ANKARA YILDIRIM BEYAZIT UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

NANOMATERIAL MODIFIED ELECTRODE SURFACE CHARACTERIZATION AND BIOSENSOR APPLICATION FOR A TERRORISM AGENT DETECTION

M.Sc. Thesis by

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NANOMATERIAL MODIFIED ELECTRODE SURFACE CHARACTERIZATION AND BIOSENSOR APPLICATION FOR A TERRORISM AGENT DETECTION

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M.Sc THESIS EXAMINATION RESULT FORM

We have read the thesis entitled "**NANOMATERIAL MODIFIED ELECTRODE SURFACE CHARACTERIZATION AND BIOSENSOR APPLICATION FOR A TERRORISM AGENT DETECTION**" completed by **MERYEM TUĞÇE ÖZEL** under supervision of **ASSIST. PROF. Dr. Nimet YILDIRIM TİRGİL** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

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NANOMATERIAL MODIFIED ELECTRODE SURFACE CHARACTERIZATION AND BIOSENSOR APPLICATION FOR A TERRORISM AGENT DETECTION

ABSTRACT

Although nerve agents are the most notable among chemical agents, they show their negative effects mainly in nervous tissue. Toxic chemical substances, which have basic properties such as killing people and other living things, destroying them with serious injury, and rendering them ineffective by disrupting their functions, have high toxic potential, are resistant to external factors, and are economical to produce, are generally defined as chemical weapons. Organophosphorus pesticide poisoning has become a global health problem affecting millions of people with acute and chronic poisonings, as the possibility of exposure to chemical nerve gases through terrorist and military activities has also become a serious threat to our soldiers. To date, many studies have been carried out for the detection of Organophosphorus pesticides, but although these techniques are selective and sensitive, disadvantages such as expensiveness, advanced personnel, and preparation processes limit the applications of on-site analysis, especially in emergency situations such as accidental pesticide release. Therefore, there is an urgent need for fast, sensitive, reliable, and cost-effective detection tools for environmental, security, military, and biomedical monitoring. In this master's thesis, the biosensor system and carbon nanotubes, reduced graphene oxide, and graphene nanomaterials, which will be the basis of the system, were used because of their unique properties. In this thesis, Carbon nanotube, graphene, and reduced graphene oxide nanoparticles were modified on the Carbon screen printed electrode surface using drop casting and electrodeposition techniques. Then, an enzyme inhibition-based biosensor system was developed for paraoxon, a terrorist agent, detection. Biosensor performance conditions under optimum conditions were investigated. It was concluded that the developed nanoparticle-based biosensor system would enable faster, cheaper, more sensitive, and real-time tests and show more linear detection range and stability efficiency than existing studies for the detection of the terrorist agent paraoxon.

Keywords: Nerve agents, organophosphorus nerve agent, biosensor system, enzyme inhibition based biosensor, nanomaterials, paraoxon analysis.

TERÖRİZM AJAN TESPİTİ İÇİN NANOMATERİYEL MODİFİYE EDİLMİŞ ELEKTROTLARIN YÜZEY KARAKTERİZASYONU VE BİYOSENSÖR UYGULAMASI

ÖZ

Sinir ajanları, kimyasal ajanlar arasında en dikkate değer şekilde etkili olmakla beraber olumsuz etkilerini esas olarak sinir dokusunda gösterirler. İnsanları ve diğer canlıları öldürme, ağır yaralama ile yok etme ve fonksiyonlarını bozarak etkisiz hale getirme gibi temel özelliklere sahip, toksik potansiyeli yüksek, dış etkenlere karşı dayanıklı ve üretimi ekonomik olan toksik kimyasal maddeler genellikle kimyasal silahlar olarak tanımlanmıştır. Terörist ve askeri faaliyetler yoluyla kimyasal sinir gazlarına maruz kalma olasılığı da askerlerimiz için ciddi bir tehdit haline geldiğinden, organofosfor pestisit zehirlenmesi, akut ve kronik zehirlenmeler ile milyonlarca insanı etkileyen küresel bir sağlık sorunu haline gelmiştir. Bu zamana kadar organofosfor tespiti için birçok çalışma yapılmıştır ancak bu tekniklerin seçici ve hassas olmalarına rağmen, pahalı olmaları, gelişmiş personel ve ön hazırlık süreçleri olması gibi dezavantajlar, özellikle kazara pestisit salınımı gibi acil durumlarda yerinde analiz uygulamalarını sınırlandırmaktadır. Bu sebeple, çevre, güvenlik, askeri ve biyomedikal izleme için hızlı, hassas, güvenilir ve uygun maliyetli tespit araçlarınaacil bir ihtiyaç vardır. Bu yüksek lisans tezi çalışmalarında, biyosensör sistemi ve sisteme temel olacak karbon nanotüpler, indirgenmiş grafen oksit ve grafen nanomalzemeler benzersiz özellikleri nedeniyle kullanılmıştır. Bu tezde, karbon nanotüp, grafen ve indirgenmiş grafen oksit nanoparçacıkları, damla döküm ve elektrodepozisyon teknikleri kullanılarak Karbon ekran baskılı elektrot yüzeyinde modifiye edilmiştir. Yüzeye daha sonra enzim inhibisyon tekniği ile asetilkolin enzimi ilave edilmiş ve sonrasında bir terorizm ajanı olan parakson eklenmiştir. Optimum koşullar altında biyosensör performans koşulları araştırılmıştır. Geliştirilen nanoparçacık tabanlı biyosensör sistemi, daha hızlı, daha ucuz, daha hassas ve gerçek zamanlı testlere olanak sağlaması ve terörist ajan olan paraoksonun tespiti için mevcut çalışmalara göre daha doğrusal tespit aralığı ve kararlılık verimliliği göstereceği düşünülmektedir.

Anahtar Kelimeler: Sinir ajanları, organofosfor sinir ajanı, biyosensör sistemi, enzim inhibisyonuna dayalı sistem, nanomalzemeler, paraokson analizi.

CONTENTS

NOMENCLATURE

Abbreviations

mL	Milliliter
µL	Microliter
M	Molar
mM	Millimolar
µM	Micromolar
mg/mL	Milligram/ milliliter
ºC	Degrees Celcius (also termed Centigrade)
g	Grams
H	Hours
I	Current
Min	Minutes
T	Temperature
Conc.	Concentration
µL	Microliter
µM	Micromolar
µm	Micrometers
pg	Picogram
pg/ml	Picogram/milliliter

Acronyms

OP	Organophosphorus Pesticides
GD	Soman
GB	Sarin
GA	Tabun
AChE	Acetylcholinesterase Enzyme
BChE	Butyrylcholinesterase Enzyme
ACh	Acetylcholine Neurotransmitter
MRI	Magnetic Resonance Imaging
NPs	Nanoparticles
CNTs	Carbon Nanotubes
rGO	Reduced Graphene Oxide
CV	Cyclic Voltammetry
DPV	Differential Pulse Voltammetry
EIS	Electrochemical Impedance Spectroscopy
XRD	X-ray Diffraction
TEM	Transmission Electron Microscopy
SEM	Scanning Electron Microscope
SPE	Screen-printed electrode
PBS	Phosphate-buffered saline
FeCN	Hexacyanoferrate (II + III) / Hexacyanoferrate(redox)
KCl	Potassium Chloride
$[Fe(CN)_6]^{3-}$	Ferricyanide
$[Fe(CN)_6]^{4-}$	Ferrocyanide
$[Fe(CN)_6]^{3/4}$	Ferricyanide
$K_4[Fe(CN)_6]$	Potassium Ferrocyanide
$K_3[Fe(CN)_6]$	Potassium Ferricyanide

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CHAPTER 1

INTRODUCTION

1.1. Literature Summary

Nerve agents are the most notable and effective class of chemical agents. They are called nerve agents because they show their negative effects mainly in the nervous tissue. Neurotoxic nerve agents are generally organophosphorus compounds that are persistent and easy to distribute. Although these substances are toxic, they are predominantly irreversible inhibitors of acetylcholinesterase. Toxic chemical substances, which have basic features such as killing people and other living things, eliminating them with severe injury, and rendering them ineffective by disrupting their functions, have high toxic potential, are resistant to external factors and are economical to produce, are generally defined as chemical weapons [1].

Organophosphate nerve agents Soman (GD), sarin (GB), VX, and tabun (GA) are among the most toxic and lethal chemical warfare agents with high acute toxicity [2]. Because of their low persistence and high insecticide activity, organophosphorus pesticides (OPs) are also widely used in modern agriculture. Because of their high neurotoxicity, OP residues found in food and the environment pose a significant risk to public health. Chemical nerve agent exposure through terrorist and military activities has also become a serious threat to our soldiers and civilians. Pesticide poisoning has become a global health issue, intoxicating millions of people and causing both acute and chronic unintentional poisoning [1]. Nerve gases affect the eyes, respiratory, cardiovascular, digestive, muscles, and central nervous systems [2]. Numerous studies have shown that these OP neurotoxins have a severe impact on the nervous system, eventually leading to death [1].

Organophosphates cause covalent modification of enzymes, mainly the serine residue responsible for catalysis, at their active site. Their main targets are cholinesterases. Acute toxic effects of organophosphate agents occur due t AChE modification in nerve tissue. This modification represses the enzyme irreversibly and the enzyme loses its

catalytic activity. Acetylcholine degradation cannot occur in the tissue and its interaction with its receptors continues [2].

Because of the high toxicity of OP pesticides and nerve agents, there is an emergency need for detection tools that are fast, sensitive, reliable, and cost-effective for environmental, safety, and biomedical monitoring [3]. In recent years, high-performance liquid chromatography, gas chromatography, mass spectrometry, enzyme-linked immunosorbent assay, capillary electrophoresis, and surface-enhanced Raman scattering spectroscopy have been developed to detect OP residues in water and food samples in order to perform various analytical techniques. Even though these techniques are selective and sensitive, drawbacks such as cost, the need for well-trained personnel, and the time-consuming pretreatment limit their use in on-site analysis, particularly in emergencies such as accidental pesticide releases [3].

As a result, simple, sensitive, portable, and low-cost methods for detecting OP residues in situ are in high demand [3]. Biosensors are widely used for OP detection. Biosensors are electronic devices that combine biological sensing material with a chemical or physical transducer to convert a signal into a measurable and accessible electrical signal. Biosensors are frequently used in various fields from medicine to food.

Numerous applications exist for enzyme-based biosensors, which use enzymes as recognition elements and combine the natural specificity of enzymes with the specific advantages of biosensors. Such biosensors have enormous potential for bioanalysis due to their specific properties such as high sensitivity, repeatability, and high specificity, and are a popular topic that constantly attracts researchers' attention. Enzyme-based biosensors consist of biocatalysts or immobilized materials, and optical and electronic electrodes usually convert the biocatalytic conversionsIn other words, any optical or electrical change in the sensing surface will reflect the biological process that is taking place at the respective electrodes [4]. Enzyme-based sensors measure the response of enzyme-catalyzed reactions; each physical measurement is related to the rate of this reaction and is used in the determination. Various procedures have been used to monitor enzyme activity using electrochemical converters. Evaluation of this activity includes directly measuring electroactive products or co-substrates in enzymatic reactions. This is detected indirectly by electron transfer between the

electrolyte species and the electrode using a synthetic mediator. There are many biosensors for pesticide detection based on catalytic activity or reaction inhibition. Organophosphorus hydrolase enzyme is frequently used to directly detect pesticides [5].

Nanotechnology is a promising technology in engineering, physics, chemistry, computer, and medicine fields. Nanotechnology is an up-to-date, usable where nano-technological products are produced. This area has a body on lives and will be constantly large. The use of nanotechnology is increasing, especially in biotechnology and biomedical sciences. It is designed in a human-enhancing manner in nano-design and has the potential to be brought back to life in practice.

Bioanalytical techniques, life technical techniques, and having well-controlled dimensions to look at viability in living organisms have less loss, selectivity, and efficiency than high-quality nanomaterials. Nanomaterials can be used for use, benefit from what they can benefit from biological use. Nanomaterials are also used as advanced technologies in advanced technologies such as MRI. Nanomaterials are similarly introduced at birth as birth and clinical nanotechnology applications [6].

Carbon nanotubes (CNTs) have become one of the most popular researched nanostructures with the advancement of technology. CNTs with one-dimensional structures differ from other carbon allotropes because they have good mechanical and electrical properties. Application areas such as engineering, aerospace industry, chip manufacturing, nanodevices, and electronic nanocircuits are quite wide. These nanomaterials, which have gotten a lot of attention because of their exceptional properties like high mechanical strength and elasticity, chemical, thermal, and structural stability, and high conductivity, are also widely used in fields like sensors and biosensors [7].

Reduced graphene oxide, which has excellent electrochemical properties, is a nanomaterial frequently used in biosensor applications. Due to its high electrical conductivity, large surface area, better electrocatalytic activity, and excellent electron transfer rates, it is a promising nanomaterial for diagnosing OPs. These nanomaterials,

mainly used to improve biosensor performance, are often preferred because of their cost-effectiveness [8].

Graphene is an allotropic type of carbon that consists of a single sp2 hybridized carbon layer in two dimensions arranged in a crystal lattice [9]. Graphene is often preferred for electrode surfaces because it has a large specific surface area, excellent conductivity, and high mechanical strength. Graphene is an essential electroanalytical material. The peak-to-peak potential separation is minimal under cyclic voltammetry (CV) conditions, which means fast electron transfer kinetics and the apparent electron transfer rate are higher [10].

1.2. Aim of the Thesis

The aim of this master's thesis is to detect various organophosphorus compounds in a faster, easier, and cheaper way in the biosensor system to be prepared with the help of nanomaterials and to bring valuable innovations to the fields of materials science, chemistry, and biosensors. With this goal, the biosensor system, which will be prepared considering the wide usage areas of biosensors, will again have a widespread use area thanks to its structure and ease of use. Carbon nanotubes, reduced graphene oxide, and graphene nanomaterials, which will be based on the system, will shed light on this target due to their uniqueness, unique chemical and physical properties, easy preparation, and cost-effectiveness. For these reasons, the aim of this thesis is; The objective is to make the detection of organophosphorus compounds faster, easier, and more cost-effective, and to prove the benefits of using nanomaterials such as carbon nanotubes, reduced graphene oxide, and graphene nanomaterials by modifying biosensors in the field of biosensors and materials science. Within the scope of the thesis studies, the nanomaterial-modified enzyme-inhibited electrochemical biosensor system is a unique system that can detect toxic substances quickly with high sensitivity and selectivity. Instead of biological structures such as antibodies, which are generally used in biosensor systems, Carbon nanotubes, reduced graphene oxide, and graphene have been preferred for reasons such as being more stable under harsh operating conditions and having unique physical and chemical properties. The benefits of these materials to the targeted biosensor system due to their advantageous usage areas will

be evaluated by considering them as work packages. One of our main objectives is to prove the original values of the thesis is to modifying nanomaterials to the electrode surface and characterizing the relevant system.

1.3. Hypothesis

Carbon nanotubes, reduced graphene oxide, and graphene offer many advantages due to their excellent properties. Compared to enzymes or antibodies, they can be produced very easily, and the manufacturing process is very cost-effective. Due to their high sensitivity and selectivity, these nanomaterials show very good results in diagnosis and diagnosis when integrated into biosensors. Due to the exceptional properties of carbon nanotubes, such as thermal and structural stability and high conductivity, they will be used for this thesis. Reduced graphene oxides and graphene are preferred due to their large surface area and electrical properties. The developed biosensor system has been characterized using various techniques and the improved analysing results have been proven.

1.4. Nanotechnology

1.4.1. The Terms of Nanotechnology and Nanomaterials

Despite being a large-scale field, nanoscience emerged with the discovery of nanomaterials. Nanotechnology studies nanostructured materials' synthesis, characterization, discovery, and application [11]. The concepts that gave rise to nanotechnology were first presented in 1959 by renowned physicist Richard Feynman in his presentation "There's Plenty of Room at the Bottom," in which he proposed the possibility of synthesis through direct manipulation of atoms. The term "nanotechnology" was invented by Norio Taniguchi in 1974, but it was not generally understood at the time. In his 1986 book Engines of Creation: The Coming Era of Nanotechnology, K. Eric Drexler suggested the idea of a nanoscale "assembler" capable of producing a copy of itself as well as other items of moderate complexity with atomic control, which has been inspired by Feynman's ideas [12–14]. As of the 1980s, with the rapid development of the world and the advancement of technology, nanoscience has become a very popular field [11]. Nanotechnology created a wide

range of materials at the nanoscale level. Nanoparticles (NPs) are a wide class of materials that comprise particulate compounds with at least one dimension of less than 100 nm [15].

Surface science, organic chemistry, molecular biology, semiconductor physics, energy storage, engineering, microfabrication, and molecular engineering are all applications of nanotechnology [16].

Even though nanomaterials have a wide range of applications due to their small size, they have recently sparked widespread interest in response to human needs. Nanomaterials, which necessitate knowledge of multiple disciplines such as physics, chemistry, electronics, computer science, biology, engineering, agriculture, and medicine, have resulted in the development of new and multifunctional nanotechnologies. Because of their specific physical, chemical, mechanical, and electrical properties, nanomaterials are used in a variety of applications, including health, food, security, transportation, sensors, and information technology [17].

1.4.2. Classification of Nanomaterials

Nanomaterials with very small dimensions are divided into several categories based on their morphology, size, and chemical properties. It can be zero-dimensional, one-dimensional, two-dimensional, or three-dimensional according to its dimensions. Common types of nanomaterials include nanotubes, quantum dots, and fullerenes. Based on their physical and chemical properties, they are divided into carbon-based NMs, organic-based NMs, inorganic-based NMs, and composite-based NMs. The nanomaterials classified in this way are mentioned below [18].

1.4.2.1. Classification of Nanomaterials by Dimensions

Nanomaterials are classified according to their size, and there are four types: zero dimension, one dimension, two dimensions, and three dimensions.

a. Zero-dimensional: In zero-dimensional (0D) nanomaterials, all three dimensions of materials exist on the nanoscale, e.g., NPs such as gold, palladium, platinum,

silver, or quantum dots. NPs can be spherical in size, measuring 1-50 nm. It has been found that some cube and polygon shapes constitute 0D nanomaterials.

b. One-dimensional: One dimension of these nanomaterials is in the range of 1-100 nm, and the other two dimensions can be on a macro scale. One-dimensional (1D) nanomaterials include nanowires, nanofibers, nanorods, and nanotubes. Some metals (Au, Ag, Si, and so on), metal oxides (ZnO, TiO2, CeO2, and so on), quantum dots, and other materials can form 1D nanostructures.

c. Two-dimensional: Two dimensions are on the nanoscale and one is on the macroscale in this class of nanomaterials. Two-dimensional (2D) nanomaterials include nano-thin films, thin-film multilayers, nanosheets, and nanowalls. The area of 2D nanomaterials can be several square micrometers while maintaining the thickness in the nanoscale range.

d. Three-dimensional: There are no dimensions on the nanoscale in three-dimensional (3D) nanomaterials, and all dimensions are on the macroscale. Bulk materials are 3D nanomaterials composed of individual blocks with nanometer scale (1e100 nm) or greater [19].

