell *via* the potentiostat. Nearly all applications rely on potentiostats to monitor current flow between the Working and Counter electrodes (Fig. **5**). In a potentiometer, the controlled variable is cell potential, while the measured variable is cell current. In order to operate a potentiostat, three electrodes are required [19].

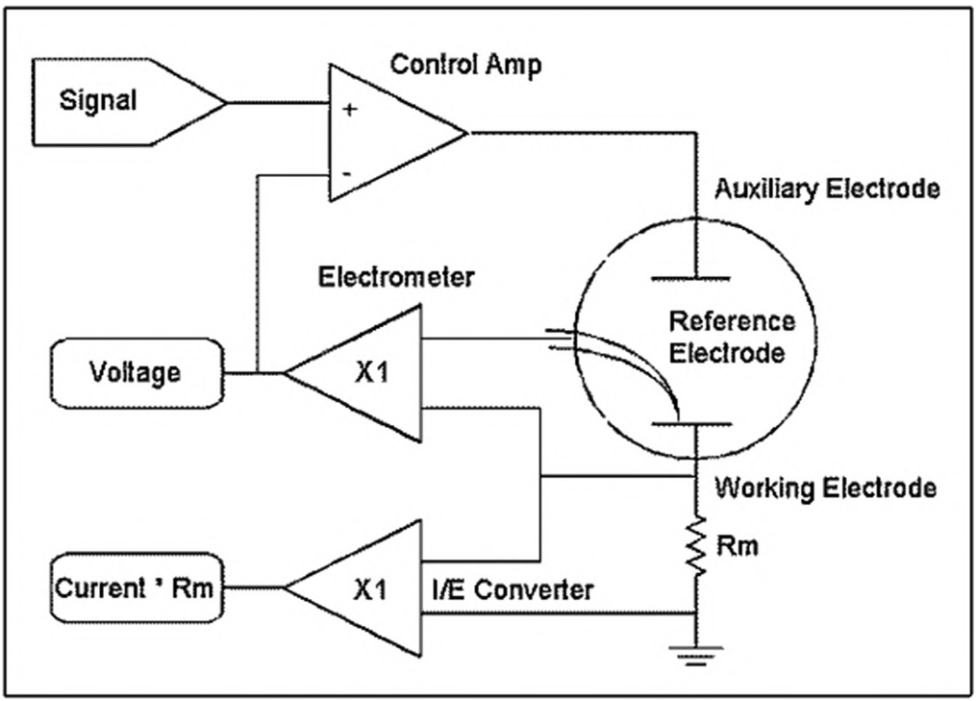

Fig. (5). Simple schematic of a potentiostat.

There are four blocks in the electrical circuit, which will be discussed in turn. Don't worry if you arenot acquainted with electronics; you can still learn from the following material.

In this design, the number 1 indicates a differential amplifier with unity gain. The difference between the voltages of its two inputs is the circuit's output voltage. The voltage and current signals transmitted to the system for A/D converters for digitization are represented by the blocks labeled Voltage and Current Rm [20].

The Electrometer

The electrometer circuit measures the voltage difference between the reference electrode and the working electrode. In addition to being the feedback signal for the potentiostat circuit, it also serves as a signal to monitor the cell voltage whenever required. An ideal electrometer has an infinite input impedance. Hence, there is no current input. The potential of the reference electrode may be changed by passing a current across it. In reality, all contemporary electrometers have input currents that are near enough to zero to allow this effect to be disregarded.

The bandwidth and the input capacitance of an electrometer are two essential features. Electrometer bandwidth can be measured as AC frequencies if powered by a low-impedance source. The electrometer's bandwidth must exceed that of the potentiostat's other electrical components. The resistance of the reference electrode and the capacitance of the electrometer input form an RC-filter. If the time constant of this filter is too high, it may restrict the electrometer's effective bandwidth and create system instability. More stable operation and high impedance electrode tolerance are possible with smaller input capacitance [19, 21].

The I/E Converter

Current-to-voltage (I/E) converters monitor the cell current in a simplified scheme. Through a current-measurement resistor, Rm, the cell current is directed. The voltage drop across Rm is used to measure the cell current. Some studies only show a small variation in cell current. During corrosion tests, the current can vary as much as seven orders of magnitude. Such a wide range of currents cannot be monitored by a single resistor. The I/E circuit can be automatically switched between a variety of Rm resistors. With this setup, a wide range of currents can be measured, each one being measured with its own resistor. A method called "I/E auto-ranging" is often used to choose the proper resistor values.

Sensitivity heavily influences the bandwidth of an I/E converter. For small current measurements, Rm should be high. I/E converters with excessive capacitance create an RC-filter with Rm, therefore limiting their bandwidth. There is no potentiostat capable of measuring 10 nA at 100 kHz because its bandwidth is too small. This can have a huge impact on EIS measurements [20].

The Control Amplifier

The control amplifier is a servo amplifier. Comparing the observed cell voltage with the target voltage, injects current into the cell to equalize the voltages. A note should be made that the measured voltage is sent to the negative input of the control amplifier. The control amplifier output is negative when the measured voltage is positive. A negative output is canceled by a positive output. Negative feedback is a kind of control system. As a rule of thumb, the cell voltage should equal the voltage of the signal source [22].

The Signal

A voltage source controlled by a computer is used in the signal circuit. Digits produced by computers are often output *via* Digital-to-Analog (D/A) converters. Proper number sequence selection enables the computer to produce voltage

ramps, sine waves and even constant voltages at the output of the signal circuit. Waveforms like sine waves or ramps may be generated with a D/A converter and are digital approximations of their analog counterparts with tiny voltage increments. Depending on the D/A converter's resolution and update rate, you may adjust the size of these steps.

Experiments with Two, Three, and Four Electrodes

In electrochemistry, the methods range from the most basic (chronoamperometry) to the most advanced (cyclic voltammetry, potentiodynamic, impedance spectroscopy, *etc.*). For each method, there may be a variety of potential experimental settings. A particular feature of these configurations will be discussed in this note: the number of electrodes used.

Potentiostat as a Four-Probe Instrument

A potentiostat is an instrument that has four probes (in certain cases). Each experiment must therefore include four relevant probes. Working Sense and Reference measure voltage (potential), while Working and Counter carry current. A four-probe device may be configurd to perform measurements with 2, 3, or 4 electrodes by changing the setup. It is important to understand when and how to use these different modes. The electrodes must be addressed in the context of n-electrode mode experiments. The term electrode refers to a (semi) conductive solid that interacts with a solution of an electrically conductive liquid. Working, Reference, and Counter are the most frequent designations (or Auxiliary).

Two-Electrode Experiments

The simplest cell setup consists of two electrodes, but the findings and analysis are usually more complex. The current-carrying electrodes in a two-electrode arrangement are also utilized for sense measurement. The current and sense leads are linked together in the physical configuration for two-electrode mode: Working (W) and Working Sense (WS) are linked to one electrode, while Reference (R) and Counter (C) are attached to a second (aux, counter, or quasi-/pseud--reference) electrode. A schematic of a two-electrode cell arrangement is shown in Fig. (**6**).

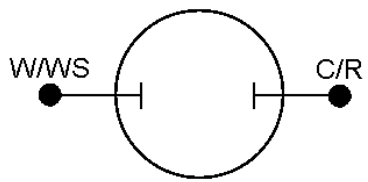

Fig. (6). Two-electrode cell setup.

In two-electrode experiments, the sense leads to detect the total voltage lowered by the current across three electrodes: the electrolyte, the counter electrode, and the working electrode. In a few typical situations, two-electrode configurations are utilized. There is one case when whole cell voltage measurement is important, such as in electrochemical energy devices (*e.g.*, batteries, fuel cells, supercapacitors). Another will be if the potential of the counter-electrode does not wander significantly during the experiment. This is most common in systems with extremely low currents or very short timeframes with a well-poised counter, such as a tiny working electrode and a much bigger silver counter electrode.

Three-Electrode Experiments

In three-electrode mode, the Reference lead is attached to a third electrode and disconnected from the Counter lead. These electrodes are often positioned to monitor a point very close to the working electrode (which is also attached to working and working sense leads, see Fig. (**7**).

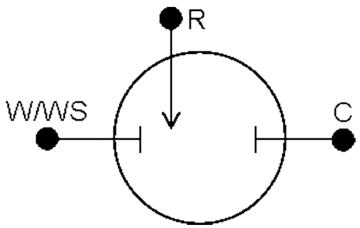

Fig. (7). Electrode cell setup.

Due to the fact that three-electrode setups only measure one-half of the cell, they have an experimental advantage over two-electrode setups. Consequently, the potential changes of the working electrode are monitored independently than those at the counter electrode. As a result of this isolation, specific responses can be evaluated with certainty and precision. As a result, 3-electrode configurations are most commonly used in electrochemical research.

Four-Electrode Experiments

In the four-electrode mode, the Working Sense lead, as well as (and in addition to) the Reference lead, are disconnected from the working electrode see Fig. (**8**). This configuration is rare in electrochemistry, although it has its uses. The potentials for any electrochemical processes happening at the working (and counter) electrode(s) are not monitored in the 4-electrode mode. The measurement determines the effect of an applied current on the solution or a barrier in the solution.

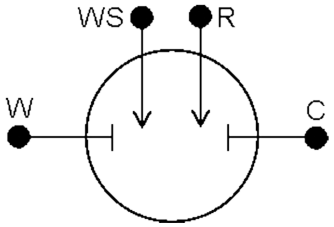

Fig. (8). Electrode cell setup.

An impedance measurement across a membrane or liquid-liquid junction is the most frequent use for this setup. For example, measuring solution resistance or material surface resistance with great accuracy may be accomplished with this setup (solid-state cells) [23].

Electrochemical Techniques

Amperometric

Amperometric transducers detect the currents generated when an electroactive species is electrochemically reduced or oxidized with the biorecognition element at a constant voltage (potential) supplied to the WE. The applied potential is what drives the electroactive species' electron transfer process. It causes the species to acquire missing electrons. The resulting current is a direct indicator of the pace of an electron transfer process that is also indicative of the procedure for recognizing and, therefore, proportional to the analyte concentration [15].

Amperometry is a subfield of electrochemistry concerned with the removal (oxidation) or addition (reduction) of electrons from an atom or a molecule. In principle, if sufficient energy is supplied, the assembly of atoms, molecules, or an atom may be reduced or oxidized. However, the range of applied energy is restricted by the experimental circumstances. Electroactive molecules are those that can be reduced or oxidized in simply accessible energy ranges. The quantity of energy needed for a redox reaction is determined by the system under study and is referred to as the redox potential. A voltage between the reference and working electrodes in reactions results in the passage of a faradaic current. This imposes a potential which promotes electron transfer at the WE, leading to a current concentration proportional to the electroactive analyte concentration. The electrode response generates a quantifiable current proportional to the concentration of the electroactive species and quantifies the pace of the electrochemical reaction [24].

$$c_O + ne^- = c_R$$

Where n denotes the number of electrons (e^-) exchanged in the middle of the reductant (R) and the oxidant (O).

The increasing rate of reduction causes the cell current to increase. The algebraic sum of the anodic and cathodic currents in a system yields the net current:

$$i = n'Fk^0\left[c_o \exp\left[-\frac{\alpha nF\left(\phi' - \phi^0\right)}{RT}\right] - c_R \exp\left[-\frac{(\alpha - 1)nF\left(\phi' - \phi^0\right)}{RT}\right]\right] \quad \textbf{(1)}$$

The kinetic constant is denoted by k^0, αis the symmetric coefficient, ϕ′is the voltage applied, R denotes the universal gas constant, andϕ0denotes the reference voltage. The current is restricted by the pace at which new electroactive species diffuse out of the bulk solution. The current is restricted by the fast depletion of the concentration c_o by the surface reaction. The diffusion-limited current is defined as follows by Fick's first law of diffusion:

$$i = n_c FD\frac{dc_o}{dn} \quad \textbf{(2)}$$

Where n_c indicates normal to the cathode surface, the current density is insensitive to the overpotential and produces the diffusion limit's highest detectable value. The following equation denotes the generalized transport equation for ionic species:

$$\frac{\partial c}{\partial t} + \nabla.\left(n'\mu E'c - D\nabla c\right) = 0 \quad \textbf{(3)}$$

Where is the electric field vector and mobility vector, we must solve the current continuity equation to get the electric field [24].

$$\nabla.j = 0 \quad \textbf{(4)}$$

The flux (j) may be represented using generalized Ohm's law in this case [24]. The system's sensitivity is determined by the electrode area and shape of the electrodes. For example, increasing the electrode's edge length while maintaining the same area may improve sensitivity by up to 12 percent. Additionally,

increasing the electrode area results in a rise in peak current. However, the production process and the system's overall size may impose size and form constraints on the electrode. While developing a sensor, the designer cannot disregard the Joule heating. Baronas and colleagues applied Fick's laws to construct a mathematical model of biosensors in two dimensions in space based on an array of immobilized enzyme microreactors [25].

Amperometric measurements are often made by keeping a constant potential at a C-based, Au, or Pt WE or electrode array with relation to a RE, which may as well function as an auxiliary electrode when currents are low (10^{-6}–10^{-9} A). The resultant current is straight proportional to the bulk concentration or generation or consumption rate of the electroactive species inside the neighboring biocatalytic layer. Due to the fact that the rates of biocatalytic reactions often depend on the first order of the bulk analyte concentration, such steady-state currents are typically proportional to the bulk concentration of the analyte. In amperometry, increasing the electrode's potential decreases the energy of the electrode's electrons. At some point, an electron may be transported from the electrode to a neighboring species's lowest vacant energy level. The redox couple's oxidized state is decreased. Alternatively, the reduced form may be oxidized by providing a sufficiently high positive potential. The flow of electrons into or out of an electrode may be quantified using a reduction or oxidation current. Amperometry is the term used to describe this method. The produced current I may be represented as follows:

$$i = \frac{-AFDnC}{\delta} \tag{5}$$

Where δ denotes the diffusion layer's thickness. It is feasible to distinguish between different redox couples by selecting an appropriately applied potential [24].

