ELECTROCHEMICAL BIOSENSORS IN PRACTICE

MATERIAL AND METHODS

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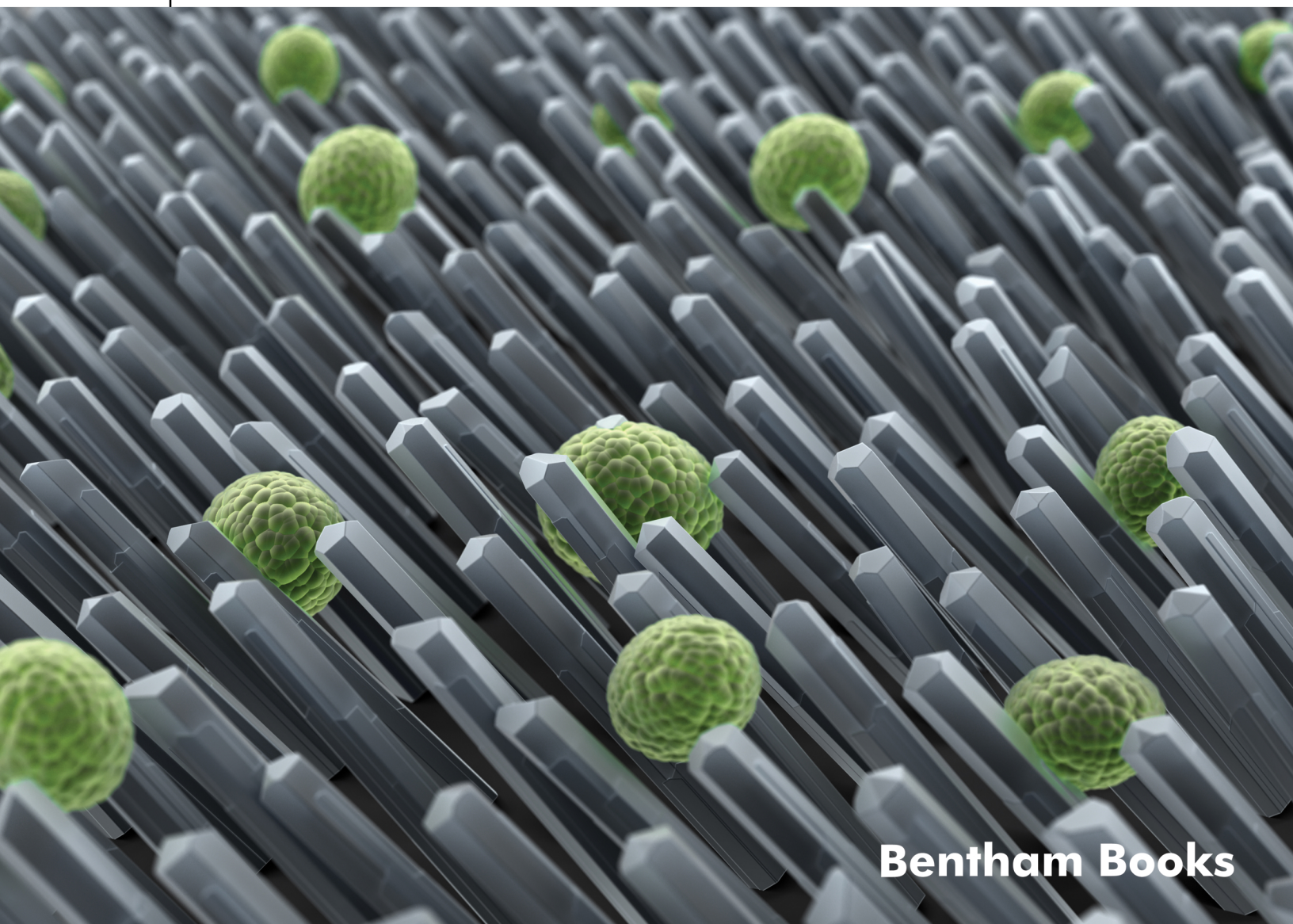

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Electrochemical Biosensors in Practice: Materials and Methods

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Electrochemical Biosensors in Practice: Materials and Methods

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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.”

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● 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].