1.4.2.2. Classification of Nanomaterials by Material

According to their natural materials, nanomaterials are examined in four categories.

a. Carbon Nanomaterials: These nanomaterials consist of carbon content and have different morphologies such as hollow carbon nanospheres or nanofibers, fullerenes, and graphene.

b. Inorganic Nanomaterials: In comparison to organic materials, inorganic nanoparticles are non-toxic, hydrophilic, biocompatible, and very stable. Because of their high cellular absorption capacity, non-immunogenic reaction, and low toxicity, inorganic nanoparticles have gotten a lot of interest as medication or gene delivery vehicles. Physical, chemical, and biological characteristics are considerably different from those of their bulk counterparts. [20]. Metals (Ag, Au, Fe, etc.), metal oxides (TiO_2, MnO_2, SiO_2, etc.), and ceramics belong to inorganic-based nanomaterials. Figure 1 and Figure 2 is describes the organic and inorganic nanomaterials.

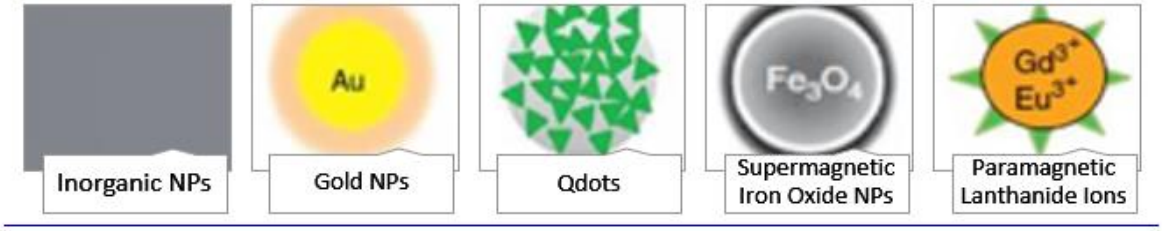

Figure 1. Inorganic nanoparticles which are Gold NP, Qdots, Supermagnetic Iron Oxide NP and Paramagnetic Lanthanide Ions [21].

c. Organic Nanomaterials: Organic-based nanoparticles are made of organic molecules such as polymeric materials, lipids, or carbohydrates.

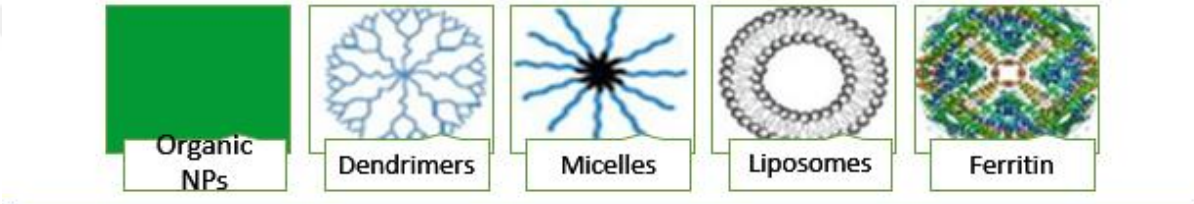

Figure 2. Organic nanoparticles which are Dendrimers, Micelles, Liposomes and Ferritin[21].

d. Composite Nanomaterials: Composite-based nanomaterials are made of two or more layers of nanoparticles which can be a form of carbon-organic, organic-organic, metal-carbon, metal-carbon-polymer, etc.

1.5. Synthesis of Nanostructured Materials

The size, shape, and surface area of the nanomaterial are critical to its function and application. In general, the process used to manufacture the material determines these properties. As such, the synthesis technique is a critical stage in establishing the traits and attributes of the nanomaterial product. Nanoparticle synthesis techniques are often classified as either top-down or bottom-up (as seen as Figure 3). In addition to the synthesis of nanoparticles by physical and chemical methods, biological synthesis and hybrid synthesis applied with enzymes, polymers, etc. are also available today. Sections 1.51 and 1.5.2 will go through these terminologies in further detail [19, 22].

1.5.1. Bottom–up approach

This method is a technology used in producing of nanostructured materials in which nanoparticles are formed from atoms and molecules; that is, upgrading from the original structural elements to nanoscale dimensions. Different types of synthesis processes are applied to bottom-up method such as chemical vapor deposition (CVD), sol-gel, hydrothermal/solvothermal, etc [23].

1.5.2. Top–down approach

Top–down approach: In this technology, nanostructured materials are produced in which nanoscale particles are obtained by grinding larger particles, powders, or solid particles. The macroscopic structure can be externally controlled during the processing of the desired nanostructures in this technique, and bulk material is restructured to convert it into a nanomaterial form; for example, it can be distributed, machined, processed, or precipitated. Ball milling and lithography are the most widely used top-down methods for nanomaterial synthesis [24].

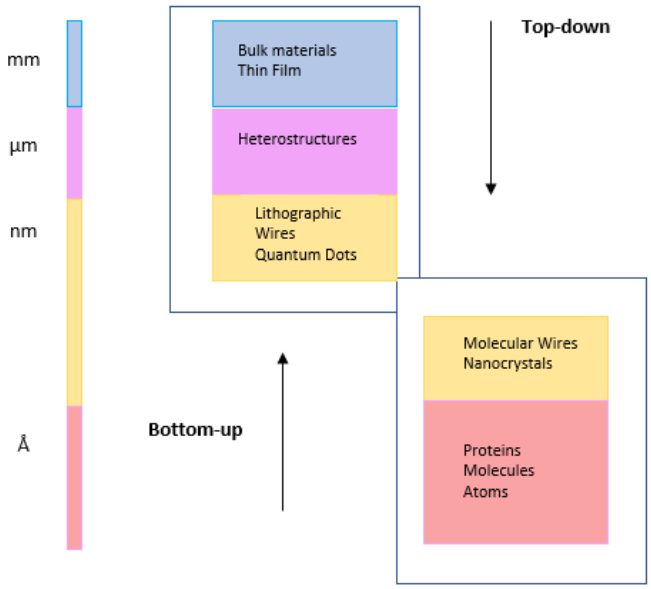

Figure 3. Top-down and Bottom-up approaches which are Nanoparticle synthesis techniques [19].

1.6. Characterization of Nanomaterials

The fundamental of nanoscience lies in seeing nanoscale objects when their size is reduced to nano size. Various microscopy techniques and some spectroscopic and diffraction methods give very important information about the nanoscale structure of the material. Nanomaterials are characterized by these parameters: particle size, size distribution, surface area, surface topography, and surface chemistry [25].

1.6.1. X-ray diffraction (XRD)

One of the most extensively used methods for the characterization of NPs is X-ray diffraction (XRD). X-ray diffraction (XRD) is a non-destructive analytical method that may reveal details about a crystalline substance's lattice structure, such as unit cell sizes, bond angles, chemical composition, and crystallographic structure, in both natural and produced materials. The concept of constructive interference of x-rays is used in XRD, and the sample must be crystalline. The x-rays from a Cathode ray tube are filtered, aligned, and then steered into the sample. Bragg's law, which links the wavelength of incoming light to the diffraction angle and lattice spacing, is employed in the XRD method. When incident monochromatic rays interact with the sample, constructive interference (and a refracted ray) results when Bragg's Law is met. The law takes the following form and in this equation (1.1) [26];

$$n\lambda = 2d\,Sin\theta \qquad (\mathbf{1.1})$$

λ: The wavelength of electromagnetic radiation

θ: Diffraction angle:

d: Lattice spacing in a crystalline sample is determined by scanning the sample through a: 2θ angle arrangement [26].

1.6.2. Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) is an electron microscopy method that uses a focussed beam of electrons to scan the topography of a surface and acquire the surface element distribution pattern. A focussed beam of electrons is used to scan the sample

surface, which interacts with the atoms in the sample and produces different signals regarding the surface topography and composition. The detector utilized to create a picture is determined by the electron beam's location on the sample. With SEM, you can get a resolution of more than 10 nm. Studies may be carried out in various settings, including high vacuum, low vacuum, wet environments, and a wide temperature range. Detecting secondary electrons generated by atoms stimulated by electron beams is the most often used scanning electron microscope mode. The number of secondary electrons identified is determined by the sample's topography [26].

1.6.3. Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) is a technique in which a beam of electrons passing through a sample creates an enlarged image or a diffraction pattern. The TEM technique is generally used with specimens thinner than 200 nm. The thinner the sample, the higher the required electron beam acceleration voltage. Resolution up to 0.2 nm can be achieved. In a transmission electron microscope, a high-energy electron beam (100-300 keV) is sent to a very thin transparent test sample (<200 nm) in a system held under vacuum; The electron beam, which interacts with the sample, magnified by electromagnetic lenses is sent to a fluorescent screen, a photographic film layer or a camera, and then an image is taken. TEMs yield significantly higher-resolution images than light microscopes. TEM has many applications in physical, chemical, and biological sciences, cancer research, materials science, environmental pollution, and nanotechnology studies [26].

1.7. Applications of Nanoparticles

Nanoparticles with exceptional properties such as vast surface area, mechanical strength, optical activity, and chemical reactivity are ideal candidates for a wide range of applications. These materials are being researched continuously for applications in physics, medicine, biomedicine, and chemistry, to developed miniaturized technologies. Nanomaterials can be used in a wide range from medical to electronics, from packaging to the energy sector. Examples of the use of nanomaterials in these areas are given in Figure 4 below [19, 27].

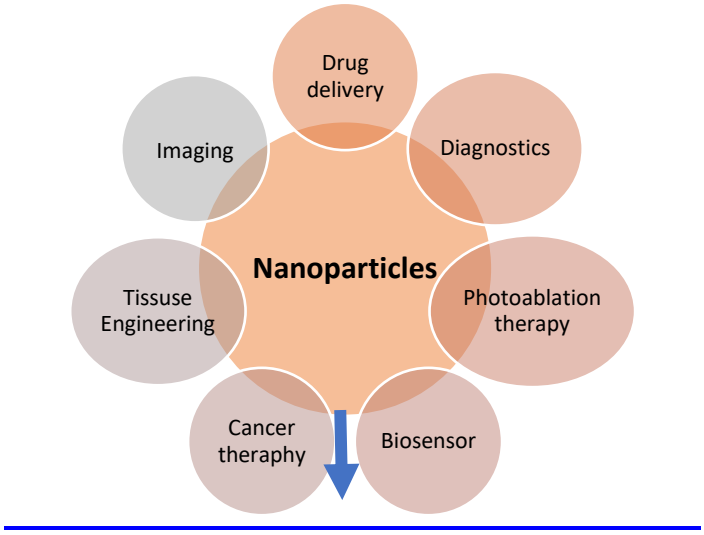

Figure 4. Nanoparticles are used in a variety of applications, including therapeutics, diagnostics, imaging, drug delivery, and tissue engineering [27].

1.8. Biosensor Applications

As nanomaterials are used as signal transducer components to indicate the identification of the analyte, applications of biosensor systems show enormous potential for detecting of chemicals and biomarkers. Detection techniques have improved significantly since introducing different nanomaterials and their unique properties. The application of various nanomaterials to biosensors has provided significant advances in biomolecule identification. Using nanomaterials in biosensors that can diagnose various diseases and chemicals is very important in sensitivity and selectivity. Through the modification of nanomaterials with biosensors, groundbreaking methods for early detection and intervention will be developed [28].

1.8.1. Biosensors

Biosensors are electronic devices that combine biological sensing material with a chemical or physical transducer to transform a signal into a quantifiable and accessible electrical signal. Biosensors are frequently used in a wide variety of fields, from medicine to food. Figure 5 depicts the general mechanism of action of a biosensor. A biosensing substance must be present in biosensors, which must recognize the chemical or analyte of importance. In addition, the biosensing substance must be near

a transducer, which can electrically translate and detect incoming signals [9]. Various compounds, like enzymes, antibodies, and whole-cell, are used to catch particular biomolecules [29]. Depending on the type of transducer utilized for signal processing and evaluation, biosensors are classified as optical, thermometric, electrochemical, or piezoelectric [30].

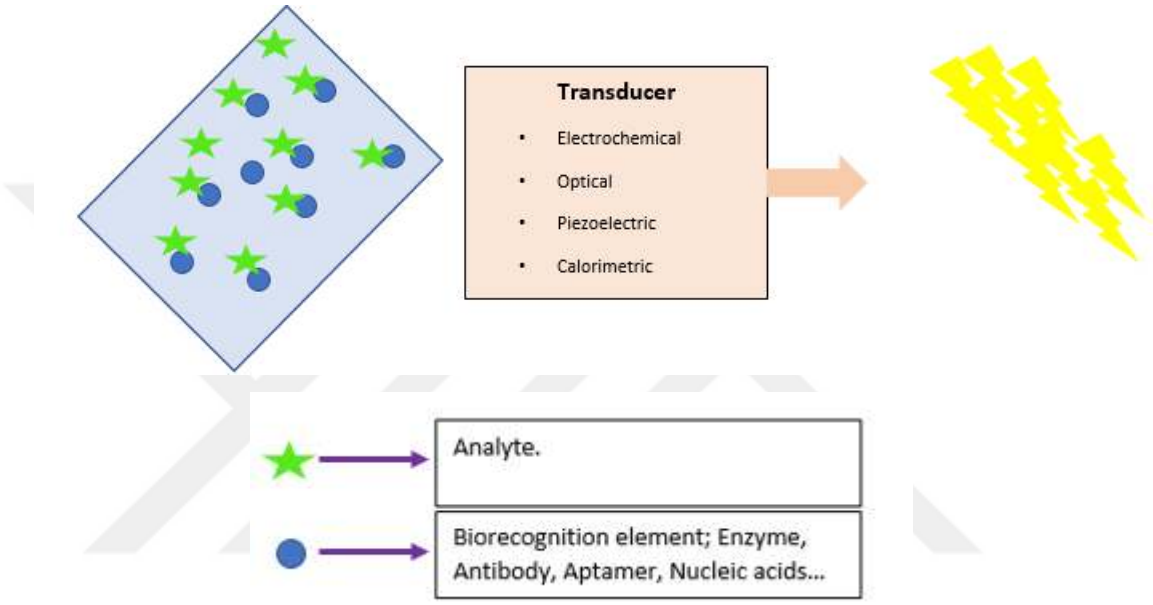

Figure 5. The basic structure of a biosensor.

1.8.2. Classification of Biosensors

Biosensor applications include biosensing materials, transducer devices and immobilization methods, which are developed as a result of multidisciplinary research in the fields of biology, chemistry and engineering. Therefore, biosensors can be classified in various ways based on the biosensing materials and the application of conversion tools. The classification of biosensor systems is shown in Figure 6 and the types of biosensors are identified in the next chapters.

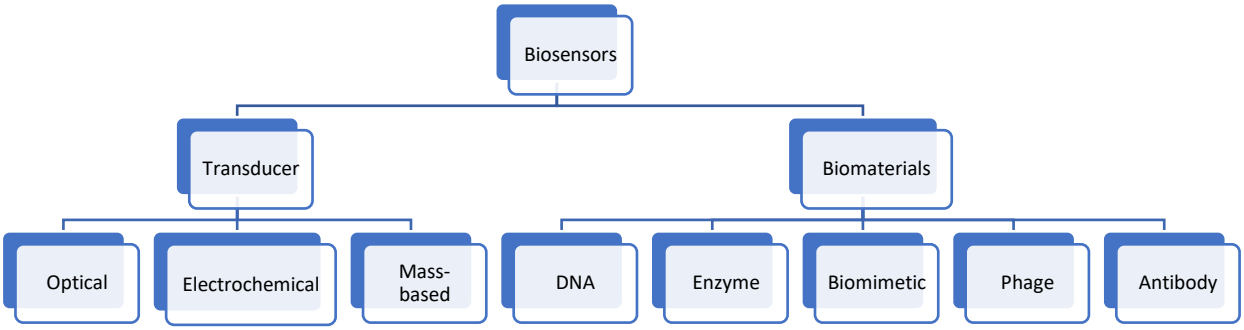

Figure 6. Classification of biosesensors.

1.8.3. Classification according to Biomaterials

1.8.3.1. Enzyme- based Biosensors

Enzymes, catalysts of biological systems, are known for their enormous catalytic power to speed up reactions by a factor of at least a million and for their high specificity in both the catalyzed reaction and reactant selection. Enzymes are the most widely used biosensing material in biosensors [9].

Enzymes accelerate reactions by stabilizing their transition state, and the first step in enzymatic catalysis is forming an enzyme-substrate complex. The mechanism of enzyme catalysis is as follows:

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad \textbf{(1.2)}$$

where;

S: Substrate

E: Enzyme

ES: Enzyme-substrate complex

P: Product

k_1: Rate of enzyme-substrate complex formation

k_{-1}: Rate of enzyme-substrate complex dissociation

k_2: Rate of the dissociation of the enzyme-substrate complex to products

Enzymatic biosensors are less stable, have lower signal intensity, are more expensive, and in some cases, require connecting the vehicle to a system. These systems, however, can be quite sensitive and selective. Surface modification via recombination or the addition of linkers can immobilize and combine biomolecules with other materials. Enzymatic biosensors are easy to use, sensitive to very low concentrations, and highly sensitive. When combined with NPs, they have great potential for miniaturization and real-time diagnostic capability, in addition to requiring minimal sample preparation and promoting high throughput [28].

The immobilization methods used for enzyme biosensors can be classified into two main groups:(1) adsorption or physical retention of the enzyme via van der Waals forces, ionic bonding or diffusion barriers; and (2) covalent attachment of the enzyme to the converter via reaction between the functional groups of the protein and the support materials. However, there is usually a loss of activity when enzymes are immobilized on the surface of a converter. As proteins, they are denatured by known factors such as temperature, acids, bases, organic solvents, detergents, salts, and others and weaken their catalytic activity. Enzyme sensors are commonly used with amperometric, potentiometric, chemiluminescence, and thermal transducers [9].

Enzymatic biosensors are the most widely studied and commercially advanced for glucose measurement. The original form involves glucose oxidation by oxygen catalyzed by glucose oxidase to give gluconic acid and hydrogen peroxide [31].

Enzymes in close contact with transducers are used in enzymatic biosensors. However, the detection of compounds using biosensors based on enzyme inhibition has recently made significant progress. The quantification of the inhibitor and the measurement of enzymatic activity in the presence and absence of the inhibitor are the underlying principles of biosensors based on enzyme inhibition. Because some drugs are based on the inhibition of key enzymes of biological pathways, while other inhibitors are

considered toxic compounds, biosensors based on enzyme inhibition can be used safely for the detection of many toxic compounds. Guilbault published the first biosensor based on enzyme inhibition in Analytical Chemistry in 1962. Because nerve agents inhibit the enzyme cholinesterase, this biosensor could detect nerve agents by using it as a biocomponent [31].