Potentiometric

The biological recognition response modulates the redox potential, the activity of an ion, or the transmembrane potential in potentiometric biosensors. Thus, potentiometric biosensors make use of the potential difference between two electrodes [26]. It consists of a a perm selective exterior layer and a membrane or sensitive surface that is selective for the target species (a bioactive substance), which is often an enzyme. A species is generated or consumed during an enzyme-catalyzed reaction, which is identified using an ion-selective electrode. Typically, a high-impedance voltmeter is used to EMF (determine electromotive force) or

the electrical potential difference between two electrodes when the current is near zero. The Nernst equation is the foundation for this kind of biosensor since it links the electrode potential (E) to the concentration of reduced and oxidized species. The Nernst equation for the reaction $aA + ne^- \Leftrightarrow bB$ is as follows:

$$E = E^0 + \frac{RT}{nF} \ln\left(\frac{[C_A]^a}{[C_B]^b}\right) \quad \textbf{(6)}$$

The concentrations of reduced and oxidized species are C_A and C_B. The number of electrons transferred in the reaction is n, the Faraday constant is F, the absolute temperature is T, the gas constant is R, and the standard redox potential is E^0, . Potentiometry's logarithmic concentration response enables the detection of very small concentration changes [15].

Additionally, the potential is produced in physical processes that do not require explicit redox reactions but have nonzero-free energy starting conditions. Ion concentration gradients over a semipermeable membrane are one example of this. This potentiometric effect is also the foundation for experiments using ISEs (ion-selective electrodes).

$$E_{mem} = (const.) - \frac{RT}{z_i F} \ln(a_i) \quad \textbf{(7)}$$

Thus, potentiometry is the process of determining the potential of a cell with zero current. Assume that an analyte interacts and with a biological component alters the number of electrons in a cell. Potentiometry may then measure the difference between the potential after the contact and the initial potential. Potentio metric measurements are made by measuring the potential difference between a reference electrode or an indicator or between two reference electrodes separated by a perm selective membrane [24].

Impedometric

Electrochemical impedance spectroscopy (EIS; which includes Faradaic impedance in the presence of a redox probe and non-Faradaic capacitance techniques) is a relatively fast technique for the function of biomaterial-functionalized and characterizing the structure electrodes. Immobilizing biomaterials on electrodes alters the electrodes' interfacial electron transfer

resistance and capacitance, resulting in alterations in impedance. Thus, this electrochemical method may be used to identify interfacial alterations caused by biorecognition processes. EIS is a commonly used method for investigating bio affinity interactions at the sensor surfaces of electrically conducting polymers. It may also be used to explore label-free analyte detection through impedimetric transduction. While electrochemical impedance spectroscopy is superior toamperometry or potentiometry in label-free detection, its detection limits are lower [15].

The resistance of a circuit element is its capacity to resist the passage of electrical current. Ohm's law defines resistance as the ratio of voltage E to current I [24].

$$R = \frac{E}{I} \tag{8}$$

While this is a well-known connection, it is only applicable to one circuit element in the form of an ideal resistance. Numerous simplifying characteristics characterize an ideal resistor:

1) Its resistance value is frequency-independent.

2) The alternating current and voltage signals are in phase with one another.

3) At all current and voltage levels, it obeys Ohm's law [24].

In the actual world, circuit components show much more complicated behavior, and the straightforward notion of resistance no longer applies. Instead, impedance is utilized, which is a more generic circuit characteristic. Impedance, like resistance, is a property of circuits that indicates their capacity to resist the passage of electrical current. Unlike resistance, impedance is not constrained by the preceding simplifying characteristics [24].

When a DC signal is delivered to an interface, Ohm's rule states that resistance equals E/I, where I and E denote the resultant current and the applied voltage, respectively. When an alternating current signal is supplied to an interface, Ohm's law applies once again. However, the amount being measured is termed the impedance Z. Z = Ep/Ip, where Ip and Ep denote the measured peak current and the applied peak voltage, respectively [24].

The AC impedance method is often used to investigate the kinetics of electrodes. The rate of the reaction is proportional to the charge transfer resistance. When determining the optimum frequency range, keep the following in mind:

1) At high frequencies, the electrical double-layer capacitors will reduce the faradaic impedance.

2) At very high frequencies, ohmic resistance takes precedence.

3) At very low frequencies, the impedance is dominated by mass transfer (diffusion resistance) [24].

Electrochemical Impedance Spectroscopy (EIS)

EIS is a versatile electrochemical method that is gaining popularity. Oliver Heaviside laid the groundwork for IS by applying Laplace to the transient response of electrical circuits. Heaviside popularized the terms impedance, capacitance, and inductance and applied them to the study of electrical circuits. Heaviside collected his writings, which began appearing in The Electrician in 1872, into a book in 1894. However, from the standpoint of application to physical systems, the history of IS starts in 1894 with Nernst's study [27].

Nernst used Wheatstone's electrical bridge to determine the dielectric constants of organic fluids and aqueous electrolytes. Others quickly adopted Nernst's method for determining galvanic cells dielectric characteristics and resistance. Finkelstein adapted the method to the investigation of oxides' dielectric response. Warburg derived equations for the impedance response related to diffusion laws, which Fick had discovered almost 50 years ago, and presented the electrolytic circuit analogy. Capacitance and resistance were frequency-dependent. Kruger used the diffusion impedance concept to study mercury electrodes' capacitive response [27].

Impedance was first used in biological systems in the 1920s, namely the capacitance and resistance of vegetal cells and the dielectric response of blood suspensions. Additionally, other biological membranes like skin tissues, muscle fibers, *etc.*, were impedance measured. The capacitance of the cell membranes was discovered to be frequency-dependent, and Fricke noticed a connection between the observed constant phase angle and impedance's frequency exponent. Cole and Cole demonstrated in 1941 that the frequency-dependent complex dielectric constant might be depicted as a depressed semicircle on a complex admittance plane plot. They proposed a formula compatible with Fricke's lawF6, which is now referred to as a constant phase element [27].

Using Lippmann's theoretical foundation's equation, Frumkin investigated surface tension, the capacitance measured using a Wheatstone bridge, and the connections between the double-layer structure on mercury electrodes in 1940. Grahame developed this approach of the mercury electrode, establishing foundational

knowledge of the electrical double-layer structure. Ershler and Dolin introduced the idea of an equivalent circuit to electrochemical kinetics, where the circuit components were frequency-independent. Randles devised an equivalent circuit for a mercury electrode that is ideally polarized, accurately describing adsorption kinetics processes [27].

Impedance was first used in a more complex reaction system in the early 1950s. Epelboin and Loric continued to investigate the function of reaction intermediates in the formation of low-frequency inductive loops in the following years. De Levie developed transmission line models for rough and porous electrode impedance responses. Newman demonstrated that disk electrodes' nonuniform potential and current distributions might result in time-constant dispersion at high frequencies [27].

Numerous writers have presented techniques for the deconvolution of impedance data in a generic manner. Stoyanov and colleagues devised a robust technique for visualizing the distribution of time constants for a particular spectrum without making an a priori assumption about the distribution function. Savova- Stoynov and Srtoyno developed a graphical technique for calculating instantaneous impedance projections from a sequence of successive impedance diagrams acquired during system evolution [27].

In 1989, in Bombannes, France, a symposium devoted to developing EIS methods was started. Following sessions were conducted in France (2007), Florida (2004), Italy (2001), Brazil (1998), Belgium (1995), and California (1992). The conference issues offer unique triennial views of the status of impedance research. One of the primary concerns expressed in these volumes is the variability of their relationship and electrode surfaces to the usage and abuse of constant phase elements. Local impedance spectroscopy, established by Lillard *et al.*, may prove to be a beneficial technique for deciphering this connection [27].

Generally, the impedance Z is calculated by applying a small amplitude voltage perturbation and observing the current response. The impedance Z is defined as its corresponding current–time function I(t) and the product of the voltage-time function V(t):

$$Z = \frac{V(t)}{I(t)} = \frac{V_0 \sin(2\pi ft)}{I_0 \sin(2\pi f + \phi)} = \frac{1}{Y} \qquad \textbf{(9)}$$

Y denotes the complex conductance or admittance, phase shift ϕ denotes the phase difference between current–time and the voltage-time curves, t denotes the time, f denotes the frequency, and I_0 and V_0 denote current signals and the maximum voltage, respectively. Since current may vary in exhibiting a phase shift ϕ and amplitude when compared to the voltage-time function, the impedance is a complicated number. Thus, the value may be represented by imaginary parts and real impedance, the phase shift , and the modulus |Z|. (Fig. **9**)illustrates this. Thus, the results of an impedance measurement may be seen in two ways: *via* a Bode plot that plots log|Z| and ϕ through a Nyquist plot, or a function of logf that plots Z_R and Z_I [28].

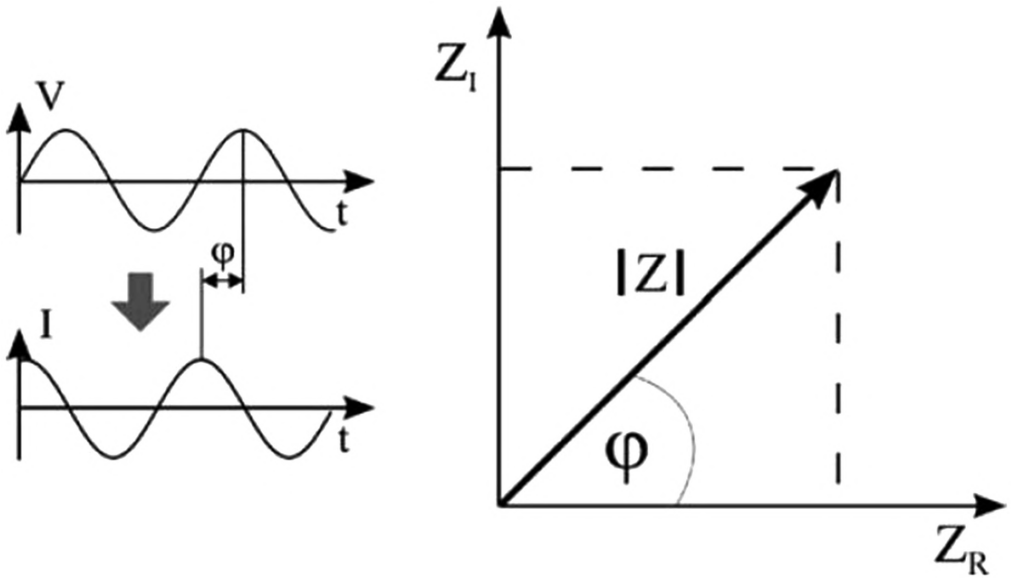

Fig. (9). Impedance is a difficult-to-define value equal to the quotient of current and voltage over time. The value of Z may be stated as the modulus of Z, also known as its phase angle ϕ, and the modulus of Z:|Z| is described as the imaginary (Z_I) and real (Z_R) portions of the impedance.

The term “spectroscopy” for impedance is derived from the fact that impedance is usually measured at several frequencies rather than just one. As a result, an impedance spectrum is produced that may be used to diffusion processes and exchange and characterize membranes, layers, or surfaces. To do this, an analogous circuit is often used to study the impedance spectrum. This circuit, which is often made up of resistances and capacitances, reflects the system's many physicochemical characteristics. Alternatively, the system may be represented using transfer functions derived from basic physical principles governing the processes involved, such as partition, diffusion, and electrokinetics [28].

Table 1. The primary impedance components used to describe the (bio)electrochemical behavior of systems, including their definitions, frequency dependency, and phase shifts.

Impedance Element	Definition	Phase Angle	Frequency Dependence
W(infinite)	$Z_W = \frac{\sigma}{\sqrt{\omega}}(1-j)$ $\sigma = \frac{RT}{n^2F^2\sqrt{2}}\left(\frac{1}{\sqrt{D_0c_0}}+\frac{1}{\sqrt{D_Rc_R}}\right)$	45°	Yes
CPE	$Z_{CPE} = \frac{1}{A(j\omega)^a}$	0-90°	Yes
C	$Z_C = \frac{1}{j\omega C}$	90°	Yes
R	Z=R	0°	No

[a] When the diffusion region is limited, a different behavior will be observed.

When EIS is used to characterize the impedance behavior in which the electrolyte solution is one of the components, four elements are typically used to describe the impedance behavior: Warburg impedance, constant phase element, capacitance, and ohmic resistance. Table **1** summarizes these components and their meanings. With these dispersed or ideal impedance components organized in parallel and series, equivalent circuits are utilized to approximate the experimental impedance data. Numerous electrochemical systems have been studied using this method. A model may describe almost every system's impedance behavior found in the literature. This may serve as a starting point for further investigation. For an electrode in contact with an electrolyte as shown in Fig. (**10**), the Randles circuit is utilized, which consists of the Warburg impedance W, the double-layer capacitance C_{DL}, the charge transfer resistance R_{CT}, and the solution resistance R_S [17, 28].