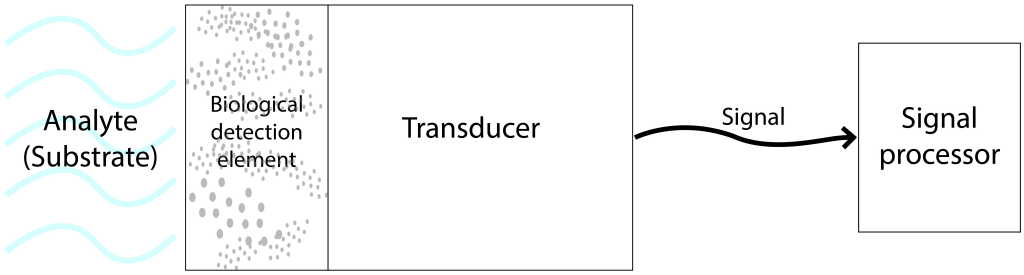

Fig. (1). A biosensor's schematic layout.

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].

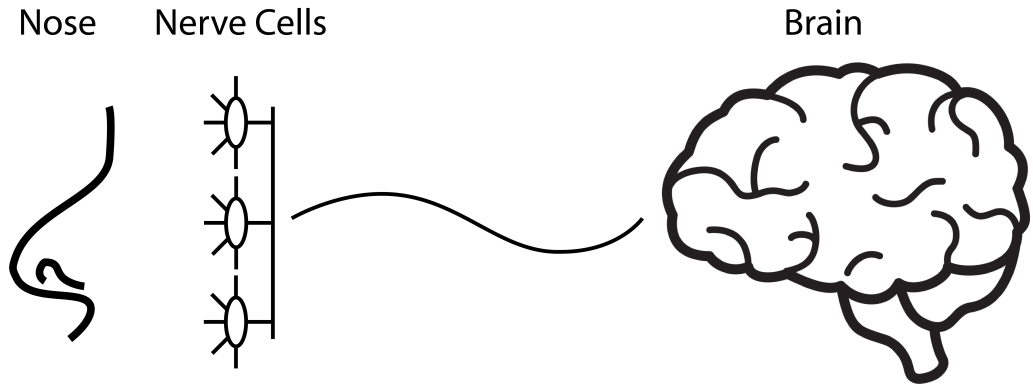

Fig. (2). Simple Biosensor in the human body is noise.

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

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

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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**).

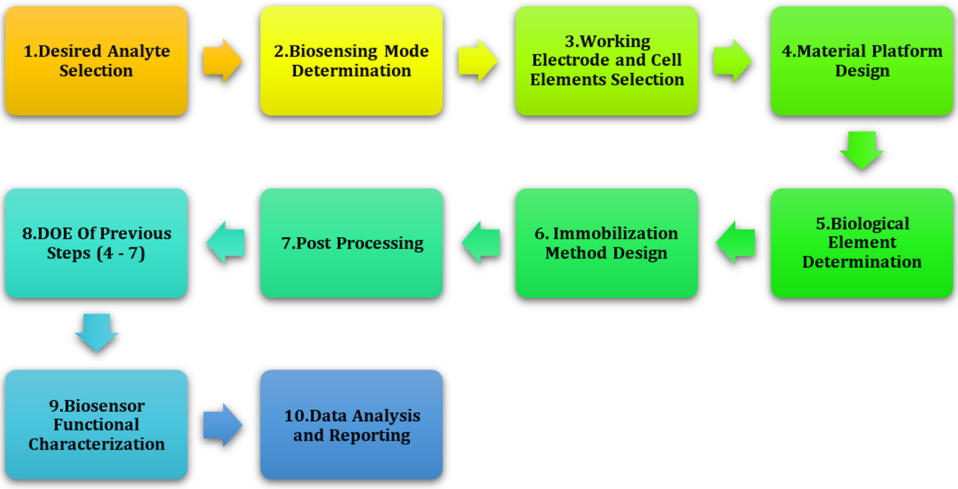

Fig. (1). Progress of biosensor design, fabrication and evaluation.

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.

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].

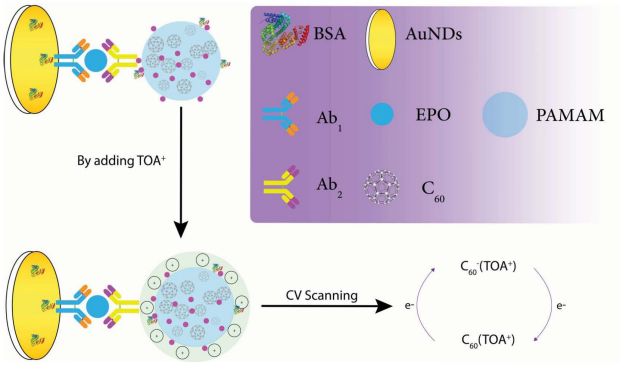

Fig. (1). An electro-ımmunochemıcal assay based on C_{60} ıs shown schematıcally.

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].

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.

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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

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

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