Typically, the interaction between enzyme and inhibitor is complex. Some key parameters, such as enzyme and substrate concentrations and incubation time, must be optimized for a highly sensitive, inhibition-based enzyme sensor. Furthermore, the type of inhibition that occurs due to the interaction between the enzyme and the toxic compound is important.

There are two types of inhibition: irreversible inhibition and reversible inhibition. The presence of a covalent bond formed between the inhibitor and the active center of the enzyme indicates irreversible inhibition. The enzyme becomes inactive as a result of covalent binding. The term irreversible refers to the fact that the enzyme-inhibitor complex decomposition only results in the destruction, hydrolysis, or oxidation of the enzyme. In the case of reversible inhibition, however, the inhibited enzyme can be restored to its original activity by simply washing with buffer or water. The enzyme-inhibitor relationship can be reversed in this type of inhibition by increasing the substrate or enzyme concentration relative to the inhibitor [31].

Furthermore, 50% of the inhibition is caused by the inhibitor concentration, and Ki is defined as the inhibition constant that measures the enzyme's affinity for the inhibitor [4].

The immobilization of the enzyme improves the biosensor's stability, reduces analysis time, and allows for iterative analysis, which is critical in the case of reversible inhibition. In the literature, various immobilization techniques have been reported. Physical capture, adsorption, covalent bonding, and covalent cross-linking are examples of these techniques.

In the case of reversible inhibition, the most commonly performed procedure is based on measuring enzyme activity in the absence of inhibitor (I0) and in the presence of inhibitör (I1), as shown in Figure 7 [31].

$((I0 - I1)/I0) \times 100$

The biosensor is washed with buffer after inhibition, and the enzyme activity is measured again. The inhibition is reversible if the enzyme restores the initial activity; otherwise, the inhibition is irreversible. In addition, an incubation time (reaction time between enzyme and inhibitor in the absence of substrate) is required in the case of irreversible inhibition (Figure 7)[31].

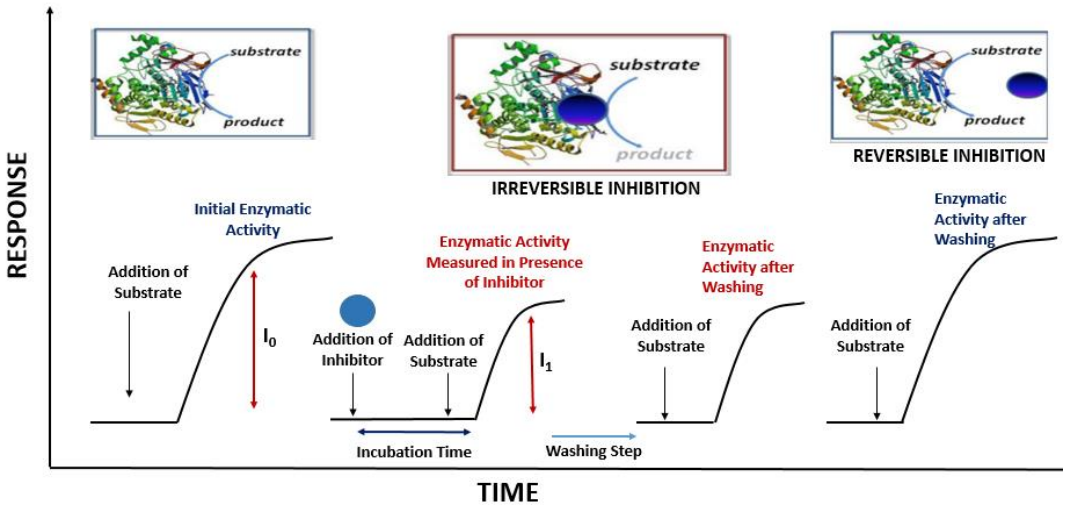

Figure 7. Different protocols for irreversible and reversible inhibitor detection using enzyme inhibition-based biosensors (adapted from Amine et al.) [31].

The cholinesterase enzyme is the gold standard biocomponent in the field of enzyme inhibition-based biosensors, as shown in Figure 7, which can be explained by taking into account various factors:

i) Cholinesterase is characterized by high turnover;

ii) are inhibited by a variety of compounds, including organophosphorus pesticides and nerve agents, and can offer fast and on-site detection for these analytes;

iii) pesticides, which are cholinesterase inhibitors, are widely distributed throughout the environment;

iv) the substrate is soluble in aqueous solution and reasonably priced;

v) Because a class of neurotoxic compounds inhibits enzymes irreversibly, the biosensor can produce a useful neurotoxic index

vi) Because this enzyme is found in insects or humans and is the exact target of organophosphate and carbamate, the bio-component was chosen by developing a bio-inspired biosensor based on natural reactions [31].

Electrochemical transducers, on the other hand, are preferred over other transducers (more than 90% of published biosensors based on enzyme inhibition use electrochemical transducers). This decision is based on several factors, including robustness, cost-effectiveness, miniaturization, and the ability to work in color solutions. Furthermore, the use of screen-printed electrodes makes such sensors suitable for easy measurement in the case of reversible and irreversible inhibitors; in the latter case, the disposable biosensor properties avoid the reactivation step [31].

Several compounds, including organophosphorus and carbamic pesticides, nerve agents, aflatoxins, and heavy metals, have been reported to inhibit acetylcholinesterase-based biosensors. The acetylcholinesterase enzyme catalyzes the following reaction:

$$\text{Acetylcholine} + H_2O \rightarrow \text{Choline} + \text{Acetic Acid} \quad \textbf{(1.3)}$$

and the amount of inhibitor is determined by measuring choline or acetic acid production before and after the inhibitor is exposed to the enzyme [31].

1.8.4. Classification according to Transducers

Transducers used for signal processing and evaluation in biosensors are classified as optical, thermometric, electrochemical, or piezoelectric, depending on the type of transducer.

1.8.4.1. Electrochemical Biosensors

Electrochemical biosensors are the oldest and most advanced biosensors compared to other types. In earlier times, biosensors were in the form of enzyme electrodes developed specifically for clinical glucose analysis. With the advancement of technology, the enzyme-linked immunoelectrochemical (IEC, Immunoelectrochemical) experiment was developed by Heinemann and colleagues to increase the sensitivity of electrochemical biosensors. The influence of the electrical properties of a buffer (caused by the enzymatic reaction or by the Ab-Ag interaction) can be measured by various electrochemical methods. Recent research on electrochemical biosensors has focused on devoloping of electrode designs [9].

1.8.4.2. Amperometric Biosensors

Amperometric biosensors can be categorized according to their current potential relationships with the electrochemical system. When electrochemical cells are subjected to a constant voltage in amperometric sensors, a reduction or oxidation process generates the corresponding current. Amperometric biosensors typically detect the current that flows through an electrochemical electrode that has been coated in a biologically active substance and is concentration-dependent. The oxidation and reduction of electroactive species on an electrode surface is the basis for amperometric conversion [9].

1.8.4.3. Optical Biosensors

Optical methods are among the oldest and most established techniques for sensing biological and chemical analytes. Biosensors have been manufactured using a variety of optical techniques. A typical optical biosensor is a light source that employs a collection of optical components to generate a light beam with specific properties and then employs this light. The steering and switching factor consists of a converted sensing head and a light detector [9].

1.8.4.4. Piezoelectric Biosensors

Piezoelectric biosensors are based on theories developed in electricity, mass, and viscoelasticity and use commercially available instruments such as quartz crystal microbalance. Piezoelectric sensors show superiority over other types of sensors in terms of sensitivity, versatile application, low cost and simplicity, and they are label-free. Piezoelectric biosensors are based on measuring the changes in the resonant frequency of the piezoelectric crystal as a result of the mass change on the crystal surface [9].

1.8.4.5. Thermal Biosensors

Thermal biosensors are also called calorimetric biosensors. They were developed by integrating a biosensing material (enzyme, organelle, microorganism, plant or animal cell, or tissue) with a physical transducer such as a thermometer, thermopile, or thermistor [9].

1.9. Characteristics of Biosensors

Each biosensor has a unique set of static and dynamic properties. The optimization of these features has an impact on the performance of the biosensor.

One of most crucial component of a biosensor is its selectivity. The ability of a bioreceptor to identify a specific analyte in a sample containing various admixtures and pollutants is referred to as selectivity. The most dramatic example of selectivity is the interaction of an antigen and an antibody. The most important factor to consider when designing a biosensor is selectivity.

The reproducibility of a biosensor refers to its ability to produce the same results under identical testing conditions. A biosensor's transducer and electronics are precise and accurate, which defines repeatability. When a sample is tested multiple times, accuracy refers to the sensor's ability to provide a mean value that is close to the true value, whereas precision refers to the sensor's ability to produce identical results each time. When biosensors' signals are reproducible, the inference built on them is very reliable and robust.

The biosensing system's stability describes how sensitive it is to environmental changes both inside and outside of it. These interruptions may cause the output signals of a measuring biosensor to wander. This could impair the biosensor's precision and accuracy, resulting in a concentration measurement error. Stability is the most important factor when a biosensor is required for long incubation times or continuous monitoring. The reactivity of electronics and transducers, which may be temperature-sensitive, could affect the stability of a biosensor. To produce a consistent response, the sensor must be properly tuned. The affinity of the bioreceptor and the degree to which the analyte binds to it may also influence stability. High-affinity bioreceptors, which encourage the analyte's covalent or robust electrostatic attachment, improve the stability of a biosensor. Stability is another factor that influences measurement results. High-affinity bioreceptors, which stimulate the analyte's covalent or robust electrostatic attachment, improve the stability of a biosensor. Another factor influencing measurement stability is bioreceptor degradation over time [32].

The linearity of the biosensor can be influenced by both the resolution of the biosensor and the range of analyte concentrations tested. A biosensor's resolution is the smallest alteration in analyte concentration required to change the biosensor's response. A high resolution may be required depending on the application, because most biosensor applications require not only analyte detection but also monitoring of analyte concentrations over a wide working range. The term "linear range," which is linked to linearity, refers to the concentration range for which the biosensor response varies linearly with concentration [32]. The feature of the biosensor is shown in Figure 8.

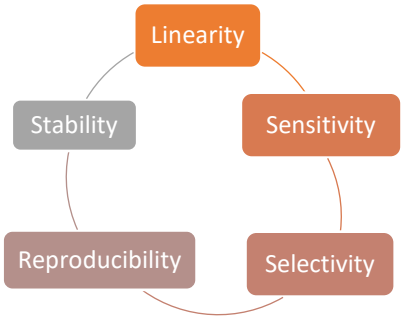

Figure 8. The features of the biosensor.

1.10. Advantages and Disadvantages of Biosensors

There are some advantages and disadvantages of biosensor systems that are frequently used today. The advantages and disadvantages of these systems and many biosensors features are given in Table 1 below.

Table 1. Advantages and disadvantages of biosensor systems.

Advantages	Disadvantages
Ease of use	Quality of results
Transportable	Clinic oriented operators
Not processed samples	Inappropriate and excessive use
Quick results	Price
Small sample volume	Conformity with regulations

1.11. Terrorism Agents

Chemicals, biological agents, radioactive materials, nuclear materials, and explosives all fall under warfare or terrorism agents. These agents can be employed to terrorize large populations of people or to wage war against enemies [33].

Terrorism agent aims to inspire fear, disease, deaths, terrorism, social disruption, or economic loss in communities or individuals through ideological, religious, or political beliefs [34].

The neurotoxins GA (tabun), GB (sarin), and GD (soman) are synthetic substances which are shown in Figure 9. The G-type agents are transparent, flavorless, and odorless liquids that mix with water and the most organic solvents. The most volatile nerve agent is the odorless sarin. Both Tabun and Soman have a faintly fruity or camphor-like aroma. Most nerve agents were initially created to find insecticides, but due to their toxicity, they were considered for use in warfare. Both terrorists and warring parties have employed nerve agents [35].

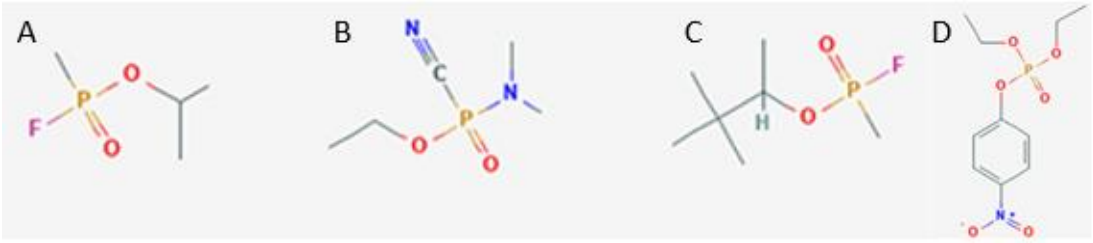

Figure 9. Structure of Organophosphorus Compound. A)Sarin, B) Tabun, C)Soman, D) Paraoxon. The chemical structures of some of the more well-known CWA nerve agents (sarin, tabun, soman) and pesticides that act as paraoxons highlight the diversity of OP compounds [35].

Bacteria, viruses, and microorganisms that cause disease or death in humans, animals, and plants are called biological agents. Biological agents have advantages over chemical agents because they are found in nature. They attract attention as biological weapons due to their infectious capacity, disease-causing effects, and their ability to be used by making various genetic changes if necessary. The expected effect from biological agents may be deadly, but it also reduces the other party's ability to fight or thwart their basic needs [36].

Biological attack, on the other hand, is the use of biological agents on humans and the pursuit of causing an epidemic. The use of biological weapons by professional armies for military purposes can be called biological warfare, and attacks by terrorist organizations targeting civilians can be called bioterrorism [36].

The common feature of biological agents used to infect the target group in biological warfare is that they can reproduce in living things. Ideal biological weapons agents are resistant, easily produced and spread (for example, in aerosol form), have good lung penetration (1-5 μm particles), are highly contagious among humans, are resistant to known antibiotics, and for which vaccination is ineffective. The properties required for an active substance to be a biological warfare agent are as follows:

- Highly contagious microbes or highly toxic substances
- The high degree of morbidity and lethality
- It is easy to distribute the active form over a wide area.

- Ease of mass production and storage
- Resistant to environmental conditions after propagation
- Designed genetically to be resistant to known antibiotics [36].

To create efficient detection strategies against biological agents, intelligence must be sufficiently thorough and precise. Rapid diagnostic equipment that is small, user-friendly, disposable, and quick to use plays a significant role in these detection methods. Biosensor systems have been developed recently thanks to various technological advancements that make it easier and quicker to detect and identify bacteria and the products they produce. Biosensors are biological agent detectors that gather potential biological warfare aerosols and check for their existence utilizing nucleic acid and immune-based identification techniques [37].

The recognition of biological threat agents includes bacteria, viruses, and toxins. Food testing, clinical and environmental samples, and nucleic acid and immune-based methods for identifying bacteria, viruses, antigens, and toxins have all found widespread application. Enhancing these applications to detect and identify biological threat agents makes sense because the basic principles are the same. These applications are distinguished by two major characteristics: (1) the development of a target-specific identification method, and (2) the formulation of an assay to run on the required sample. An enzyme, an antibody, a cell (bacteria or fungus), a tissue slice, a receptor, nucleic acid, or an organelle can all be bioactive ingredients. It is used to identify and interact with the analyte under consideration [37].

CHAPTER 2

METHODS

2.1. Electrochemical Techniques and Electrochemistry

The subject of electrochemistry is the creation of electrical energy as a result of chemical alterations and processes brought on by the movement of an electric current. Electrolytes are substances that can conduct electrical currents. The electrodes are the devices that allow phases to change in an electrolyte environment. Electrodes are frequently referred to as cathode and anode. The cathode is the electrode connected to the (-) pole of the generator and is where the reduction event occurs. The anode, which is where oxidation occurs, is the electrode that is fastened to the generator's (+) pole. Species that give electrons oxidize, whereas electron acceptors decline. An electric current is produced when electrons move over a conductor [38].

Electrochemistry requires a three-electrode setup. It includes a working electrode, a counter electrode, and a reference electrode, the three-electrode system. The reference electrode, which does not conduct any current, acts as a reference for monitoring and correcting the working electrode potential. An electrical effect is applied to the electrode-solution system in electrochemical techniques, and the system's response is measured. More like a stream, this answer gives information about the system's features. All electrochemical techniques have potential, current, and time parameters. These parameters are included in the name of the method. Commonly used electrochemical techniques include Amperometry and Cyclic Voltammetry [38].

2.1.1. Differential Pulse Voltammetry

Differential pulse voltammetry (DPV) is a technique that involves applying amplitude potential pulses over a linear ramp potential. In DPV, a base potential value without faradaic reaction is selected and applied to the electrode. The base potential is increased between pulses in equal increments. The current is measured just before and after the pulse, and the difference between them is recorded [39].

2.1.2. Electrochemical Impedance Spectroscopy (EIS)

By adjusting the alternating current potential frequencies, the electrochemical method known as electrochemical impedance spectroscopy (EIS) may determine the impedance of a system. Using measurements of alternating current impedance with an external electric pulse acting as the excitation force, this approach investigates the electrical characteristics of conductive materials and their interfaces. A technique for figuring out how many new materials or gadgets can restrict the flow of electricity is called electrochemical impedance spectroscopy. To achieve this, an alternating current signal is sent through electrodes connected to the sample. Electric current is measured after applying an alternating current voltage at various frequencies to a sample. Compared to conventional techniques, it involves the concentration of electroactive species, charge transfer, and mass transfer from the bulk solution to the electrode surface. This method is represented by an electrical circuit consisting of resistors, capacitors, or stationary phase elements connected in parallel or series. As a result, EIS can investigate mass, charge, and diffusion processes, intrinsic material properties, or specific processes that can affect the conductivity, resistance, or capacitance of an electrochemical system. Since the resistance observed in DC circuits directly obeys Ohm's Law, impedance differs from resistance. Impedance response is measured using a modest signal excitation [40].

2.1.3. Cyclic Voltammetry (CV)

Electrochemical measurements using potentiodynamic include cyclic voltammetry (CV). The working electrode's potential ramps linearly with time during a cyclic voltammetry experiment. In contrast to linear sweep voltammetry, a CV experiment ramps the working electrode's potential in the opposite direction from the set potential to return to the initial potential. The number of times these potential ramp cycles can be performed is up to you. The working electrode's current is plotted against the applied voltage (i.e. the potential of the working electrode) to produce the cyclic voltammogram trace. In most cases, cyclic voltammetry is employed to investigate the electrochemical characteristics of an analyte in a solution or of a molecule adsorbed onto the electrode [41].