The impedance may be determined by measuring the current in response to the potential waveform. At least two electrodes are needed for impedance measurement; however, three electrodes are often employed. The RE is employed to keep the electrodes at a constant potential with relation to the fluid in which they are submerged. A second electrode, the WE, is the electrode that is immobilized with the sensing film. Calomel and silver/silver chloride electrodes are often used as RE. Finally, a CE is used to exchange current with the solution,

maintaining the reference electrode's constant potential. Numerous materials, including metals and carbon, have been used as counter- and working electrodes. A critical aspect is that the distance between the electrodes remains constant; otherwise, the solution impedance becomes another variable. One commonly used technique is to screenprint the electrodes onto appropriate ceramic or plastic substrates; this allows the fast fabrication of large numbers of identical electrode arrays at a cheap cost [29].

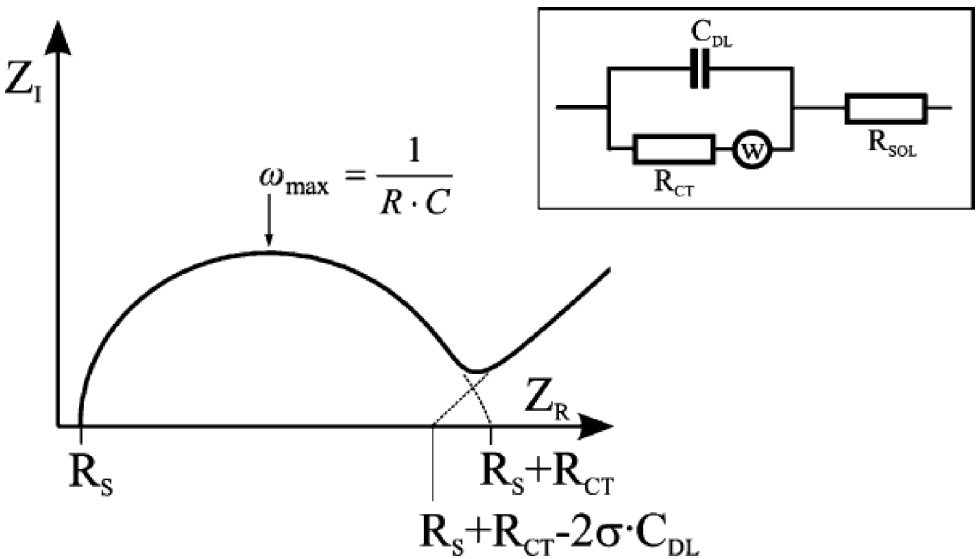

Fig. (10). For an electrode in contact with an electrolyte, Randles' equivalent circuit is shown. The charge accumulated in the double layer at the interface causes the capacitance. The charge transfer resistance R_{Ct} denotes the current flow generated at the contact by redox processes. The Warburg impedance is determined by the current impedance of the bulk solution diffusing to the interface. R_s and R_{Ct} can be readily calculated using the Nyquist plot. R_s denotes the solution resistance determined by the ion concentration and the shape of the cell. However, the Warburg impedance value may also be assessed.

Impedance techniques are advantageous because they can measure electron and mass transfer at high and low frequencies, respectively [30]. One advantage of AC over DC measurements is that. In contrast, in DC, only the potential can be varied; in AC measurements, the potential waveform frequency can also be varied: A typical range can be from less than 1 Hz up to megahertz, although single-frequency measurements can also be made. Since the voltages are commonly tiny, a few millivolts usually do not significantly affect the sensing film itself. Higher voltages, such as those often used in DC measurements (amperometry or cyclic voltammetry) could potentially interfere with the interactions between antibodies and antigens, perhaps repelling charged species from the electrode surface or otherwise disrupting its structure. The use of DC can also be combined with AC in that a bias can be applied to the electrode, with the

AC waveform being superimposed on this potential. Care must be taken that this does not disrupt the binding interactions or damage the film [29].

One method for increasing the sensitivity of the AC impedance approach is to use a redox couple in solution; common redox couples include ferrocene derivatives, Ferri-/ferrocyanide combinations, and ruthenium salts. When used in conjunction with an appropriate DC bias, this pair may conduct reversible redox reactions on the electrode surface, resulting in electron transfer between the electrode and the probe. This is referred to as faradic impedance spectroscopy; however, employing such a probe complicates the method in point-of-care applications [29].

Two distinct scenarios may arise in analytical applications:

1- Alternatively, the biological component is fixed on the WE and detected *via* its interaction with an analyte molecule. This is an illustration of a common biosensor application. The impedance of the sensing electrode (the WE modified with the biological component) determines the total impedance in this case. As a result, the counter electrode's impedance must be considerably lower. This is often accomplished by using an electrode with a surface area of at least 10 times that of the sensing electrode. Additionally, roughening the surface through electrochemical metal dissolution or metal deposition is possible. The solution resistance may be changed without limiting the current responsiveness by using a supporting electrolyte (0.01–1 M) [28].

2- The impedance of biological material is determined as a function of either the concentration of time or a particular analyte in solution. This is comparable to the study of solution resistance as a function of solution ion concentration (conductivity measurements). In all instances, the two electrodes' impedance must be negligible compared to the desired impedance. Utilized electrodes accomplish this with a relatively high surface area. Additionally, it is necessary to prevent nonspecific adsorption of biological material from the solution since this would raise the interfacial impedance. Determining cell mass during a fermentation process is an example of this kind of measurement. The growing density of cells inside the solution produces a readily detectable change in impedance [28].

As a result, we will concentrate on the second; we will evaluate the breadth of biological applications that use EIS and the range of information inferred from EIS in biological applications. Samanta and Sarkar's study examines the literature on biomacromolecule immobilization on discusses and substrates the various methods' relative advantages; such techniques will become more prevalent in the future.

Impedimetric detection is a technique that is mainly utilized with affinity

biosensors [30]. EIS has been extensively utilized to evaluate biosensor manufacturing processes. It is also critical in the analysis of a variety of bioanalytical species, including antibodies, antigens, nucleic acids, bacteria, proteins, and entire cells, which were examined using the EIS method to analyze clustered mineral ions. This substance is known to produce a significant rate of death in the pathological calcification of the cardiovascular system. Thus, electrical impedance alterations are seen as a result of mineral ion aggregation in supersaturated blood (Fig. **11**). Ectopic calcification is inhibited in 2 ways: (1) stability of calcium phosphate prenucleation clusters utilizing a fetuin-A monomer, and (2) Production of fetuin-A, nano spherical calcium phosphate mineral complexes, calciprotein particles, and different acidic serum proteins [17].

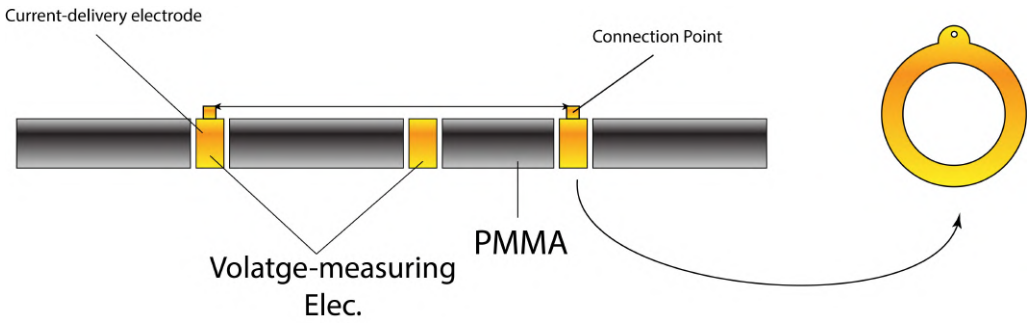

Fig. (11). A) The four electrodes in the EIS-determination cell, which holds one milliliter of fluid, are the sensing and stimulating electrodes. B) The EIS data analysis model uses the electrical equivalent circuit.

To begin, EIS has been utilized to create DNA biosensors. A study by Hlavata *et al.* demonstrates the application of EIS in detecting DNA damage. SPE (Screen-printed carbon electrodes) are used in their study, which is uncommon in the literature for EIS. On the surface of the SPE, a composite is applied to consist of SWCNT (single-walled carbon nanotubes), which have been shown to be helpful for DNA detection, chitosan (CHIT) derived from shrimp shells (which is often employed as a blood-clotting agent), and DNA with a known base sequence. A Nyquist plot comparing several electrode modifications in 1 mM $[Fe(CN)_6]^{3/4-}$], demonstrating that the addition of CHIT and SWCNT to the electrode surface increases charge transfer resistance, as shown by a bigger semi-circle in the figure Comparing (c) to (d), where the only difference is that(d) contains the binding agent while (c) includes no DNA binding agent (thioridazine), there is a slight

difference in the charge transfer resistance. This is attributed to the combination of the DNA and the binding agent on the electrode surface. As a result, it behaves like a molecular wire, promoting electron transmission, decreasing charge transfer resistance, and improving conductivity. Although the image does not include a matching circuit, we may infer from prior instances and examine the form of the Nyquist plot. To begin, given the form of the plot, with a tiny linear section and a semi-circle, we would anticipate a basic Randles circuit with a minor contribution from the Warburg impedance in the frequency range examined (which would be more prevalent if the frequency range extended to lower frequencies). Surprisingly, there is no evidence of a tiny semi-circle in the high-frequency region, as one would anticipate, given the electrode's composition. This may be because the film has a low impedance, which is a reasonable conclusion considering the high electron transfer rate of carbon nanotubes. It would be better to increase the high-frequency range to examine the film's impedance contribution [31].

The most recent development of biological substrates in combination with Electrochemical Impedance Spectroscopy involves label-free biosensors, which have been researched in conjunction with EIS for over a decade [31]. Impedance measurements have grown in popularity over the last several years as a label-free detection technique for a variety of various kinds of biosensors. In impedance measurements, the biosensor is constructed on an electrode so that the analyte controls the electron transfer rate selectively. While many chemical processes may alter the electrode surface, SAMs with suitable functional groups that selectively capture particular analytes are commonly used due to their ease of immobilization and full blocking capability. A typical biosensor is produced by immersing a gold electrode in a solution containing (a) a functional group capable of selectively collecting a target analyte on one side and (b) an organic molecule having a thiol group on the other side. The SAM that develops on the gold electrode through covalent bonding with the thiol group modifies the interface's electrochemical and electrical habitats; changes in electrochemical environments and the interface's electrical are measured as changes in charge-transfer rates or capacitances. These characteristics are further modified by the analyte being captured by the functional group that faces the solution. Voltammetric, amperometric, or impedimetric measures may be used to detect the changes. A functional group supplies selectivity to the molecule that forms the SAM. Electrochemical detection of the analyte is not required. Prior to analysis, an effectively fluorescing molecule or group and a conventional fluorometric detection technique must be tagged onto the analyte. The tagged analyte's fluorescence intensity is proportional to its concentration [32].

Mathebula *et al.* published further interesting work on antibody/antigen

recognition, in which they utilized EIS to identify tuberculosis (TB). In their paper, they provide a new technique for self-assembling an antigen layer on a gold electrode. To begin, a coating of a long-chain organic molecule (N-(2-mercaptoethanol)octadecanoid, MEODA) is formed on the electrode surface by immersing it in cysteamine first and then adding stearic acid. After that, the electrode is incubated for 48 hours in a dry dimethylformamide solution (DMF) containing mycolic acids (MA). Take note that mycolic acids are antigens that serve as recognition molecules for tuberculosis. After immersing the electrodes in SAP (saponin), a blocking agent was used to completely block all nonspecific adsorption/binding sites. As a consequence, the impedance measurements obtained after the TB binding process may be attributed to the bound TB. These electrodes were then utilized in EIS studies on human sera, both negative for tuberculosis (HIV^+TB^+) (HIV^- TB) and HIV and positive for tuberculosis (HIV^+TB^+) and HIV. Their research focuses on fighting tuberculosis in HIV/TB co-infected people since tuberculosis is the leading cause of death among HIV^-infected individuals. Their EIS results unequivocally demonstrate that when the concentration of TB rises, the charge transfer resistance increases compared to an electrode that has not been immersed in HIV^+TB^+. This study demonstrates how beneficial an appropriate EIS might be for medical technology [31].

EIS offers several benefits over the conventional amperometric detection method. The active site for the biologically induced redox reaction must be accessible to the analyte solution and located near the electrode surface. As previously stated, redox mediators have been utilized to proximity constraints and circumvent accessibility, but the mediator's mass transfer rate constraints their detection. Additionally, suppose the detecting potential is not properly selected. In that case, other active-redox species, such as ascorbate and urate, often present in the sample matrix, may contribute to the amperometric signal. Being able to monitor Ag–Ab directly binding impedi metrically helps circumvent the constraints described before. However, biosensors that use impedance detection must be carefully constructed to reduce analyte nonspecific binding. Additionally, EIS is insensitive to the majority of environmental disturbances [30].

Working with Biological Elements

What is Cell Culture?

An animal or plant's cells can be cultured in an artificial environment by removing cells from the affected organism. The cells may be extracted directly from the tissue and disaggregated by enzymatic or mechanical methods before culture, or they can be generated from a previously established cell line or cell strain.

Primary Culture

Cells are called primary cultures after they have been separated from their tissue and multiplied under the proper conditions until they have filled their substrate (*i.e.*, reached confluency). Cells must be passaged (*i.e.*, subcultured) by moving them into a new vessel with fresh growth media to allow them to continue developing.

Cell Line

After the first subculture, the main culture is referred to as a cell line or subclone. Resulting of the passage of primary cultures through populations of cells, the cells with the strongest growth capacity thrive, leading to a degree of genetic homogeneity and phenotypic uniformity.

Cell Strain

By cloning or using another technique, a subculture of a cell line can be transformed into a cell strain. Following the start of the parent line, a cell strain often accumulates further genetic modifications.

Finite vs. Continuous

In the absence of senescence, normal cells divide a few times before losing their ability to multiply; these cell lines are referred to as finite. The process of metamorphosis, on the other hand, can make some cell lines immortal, either naturally or through chemical or viral manipulation. A finite cell line becomes a continuous cell line when it undergoes transformation and has the capacity to divide forever [33].

Cell Culture Conditions

In artificial cells culture environments, different cell types require different types of vessels and substrates that provide nutrition (amino acids, carbohydrates, vitamins, minerals), growth factors, hormones, oxygen, and carbon dioxide, in addition to regulating the physicochemical environment (pH, osmotic pressure, temperature). Some cells can be grown in suspension (adherent or monolayer culture), but most must be anchored to a solid or semi-solid substrate (adherent or monolayer culture) [34].

Morphology of Cells in Culture

The morphology of cells in culture can be categorized into three basic groups:

● A fibroblast (or fibroblast-like) cell has elongated morphology, tends to be bipolar or multipolar, and grows attached to a substrate.

● Epithelial-like cells are shaped in polygonal shapes with regular dimensions and grow in isolated patches.

● Cells with lymphoblast-like characteristics have a spherical shape and grow in suspension without attachment to anything [35].

What is Subculture?

Passaging, also known as subculturing, is a method of propagating cells by removing the media from previous cultures and transferring the cells into a new growth medium. This allows the cell line or cell strain to grow further. After seeding, cells go through the lag phase before moving on to the log phase, when they multiply exponentially. When cells in adherent cultures are fully occupied and have no space to expand, or when cells in suspension cultures exceed the medium's ability to maintain further growth, proliferation is significantly decreased or stopped see Fig. (**12**) below. The culture must be split and new media is given to maintain them at an optimum density for ongoing development and stimulation of future proliferation [33].

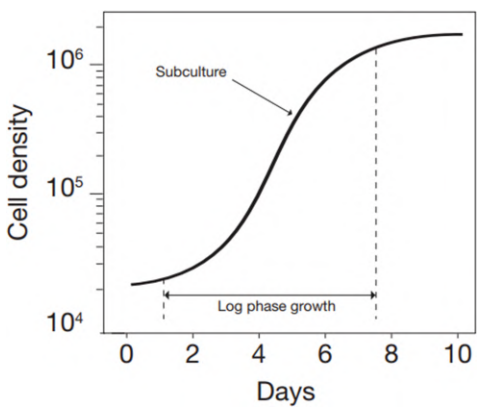

Fig. (12). Cultured cells have a unique growth pattern. Cell density is plotted as a function of culture time in the semi-logarithmic figure Cultured cells generally multiply according to a set pattern. The lag phase refers to the initial phase of development after the cells are seeded, and it is characterized by sluggish g Cells in an exponential growth phase are known as “logarithms,” and they rapidly absorb nutrients from the growth medium. When all of the growth media has been used up (*i.e.*, one or more nutrients have been depleted) or the cells have occupied all of the available substrates, the cells enter the stationary phase (*i.e.*, plateau phase), during which their proliferation is substantially slowed or completely stopped.

Applications of Cell Culture

As one of the most important methods in cellular and molecular biology, cell culture can be used to study both cell physiology and biochemistry (*e.g.*, metabolism, aging), pharmacological and toxic chemical effects on cells, and even mutagenesis and cancer development. In addition to being used in medication testing and research, it is also used in large-scale biological chemical production (*e.g.*, vaccines, therapeutic proteins). A key advantage of using cell culture for any of these applications is the uniformity and repeatability of findings [36].

Biologically Important Molecules

The three types of molecules of biological relevance are ions, tiny molecules, and macromolecules. An example of a organic small molecule (inhibitor, drug) would be an enzyme ligand or a substrate, such as adenosine triphosphate (ATP). Signaling is greatly influenced by ions such as Ca^{2+}. The building blocks of biological macromolecules are covalently bonded monomers [37].

Proteins

A protein is derived when an amino acid's amino group (or an amino group containing proline) reacts with another amino acid's -carboxyl group. At the same time, a peptide bond is formed and a water molecule is lost. In the peptide bond, rotation around the C-N bond is prevented by its partial double bond. As numerous amino acids gradually combine, an unbranched polypeptide chain is formed. Due to the fact that protein synthesis begins with the N-terminal amino acid and ends with the C-terminal amino acid, the N-terminal amino acid is considered the starting point and the C-terminal amino acid the finishing point. The difference between proteins and peptides is that peptides have less than 50 residues, while proteins have more than 50 residues. A typical protein contains hundreds of amino acids; for instance, ribonuclease is a very small protein with only 103 residues. The majority of physiologically active peptides have fewer than 20 amino acids [37].

Nucleic Acids

In macromolecular structure, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are chains of nucleotide polymers that repeat in a regular pattern. In essence, nucleosides are the fundamental building blocks of nucleic acids; they are composed of two elements: a pentose sugar (DNA is 2-deoxyribose, while RNA is ribose) and a nitrogenous base. As the sugar's carbon atoms are labelled as “prime” (1′, 2′, 3′, *etc.*) they are distinguished from the nitrogenous bases' carbon atoms, which are divided into purine and pyrimidine. The attachment of a

phosphate to the 5′ positions of a nucleoside by an ester bond forms a nucleotide or nucleoside phosphate. The phosphate of one nucleotide can be linked together with the 3′-hydroxyl of another nucleotide to form a second ester bond, which can be repeated endlessly to form polynucleotide molecules. The polynucleotides in DNA have polarity or directionality because they both have free 5′-hydroxyl groups at one end and a 3′-hydroxyl group at the other end. In DNA, the two strands of the molecule are polarized in opposite directions, resulting in an antiparallel structure [37].

CONCLUSION

In order to conduct successful and reliable research in the field of electrochemical biosensors, a variety of topics must be considered. In this chapter, we reviewed three essential practical topics for biosensor research. For an in-depth understanding of electrochemical biosensor investigations, it is necessary to be conversant with a number of different engineering disciplines, including experimental design, electrochemical lab tools, primary biology literature, and biological elements. The design of experiments is systematic approach researchers use to examine the relationship between a variety of input variables and output variables. Researchers use this approach to gather and analyze data. Working with electrochemical tools and biological elements is crucial to reducing costs and ensuring project success in the field of electrochemical biosensors. It was the goal of this chapter to give you a quick review of these subjects. This chapter was the beginning step to being a professional researcher in a lab.

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CHAPTER 5

Biosensor Application

Abstract: In Chapter 5, we want to focus on biosensors application in different fields and Focus on various newest research related to electrochemical biosensors in the fields of medical diagnosis, environmental monitoring, and food quality. In the medical diagnosis section,, the research done on HIV-1 is examined. Then hepatitis B, hepatitis A, Ebola, Zika, murine norovirus, influenza A, dengue serotype 2, adenovirus, enterovirus 71, Epstein-Barr virus, the apple steam pitting virus, papillomavirus, and phinovirus, are examined, respectively. In addition, in the monitoring environment section, research conducted on heavy water and pesticides is reviewed. In the food quality analysis section, research conducted on food toxicity and Antibiotic residues are reviewed.

Keywords: Biosensor, Food, Industry, Environmental, Medical, Heavy metal.

INTRODUCTION

Biosensors are integrated receptor-transducer devices in which the transducer (detector) dictates the device's sensitivity, while the receptor (sensing surface) regulates the biosensor's selectivity and specificity. To fully harness the potential of a biosensor, it is necessary to integrate it into an electrochemical biosensor to maintain the device's integrity, sample solution accessibility, and proper sample management. The requirements for electrochemical biosensors are very application-dependent. Sample preparation stages such as separation, enrichment, mixing, or dilution may need to be added to sample transit when testing actual samples. Nevertheless, small sample quantities and low-cost devices are sought, especially for point-of-care applications. Recently, electrochemical detection approaches were detailed in-depth and compared. This chapter will look at how electrochemical biosensor applications that use real-world sample media have changed recently [1].

Medical Diagnosis

Genetic Disorder

A mismatch in base pairing causes numerous genetic diseases. A single base-pair mismatch is a single nucleotide polymorphism (SNP), and it may be detected

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using particular DNA sequences. Pathogenic bacteria are also detected using a similar method based mostly on DNA hybridization. Millan and Mikkelsen pioneered electrochemical DNA biosensors, which enabled the development of accurate point-of-care, sensitive, portable, and small diagnostic devices by exploiting the particular affinity of peptide nucleic acid (PNA), or ss-DNA, for its complementary strand. Typically, the recognition interface is created by immobilizing PNA or ss-DNA onto the electrode surface through chemisorptive or electrostatic adsorptive immobilization. Considerations for such sensors include the solution environment, the probe's surface coverage, the linker length used to bind the nucleic acid to the surface, the immobilization technique used, and the kind of probe nucleic acid to be immobilized and its interaction with the surface [2].

DNA Fragmentation

The conventional methods for detecting DNA damage rely on time-consuming and costly chromatographic and electrophoretic tests. On the other hand, electrochemical gene sensors provide a fast and cost-effective method for determining DNA damage induced by radioactive agents, chemicals, or pharmacology. Several organizations have reported fast detection of irreversible toxicants or identifying the substances and DNA damage that cause it by monitoring the oxidation peaks of DNA bases adenine and guanine. In comparison, several other publications have suggested using guanine signals to monitor drug-DNA and radiation-DNA interactions. Ozsoz *et al.* devised a label-free electrochemical sensing method based on differential pulse voltammetry to detect conformational damage to fish sperm double-stranded DNA induced by radioactive iodine (I131) and technetium (99mTc) [2].

Detection of Pathogenic Microbes

Our capacity to quickly screen nucleic acids generated enormous interest in creating gene sensors for bacterial infections or particular viral, with some success previously achieved. Nonetheless, one inherent weakness of such detection is the microscopic amount of nucleic acid produced by microbes, necessitating prior nucleic acid amplification *via* PCR (polymerase chain reaction), which adds time and cost to the analysis but increases sensitivity by at least three orders of magnitude. Gau *et al.* demonstrated that integrating nanoscale chemical structures such as SAM (self-assembled monolayers) with a rapid, ultralow concentration and an electrochemical sensing system can be achieved in ionic assays, clinical chemistry, and protein assays without the need for PCR amplification. *Mycobacterium tuberculosis*, Giardia, *Escherichia coli*, and Cryptosporidium have all been detected using electrochemical DNA hybridization sensors.

Hepatitis B virus, *Streptococcus sobrinus*, and *Salmonella enteritidis* were detected electrochemically. Due to its interaction with guanine, methylene blue was employed as a redox indicator for the electrochemical detection of mismatched bases in hepatitis B viral DNA. *E. coli* was quantified in urine samples using cyclic voltammetry and a basal-plane pyrolytic graphite electrode, quantified in urine samples with a 5102 cells/mL detection limit [2].

Autoimmune-mediated Inflammatory Disease

Apart from hereditary reasons, autoimmune disorders may be caused by environmental causes, most notably when bacteria, viruses, or other infectious pathogens interact with the host. In this scenario, we may refer to the resulting illnesses as autoimmune infectious diseases. Hepatitis is the most serious autoimmune disease induced by viral contact [3].

Tang *et al.* described developing a novel electrochemical biosensor to detect hepatitis C virus (HCV) using BamHI (a type II restriction endonuclease extracted from Bacillus amyloliquefaciens) enzymatic signal enhancement using HRP encapsulated nanogold hollow spheres and thionine [4]. Liu *et al.* presented an electrochemical biosensor for detecting the hepatitis B virus (HBV). The biosensors were created by a mismatched DNA capture probe and modifying a 16-electrode array with a complimentary. Thiolated oligo(ethylene glycol) was employed as a fouling agent to minimize nonspecific adsorption on the electrode surface. A biotin-modified DNA detection probe was employed following the hybridization process, followed by denaturation and ligase methods. Ligation between detection probes and the tandem capture is possible only when the target DNA sequence is complementary to the capture probe. Finally, the test was completed by incubating with the avidinHRP (avidin horseradish peroxidase) enzyme and performing an amperometric measurement [5]. Shakoori *et al.* suggested that gold nanorods and nanostructured electrochemical biosensors be developed for HBV detection [6]. Zheng *et al.* described another HBV biosensor (Fig. **1**) [3].

Chronic Autoimmune Disease

SLE (systemic lupus erythematosus), rheumatoid arthritis, celiac disease, multiple sclerosis, and type 1 diabetes are all autoimmune illnesses when the body's immune system targets its components and tissues. These illnesses are defined by the generation of high-affinity autoantibodies associated with and identified with the disease's existence. However, no reference techniques for detecting autoantibodies have been developed due to a lack of standardization and low projected negative results. Electrochemical biosensors, on the other hand, are a viable alternative because they provide critical information on

antigen–autoantibody affinity reactions [3].

An article on specific biosensors for various diseases and disorders is in Table **1**.

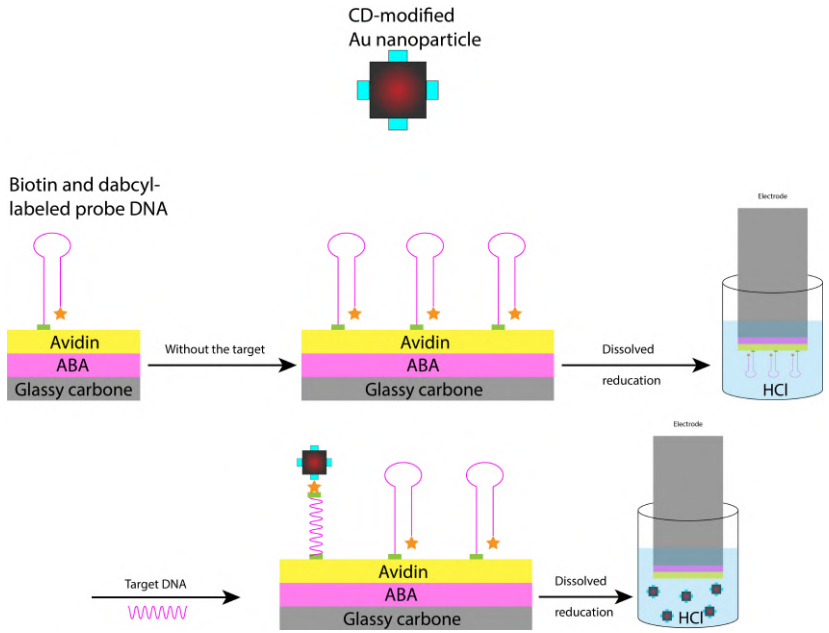

Fig. (1). Hepatitis B virus detection using an electrochemical biosensor based on a molecular beacon.