In cyclic phases, as shown in Figure 10, the electrode potential rises linearly against time in cyclic voltammetry (CV). The sweep rate (V/s) of the experiment is the rate at which the voltage changes over time throughout each of these stages. The working electrode and the reference electrode are used to measure the potential, while the counter electrode and working electrode are used to measure the current. hese data's current I and applied potential are displayed (E). The cathodic current will grow throughout this time, at least initially, provided reducible analytes are present in the system, as shown in Figure 10 where a rising reduction potential is supplied during the first forward scan (t0 to t1). After the analyte reaches its reduction potential, the concentration of the reducible analyte will eventually run out, which will cause the cathodic current to drop. If the redox pair is reversible, the reduced analyte will begin to oxidize once more during reverse scanning (t1 to t2), resulting in an anodic current with a different polarity from the one before. The more reversible the redox pair is, the more similar the oxidation peak will be to the reduction peak. Therefore, CV data provides information about redox potentials and electrochemical reaction rates. The Randles-Sevcik equation is generally used in the CV method [41].

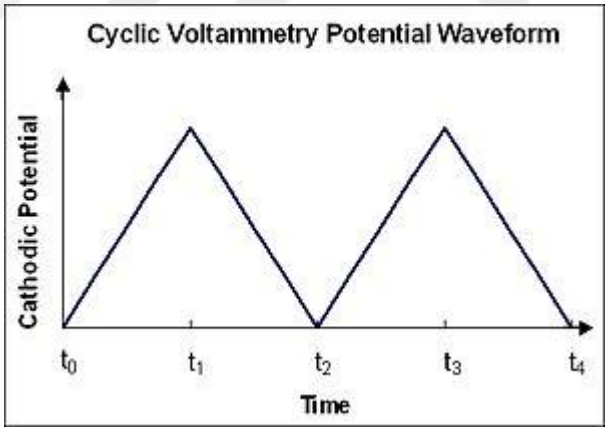

Figure 10. Cyclic Voltammetry waveform [42].

2.1.4. Electrodes

An example of an inert electrode is a screen-printed (SPE) disposable, low-cost, portable device containing a miniature image of the working, reference, and counter electrodes (as shown in Figure 11). These devices are prepared to work with samples with micro volumes of this configuration on conductive substrates such as carbon nano

allotropes (eg, graphite, Graphene) and metals (eg, Au, Ag, Pt). Application areas include electrochemical analysis in the environmental, clinical, and agri-food fields [43]. Screen-printed electrodes (SPEs) appear as a single device with three different electrodes:

Working Electrode: Analyte concentration affects response.
Reference Electrode: A reference electrode, in contrast to the analyte and other ion concentrations, allows the application of a known potential. It has a constant potential and is measured in relation to the working electrode.
Counter Electrode: The auxiliary or counter electrode completes the circuit of the three-electrode cell because it is suitable for current flow. It examines of the processes involved in electronic transfer [44].

The three electrodes can be printed on various surfaces (plastic or ceramic) and researched and developed using a wide range of inks. Silver and carbon inks are the most common, but other metals such as platinum, gold, palladium, or copper can also be used. Screen-printed electrodes have many advantages, including low cost, design flexibility, significant repeatability of the process and the resulting electrodes, ease of manufacture with different materials, and the ability to vary the work surface. Furthermore, screen-printed electrodes do not require tedious cleaning and can be finished simply [45].

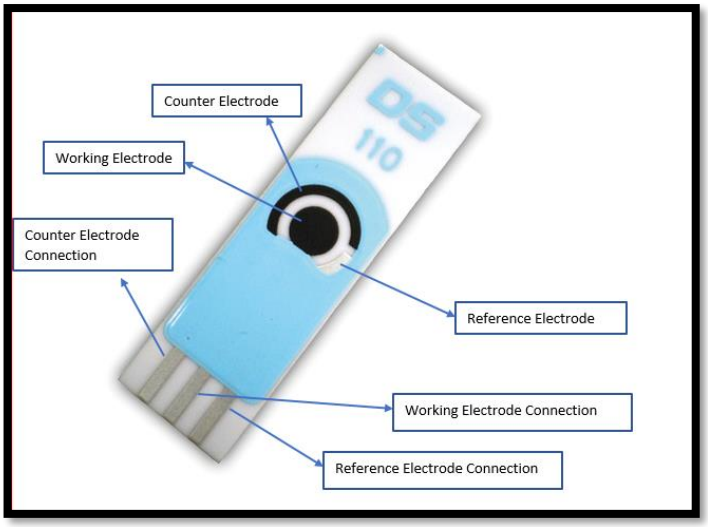

Figure 11. Picture of the screen-printed electrode and its sections.

2.2. Electrode Surface Modification

The modification forms a layer on the surface of the chemical compounds while adhering to the electrode surface. Since the electrodes used in voltammetry have limited working conditions, modified electrodes are created due to changing the chemical or electrochemical properties of the electrodes to improve the working conditions. Unset solid electrode surfaces are unstable and have the disadvantage of differentiation over time. The desired reaction on the glassy carbon electrode surface may be blocked or proceed differently as time passes due to surface oxidation and contamination. To prevent this, the surfaces of the solid electrodes are modified. Chemically modified electrodes are crucial in developing of analytical techniques to selectively and sensitively detect very small ion concentrations [46]. This study used two different techniques to modify nanomaterials on the electrode surface. Drop Casting and electrodeposition are these techniques.

2.2.1 Modification of Carbon-Based Nanomaterials to the Electrode Surface

Various types of nanomaterials are frequently used on electrode surfaces in biosensor designs, as their structure and dimensional effects add new properties to the electrode. Modification of the applied nanomaterials to the electrode surface with their unique properties and nanoscale dimensions creates a nanostructured surface. Carbon nanomaterials have been a type of electrode surface modification that has been widely studied in recent years due to their unique structures, physical and chemical properties, and biocompatibility. It provides a high surface area for many redox-active systems, a wide operating potential window in both aqueous and non-aqueous environments, robust electrocatalytic activity and chemical inertness. Moreover, the surface of carbon nanomaterials can be formed and further described, that is, used as a platform to integrate other modifiers, expanding the variety of uses of the formed electrodes. Carbon nanotubes (CNTs), fullerenes and Graphene are the most widely used carbon nanomaterials that shown in Figure 12.

Improved electrical characteristics, a better broad-sided plane to basal plane ratio, and a quicker rate of electron transfer processes are all displayed by electrodes augmented with carbon nanomaterials. Compared to conventional carbon electrodes, carbon

nanomaterial-based electrodes offer better sensitivity, lower detection limits, and quicker electron transfer kinetics at low concentrations or complex structures. Electrode performance is significantly influenced by surface alterations, electrode bonding techniques, and the presence of electron mediators. These factors make it possible to determine the ideal electrode response circumstances for the target analyte [47].

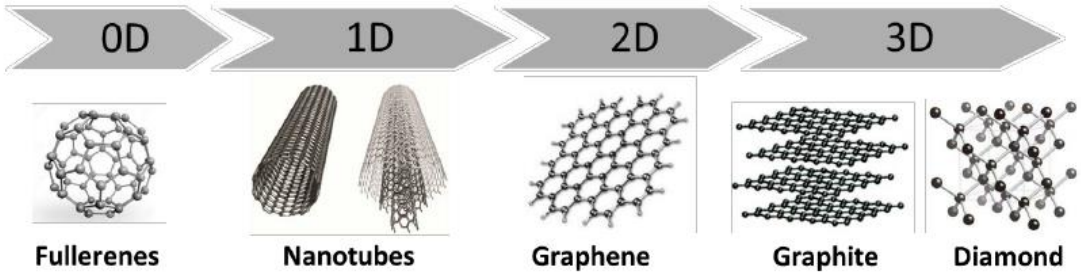

Figure 12. Carbon nanomaterials [48].

- Graphene

Graphene, the most recent allotrope of elemental carbon, is a one-atom-thick layer of hexagonally arranged sp^2-bonded carbon atoms. It is also the world's thinnest material and, in theory, a crucial building component for many other carbon materials. Graphene is employed in various sorts of nanotechnology research because to its exceptional electrical, mechanical, and chemical capabilities. Graphene can be used in sensors, batteries, supercapacitors, solar and fuel cells, and biotechnology. It can be rolled into 1D CNTs and layered into 3D graphite. In terms of electronic characteristics, graphene is a zero-gap semiconductor with unique electronic properties due to the charge carriers in graphene being specified by a Dirac-like equation. More importantly, graphene has better mechanical, optical, thermal, and electrochemical capabilities than other carbon allotropes such as graphite, diamond, fullerene, and CNTs [49].

Graphene is preferred for electrode surfaces because to its enormous specific surface area, great conductivity, and strong mechanical strength. Graphene is categorized as single-layer, multilayer (2-10 layers), or multilayer based on the number of stacked

layers. Only single or multi-layer topologies preserve the usual features of graphene. Graphene has a vast surface area (2,630 m^2/g for single-layer Graphene) and a small unit size. All graphite forms, including one-dimensional carbon nanotubes and three-dimensional graphite, are made up of zero-dimensional fullerenes. Because of its superior conductivity, large electrochemical potential window, atomic thickness, and low electrical resistance in comparison to well-defined redox peaks, graphene is a particularly useful electroanalytical material. Under cyclic voltammetry (CV) conditions, the peak-to-peak potential separation is limited, indicating rapid electron transfer kinetics and a greater apparent electron transfer rate [10].

Furthermore, when high-density edge plane defect regions are introduced to the graphene surface, the electron transfer rate improves considerably, producing many electrochemically active areas. At the margins of graphene sheets, several oxygenated species may be discovered, which can aid to efficiently connect redox centers of molecules to the electrode and boost molecule adsorption and desorption. The majority of graphene utilized in electrochemistry is derived from graphene oxide that has been chemically, thermally, or electrochemically reduced. The graphene oxide structure is not fully planar due to damage to the sp^2 carbon network. It has several oxygen-containing groups, which can assist the material in becoming more functional [50].

Graphene formed through graphene oxide's chemical/thermal reduction is referred to as chemically reduced graphene oxide. It has a lot of structural defects and functional groups, which makes it ideal for electrochemical applications. Electrochemically reduced graphene oxide outperforms chemically reduced graphene oxide for electrochemical applications. Graphene-modified electrodes are commonly utilized in organic chemical electroanalysis [51].

- Carbon Nanotubes (CNTs)

Carbon nanotubes have been one of the most widely explored nanostructures in recent years. They are only one dimension. They are distinguished from other carbon allotropes by having distinct mechanical and electrical characteristics. Engineering, aerospace, chip fabrication, nanodevices, and electrical nanocircuits are some of the many application fields. Carbon nanotubes' extraordinary qualities, including great

tensile strength and elasticity, chemical, thermal, and structural durability, and high conductivity, have sparked considerable interest. CNTs, also commonly employed in sensors and biosensors, can assist in detecting illnesses, controlling medicine distribution, and process biomaterials [49].

Carbon nanotubes are divided into two types according to their size and structure. Multi-walled carbon nanotubes (MWNTs) are composed of several concentric tubes, whereas single-walled carbon nanotubes (SWNTs) are composed of a single graphite sheet. The double-walled carbon nanotube is a subset of the multi-walled carbon nanotube that comprises only two concentric cylinders [50].

Carbon nanotubes have very large specific areas, high sensitivity, and chemical stability, allowing the fabrication of ultrasensitive tubular sensors. They are also composed of only one nanotube, making them ideal nanomaterials for electrode modification [51].

- Reduced Graphene Oxide (rGO)

Graphene oxide (GO) as a nanomaterial support is currently gaining popularity among researchers due to its structural and electrochemical properties. Although GO can be modified to have many functional groups on the surface capable of binding any biomolecule, the presence of oxide layers on the surface can reduce electrochemical sensing performance in redox activities. As a result, in order to remove the oxide layers, GO must undergo the reduction process to form reduced graphene oxide (rGO). Because of its unique properties, such as high surface area, low production cost, good biocompatibility, superior mechanical flexibility, and high thermal and electrical conductivity, rGO is frequently used in the fields of electrochemical sensing [52].

2.2.2. Drop Casting Method

This is the simplest approach to replacing SPE electrodes. It is applied by dripping on the electrode. The amount of final drop on the electrode is the only parameter that needs to be optimized. Droplet size and concentration of metal Nanoparticles dispersed in the solution can affect this. Sensors created using this technology use one of two

methods: direct modification of selected nanoparticles to the working electrode. This method is easier to implement as there is no functionalization step. The metal nanoparticle solutions used are bismuth, platinum, rhodium, gold, silver, copper and nickel. In these cases, the most important disadvantage is agglomeration. Another method is ex-situ formation of nanoparticle composites and carbon nanomaterials. As the nanoparticles are poured onto the working electrode and dried, they tend to aggregate during drying and give the final material applied to the surface a microparticle-like appearance even if a nano-sized material is spilled. The latter technique is widely used to overcome this challenge as more reproducible nano-sized metallic cores are created [46]. Figure 13 is shown in drop casting technique onto SPE.

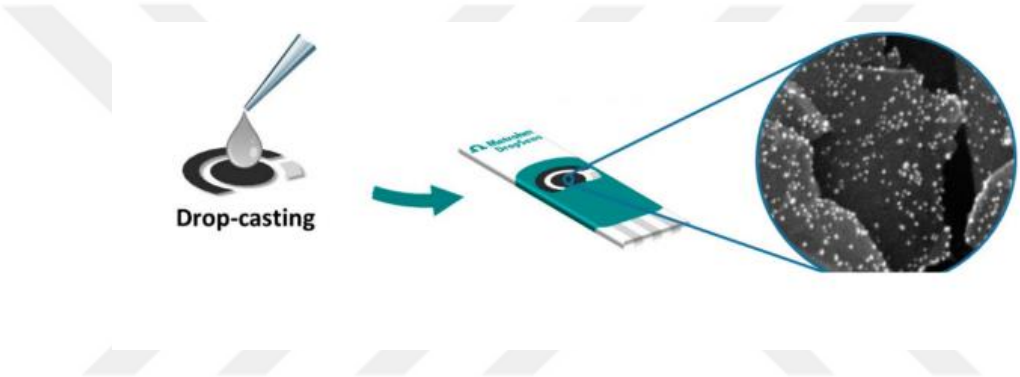

Figure 13. Drop casting on Screen-Printed Electrodes.

2.2.3. Electrodeposition

Electrochemical deposition is the most commonly used method for modifying SPEs with NPs because it allows for precise control of NP morphology. This method produces tailor-made metal particles on conductive substrates by reducing oxidized species, typically metallic water-soluble salts, at a fixed potential or current. Typically, the parameters that are optimized are divided into two groups: those related to the precursor solution, which includes the salt type and concentration, and those related to the electrochemical deposition conditions.

Although larger precursor concentrations allow for larger particles, size and shape are typically electrochemically controlled. As a result, the precursor concentration is usually high enough to have sufficient deposition-sensitive material and is rarely adjusted. The applied potential or current and the deposition time are critical to

controlling the size and structure of growing nanoparticles. It regulates the amount and size of metal on the electrode, resulting in more nanoparticles with more significant particle sizes for a longer period of time. Potentiostat techniques are based on potential changes, while galvanostatic techniques are based on current changes [46]. The basic system of the electrodeposition technique is seen in Figure 14.

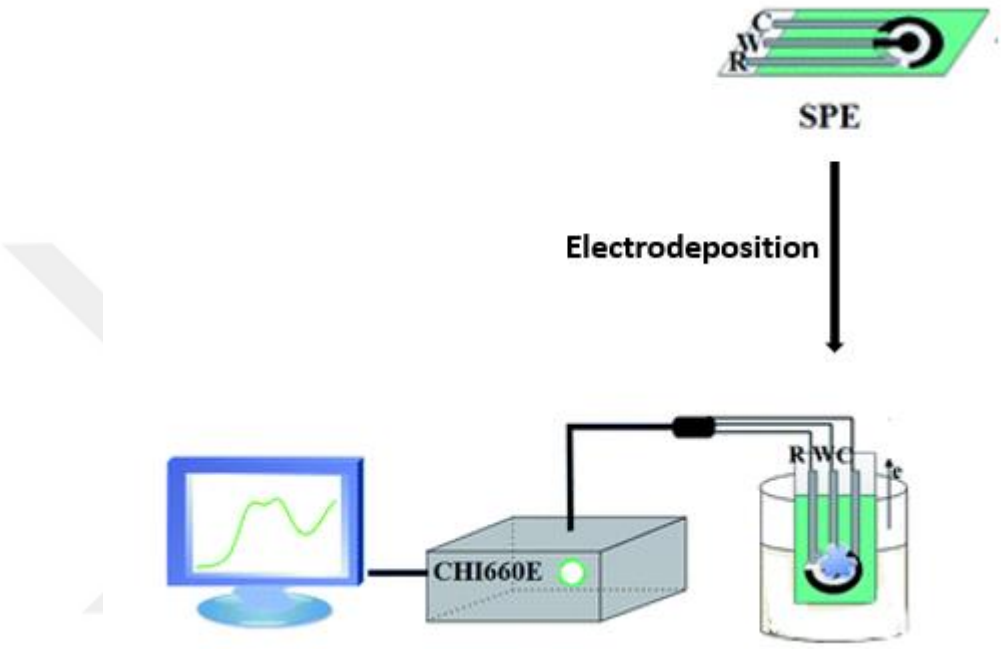

Figure 14. Electrodeposition method applied to the SPE electrode for sensor fabrication [53].

2.2.4. Randles-Sevcik Equation

Electrochemical redox processes may be shown to be reversible or irreversible using the Randles-Sevcik equation. Diffusion coefficients and stoichiometric calculations may be performed using the Randles Sevcik equation (2.1) [54].

$$i_p = k.n^{3/2}.A.\sqrt{D.v.}\ C_{FC} \quad \textbf{(2.1)}$$

- n is the number of electrons,
- A is the electrode area (cm^2),
- D is the analytes diffusion coefficient ($cm^2.s^{-1}$),
- v is the rate at which the potential is swept in this equation ($V\ s^{-1}$) [54].

2.3. Enzyme Immobilization Technique

There are many methods used for immobilization in biosensor systems. In biosensors systems, three main methods can be used as immobilization of biosensing materials, surface immobilization techniques and electrode immobilization techniques.

Biosensing materials need to be connected to a transducer in order to get a biosensor's response signals. Adsorption, micro-encapsulation, entrapment, cross-linking, and covalent bonding are common physical and chemical techniques used to immobilize biosensing materials onto transducers. The characteristics of a transducer's surface, the characteristics of biosensing materials, and the structure of the biosensor all affect the immobilization techniques that may be used [9].

Adsorption is the simplest fixation method with less degradation on biosensing materials. However, the bonding is weak and the service life is short. The surface-attached biosensing material is sensitive to changes in temperature, pH, ionic strength, flow rate, and substrates. Also, the attachment does not support the molecular focusing of biosensing materials for optimal efficiency in chemical conversion [9].

In the entrapment method, the biosensing material is trapped in a gel, paste, or polymer matrix near the transducer surface. Gels include polyacrylamide, starch gel, nylon, and elastic gels. Polymetric materials can impart many functions such as a well-structured skeleton, selective ion permeability, enhanced conductivity, and mediating the electron transfer process. The entrapment method cannot ensure the continuous positioning of macromolecules on the transducer surface, and its lifespan is relatively short, generally only a few weeks [9].