Table 1. An electrochemical biosensor for detecting indicators for infectious and autoimmune illness.

Biomarker	Electrochemical Procedure	Range Linear	Limit of Detection
Hepatitis B virus	Amperometry	Single-nucleotide	polymorphism
Hepatitis C virus	DPV	0.01–8 µM	1.0 pM
Anti-citrullinated auto-Ab	Amperometry	0–0.005 serum Dilution	----------
Transglutaminase auto-Ab	CV	0–40 U mL-1	----------
Anti-DNA auto-Ab	Amperometry	----------	0.04 µg IgG
Anti-chromatin auto-Ab	Pulse amperometry	Correlation with ELISA	----------

Application for Diagnostics

Certain electrochemically detectable peptides and proteins found in the extra- and intracellular matrix as a result of particular illnesses or disease states serve as biomarkers for these disease states or diseases. Electrochemical sensors showed enormous promise for the biochemical analysis of blood and other analytes.

Glucose sensors are the most successful use of such sensors. Such sensing systems have been reported for cytochrome c, hemoglobin, neurotensin, bombesin, myoglobin, insulin, and a-synuclein, among others. Potentiometric stripping *via* adsorbed anti-HSA IgG onto a thick-film carbon working electrode was used to test human serum albumin (HSA). Our group has described using adsorptive transfer differential pulse anodic stripping voltammetry to detect human telomerase reverse transcriptase (hTERT) for cancer diagnosis utilizing cell lysates generated from adult urine samples. Additionally, we developed a label-free electrochemical sensor (Fig. **2**), a cancer marker, for the detection of total prostate-specific antigen (T-PSA) using SWCNT-modified microelectrode arrays. The oxidation signals of tryptophan (Trp) and tyrosine (Tyr) residues were enhanced during their contact with T-PSA from a covalently bound T-PSA-mAb. The sensor's specificity was validated using bovine serum albumin (BSA) [2].

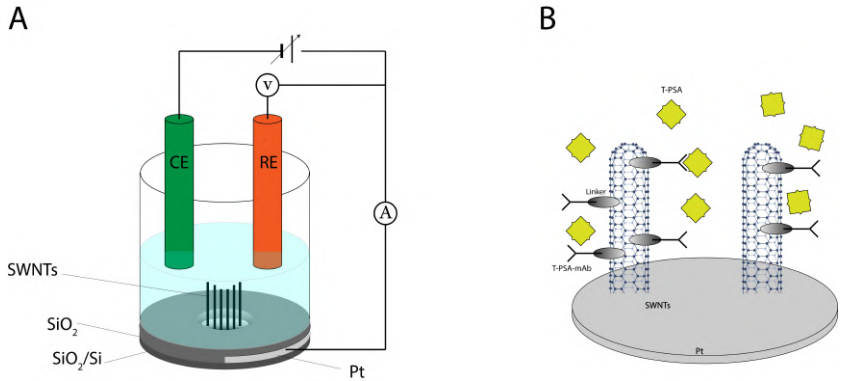

Fig. (2). A) SWNT-modified platinum microelectrode connected to a platinum wire. The label-free electrochemical immunosensor concept is illustrated in B) After coating SWNTs with T-PSA-mAb monoclonal antibodies coupled to the pyrenebutanoic acid succinimidyl ester, the fibers were capped with 1-pyrenebutanoic acid succinimidyl ester (Linker) when the antigen-antibody combination is formed, the peak current for intrinsic protein oxidation increases.

Pathogen Diagnostics

Numerous techniques may be used to enhance these advantageous features, including redox labeling, immobilization approaches, recognition probes, and material modification. For example, Kang *et al.* found that the redox label on ferrocene results in a high affinity for the target, while methylene blue improves long-term stability. Additionally, nanotechnology has impacted all electrochemical biosensors significantly. Nanoparticles may be employed as the surface area of the solution interface or to improve the electron transfer efficiency or electrochemical labels [7]. For example, Steinmetz *et al.*, Lee *et al.*, and Khater *et al.* utilized gold nanoparticles to enhance the responsiveness of their sensors while detecting viral infections. The use of nanotechnology is consistent with mobility and the current trend toward downsizing in biosensors [8, 9]. Similarly,

advances in isothermal amplification techniques have resulted in the creation of ultrasensitive electrochemical sensors capable of detecting a wide variety of pathogens [10, 11].

Enhancing characteristics such as portability while maintaining maximum sensitivity is critical during the COVID-19 pandemic and may be accomplished *via* the use of these new methods [12]. A variety of new testing platforms are discussed in this section and summarized in Table **2** [13].

SARS-CoV-2 may be identified in a variety of methods, including by analyzing serological antibody testing, viral antigens, or the virus's genetic material. The lack of information about SARS-CoV-2 and its development was a major factor in the pandemic's severity. Throughout the pandemic, scientific knowledge of the virus has increased rapidly, owing in part to comparisons between morphologically and the new SARS-CoV-2 and genetically related viruses such as influenza, MERS-CoV, or SARS-CoV. For instance, one of SARS CoV-2's entrance pathways was identified *via* previous research on SARS-CoV. This technique may also be used to detect systems. By examining existing platforms capable of identifying well-studied viruses, it is possible to develop more effective techniques for detecting SARS-CoV-2 (Fig. **3**) [13].

Table 2. Among the electrochemical biosensor classification schemes studied in this book are: SWV denotes square wave voltammetry and CV cyclic voltammetry. Electrical impedance spectroscopy follows.

-	Author	Objective	Send the signal	Credibility Embodiment	Limit of Detection (LOD)	Linear Range
Detection of Viral Antigens	(Hassen *et al.* 2011)	Type A IV	Activation of the EIS signal	Polyclonal antibodies	8 ng/mL	0 – 64 ng/mL
	(Seo *et al.*, 2020)	SARS-CoV-2	FET	SARS-CoV-2 spike antibody	1 fg/mL and 1.6×10^{1} PFU/mL	1.6×10^{1}-1.6×10^{4} PFU/mL
	(Layqah and Eissa 2019)	MERS-CoV And H-CoV	SWV signal-on	Antibodies specific for MERS-CoV and human coronavirus	0.4 pg/mL and 1.0 pg/mL	10^{-3} – 10^{2} ng/mL and 10^{-2} – 10^{4} ng/mL
	(Singh *et al.* 2017)	H1N1 IV	CA signal-on	Target specific Capture antibodies	0.5 PFU/mL	1 - 1×10^{4} PFU/mL
	(Han *et al.* 2016)	H1N1, H5N1, H7N9 IV	CA signalon	Target specific Capture antibodies	1 pg/mL	1 – 10 ng/mL

(Table 2) cont.....

The analysis of nucleic acid content	(Jampasa *et al.* 2014)	HPV	Deactivation of the SWV signal	Anthraquinonelabeled Pyrrolidinyl peptide nucleic acid probe	4 nM	0.02 -12 µM
	(Ju *et al.* 2003)	HBV	CV Signal-on	HBV Complementary ss-DNA fragments	the 10^{-21} moles of the original material are included in the original item.	*Not Reported*
	(Chung *et al.* 2011)	Type A IV	CV Signal-off	Biotinylated Complementary DNA probe	(85.1 fM) 8.51 $\times 10^{-14}$ M	1 $\times 10^{-13}$ - 1$\times 10^{-10}$M
	(Malecka *et al.* 2016)	Avian IV H5N1	SWV Signal-off	Thiolated ssDNA Probe	1 pM	*Not Reported*
	(Díaz González *et al.* 2008)	SARSCoV (30-bp of genome)	CA Signal-on	Complementary DNA probe (electrostatic immobilization)	0.5 nM	20 – 200 pM
	(Martínez Paredes *et al.* 2009)	SARS-CoV (30-bp of the genome)	CV Signal-on	the target-matching DNA probe was conjugated with alkaline phosphatase	2.5 pM	2.5 – 50 pmol/L
	(Abad-Valle *et al.* 2005)	SARS-CoV (30-bp of the genome)	SWV signal-on	a DNA probe complementary to a thiol-modified target	6 pM	0.102– 5.10 NM

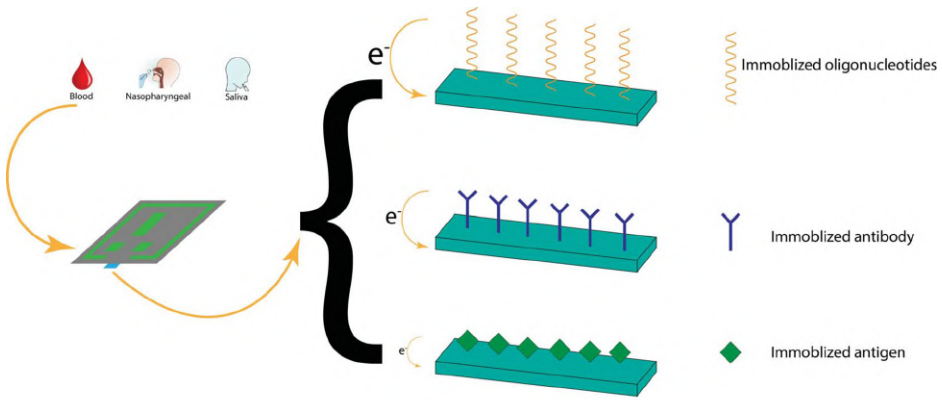

Fig. (3). Electrochemical biosensors can detect analytes in saliva, nasopharyngeal, and finger-prick blood. The platforms include quantifiable antigens, antibodies, and antigen recognition elements to detect target oligonucleotides, a set of electrodes with complementary oligonucleotides, antibodies, and electronic leads to transfer responsibility for the electrodes to the readout device; and a biosensing chip with a sample delivery system.

Tumor Markers

A critical aspect of immunosensors used in monitoring, screening, and clinical diagnosis is that the biomarker employed should be produced in bodily fluids capable of detecting limitations. According to the FDA's (Food and drug administration) list of approved cancer biomarkers, some biomarkers for prostate cancers, ovarian, and breast may be used for drug development, staging (human chorionic gonadotropin in testicular cancer), prognosis (cytokeratins in breast tumors), monitoring and screening (PSA complex, PSA total, and PSA), or monitoring (such as CA125, CA2729, and CA153) [3].

With cancer being one of the major causes of death, early clinical identification is critical for effective disease treatment. Combining two or more biomarkers may improve the accuracy of detecting cancer in its early stages compared to utilizing a single biomarker. Numerous biosensors for detecting these biomarkers have been described in the literature (Table **3**) [3].

Table 3. Biosensor for the detection of biomarkers associated with cancer.

Biomarker	Electrochemical Technique	Linear Range	Detection Limit
CEA	DPV	0.02–20 ng mL^{-1}	7.0 pg mL^{-1}
PSA	EIS	0–25 ng mL^{-1}	0.5 ng mL^{-1}
	CV	0–80 ng mL^{-1}	1 pg mL^{-1}
HER2	EIS	0–40 μg mL^{-1}	6 μg mL^{-1}
	EIS	10^{-5} to 10^{2} ng mL^{-1}	5 ng mL^{-1}
	Amperometry	1–200 μg mL^{-1}	1 μg mL^{-1}
HE4	SWV	0–400 pM	6.8 fM
CA125	Amperometry	0.002–20 U mL^{-1}	0.001 U mL^{-1}
	DPV	0.05–20 U mL^{-1}	0.002 U mL^{-1}
	DPV	0–25 U mL^{-1}	2 U mL^{-1}
	Amperometry	5–1000 U mL^{-1}	40 U mL^{-1}
	EIS	0–0.1 U mL^{-1}	0.0016 U mL^{-1}
	EIS	0–150 U mL^{-1}	1.03 U mL^{-1}
	EIS	0–100 U mL^{-1}	0.1 U mL^{-1}
	EIS	0–100 U mL^{-1}	6.7 U mL^{-1}
	SWV	0.001–25 ng mL^{-1}	0.25 pg mL^{-1}

Abbreviations: CEA: Carcinoembryonic Antigen, PSA: Prostate-specific antigen, HER2: Human epidermal growth factor receptor-2, HE4: Human Epididymis Protein 4, CA125: Carbohydrate Antigen 125, DPV: Differential pulse voltammetry, EIS: Electrochemical impedance spectroscopy, CV: Cyclic Voltammetry, SWV: Square Wave Voltammetry

Cardiac Marker

Existing techniques for diagnosing CVD (Cardiovascular diseases) depend largely on traditional procedures based on tests performed in central labs that may take several hours or even days from ordering to receiving findings. Laboratory testing is critical in detecting myocardial infarction, and rapid and cost-effective diagnostic methods are required. Three of the most informative indicators now available are natriuretic peptides, myoglobin, and cardiac troponin T or I (cTnT/I), especially those of the B type (BNP). Cardiovascular biomarkers are classified according to the kind of disease. Different immunoassay techniques, including ELISA, radioimmunoassay, and immunochromatographic assays, are used to identify these markers [3].

As shown in Table **4**, several biosensors have been created to detect cardiac biomarkers.

Table 4. Electrochemical biosensors for the detection of cardiac biomarkers are being developed.

Biomarker	Electrochemical process	Linear Range	Detection Limit
miRNA 499	CV	10^{-6} to 1 μM	0.3 pM
MPOmass	Amperometry	0.004 ng mL^{-1}	0–16 ng mL^{-1}
MPOactive	Amperometry	0.200 ng mL^{-1}	0–16 ng mL^{-1}
Protein	Chronoamperometry	2.2–200 ng mL^{-1}	2.6 ng mL^{-1}
C-reactive	EIS	0.5–5 nM	176 pM
Troponin T	OCP	10^{6} magnitude	<5 pg mL−1
	Amperometry	0.1–10 ng mL^{-1}	0.2 ng mL^{-1}
Cardiac	CV	0.009–0.8 ng mL^{-1}	9 pg mL^{-1}
Myoglobin	EIS	0.582–4.24 μg mL^{-1}	2.25 μg mL^{-1}
	SWV	1.1–2.98 μg mL^{-1}	—

Analyses of Drug Action

Serrano *et al.* reported on the electrochemical biosensor's potential for drug action investigation. The mechanism of the interactions should be better understood to better comprehend the electrochemical reactions before and after the DNA-drug interaction. This knowledge may aid in the quantification of medicines and the screening of new treatments that are more effective at targeting and interacting with other biomolecules or DNA. Bagni *et al.* have written an excellent overview of this feature of electrochemical sensors.

SWV (Square Wave Voltammetry) Diagnostic

Rapid and sensitive detection of disease-related biomarkers is critical for diagnostic applications and assessing pharmacological responses to therapy. Numerous methods have been used in recent years for sensing/biosensing for a various illness biomarkers. The primary methods include immunoassays, magnetic beads functionalized with antibodies, and electrodes coated with DNA. Numerous research has been conducted utilizing SWV to investigate electrochemical sensing/biosensing of different analytes for diagnostic applications [14].

Other Works

Babamiri and colleagues created an imprinted-based electrochemiluminescence biosensor for HIV-1 (Human Immunodeficiency Virus) gene recognition as part of their work in medical diagnostics [15]. They began with an HIV aptamer template and ended with OPD (o-phenylenediamine) as a functional monomer (Fig. **4a**). Following the tests, they found a substantial increase in responsiveness following the hybridization process. They detected HIV genes with a sensitivity of 0.3 fM in a concentration range of 3.0 fM to 0.3 nM [16].

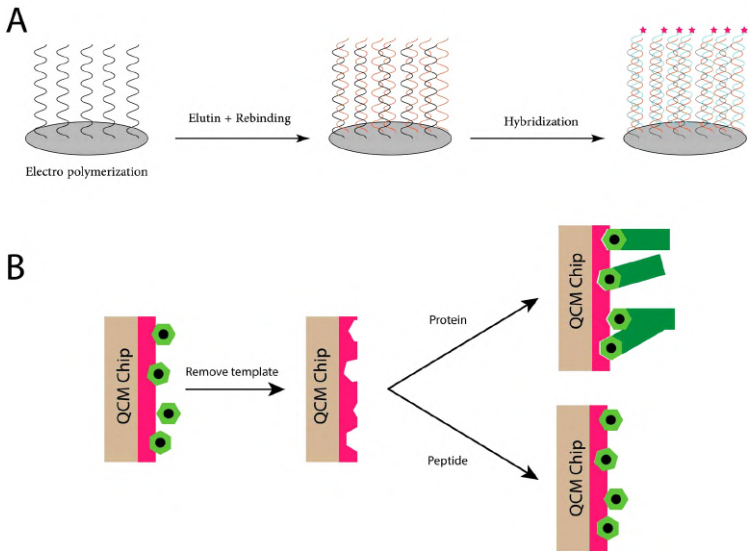

Fig. (4). Electro-chemiluminescence (**A**) and quartz crystal microbalance schematic representations; (**B**) HIV-1 detection biosensors.

Lu and colleague created a biosensor to detect human immunodeficiency virus-1-associated glycoprotein 41 [17]. They used an epitope imprinting technique to modify the surface of a quartz crystal microbalance biosensor with a synthetic

peptide similar to the 579–613 residues of Gp41 (Fig. **4b**) [16]. The findings indicate that an imprinted film may preferentially bind Gp41 protein and a high affinity for the target peptide. Additionally, they determined that the LOD (Limit of detection) was 2 ng/mL [16, 17].

Shafiee and colleagues showed the detection of HIV-1 in biological samples using an optical sensor device [18]. This research discovered that when the surface adsorbed the complete virus, this resulted in a change in the resonant peak wavelength amount. This amount may be observed with a precision of 10 pm wavelength. This biosensor is capable of detecting both biomolecular layers and even low viral quantities. Additionally, they investigated HIV-1 recognition in PBS and serum samples with 10^4 to 10^8 copies/mL viral loads [16].

Hassen and colleagues described a technique based on DNA hybridization for non-faradic electrochemical impedance spectroscopy recognition of the hepatitis B virus [19]. They began by modifying DNA probes with biotin and encapsulating them in streptavidin-based magnetic nanoparticles. They then used a magnet to attach nanoparticles onto the bare gold electrode after successful immobilization of hybridization and DNA probes with various amounts of complementary DNA.

Additionally, Tam *et al.* examined the potential of an SPR (Surface plasmon resonance) biosensor to recognize hepatitis B surface antigen-antibody capability [20]. They achieved a linear performance of 0.00098–0.25 mg/L, a sevenfold increase in the limit of detection, and a twofold rise in the coefficient of variation of repeated findings when compared to the enzyme-linked immunosorbent assay.

Further, Uzun *et al.* identified a hepatitis B surface immunoglobulin in human serum using an SPR biosensor for detecting hepatitis [21]. They conducted kinetic tests on human blood samples positive for hepatitis B surface antibodies [21].

In contrast, Li and colleagues created an impedimetric biosensor enhanced with Au NPs for hepatitis B virus-specific DNA hybridization [22]. They demonstrated that the biosensor exhibited a good correlation coefficient across a wide surface-suited, reproducible responses, and concentration range for increased DNA binding [21].

Zengin and colleagues described a biosensor for detecting the DNA sequence of the hepatitis B virus that relied on SERS (surface-enhanced Raman scattering) and a sandwich test. To begin, they created a temperature-responsive hybrid silicon substrate on which DNA strands could be immobilized on the Au nanoparticle surface. The sandwich technique was then used to identify target DNA using increased Raman scattering signals from the surface [23].

Istek and colleagues developed an electrochemical biosensor based on paper to recognize DNA from the hepatitis B virus [24]. They claimed that this biosensor had 4 critical characteristics. To begin, paper folding and combined design to determine the incubation time. In the second section, 2 amplification steps were performed: a silver nanoparticle tagged with the highest amplification factor was used, and magnetic microbeads were used to collect the probes. Third, the absence of an enzyme or immunoglobulin enhanced the biosensor's stability, speed, and robustness. Finally, just one sample incubation step was required before initiating detection. They determined the detection limit to be 85 pM [24].

Liu and colleagues developed a thermosensitive polymer-based biosensor for detecting the hepatitis A virus. They controlled the biosensor's detection performance using temperature control for viral collection. Additionally, they estimated a very low detection limit (1.1 pM). Additionally, they effectively utilized this biosensor to identify different hepatitis A viruses in a dilution of human serum, with great recoveries ranging from 90.8 to 108.3 percent at three viral concentrations. Finally, they addressed the issues of the detection process's length and high non-specific adsorption [25].

Ilkhani *et al.*, for example, developed an electrochemical biosensor for Ebola virus DNA diagnostics using enzyme-amplification recognition [26]. They tagged the biotinylated hybrid using a streptavidin–alkaline phosphatase combination, as shown in Fig. (**5**) [27].

Additionally, Yanik *et al.* showed the direct detection of real viruses in a biological medium using optofluidic biosensor technology [27]. The detection may be performed at clinically relevant concentrations without the requirement for sample preparation. The biosensor was constructed using group-specific antibodies based on the light transmission effect in plasmonic nanoholes. They Covered the recognition of small RNA viruses (pseudo-typed Ebola and vesicular stomatitis virus) within a dynamic range spanning three orders of magnitude [27].

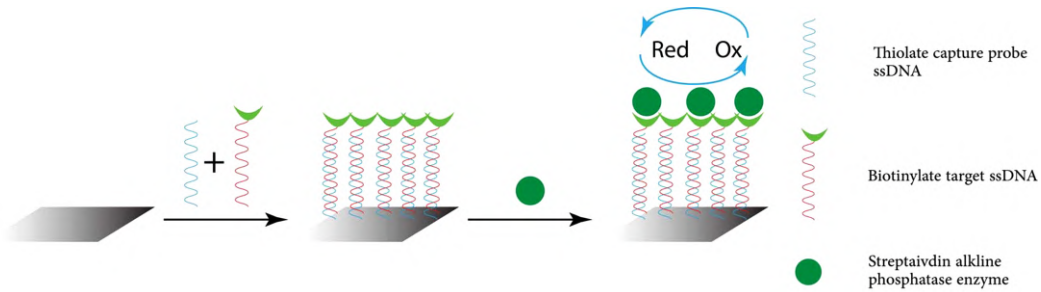

Fig. (5). The diagrammatic representation of the various steps included in the fabrication of an electrochemical biosensor.

Cai and colleagues also stated a study about quantification and amplification-free recognition of the Ebola virus on clinical samples [28]. For sample processing and viral pre-concentration, a microfluidic chip was utilized.

Afsahi and colleagues created a moveable and Affordable Gr-enabled biosensor for the recognition of the Zika virus using an immobilized monoclonal immunoglobulin [29]. They covalently attached monoclonal immunoglobulin to Gr for the detection of natural Zika virus antigens. They determined the concentrations of common antigens (450 pM) [29].

Additionally, Kaushik and colleagues demonstrated an electrochemical biosensor for the recognition of Zika virus proteins. They used EIS to determine the biosensor's electrical response as a subordinate of protein concentrations [30]. They demonstrated that their biosensor identified Zika virus protein preferentially in a LOD value of less than 10 pM and a recognition range of 10 pM to 1 nM.

Song *et al.* have described a disposable cassette-based RTLAMP (reverse transcription loop-mediated isothermal amplification) test for identifying the Zika virus [31]. Thermal control of the cassette was accomplished using a chemically heated cup. As a result, no electrical power was required.

Ashiba *et al.*, for example, explored an SPR biosensor for the recognition of norovirus-like particles [32]. They created this biosensor using a chip with a V-shaped trench Fig. (**6**). They chose a wavelength of 390 nm to induce SPR on the biosensor's Al layer [32].

Additionally, Weerathunge and colleagues have presented a colorimetric biosensor platform for the ultrasensitive and fast recognition of the pathogenic MNV (Murine norovirus) [33]. They created a biosensor by combining the enzyme-like catalytic activity of Au NPs with the great target selectivity of an MNV aptamer. They discovered that when norovirus was present, this biosensor generated a blue hue [32].

Also, Lee *et al.* produced CNT coated with binary NPs and utilized them as a biosensing platform [34]. They began by aligning gold/magnetic nanoparticles-carbon nanotubes on a platinum-interdigitated electrode. They then bonded a probe DNA with a thiol group to the Au NPs surface to create this hybrid structure. Target DNA concentrations ranging from 1 pM to 10 nM. in addition, they verified specificity by comparing it to other mismatched DNA sequences.

Influenza is a viral infectious illness that is associated with a variety of medical complications and a significant cost impact.

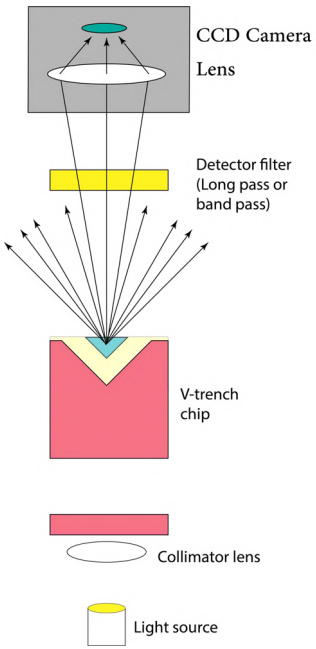

Fig. (6). A schematic representation of a V-trench biosensor.

For instance, Sayhi *et al.* devised a technique for isolating and detecting influenza A virus subtype H9N2 [35]. They initially coated iron magnetic nanoparticles with an anti-matrix protein and two antibodies and utilized them to separate the influenza virus from the allantoic fluid. Following that, Fetuin-A was conjugated to an electrochemical recognition label, Au NPs, in order to quantify the viral tacking benefit conferred by the Fetuin-HA association (Fig. **7a**) [36].

Pang and colleagues recognized a fluorescent biosensor to recognize the H5N1 influenza virus's recombinant HA protein in human serum [37]. They constructed a fluorescent biosensor in many stages, as shown in Fig. (**7b**) [36]. To begin, they immobilized guanine-rich anti-recombinant HA aptamers on the silicon–Ag_2O NPs using SELEX. Next, they added a fluorescent tag called thiazole orange.

Additionally, Tam *et al.* reported a research on the immobilization of DNA using MWCNT to detect influenza viruses [38]. They used Fourier transform Raman spectroscopy and infrared to analyze the interaction and connected the DNA probe to the biosensor. They identified hybridization in the middle of the target DNA and DNA probe by observing changes in the conductance on the surface of biosensors, which determined the detection limit to be 0.5 nM. They ensured a change in the system's output signal.