In the cross-linking method, bifunctional agents are generally used to form intermolecular bonds between biomolecules that stabilize the layers and prevent leakage from the reaction layers in fixing the biosensing materials on the transducer. This method does not provide high mechanical strength and may limit substrate diffusion [9].

In the covalent bonding method, chemical bonds are formed between the biosensing material and the transducer surface. Compared to the tack, trap, and crosslink methods, covalent bonding provides a better surface structure that lasts for months [9].

The basis of enzyme immobilization is the retention of enzyme molecules on the surface of a reliable support that is different from the substrate or products in it. Immobilized enzymes have higher activity than dissolved enzymes and can be reused multiple times. A high enzyme-substrate ratio also ensures efficient assimilation and safe use. The intrinsic structure of free enzymes determines their stability, whereas the stability of their immobilized counterparts is highly dependent on several other factors, as shown in Figure 15. These factors are in charge of immobilized enzyme stability at various temperatures and storage conditions. During the immobilization process, experimental variables are expected to increase or decrease.

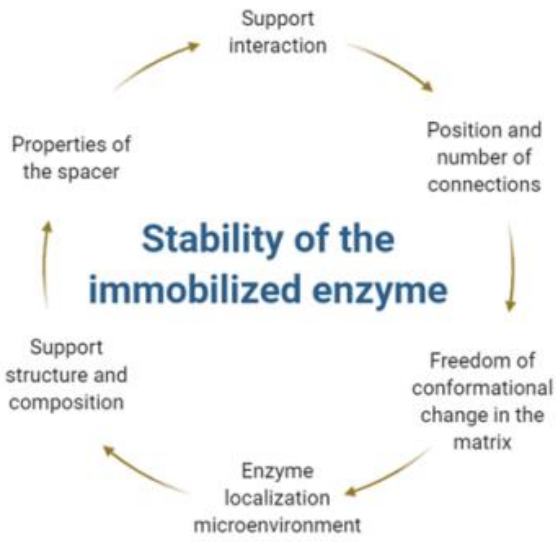

Figure 15. Factors influencing the stability of immobilized enzymes [28].

CHAPTER 3

EXPERIMENTAL

This thesis study includes three different studies: nanomaterial modification, enzyme inhibition, and biosensor system development. Each run has some steps such as electrodeposition and drop casting, enzyme immobilization, biosensor development, and actual sample testing as shown in Figure 16.

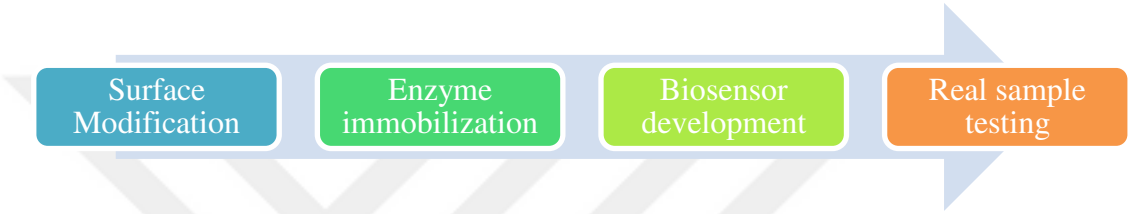

Figure 16. The flow chart for the experimental steps of the thesis.

3.1. Chemicals and Reagents

The chemicals used in the studies are as follows; CNT, reduced Graphene Oxide, Chitosan, Acetylcholinesterase enzyme, Acetylcholine chloride (ACH) enzyme, choline chloride enzyme, KCl, Ksantin and Paraxon were purchased from Sigma Aldrich. Phosphate buffer (PBS) was prepared using di-potassium hydrogen phosphate and potassium dihydrogen phosphate. 5 mM $[Fe(CN)_6]^{3/4}$, which contained 0.1 M KCl solution, was prepared in the laboratory to ascertain the surface activity and conductivity of the electrode.

3.2. Instruments and Electrochemical Methods

Electrochemical techniques which are Electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), and differential pulse voltammetry (DPV) measurements were carried out using an electrochemical workstation CHI-760E (PalmSens BV, Netherlands). Screen printed electrodes (Carbon SPE) were purchased from DropSens (Switzerland). EIS was performed in 0.1 M KCl containing 5 mM $K_3[Fe(CN)_6]$ and 5

mM $K_4[Fe(CN)_6]$ by applying different frequencies of AC impedance from 10 Hz to 1,000,000 Hz with 50 mV of amplitude at a constant potential of 0 V. CV techniques for electrode surface characterization depends on the electroactive surface area were performed by scanning the surface with different scan rates between 10 mv/sec and 250 mV/sec. DPV techniques were performed during the biosensor development and performance optimization steps. Surface electrochemical impedance spectroscopy measurements were also conducted after each modification of the SPE in KCl solution (0.1 M) containing mM $[Fe(CN)_6]^{¾}$,10 mV amplitude, and a frequency range of 0.1–100.000 Hz. The surface conductivity of each SPE electrode's surface after nanomaterial modification was observed and analyzed using this method.

3.3. Preparation of Modified Electrode with Nanomaterials

Surface modifications of carbon SPEs were made by drop casting and electrodeposition techniques using carbon nanotube (CNT), reduced Graphene Oxide and graphene as seen in Figure 17 Step A.

3.3.1. CNTs Modification

0.1 mg/ml of Multi Walled Carbon Nanotube (MWCNT) was mixed with distilled water and sonicated (10 minutes) to make an aqueous solution and provide a homogeneous appearance. The prepared solution was dripped onto the Carbon SPE electrode surface with the help of a dropper and left to dry at room temperature.

The CNT solution, which was converted into an aqueous solution by the Electrodeposition method, a second modification technique, and had a homogeneous appearance, was electrodeposited on the carbon SPE surface for 10 minutes. The electrode, which was modified with CNT for 10 minutes, was then subjected to the electrodeposition method for another 10 minutes and electrodeposition was applied for a total of 20 minutes (10+10). By performing a voltammetric analysis of the applied sample, the effect of time on oxidation-reduction values was observed in the electrodeposition method.

3.3.2. rGO Modification

Different concentrations of rGO were mixed with distilled water and sonicated (10 minutes) to make rGO an aqueous solution and provide a homogeneous appearance. The prepared solution was dripped onto the Carbon SPE electrode surface with the help of a dropper and left to dry in the oven. For rGO's adhering to the dried surface, analysis was made with the CV, EIS techniques and morphology of the surfaces were observed by SEM images.

3.3.3. Graphene Modification

Different concentration of graphene was mixed with distilled water and sonicated (10 minutes) to make graphene an aqueous solution and provide a homogeneous appearance. 10 µL of the prepared aqueous conductive graphene sample was dripped onto the Carbon SPE electrode surface with the help of a dropper and left to dry in the oven. For graphene adhering to the dried surface, voltammetric analysis of the sample was performed after each step.

Aqueous conductive graphene solution was prepared with a stirrer. Using the prepared sample, the electrodeposition method was applied to the blank carbon SPE surface in two steps, 10 cycles and 10+10 cycles, respectively.

3.3.4. Calculations

The experimental portions of this thesis also use the Randles-Sevcik equation, which is employed in cyclic voltammetry to demonstrate the link between peak current (ip) and scan rate (v). This computation utilized the slope value from the peak height-scan rate graphs of reduced graphene oxide (rGO), carbon nanotubes (CNT), and graphene, as well as the concentration amount (0.000005 mol/cm^3) and other constant variables. With this calculation, the active surface area of the electrode was calculated after each carbon-based nanomaterial modification.

$$\text{Slope} = \left(\frac{i}{\sqrt{v}}\right) = \text{k}.\, n^{3/2}.\, \text{A}.\, \sqrt{\text{D}}.\, C_{FC} \quad \textbf{(3.1)}$$

where;

n=1

k= 2.69 x 10^5 $Cmol^{-1}$ $V^{-½}$

D= = 0.16 x 10^{-6} cm^2 s^{-1}

A= Electrode Area (cm^2)

C_{FC}= 0.000005 mol/cm^3

V= 10 mV/sec to 250 mV/sec.

3.3.5. Enzyme Immobilization

Acetylcholinesterase and colin oxidase enzymes, which are purchased ready-made and stored at -20 °C degrees, were prepared in PBS buffer solution in the following ratios: 1 mg/ml acetylcholinesterase in PBS and 5 mg/ml colinoxidase in PBS.

20 µL colin oxidase, 10 µL acetylcholinesterase, and 20 µL chitosan were combined in an empty tube. 15 µL of the prepared enzyme mixture was added to the modified electrode surface and dried in an oven at 35-40 ° C degrees for 20 minutes (as seen Figure 17, Step B). Chitosan ensures the immobilization of enzymes and their attachment to the surface. EIS, DPV analysis of the prepared surface was performed. Enzyme immobilization on the carbon SPE surface is referred to as an entrapment method in the literature.

3.3.6. Biosensor Preparation and Assay Procedure

After enzyme immobilization onto the modified electrode surface 50/100/200/400/600/800 µM acetylcholine chloride was injected to the surface, and DPV analyzes were performed as seen in Figure 17, Step B. As a result of the analysis, the peak height was calculated and a calibration graph was created as shown in Figure 17, Step C. The saturation of the enzyme-immobilized electrode surface determined the optimum level. This is how the optimized concentration of acetylcholine chloride was discovered. Acetylcholine enzymatic reaction is represented in Figure 18.

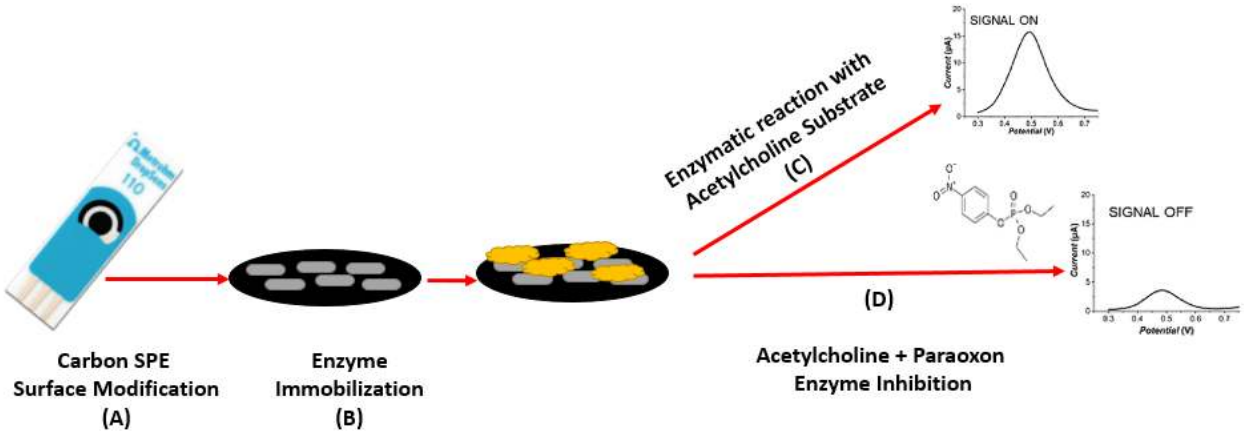

Figure 17. General working principle of the biosensor method including enzyme inhibition-based paraoxon analysis. A is the surface modification, B is enzyme immobilization, C is the calibration graph of the enzyme (acetylcholinechloride) added to the modified electrode surface, and D is the paraoxon calibration graph, which is a terror agent added to the surface of the enzyme-modified electrodes.

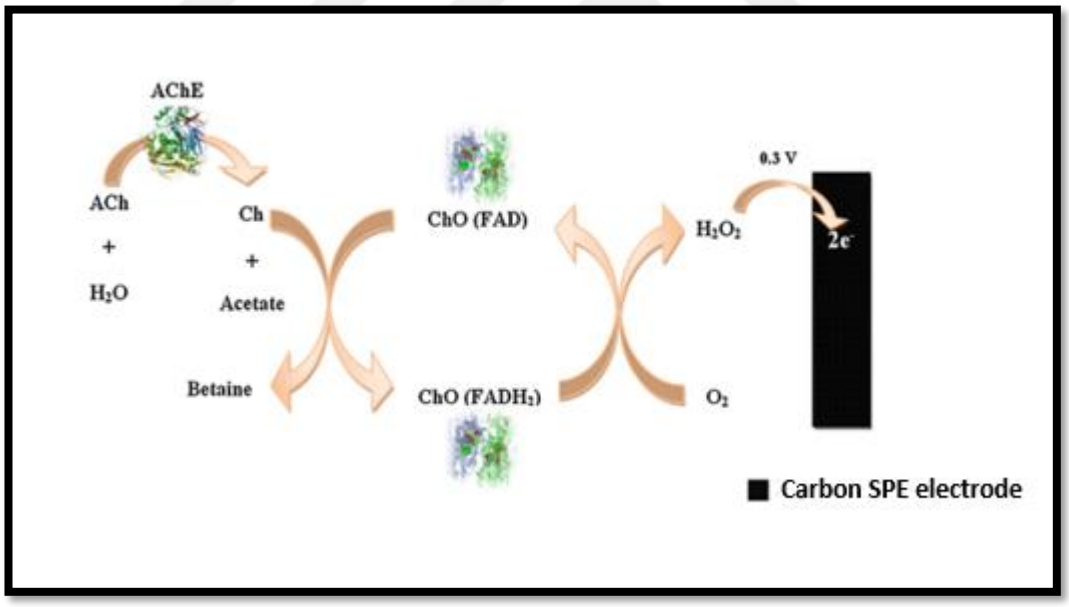

Figure 18. Reaction scheme for the ACh determination [55].

In the literature, many different enzyme sensors have been developed with different techniques. It is notable for detecting of choline, acetylcholine, bacterial toxins, nerve agents, and pesticides. Determination of acetylcholine is important because it plays a role in the detection of terrorist agents. Cholinesterase-based biosensors are effective

and sensitive in monitoring toxicity in environmental, agricultural, defense, and food samples. Organophosphate pesticides, for example, inhibit cholinesterase enzyme activity. Following the decrease in enzyme activity, this inhibition is used in biosensors. Although many biosensors based on cholinesterase inhibition have been reported, electrochemical techniques were used in this thesis. In the equations below, it is explained step by step how acetylcholine is converted to peroxide by electrochemical techniques. A second enzyme, choline oxidase, is used for electrochemical detection. The AChE hydrolysis product choline (Ch) reacts with ChO to form hydrogen peroxide (Eq. (2)). Within a suitable potential, hydrogen peroxide is detected electrochemically (Eq. (3)). The electrochemical AChE-ChO enzyme biosensor is thought to be the most promising AChE-based biosensor due to its potentially high sensitivity, good selectivity, and reproducibility [55].

$$\text{Acetylcholine} + H_2O \xrightarrow{\text{AChE}} \text{Acetate} + \text{Choline} \quad (1)$$

$$\text{Choline} + 2O_2 + H_2O \xrightarrow{\text{ChO}} \text{Betaine} + 2H_2O_2 \quad (2)$$

$$H_2O_2 \xrightarrow{\text{electrode}} O_2 + 2H^+ + 2e^- \quad (3)$$

[55].

After the experiments, paraoxon, a terrorism agent, was added to the surface of enzyme-modified electrodes in different concentrations, and the change of enzyme-substrate pair detected signal was investigated. Different concentrations of paraoxon (2 pg/ml to 100 pg/ml) were tested by the developed biosensor system as shown in Figure 17 Step D, and a calibration curve was observed.

- Signal measurements of reversible inhibition

The degree of inhibition can be used to assess the decrease in enzymatic activity. In the case of reversible inhibition, the most common method is to measure enzyme activity in the absence (I_0) and presence (I_1) of the inhibitor, as shown in the equations.

I_0: Peak height with optimum Acetylcholine concentrations

I_1: Peak height with added paraoxon

ΔI: I_0-I_1

ΔI/I0 (relative signal difference): The data which in used in the biosensor performance optimization and sample testing.

3.3.7. Optimization of the Performance Conditions

3.3.7.1. pH optimization

The pH of immobilized enzymes is an important parameter in their characterization and optimization. The activity of an enzyme attached to any immobilization matrix or the electrode surface in biosensors has a significant impact on the biosensor's performance. The performance of a biosensor based on enzyme inhibition is evaluated by testing different pH values. An enzymatic biosensor for paraoxon detection was developed in this thesis, and the effect of optimum pH was investigated by varying the pH between 6.0 and 8.0.

3.3.7.2. Selectivity

Selectivity is one of the most important properties of a biosensor. Selectivity refers to a bioreceptor's ability to detect a specific analyte in a sample containing other additives and contaminants. The interaction of an antigen and an antibody is the best example of selectivity. Traditionally, antibodies are bioreceptors and immobilized on the transducer's surface. The transducer is then exposed to a solution containing the antigen, where the antibodies only interact with the antigens.

In order to prove the selectivity in the prepared biosensor system, phenol, catechol, gallic acid, and hypoxanthine solutions were prepared together with paraoxon and dropped onto the optimized acetylcholine chloride injected electrode surface. The results of these experiments were compared with the DPV technique.

3.3.7.3. Repeatability

Reproducibility is the biosensor's ability to produce similar responses for the same experimental setup. Traditionally, antibodies are bioreceptors and immobilized on the transducer's surface. Precision refers to the sensor's ability to produce consistent results each time a sample is measured, whereas accuracy refers to the sensor's ability to provide an average value that is close to the true value when a sample is measured

multiple times. Reproducible signals provide high reliability and robustness in predicting a biosensor's response. In order to prove the reproducibility in these experimental studies, 10 different experiments were carried out by adding to optimized nanoparticles and paraoxon. The results were compared with the DPV technique.

3.3.7.4. Stability

The biosensor system modified with fixed paraoxon and optimized nanoparticles were stored at 4 °C for 45 days and tested at regular intervals with different paraoxon concentrations. The stability of the system was determined by comparing the results obtained daily with the results obtained on the first day.

3.3.7.5. Real Sample Testing

The prepared biosensor system was tested with wastewater and tap water samples. Experiments were carried out for polluted water on the nanoparticle-modified carbon electrode surface. Paraoxon 5 pg/ml, 20 pg/ml, and 80 pg/ml were added to the dirty water, and dropped onto the electrode surface. The results were analyzed by the DPV method. For tap water used as another example, 5 pg/ml, 20pg/ml, and 80 pg/ml paraoxon were added to the modified carbon electrode surface, respectively.

CHAPTER 4

RESULTS and DISCUSSION

4.1. Surface Modification and Characterization

The cyclic voltammetry (CV) technique was preferred to analyze the charge transfer mechanisms and surface characterization of electrodes modified using two different techniques with CNT, Graphene, and rGO prepared under optimum conditions. The CV technique is required to observe the changes in surface area conductivity after the treatments and the change in peak height values due to voltammetric analysis. An unused (blank) carbon SPE electrode analysis was performed for CV experiments were performed in the presence of 5 mM $[Fe(CN)_6]^{3/4}$ to examine the electron transfer mechanism between the species in solution and the electrode surface. The cyclic voltammetry graph obtained from the volumetric analysis is shown in Figure 19.

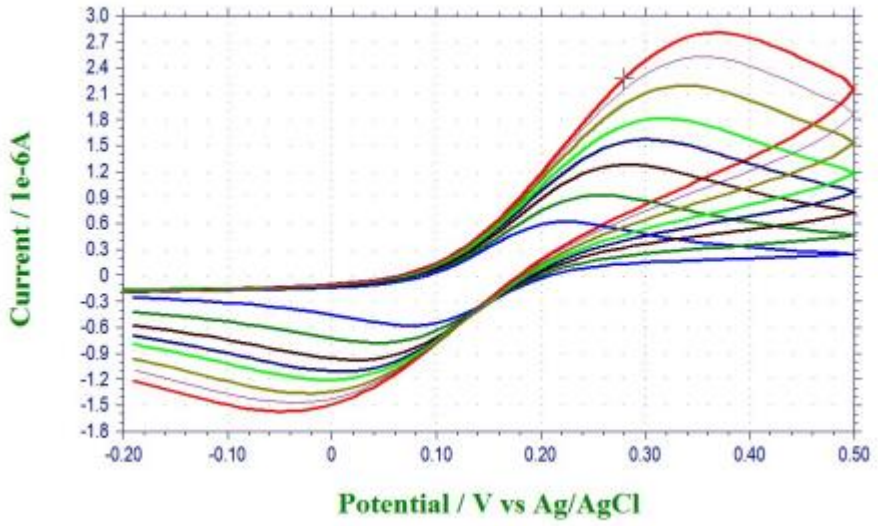

Figure 19. Cyclic voltammetry graph of Blank carbon SPE electrode with scan rates from 10 mV/sec to 250 mV/sec with 5 mM FeCN solution in 0.1M KCl.

4.1.1. Carbon Nanotubes (CNTs) Modifications

CNT modification was made on the Carbon SPE electrode surface using two different techniques such as drop casting and electrodeposition. Cyclic Voltammetry graphs of surface-modified Carbon SPE electrode with CNT show the electrodeposition method in Figure 20 and the analysis of the drop casting technique in Figure 21. When the results were examined, it was seen that the CNT-modified Carbon electrode with the Drop Casting technique gave a higher peak height than the CNT modified Carbon electrode with the electrodeposition method.

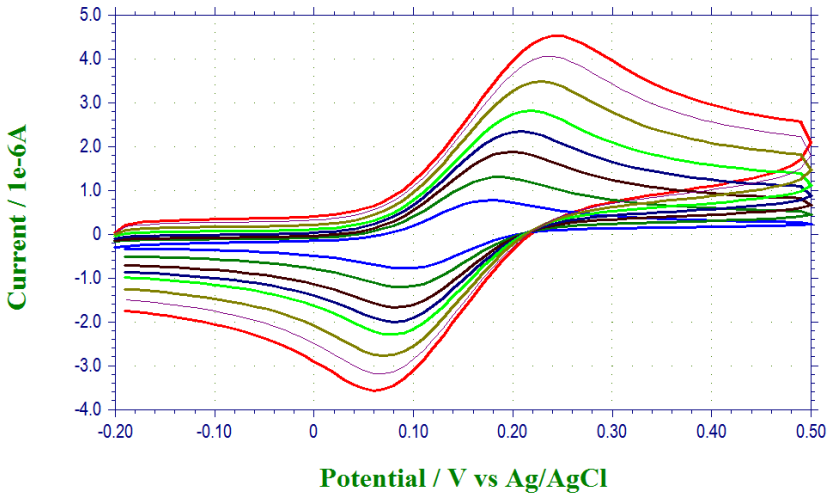

Figure 20. Voltammetry plot of electrode modified with CNTs using electrodeposition technique, with scanning rates from 10 mV/s to 250 mV/s with 5 mM FeCN solution in 0.1 M KCl for 5 min.

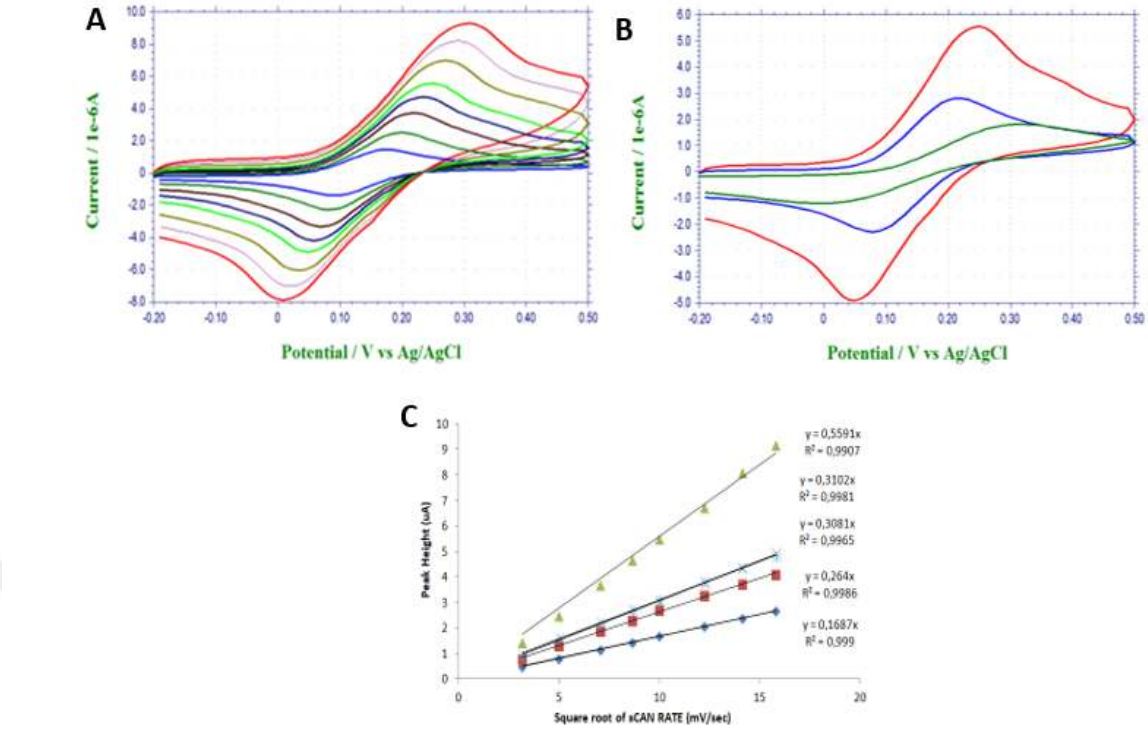

Figure 21. A: Modified electrode containing 0.1 mg/mL CNT with a 5 mM solution of FeCN in 0.1 M KCl with scanning rates of 10 mV/s to 250 mV/s with drop casting technique. B: Peaks of CNTs modified electrodes which have been used by Electrodeposition method and Drop casting method and blank carbon SPE's Cyclic Voltammetry graph were taken in 5 mM FeCN solution in 0.1M KCl at 100mV/sec. (Red peak = modified electrode by drop casting method, blue peak = modified electrode by electrodeposition method, green peak = Empty carbon SPE.) C: Graph of Peak Height (µA)/scan rate square root (mV/s) of blank carbon SPE sample vs. CNT samples. (Green line = Drop casting method with CNTs, Blue line = Electrodeposition method with CNTs total 20 minutes, Red line = Electrodeposition method with CNTs 10 minutes, Dark Blue line = Empty carbon SPE.)

4.1.2. Graphene Modifications

Graphene modification was made on the carbon SPE electrode surface by drop casting and electrodeposition methods. Graphene surface modification was made on the Carbon SPE electrode surface for 20 minutes and 30 minutes using the electrodeposition method. Cyclic voltammetry graphs of the surface-modified samples are given in Figure 22. The graphs show that the obtained peak height value is lower than the sample with 30 min modification.

Cyclic voltammetry graphs of electrodes modified with 1 mg/ml and 2 mg/ml graphene aqueous solution were obtained by the Drop Casting technique. For each sample graph, scan rate values are available in the range of 10 mV/s – 250mV/s. The peak height values of the electrode modified with 2 mg/ml are lower than that of the electrode modified with 1 mg/ml. This may be due to the high density of graphene solution on the electrode surface modified with 2 mg/ml, which makes electron transitions difficult. In addition, the lack of homogeneous distribution on the surface in the modifications with the drop-casting method may cause the formation of undesirable dense regions during the surface density modification. Although this is a disadvantage of the drop-casting technique, the drop-casting technique still produced better results than the electrodeposition technique. The peak height values on the electrode surface modified with 2 mg/ml were lower than the electrode modified with 1 mg/ml. The graphs show that (as seen in Figure 23) the use of m1 g/ml graphene is an ideal amount for SPE surface modification.

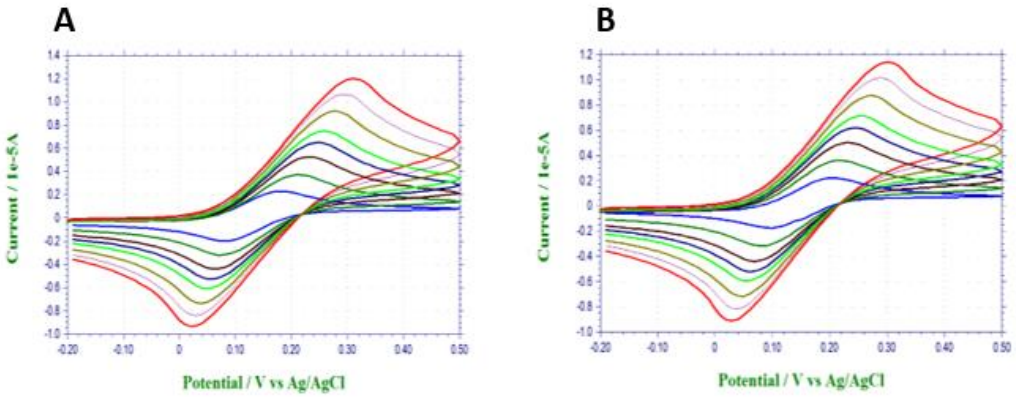

Figure 22. A. The modified electrode with Graphene with electrodeposition technique for 20 min with scan rates from 10 mV/sec to 250 mV/sec with 5 mM FeCN solution in 0.1M KCl. B. The modified electrode with Graphene with electrodeposition technique for 30 min with scan rates from 10 mV/sec to 250 mV/sec with 5 mM FeCN solution in 0.1M KCl.

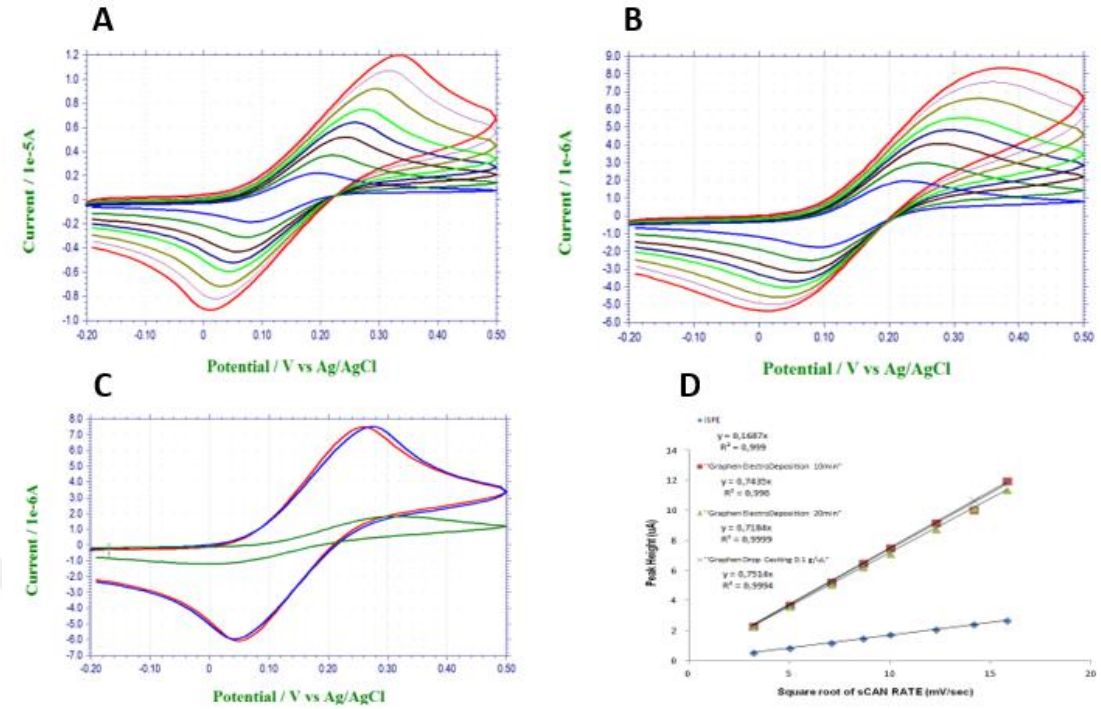

Figure 23. A. Electrode containing 1 mg/ ml graphene with a 5 mM FeCN solution in 0.1 M KCl and 10 mV/sec to 250 mV/sec scanning rates and modified by dropping technique. B. Electrode containing 2 mg/ ml graphene with a 5 mM FeCN solution in 0.1 M KCl and 10 mV/sec to 250 mV/sec scanning rates and modified by dropping technique. C. The peaks were obtained from the electrodeposition method of graphene-modified electrodes and the drop-casting method, and the peaks were obtained from blank carbon SPE in 5 mM FeCN solution in 0.1M KCl at a scanning rate of 100 mV/s. D. Peak Height (μA)/scan rate square root (mV/s) graphs of graphene-modified samples using electrodeposition and drop casting methods at different electrodeposition times and amounts.

The 100 mV/sec scanning speed peaks of the samples whose surface modifications were made with Graphene using electrodeposition and drop-casting methods are also included in Chart C together with the empty carbon SPE. When the graphic results are examined, the peak height values obtained by the Electrodeposition method and the drop-casting method are very close to each other. Therefore, the results to be achieved with both techniques will be similar.

Considering the results of both electrodeposition and drop casting, the samples with surface modification with graphene gave the highest surface area. In addition, the surface area obtained by the drop-casting method for CNT and graphene materials is larger than that obtained from the electrodeposition method. In the drop casting

technique, the surface is more electroactive. That is, experiments with the drop casting technique provided both more surface area and a more electroactive surface. For this reason, experiments were continued with rGO, another Carbon-based nanomaterial, using only the drop casting technique. As shown in the Figure 23, different concentrations of rGO experiments were tested.

4.1.3. Reduced Graphene Oxide Modifications

Surface modification of the carbon SPE electrode with differetn concentrations of reduced graphene oxide was carried out by drop casting technique only.

Cyclic voltammetry graphs of electrodes modified with 1 mg/ml, 2mg/ml, and 3 mg/ml reduced graphene oxide aqueous solution was obtained by the Drop Casting technique. Scan speed values are available for each sample graph in the range of 10 mV/s – 250mV/s. The peak height values of the electrode modified with 2 mg/ml were higher when compared to the other concentrations and the blank electrode. Thus, it can be interpreted that 2 mg/ml rGO is at the optimum level in these thesis studies. In Figure 24, different concentrations of rGO nanomaterials scan rates CV graphs are given.

In areas A and B of Figure 25, the graph of the SPE electrodes modified using the drop-casting method with different concentrations of graphene oxide and the different scanning speeds and peak heights for the empty carbon SPE and the CV graph of these electrodes are given. According to the results derived from this figure, the rGO-modified electrodes provide much higher electron transfer than the blank electrode. The reason for trying different values at different concentrations was to determine the optimum amount of rGO and to continue the experiment processes with the optimum amount. Figure 25- B step Show that, 2 mg/ml rGO gave the highest peak value. For this reason, 2 mg/ml rGO was used in the experiments.

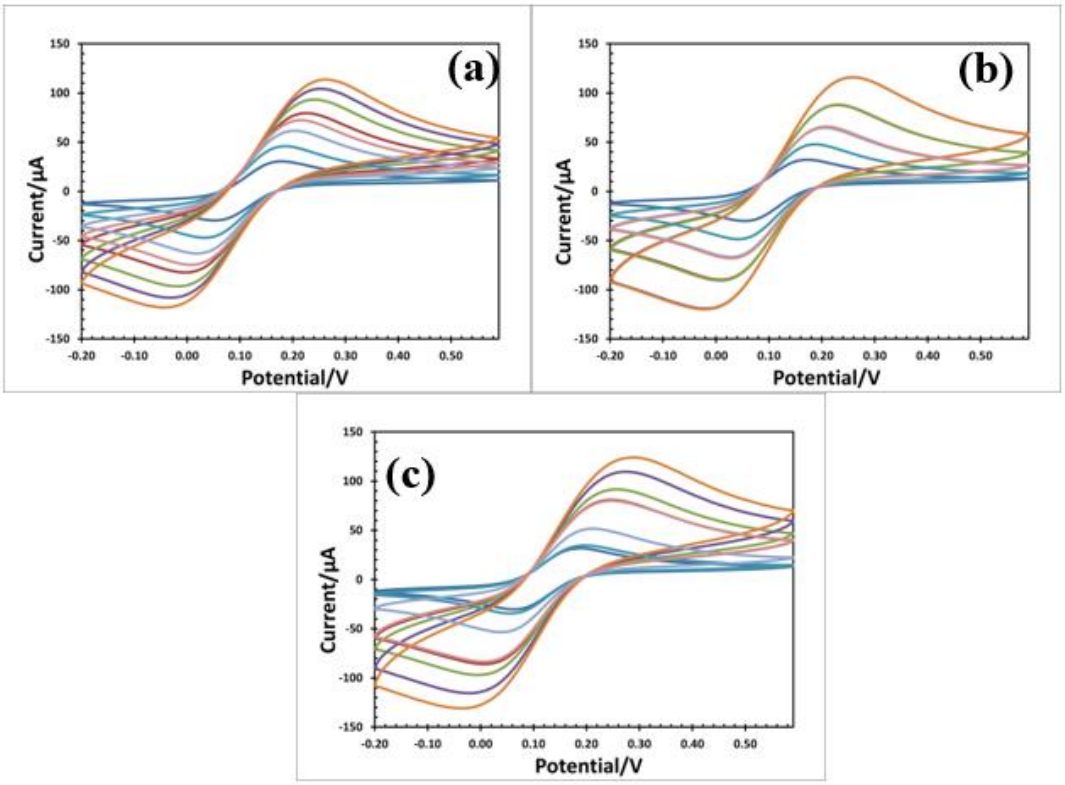

Figure 24. A is the 1 mg/ml rGO added surface, B is the 3 mg/ml rGO added surface, C is the 2 mg/ml rGO added surface.