Furthermore, Vollmer and colleagues developed an optical technique for detecting the Influenza A virus [37]. They found that discrete variations in the resonance frequency of a whispering gallery mode generated in a microspherical cavity resulted in the binding of single virions. Additionally, they discovered that by shrinking the microsphere size, the amplitude of the discrete wavelength-shift signal might be adequately increased.

Using an aptamer, Bai and colleagues developed a portable SPR biosensor to recognize the avian influenza virus H5N1 in chicken swab samples [36]. They constructed the biosensor utilizing immobilized and biotinylated aptamers on the Au surface of the biosensor coated with streptavidin.

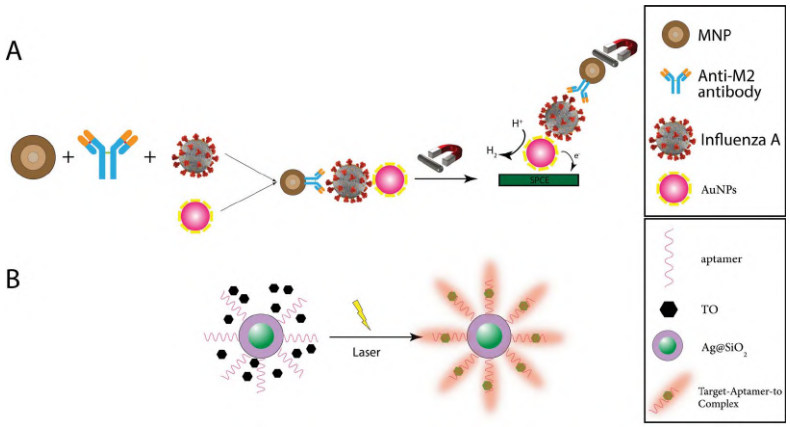

Fig. (7). The development approach for the Au NP-based chronoamperometric biosensor for influenza virus (a), the production of the determination of the H5N1 rHA protein, and the aptamer-based biosensor are shown schematically (b).

For instance, Zhang and colleagues established the detection of dengue serotype 2 using a silicon nanowire biosensor [39]. The researchers began by covalently attaching a peptide nucleic acid to the surface of a silicon nanowire. Following that, a corresponding dengue serotype produced two fragments and immobilized on the silicon nanowire functionalized with peptide nucleic acid. They confirmed hybridization by comparing the resistance of the Si nanowire biosensor after and before DEN-2 was bound to the PNAs sequence (Fig. **8**) [40].

Additionally, Deng and colleagues demonstrated a DNA detection technology based on anodic aluminum oxide membrane sensing [41]. They monitored the impedance changes inside the nanopores following DNA attachment using the electrochemical impedance method and covered the Al_2O_3 membrane with Pt electrodes to remove solution resistance outside the nanopores.

Lim *et al.* determined the affinity of peptides for the NS1 protein using polyvalent

phage [42]. They determined that the peptide had a high concentration of basic residues *via* amino acid sequence analysis. Between all the peptides examined, the chosen phage demonstrated the higher rise in impedance and the greatest reduction in current in cyclic voltammetry in EIS following binding to NS1 proteins. Additionally, they demonstrated that phage clones had a higher affinity for NS1 proteins than the M13 wild-type or bovine serum albumin [40].

Jahanshahi and colleagues characterized the development of an SPR biosensor to recognize DENV (Dengue virus) antibodies in human blood samples [40]. On a biosensor, they utilized four DENV serotypes as ligands.

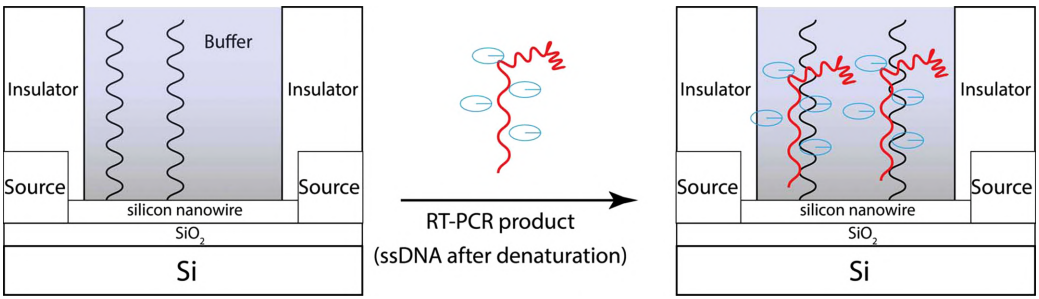

Fig. (8). A illustrates a diagram of the sensor.

Jin *et al.* described a viral diagnostic system for human adenovirus detection that is based on a microfluidic sample processing and optical biosensor [43, 44]. They began by extracting viral DNA from human adenovirus samples utilizing a distinct method. They then discovered that the optical biosensor was capable of detecting 10 copies of human adenovirus in clinical samples in less than 30 minutes. Lastly, they verified the viral diagnostic system's clinical usefulness using 13 human samples (three of them from another pathogen and ten of them by human adenovirus).

Using a portable surface plasmon resonance device, Prabowo *et al.* demonstrated the fast quantification technique and label-free detection for human enterovirus 71 [45]. They used human enterovirus 71's main capsid protein as a biomarker. They decreased the time needed for the measurement of human EV71 from 6 days to a few minutes [43].

Riedel and colleagues developed an SPR-based biosensor platform for diagnosing various EBV (Epstein-Barr virus) infection stages in clinical blood samples [46]. This was accomplished by attaching the biosensor through complementary oligonucleotide hybridization and concurrently detecting immunoglobulin against 3 different immunoglobulins contained in the virus [43].

Bai *et al.* used imprint-lithography methods to create a double imprinting method for embedding a virus-imprinted hydrogel into a sensor [43]. They detected the apple stalk pitting virus at concentrations as low as (10 ng/mL) using a simple laser transmission device that measured diffraction and could be viewed with the naked eye [43].

Inan and colleagues developed a microfluidic filter system to recognize and quantify HPVs 16 E7 immunoglobulins in whole blood as a non-invasive aid in detecting human papillomavirus-associated cancers. They identified antibodies to the human papillomavirus 16 E7 at a concentration of 2.87 ng/mL [47].

Birnbauer *et al.* published research on developing a micro total analysis biosensor device for continuous monitoring of virus contamination. They used microfluidics with imprinted polymer and integrated native in conjunction with contact-free dielectric biosensors to detect HRV2. They demonstrated that particular frequencies of virus and dissociation binding might be easily identified. They successfully removed the virus to show the biosensor's reusability after a fiftyfold rise [48].

Environmental Monitoring

There is a growing need for monitoring natural and harmful pollutants in the environment and enforcing tight control over them in the modern-day. The food, water, and air supply are the primary environmental components routinely exposed to various naturally occurring and unwelcome harmful chemical pollutants. These pollutants have the potential to cause serious health problems and diseases in living organisms. Additionally, different techniques such as wet chemistry, FT-IR (Fourier-transform infrared spectroscopy), GC (Gas chromatography), and HPLC (High-performance liquid chromatography) are utilized to identify a variety of contaminants such as organophosphate insecticides, heavy metals, and phenolic compounds. These approaches have a number of disadvantages, while electrochemical methods have many benefits, including fast and sensitive detection and low cost [14]. Used to detect algal toxins [49].

Heavy Metal

Heavy metals are significant environmental contaminants that have a harmful impact on soil microorganisms through the biological activity of microbial communities, population size, and altering the variety [50]. Se (selenium), Ag (silver), Zn (zinc), Cr (chromium), Cu (copper), Ni (nickel), Cd (cadmium), As

(arsenic), Pb (lead), and Hg (Mercury) is among the most often polluted heavy metals [51].

Electrochemical biosensors have established a novel technique for screening heavy metals in ecological samples by using aptamers as bio-receptors within the last several years. Among the various heavy metals that are frequently encountered in environmental commodities, Hg (mercury) is the most widely investigated analyte. Lately, an aptasensor for the selective recognition of mercury ions has been created [52]. The reported aptasensor used T–Hg^{2+}–T (thymine-Hg^{2+}-thymine) chemistry in conjunction with nanoporous Au particles for signal amplification. The designed aptasensor detected Hg^{2+} at concentrations ranging from 0.01 to 5000 nM with a detection limit of 0.0036 nM. The authors successfully used a designed aptasensor to detect mercury ions in water samples and asserted a possibility for on-site Hg^{2+} recognition [53].

In several other instances of aptasensors for detecting mercury ions, writers have used electrochemical and photoelectric techniques. for example, Han and colleagues developed an N-doped TiO_2 (titanium oxide) based visible light-activated PEC (photoelectrochemical) aptasensor for label-free designation of mercury ion *via* quenching photogenerated electrons [54]. Likewise, Li and colleagues developed a selective PEC aptasensor and an ultrasensitive to recognize mercury ion ions at fM (femtomole) levels for the first time using the quercetin-Cu^{2+} combination as the DNA intercalator [55]. Other publications created label-free and an enzymatic electrochemical aptasensor for low-level detection of Pb^{2+} (lead ions). The proposed approach achieved a LOD of 0.032 pM by loading a MOF with Silver-Platinum particles that served as electrocatalytic enhancers [56].

Yang *et al.* developed an electrochemical nano biosensor to recognize mercury ions in an aqueous solution using a 3D-Reduced graphene oxide/ Polyaniline composite as the sensitive layer. The T-rich DNA strands were fixed onto the electrode surface using an amino group-rich 3D-Reduced graphene oxide/ Polyaniline that binds to mercury ion preferentially and forms T-Hg^{2+}-T coordination. The scientists asserted that the rGO/PANI composite enhanced the given electrochemical performance, electrical conductivity, and biosensor's specific surface area (Fig. **9a**) [57, 58].

Tang and colleagues created a reusable and ultrasensitive electrochemical DNA biosensor for Hg^{2+} recognition on GCE utilizing a DNA anionic intercalated as the signal indication and AuNP composite/ SPAN (self-doped polyaniline nanofiber)/mesoporous carbon (OMC) as the platform. The GCE surface was initially changed with OMC and SPAN nanofibers through layer-by-layer self-

assembly, as shown in Fig. (**9b**) [58]. The electrode surface treated with SPAN/OMC was then electrodeposited with AuNPs. Finally, the electroactive signal indication was the AQDS. This DNA electrochemical sensor has been reported to have a reasonably broad dynamic range of 10 fM to 1M and a LOD of 0.6 fM (S/N = 3) [58].

In 2017, Barrosa *et al.* used AuNPs, Na^{+}-MMT (sodium montmorillonite clay mineral), and PANI-ES (polyaniline emerald salt) to concurrently detect Cd^{2+}, Pb^{2+}, and Cu^{2+} ions. For copper (II), Lead(II), and Cadmium(II) ions, the sensor reached detection limits of 0,02, 0, 009, and 0.001 g. L^{-1}, respectively [59].

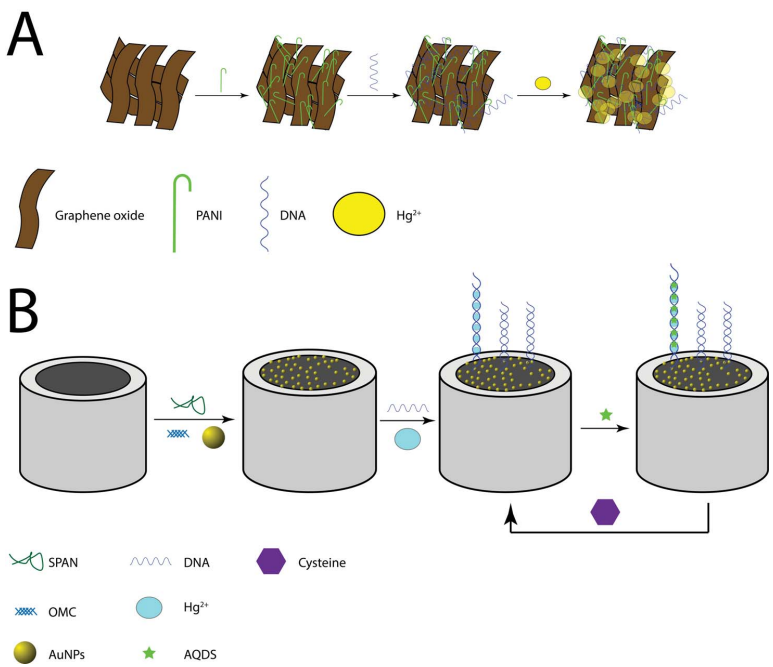

Fig. (9). Examples of biosensors based on PANI/nanoparticles for the sensitive recognition of Hg^{2+}. (A) illustrate the electrochemical DNA biosensor created on the basis of 3DrGO@ PANI nanocomposite for the recognition of Mercury ions. (B) illustration depiction of a DNA nano biosensor for the measurement of mercury ions based on a DNA anionic intercalated as the signal indication and AuNP composite/SPAN (self-doped polyaniline nanofiber)/mesoporous carbon (OMC) as the platform.

In 2014, Dong *et al.* developed a ternary nanocomposite of GNPs, PANI, and GR on the Glassy Carbone electrode surface to develop a sensitive biosensor to determine Lead(II). The results showed that triplex nanocomposite might be utilized for heavy metal analysis owing to its advantageous properties, including high electrical conductivity and an apparent synergistic impact on metal ion detection [60].