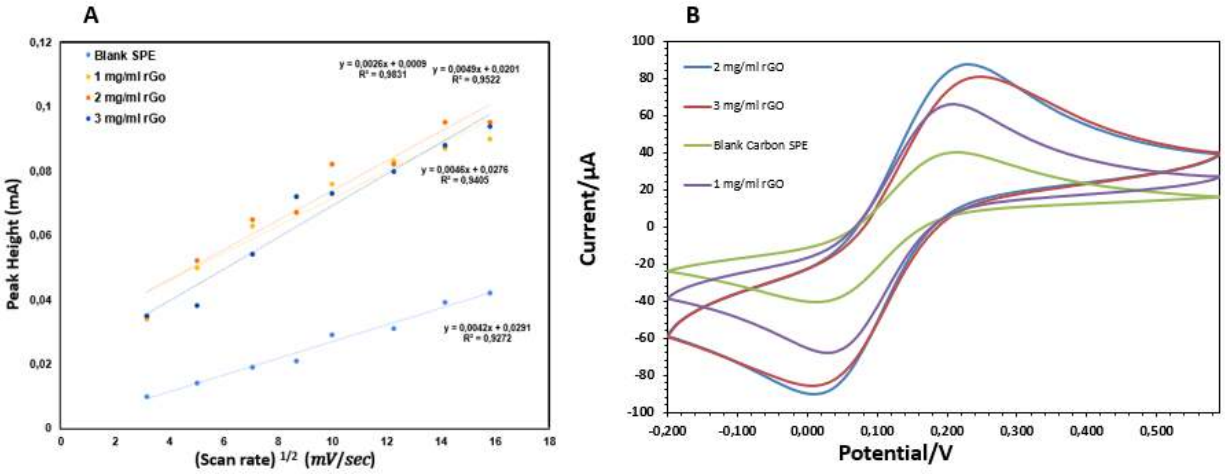

Figure 25. A. The graph of different scan rates and peak heights for different concentrations of reduced Graphene oxide modified electrodes and blank carbon SPE which is used in the drop-casting technique. B. Peaks of modified electrodes with different concentrations of reduced Graphene oxide at 100 mV/sec scan rate obtained from drop casting method and blank carbon SPE with 5 mM FeCN solution in 0.1M KCl.

The calculated surface area values of Carbon SPE electrodes prepared with different nanomaterials at different concentrations and surface modifications prepared with different techniques during the experimental processes of this thesis are given in Table 2. In electrodeposition techniques performed at different times, the surface area covered increases as time increases. When we compare the drop-casting and electrodeposition techniques, a higher surface area was obtained in the experiments performed with the Drop Casting technique on the electrodes with surface modification. When nanomaterials were compared, reduced graphene oxide gave the highest surface area. By looking at Table 2, the following conclusion can be drawn; Electrodes modified using reduced graphene oxide with Drop Casting technique have the highest surface area. As a result, this technique and nanomaterial is the most appropriate method to use in the biosensor system prepared for the thesis.

Table 2. Surface area changes of various nanomaterials were obtained as a result of measurements made at different concentrations and times using different modification methods.

Materials	Modification Method	Surface Area
Blank carbon SPE	-	0.3136 cm²
CNT (10 min)	Electrodeposition	0.4907 cm²
CNT (20 min)	Electrodeposition	0.5726 cm²
CNT	Drop Casting	1.039 cm²
Graphene	Electrodeposition	1.382 cm²
Graphene	Drop Casting	1.2cm²
Reduced Graphene Oxide	Drop Casting	1.425 cm^2

4.2. CHARACTERIZATION

4.2.1. EIS Measurements with Different Nanoparticle Modifications

With the impedance measurement, the surface resistance and, accordingly, the conductivity values were calculated. As seen in Table 3, 233 ohm Rct value was found in the blank electrode. When the electrode surface was modified with CNT, it was observed that the Rct value was less than the empty electrode. Considering the results, since the conductivity value was 1/Resistance, the Rct value decreased and the

conductivity value increased in the blank electrode, CNT, graphene, and rGO modified electrodes. Looking at the graphene and rGO values in the table, the Rct value for rGO decreased to 56 ohms, while the result for graphene was 72 ohms. Considering this, it is interpreted that the lowest Rct value was recorded in the rGO modified electrode, and accordingly, the highest conductivity value was in the rGO modified electrode.

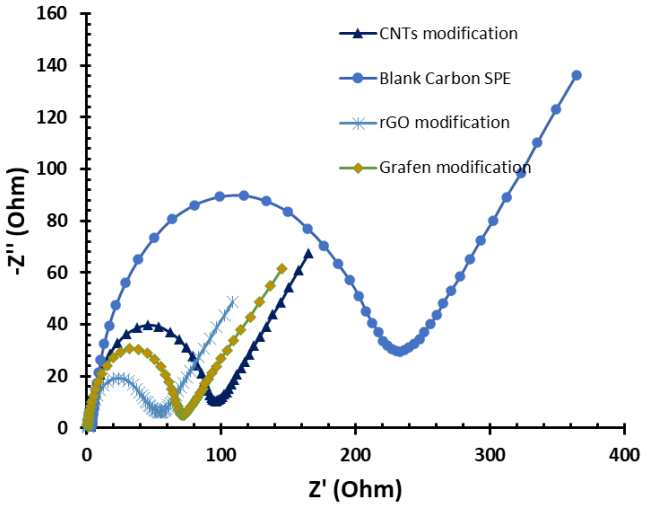

Figure 26. Electrochemical impedance spectra after each nanomaterials modification in KCl solution (0.1 M) containing 5.0 Mm $[Fe(CN)_6]^{3/4}$, open circuit mode, 10 mV amplitude and frequency range of 0.1–100.000 Hz.

Table 3. Rct and conductance values for each nanomaterials modification of SPE electrodes.

Modification	**Rct value (Ohm)**
Blank SPE	233
CNT modification with drop casting	98
Graphene modification with drop casting	72
rGO modification with drop casting	56

4.2.2. Scanning Electron Microscope (SEM)

A scanning electron microscope (SEM) is an electron microscope that uses a focused beam of electrons to scan the sample surface and produce images. Electrons interact with atoms in the sample, producing a variety of signals that carry information about the sample's topography and composition. The surface morphologies and surface homogeneity of the samples were observed using different modifications with the

SEM imaging device in the AYBU central laboratory. Two different scaled images, 5.00 µm and 50.0 µm, were taken at the SEM imaging center.

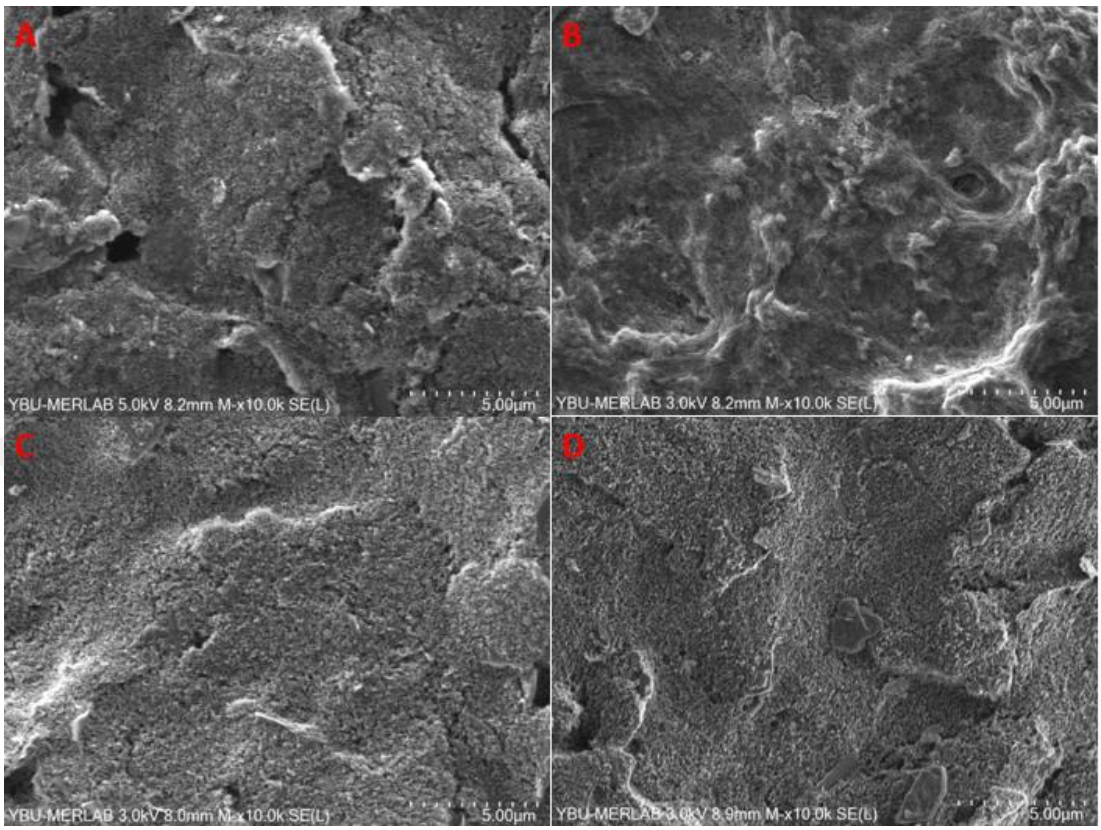

Figure 27. SEM images of carbon-based nanomaterial modified Carbon SPE electrodes by drop casting technique; A) Blank, B) CNT C) Graphene D) rGO

According to SEM images, changes in the surface morphology and homogeneity of the samples with different modifications were observed. As ssen in Figure 27, a more porous structure was observed on the surface of the unmodified (blank) Carbon SPE electrode sample compared to the surfaces of the CNT, Graphene, and rGO-modified samples using drop casting and electrodeposition methods. The surface porosity of the samples with surface modifications appears to be much less. The samples with the highest peak height values, whose surface modifications were provided by electrodeposition and drop-casting methods at different times and amounts, were determined. The best values determined for each sample are shown in the CV graph and the peak-height-square root of the scan rate graph. When the graphs are examined, it is seen that the samples with the highest peak height value are the samples with

surface modification with rGO nanomaterial. The high surface area and high conductivity value of rGO nanomaterial at room temperature make it a preferred nanomaterial for surface modification to increase the conductivity of the Carbon SPE electrode and reduce the impedance value.

During the experiments, the calculated surface area values of the samples prepared with different methods, nanomaterials, quantities and times and surface modifications were calculated. In both methods, the samples with surface modification with rGO gave the highest surface area. In addition, the surface area obtained by the drop-casting method for CNT and graphene materials is larger than the surface area obtained by the electrodeposition method.

4.3. Biosensor Performance Assessment

4.3.1. Enzyme immobilization

As seen in the graph below, DPV analyses were carried out under optimum conditions with the experiments on enzyme immobilization on rGO coated SPE electrodes. As a result of the analysis, the peak height was calculated and a calibration graph was created.

The Nyquist plots of each electrode surface modification in the frequency range of 0.1-100.000 Hz were obtained using $Fe(CN)_6^{3/4}$ redox couple as an electrochemical probe (Figure 28). The Nyquist plots (Figure 28) fit the Randles equivalent circuit model, which is shown in the inset of Figure 28. Figure 28 shows that each nanomaterials modification resulted in a significant decrease in Rct (charge transfer resistance) values, indicating that the nanomaterials were successfully accumulated and surface conductance increased. Considering that there is an inverse ratio between resistance and conductivity, it was concluded that the conductivity of electrode surfaces with a high Rct value is lower. Table 3 represents Rct and SPE surfaces' conductance values after each modification of nanomaterials. As seen from the results rGO modification with the drop-casting method provided the most conductive surface.

While the Rct value was the highest in the blank SPE, the Rct value of the rGO-modified surface was the lowest. When the enzyme is immobilized, the Rct value rises again because a non-electroactive material is added to the surface.

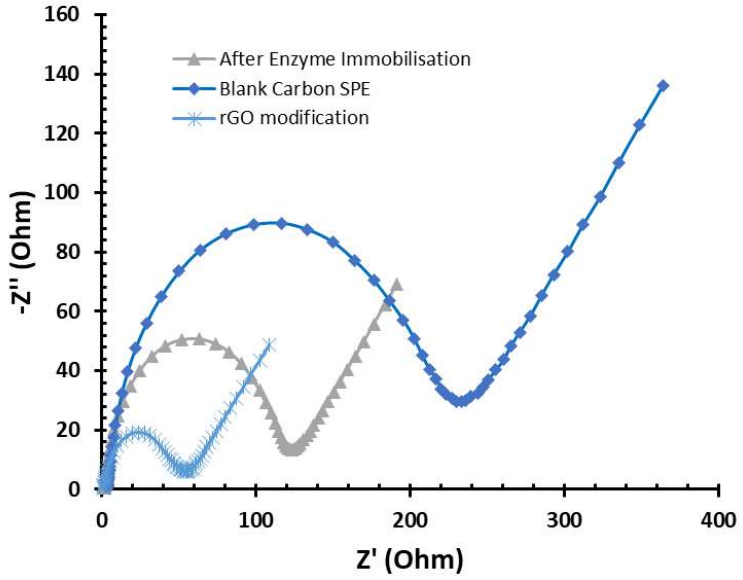

Figure 28. Electrochemical impedance spectra after each nanomaterials modification in KCl solution (0.1 M) containing 5.0 Mm $[Fe(CN)_6]^{3/4}$, open circuit mode, 10 mV amplitude and frequency range of 0.1–100.000 Hz.

Rct values of the blank electrode, rGO-modified electrode, and enzyme-immobilized electrode were compared, as seen in Figure 28. The conductivity of the blank electrode is the least, while the conductivity of the rGO-modified electrode is the highest. Enzymes are non-electroactive molecules. Therefore, after the enzyme immobilization on the surface, the conductivity of the surface decreased.

As seen in Figure 29, there are SEM images of the enzyme-immobilized surface of blank Carbon SPE, rGO coated surface and rGO coated surface with drop casting. According to the results obtained, enzyme immobilization was more strongly attached to the rGO-coated SPE electrode surface with the drop casting technique. This proves that in addition to the unique properties of rGO nanoparticles on the surface, it also gives successful results in enzyme immobilization.

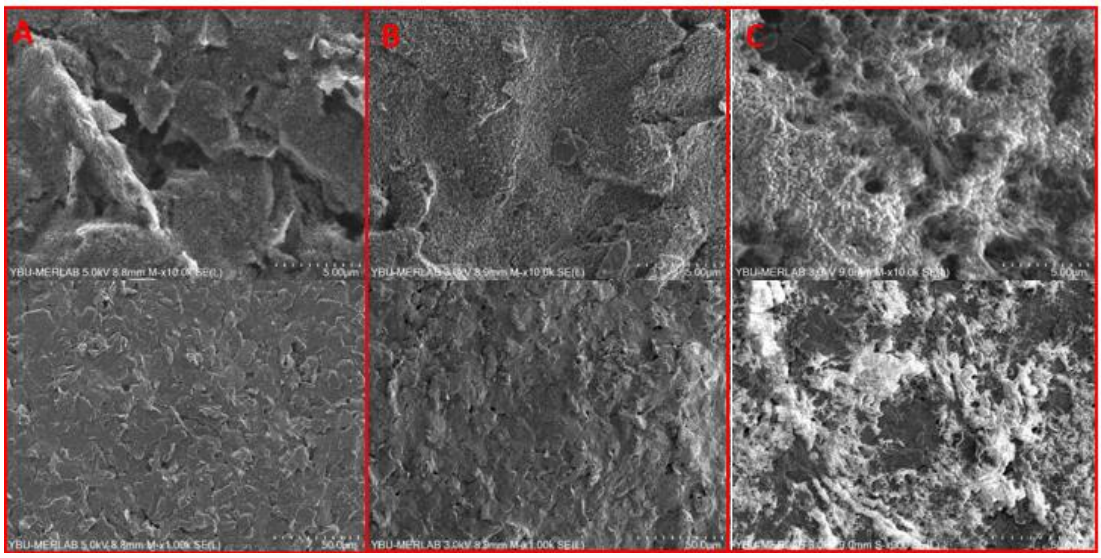

Figure 29. A) SEM image of blank Carbon SPE surface, B) SEM image of Carbon SPE surface coated with rGO, C) SEM image when enzyme immobilization is performed on Carbon SPE surface coated with rGO.

4.3.2. pH optimization

The prepared biosensor system with acetylcholinesterase/rGO/carbon SPE electrode shows an excellent affinity for acetylcholine chloride. Acetylcholinesterase bioactivity was greatly affected by pH. Results from pH optimization experiments for the operation of this biosensor system containing 800 µM acetylcholine are like the graphic below (as seen in Figure 30-A). This system performs its most efficient operation in the ph range of 7.5 (as seen in Figure 30-B).

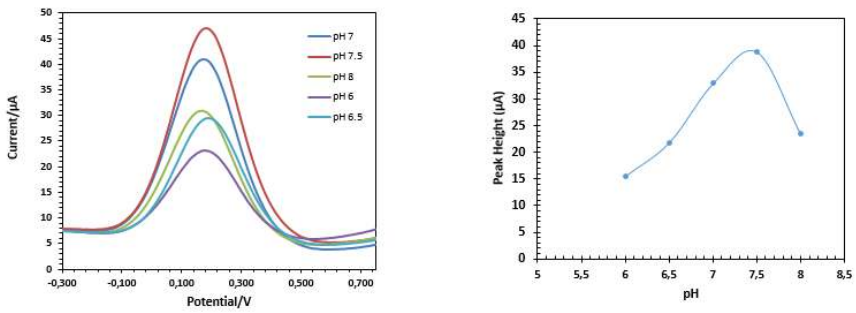

Figure 30. A. DPV measurements at different pH ranges of rGO-modified carbon SPE electrode containing 800 µM acetylcholine. B. Peak height indicating pH determination for optimum operating conditions.

4.3.4. Acetylcholinechloride Calibration Curve

Different concentrations of acetylcholine chloride were added to the electrodes prepared for optimum acetylcholine chloride determination. As seen in Figure 31, it was observed that the biosensor prepared with 800 µM acetylcholine gave better results than other concentrations. For this reason, paraoxon experiments were continued with the optimum 800 µM acetylcholine enzyme.