Kong and colleagues (2018) developed a simple-to-use electrochemical sensor utilizing shell-core Fe_3O_4@PANI (ferroferric oxide@polyaniline) NPs as a sensitive system for the quantitative recognition of $Cd^{2+}$and Pb^{2+} ions. The linear from 1.0 to $0.9*10^4$ and 0.1 to 10^4 nmol under optimum experimental conditions. LOD of 0.3 and 0.03 nmol for L^{-1}. Cd^{2+} and Pb^{2+} ions were detected using L^{-1}, respectively [61]. In the same year, Deshmukh and colleagues developed an electrochemical sensor for measuring the concentration of Cu^{2+} ions based on an SWNT/EDTA-modified PANI nanocomposite on SSE (a stainless steel electrode). The sensor has a linear range of 2 mM to 4 M and a LOD of 1.4 M [62].

Pesticides

Pesticides are among the most significant environmental pollutant owing to their widespread utilization. For example, organophosphorus insecticides are widely used in agriculture and are considered to be the most environmentally hazardous pesticides owing to their high toxicity. Thus, as detailed in the following, miniaturized, sensitive, and simple *in situ* methods like biosensors have been created as analytical strategies for recognizing and monitoring, minus the requirement for significant sample pretreatment [63].

Sun and colleagues (2013) developed a low-cost, highly sensitive, and stable chlorpyrifos immunosensor based on GCE and CH/MWCNT/PANI/AuNP. The electrode was coated with the chemically produced PANI/MWCNT/CH nanocomposite and AuNPs. Owing to their great surface-free energy, large specific surface area, and excellent biocompatibility, AuNPs were employed as the linker and securely adsorbed antichlorpyrifos. Additionally, MWCNTs' conductivity and large surface area enhance the redox characteristics of promoting electron transport and conducting polymers among the electrode surface and the redox center. The suggested immunosensor assessed real-world samples of leek spiked lettuce, pakchoi, and cabbage with various chlorpyrifos doses. The findings indicated that the linear range was between $40*10^{-6}$ and 0.1 $mg.mL^{-1}$ and between $500*10^{-6}$ $mg.mL^{-1}$ and $40*10^{-6}$ $mg.mL^{-1}$, with a LOD of $0.06*10^{-6}$ $mg.mL^{-1}$ (Fig. **10**) [64].

He and colleagues recently reported the identification of numerous aptamers for the selective screening of acetamiprid. The stated aptamer has a dissociation constant (K_d) of 4.98 M [65]. Wang *et al.* also stated the development of a DNA-based aptamer to recognize several organophosphate pesticides, including omethoateas, isocarbophos, profenofos, and phorate [66].

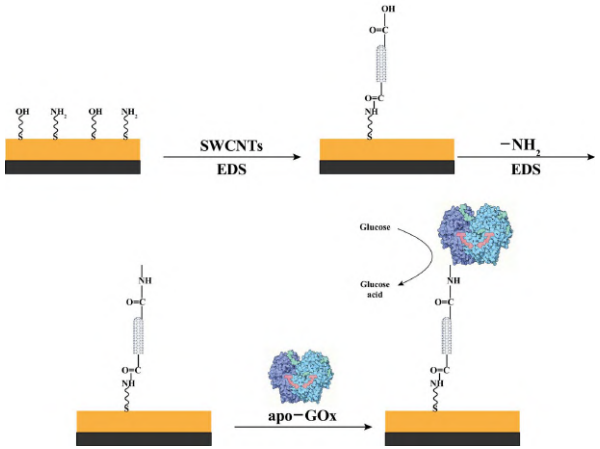

Fig. (10). The chemical process used to fabricate the PG/PANI/CuNP/cMWCNT/Hb electrode is shown schematically.

Eissa and colleagues [67] created an electrochemical carbendazim aptasensor in which the aptamer was immobilized on a gold surface. Carbendazim's precise identification by the aptamer will result in conformational alterations in the aptamer structure. This conformational shift changes the aptasensor surface's accessibility to a ferrocyanide/ferricyanide redox pair. The reaction was then quantified using EIS. The schematic shown in Fig. (**11**) illustrates the concept of a structure-switching aptamer-based assay [67].

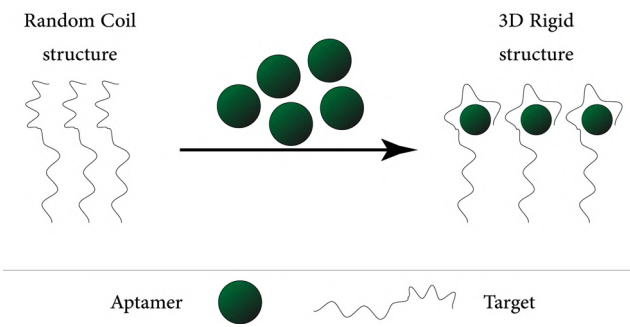

Fig. (11). The concept of the structure switching aptamer-based test is shown schematically.

Cesarino *et al.* used MWCNTs and PANI to fabricate sensitive electrochemical AChE (acetylcholinesterase) nano biosensors for carbamate pesticide detection in vegetables and fruits (cabbage, broccoli, and apple). The obtained findings were completely in conformity with those obtained using the High-performance liquid chromatography method [68]. He and colleagues, In 2018, developed a new electrochemical biosensor for fast identification of malathion by AChE (anchoring

enzyme) onto a shell-core nanostructured composite of hollow carbon spheres coated in HCS@PANI (needle-like polyaniline). The (AChE) enzyme was absorbed onto the HCS@PANI nanocomposite after its synthesis. In the presence of malathion, the electrochemical catalysis of an acetylcholinesterase/ HCS@Polyaniline -based biosensor towards ATCl (acetylthiocholine chloride) was suppressed. This phenomenon was used to identify malathion. Additionally, the High carbonized nanospheres@Polyaniline nanocomposite may serve as an appropriate layer for catalysis of ATCl and AChE immobilization, which has a broad potential use in high-sensitivity pesticide recognition [69].

Khan and colleagues developed a selective and sensitive electrochemical sensor based on modified MWCNT/Silver Nanoparticle/PANI GCE for evaluating and measuring fenitrothion in surfactant solutions [58]. Zhang and colleagues created an electrochemical sensor for 4-NP recognition in 2017 by coating GCE with AgNW/PANI (a silver nanowire-polyaniline) composite. The sensor was used to Diagnosis 4-NP in samples of natural water. The findings showed that the developed sensor is mighty for diagnosing 4-NP in real-world water samples [70].

Food Quality

Human health is at risk from a variety of food-borne contaminants that enter the food chain through the water supply. Natural poisons (mycotoxin), infections (microbial), and allergies generated naturally are all examples of food-borne chemicals. Human progress and activities produce a large number of new chemicals with little concern for human health or long-term effects on the environment. A number of them, including pesticides, antibiotics, and food additives, are synthesized synthetically. Electrochemical aptasensors are mostly used in the food industry to determine naturally occurring or synthetically manufactured food-borne chemicals. Electrochemical determination concepts and forms are inextricably linked to the applications of electrochemical diagnostics [52]. Electrochemical Biosensors are used in Food analytes like Microbial Adulteration of Food, Food Freshness, and Taste, Food Ingredients, GMO Content [2], and Salmonella control in food products [71].

Food Toxins

Castillo and colleagues [72] created a multilayer AFB1 sensor. The PAMAM G4 (fourth-generation poly (amidoamine) dendrimers) was immobilized on an Au electrode coated with cystamine and used to covalently link single-stranded amino-modified DNA aptamers specific for AFB1. AFB1 was detected using the CV and EIS methods.

Nguyen *et al.* recently announced the development of an electrochemical

aptasensor for AFM1 based on SWV and CV. Aptamers specific for AFM1 were immobilized on IDE (an interdigitated electrode) polymerized with Iron (II, III) oxide-incorporated PANI. The aptasensor described here demonstrated excellent sensitivity, repeatability, and stability (0.00198 g/L) for AFM1 detection [73]. Mishra and colleagues have revealed for the first time a sensitive recognition method for OTA (Occupational Therapy Assistant) in cocoa beans using a competitive aptasensor based on Differential pulse voltammetry [74]. Catanante *et al.* previously described an aptasensor for OTA recognition based on a folding process by examining MB-tagged anti-OTA aptamers [75]. Likewise, Gaud and colleagues improved a label-free impedimetric electrochemical aptasensor to recognize Aflatoxin B1 in alcoholic drinks [76]. Fetter and colleagues [77] recently created a ricin biosensor by covalently linking an aptamer to the surface of a gold electrode. The electrochemical signal of the labeled redox probe methylene blue was then analyzed using the Square Wave Voltammetry technique to estimate the nanomolar concentrations of botulinum neurotoxins and ricin in diluted serum.

Shi [78] created an aptasensor that utilizes both graphene/thionine nanocomposites and Au NPs to recognize fumonisins B1 (FB1). The recognition method for electrochemical aptasensors using enzyme catalysis and redox probe-attached aptamer for food toxins is shown in Fig. (**12**) [52].

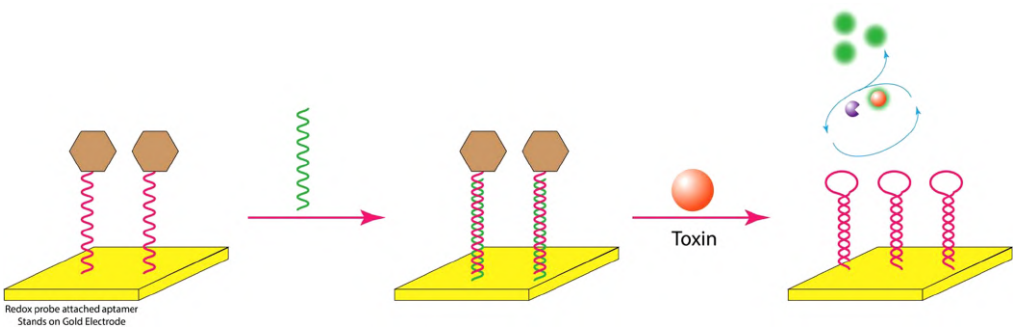

Fig. (12). The electrochemical aptasensor is designed to detect food toxins using enzyme-induced catalysis and a redox probe-attached aptamer test.

Antibiotic Residues

Antibiotic resistance to antibiotic buildup and pathogenic bacteria in food components have resulted from the growing use of antibiotics in conjunction with other medicines to feed additives or treat inflammatory illnesses. Antibiotic residues in food are essential for monitoring human consumption; even a trace of these remaining parts may cause an allergic response in hypersensitive people [79].

Zhou and colleagues explained the utilization of an aptasensor to determine kanamycin in milk without using a label. The scientists used the SWV method to measure kanamycin residues through a current response induced by a conformational change in a particular aptamer linked to an Au-electrode [80]. Similarly, Liu and colleagues described the development of a sandwich-type electrochemical aptasensor for the determination of OTC residues [81]. Kim and colleagues described the development of a tetracycline-specific aptasensor using a single-stranded DNA aptamer as the bioreceptor. SWV and CV methods were used to determine a biotinylated single-stranded DNA aptamer's binding immobilized on a streptavidin-modified SP-Gold-electrode to tetracycline [82]. Additionally, another aptasensor based on an ssDNA-binding protein was lately described to detect ciprofloxacin at an ultrasensitive level. The developed aptasensor used SSB (an ssDNA-binding protein) on showed good and Gold electrode specificity for recognizing ciprofloxacin in milk samples with an outstanding Limit of detection of 263 pM [83].

A portable and disposable impedimetric aptasensor for the recognition of kanamycin residual in milk samples was recently described. The identification of the target was predicated on its precise detection by a covalently immobilized kanamycin-aptamer on Solid-phase extraction [84].

Study of Enzyme Kinetics

Electrochemical biosensors are being used in a new and successful way in the field of enzyme kinetics research. Apart from their function as essential components of many industrial processes, enzymes also play a critical role in the pathophysiology of numerous illnesses. A biosensor that is capable of determining enzyme activity may have several advantages [14].

Table **5** summarizes recent developments in electrochemical sensors/biosensors for determining enzyme kinetics.

Table 5. SWV-based electrochemical and biosensors for the investigation of enzyme kinetics.

Enzyme	Electrode	Analytical Parameters
Trypsin	Electrodes covered with gelatin and screen printed	Linear range: 0.75–7500 U mL^{-1} Limit of Detection: 0.075 U mL^{-1}
Extracellular signal-regulated kinase 1, sarcoma-related kinase, and cyclin A-dependent kinase 2	Peptide-modified sprayed gold electrode (on silicon chip)	—

(Table 5) cont.....

Enzyme	Electrode	Analytical Parameters
Protein kinase associated with sarcomas	Gold rod electrode modified with peptide (EGIYDVP)	K_m: 200 mM V_{max}: 115 mA cm^{-2} min^{-1}
Cytochrome P450 17A1	Graphite electrode with immobilized hemoprotein modified with didodecyldimethylammonium bromide (surfactant) and gold nanoparticles	Km (pregnenolone): 5.0 _ 0.7 mM

CONCLUSION

So, this section shows that electrochemical biosensors have many advantages and useful applications. For this reason, it can be economically viable and has vast market potential in this field, especially in medical applications such as HIV-1, Virus-1-associated glycoprotein, Hepatitis B, Hepatitis A virus, Ebola, Zika, MNV (Murine norovirus), Influenza, DENV (Dengue virus), EBV (Epstein-Barr virus), HPV, *etc*. (which are widely used in industrial applications today), environmental monitoring such as Selenium, Silver, Zinc, Chromium, Copper, Nickel, Cadmium, Arsenic, Lead and Mercury, and in the pesticide field, it can detect organophosphate pesticides including omethoateas, isocarbophos, profenofos, and phorate and can identify and check the food quality.

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SUBJECT INDEX

A

B

C

D

E

F

G

H

I

L

M

N

O

P

Q

R

S

T

U

V

W

Z