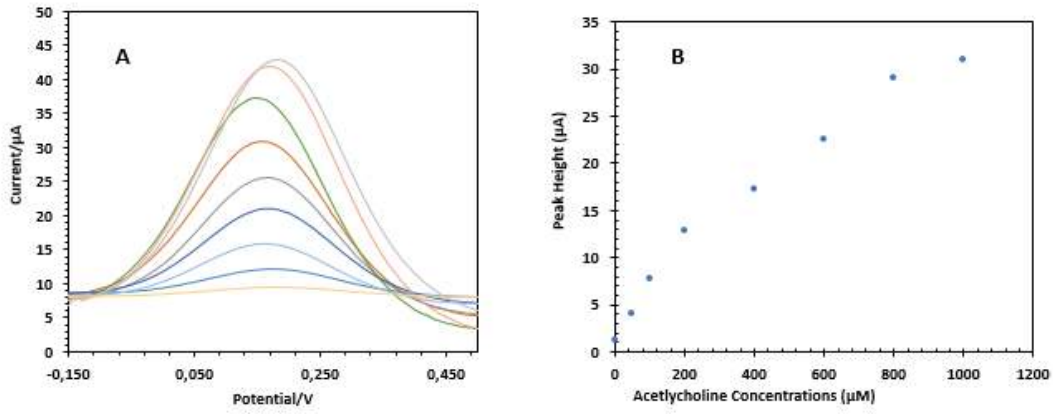

Figure 31. A. DPV measurements made by adding different concentrations of acetylcholine chloride to the prepared electrodes to find the optimum acetylcholine chloride determination. B. Calibration curve showing the optimum Acetylcholine concentration of 800 µM.

Considering the calibration graph, the saturation value of the acetylcholine enzyme added to the modified surface was 800 µM. The peak height started to stabilize after 800 µM (as seen in Figure 31). Thus, it can be interpreted that 800 µM is optimum for the rGO-modified electrode surface. 800 µM acetylcholine enzyme was used for the subsequent paraoxon analysis experiments.

4.3.5. Paraoxon Detection

DPV studies were carried out by adding different concentrations of paraoxon, a terrorism agent, on rGO modified Carbon SPE electrodes prepared with 800 µM acetylcholine (as seen in Figure 32-A.). Considering the DPV results in the tests performed with different concentrations of paraoxon, it was determined that the amount of peak height observed by acetylcholine was decreased with the increase of

paraoxon concentration. Figure 32 was formed by adding different paraoxon concentrations over the optimum acetylcholine concentration (800 µM), and a linear calibration curve of Paraxon was drawn according to Figure 32-B under optimum conditions. According to Figure 32-B internal figure; Paraoxon concentrations from 1 pg/ml to 40 pg/ml added to 800 µM acetylcholine concentration were linear. That is, up to the amount of paraoxon added to 40 pg/ml, the optimum amount of acetylcholine decreased linearly. With the increase in the amount of paraoxon, the existing acetylcholine is depleted. Thus, paraoxon inhibits the acetylcholine chloride enzyme. The limit of detection of developed enzyme inhibition based biosensor system was also calculated as equal to 0.56 pg/ml (S/N=3) by calculating with the 3 times the standard deviation of the signals obtained from the blank standards rule [56].

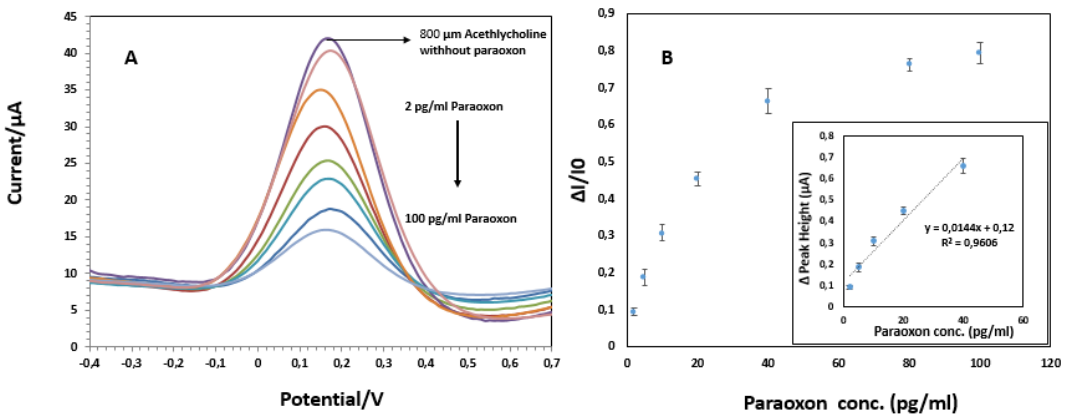

Figure 32. A. DPV results by adding different paraoxon concentrations above the optimum acetylcholine concentration (800 µM). B. Paraxon linear calibration curve under optimum conditions, inner figure; Linear range for paraoxon concentration from 1 pg/ml to 40 pg/ml.

4.3.6. Stability, Repeatability, and Selectivity

4.3.6.1. Selectivity

One of a biosensor's most crucial characteristics is selectivity, which is the capacity of a bioreceptor to identify a specific analyte in a sample that contains other additives and contaminants. The interaction of an antigen and an antibody is the best illustration of

selectivity. Traditionally, antibodies are immobilized on the transducer's surface where they function as bioreceptors. The transducer is then exposed to a solution containing the antigen, where the antibodies only interact with the antigens. Selectivity is the primary factor to be taken into account when selecting bioreceptors for a biosensor.

In the biosensor system, where acetylcholine was added to the rGO electrode, which was developed to detect the chemical paraoxon, which is a terrorist agent, the analyte was the paraoxon chemical, while the acetylcholine chloride solution acted as a bioreceptor. While performing selectivity experiments, gallic acid, phenol, hypoxanthine, and catechol chemicals were used as well as paraoxon. As seen in Figure 33-A, the DPV technique was used to understand how gallic acid, phenol, hypoxanthine, and catechol chemicals can act as bioreceptors in this system. When the results are compared according to Figure 33-B, the electrode containing the electrode is 800 µM acetylcholine chloride target paraoxon, and a maximum of 15% detection can be made from other chemicals. Therefore, it can be interpreted that paraoxon is the target material for this biosensor system and it is the chemical that reduces the amount by interacting with acetylcholine chloride the most. Paraoxon is selective in the biosensor system with acetylcholine added to the rGO electrode.

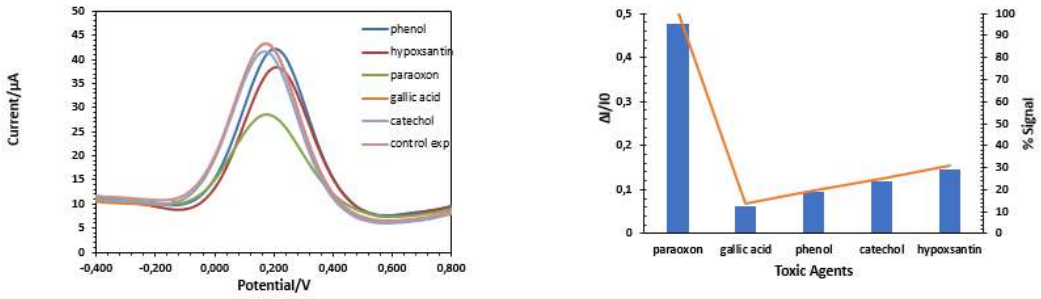

Figure 33. A. DPV results of the biosensor's selectivity with phenol, hypoxanthine, paraoxon, gallic acid, catechol and control experiment. B. Evaluation of the variation of Peak height with %signal for selectivity of different terror agents added under optimum conditions.

4.3.6.2. Stability

The biosensor system prepared with rGO modified, acetylcholine chloride immobilized based on paraoxon detection was stored at 4 °C for 45 days and tested with paraoxon at regular intervals. The stability of the systems was determined by comparing the results obtained depending on the days with the results obtained on the first day. As seen in Figure 34, the stability of the results obtained with the developed biosensor system started to fall below 80% signal after 21 days.

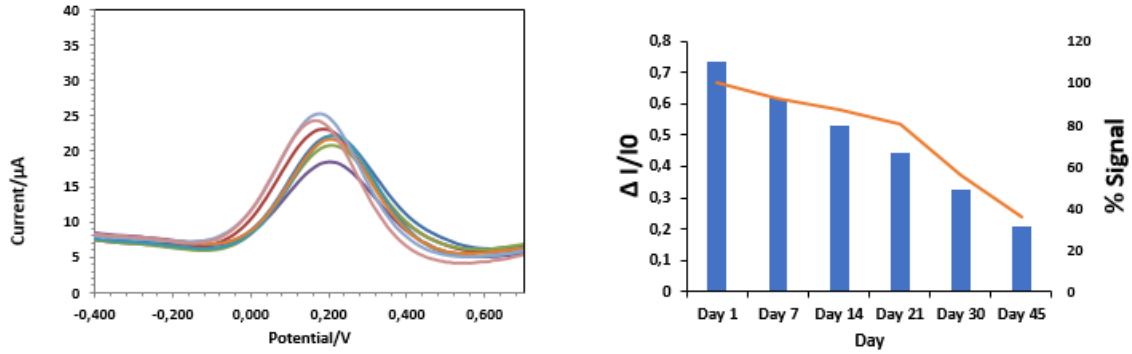

Figure 34. A. DPV results tested under the same conditions but on different days to measure the stability of the same biosensor system. B. Graph showing the varying stability of the biosensor system from day 1 to day 45 under the same conditions.

4.3.6.3. Repeatability

The capacity of a biosensor to produce the same results for an experimental setup that has been replicated is known as repeatability. The precision and accuracy of the transducer and electronics in a biosensor are what define repeatability. When a sample is measured multiple times, accuracy refers to the sensor's capacity to provide an average value that is reasonably close to the true value. Precision is the sensor's capacity to produce similar results each time. High reliability and robustness are provided by repeatability signals when inferring a biosensor's response. In Figure 35, DPV results showed the biosensor's reproducibility by performing the same biosensor trial on the same day.

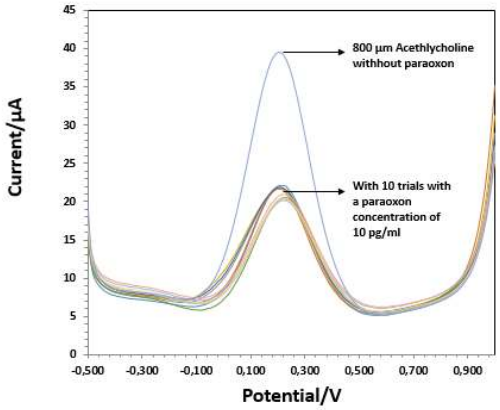

Figure 35. DPV results in which the biosensor's reproducibility was seen by performing the same biosensor trial on the same day.

Ten different trials were made on the same day with the produced biosensor, and a standard deviation calculation was made using the DPV technique results.

After 10 repetitive experiments, 0.05 sd and 5.45% sd values were found for the system. Accordingly, the system can perform reproducible analysis with a 5% margin of error.

4.4. Real Sample Analysis

As a result of the experiments, real sample analyzes were made in the biosensor system with acetylcholine added on the rGO electrode developed to detect the paraoxon chemical prepared. In the system tested with dirty water and tap water samples, paraoxon was added at 5 pg/ml, 20 pg/ml, and 80 pg/ml respectively, and tested with the developed system. In the mentioned biosensor performance test step, DPV measurements were made for each added sample, respectively, and ΔI/I0 values were calculated. The amount of paraoxon in each added sample was calculated using the calibration curve drawn earlier. The amounts of paraoxon added and calculated for each sample were compared and the percentage of recovery values was calculated throught Table 4. Accuracies of all measured samples ranged from 84% to 115%, with cv (Coefficient of Variation) results of 5.6 to 9.5, respectively.

Table 4. Trial results of improved rGO-based enzyme inhibition-based biosensor system in contaminated water and tap water.

	Spiked Paraoxon Concentration	Calculated Paraoxon Concentration	% Recovery	%cv
Tap water	5 pg/ml	4,2	84	5.6
	20 pg/ml	21.2	106	7.5
	80 pg/ml	83.3	104	6.8
1.contaminated water	5 pg/ml	4.3	107	6.8
	20 pg/ml	22.1	110	9.3
	80 pg/ml	84.6	106	8.9
2.contaminated water	5 pg/ml	4.6	115	7.3
	20 pg/ml	22.3	111	9.3
	80 pg/ml	84.6	106	9.5

4.5. Comparison

Studies on biosensor systems that detect nerve agent agents have been carried out for many years. In the table below, nine different studies are analyzed and summarized separately from this thesis. Different limit of detection, Detection range, Repeatability, and Stability values was found in each study. Similarly, real sample tests were also carried out in related studies and detection limits were calculated.

The gold electrode surface of the quartz crystal microbalance (QCM) is covered with AChE immobilized nanofiber in the sensor system that uses QCM to selectively identify and bind the paraoxon molecule. The electrode was examined in the range of 0.1 ppm to 10 ppm paraoxon, and it was observed that the frequency value decreased when the electrode interacted with the paraoxon. When the calibration chart of this study was obtained, the LOD value was found to be 4.57×10^{-8} M. These results show that the developed method can analyze very small amounts of paraoxon samples.

The optical biosensor system designed for detecting organophosphate compounds—which are nerve agents—investigated label-free detection of methyl-parathion (MPT) as an organophosphate pesticide and online monitoring of its binding to the enzyme. In the related study, the detection limit of the biosensor was found to be as low as 2.4 $X10^{-10}$ M for the concentration range of 10^{-10} M to 5x 10^{-5} M for methyl parathion.

This system has a highly reproducible response to pesticides and reactivators with a standard deviation (RSD) of 2.44%. The proposed sensor proposes a new tool for fast, sensitive, cost-effective, real-time screening of organophosphate pesticides.

The basic calorimetric biosensor structure of Detehit was used to provide quick, easy, and reliable detection of cholinesterase inhibitors. Sarin, Soman, Cyclosarin, and VX, four military-grade organophosphorus inhibitors, were used to validate the performance of the biosensor in water and the carbamate inhibitor physostigmine in particular. The Detehit biosensor and other technical detection tools based on the enzymatic method had detection limits for the aforementioned organophosphates of about 0.001 μg/mL. The clear green-white color gradient of the cutting-edge biosensor's main benefit allows for simple visual detection even with the eye open. This is particularly true when waiting for weak or light periods in the field before using the biosensor. Another benefit of the biosensor is its resistance to high ambient temperatures; even with a restriction, it maintains its display after 10 days of exposure to 60 °C. A 25% performance in inhibition was recorded. In a different study, real-time detection of the pesticide methylparathion (MPT) was accomplished using an enzymatic and optical-based system in the presence of acetylthiocholine iodide (ATCh). In the related study, the limit of detection value was found to be 23×10^{-9} M. With this study, low levels of MPT were detected in tap and river water, and the specificity of the sensor against different substances was investigated. Based on the results obtained, the prepared biosensor will be able to give good results in the low-cost, real-time, label-free, and quantitative determination of organophosphate pesticides in real samples.

In another study in the table, the characterization of an economical and easy-to-use carbon paste acetylcholinesterase (AChE) based biosensor to determine the concentration of Paraoxon pesticides was also investigated. Linear calibration curves were obtained for the determination of Paraoxon, which resulted in 0.86 ppb.

Since consuming food contaminated with carbosulfan has harmful effects on human health, another biosensor system for carbosulfan detection has been created. It is a highly sensitive acetylcholinesterase (AChE) biosensor based on a modified platinum (Pt) electrode with zinc oxide (ZnO) nanocuboids. The electrochemical detection of

carbosulfan in the rice sample was carried out using the Pt/ZnO/AChE/Chitosan bioelectrode. Under ideal conditions, the bioelectrode can detect carbosulfan at concentrations between 5 and 30 nM with a limit of detection (LOD) of 0.24 nM. A promising tool for carbosulfan analysis in rice sample, the developed Pt/ZnO/AChE/Chitosan bioelectrode demonstrated good recovery (99.06%-100.96%).

A conductive polymer and silver nanowires were used in a different study to create a biosensor using a novel method. By depositing silver nanowires on polymer-coated surfaces, which increased the charge transfer rate, the studied Biosensor's performance was enhanced. As a result, amperometric sensors that are quick, highly sensitive, and stable have been created to measure the organophosphorus pesticide paraoxon quantitatively. The manufactured biosensor had a low 0.212 μM detection limit.

In this study, a simple, sensitive, fast, and useful biosensor based on aggregation-induced emission (AIE) was developed for the detection of trypsin and organophosphorus pesticides. In the biosensor system, the Detection system is highly sensitive in the range of 0.22 nM detection limit to 0–16 nM linear trypsin concentration. Moreover, the detection platform also showed excellent selectivity towards trypsin. More importantly, based on trypsin inhibition, the Su-TPE/PrS platform is also sensitive to organophosphorus pesticide detection. The commendable performance of this studied biosensor, together with rapid and high-throughput screening of organophosphorus pesticides, will enhance the future development of rapid trypsin detection.

The enzyme acetylcholinesterase (AChE) was immobilized on the Pt/PPy/Chi electrode surface to form a voltammetric biosensor (Pt/PPy/Chi/AChE) in a voltammetric biosensor study designed for the detection of acetylthiocholine (ATCh) and paraoxone. The biosensor's operating stability was 94% after 20 consecutive measurements, and its storage stability was determined to be 72% even after 60 days. The limit of detection (LOD) was established as 0.45 μM for ATCh and 0.17 nM for paraoxon, with the linear range for the biosensor being 30-50 μM for ATCh and 0.46-1.84 nM for paraoxon.

All the biosensor systems mentioned above were compared with the system produced in this thesis. Although the methods used are different, paraoxon was added at different concentrations from low to high in the sensor system studied for this thesis, and the reduction in the presence of acetylcholine enzyme immobilized on the rGO nanoparticle-coated electrode surface was investigated. The limit of detection value is also calculated within this system. The system, which showed recovery with a standard deviation of 5%, continued its experiments for 45 days and it was seen that it showed 85% stability at the end of 15 days. Experiments were also tried on real samples and margins of error were calculated. In real samples, our system can analyze with 5% to 10% error margin and %84 to %115 recovery values.

Table 5. Comparison of different biosensor techniques to detect organophosphorus nerve agents.

Biosensor Method	Detection limit (LOD)	Detection range	Repeatability	Stability	Real Sample testing	Selectivity	Ref
Quartz Crystal Microbalance (QCM) based sensor	4.57 × 10−8 M	0.1 ppm to 10 ppm of paraoxon	5 times with 4.203×10^{-3} %sd	#NA	As response time and performance are very good.	Phenmedipham	[57]
Optical biosensors	2.39×10-10 M	10−10 M to 5×10−5 M	6 times with 2.36%sd	0.05 M phosphate buffer solution at ph 7.4	In situ, real time monitoring of proteins, toxins, DNA, and bacteria	Methyl-parathion (MPT)	[58]
Calorimetric biosensors	0.001 µg/ml	0.012 µg/ml nerve agents	#NA	10 days of storage at 60 °C, %27	Drinking water	Sodium bisulfite and sodium sulfide	[59]
Enzymatic-Optical biosensors	23×10 -9 M	10 nm up to 50 µm MPT	Recoveries ranging from 96.5% to 103%	#NA	1 µm and 10 Mm MPT detection in tap and river water	#NA	[60]
Electrochemical biosensors	0.86 ppb	0-3 ppm paraoxon	Reproducibility was around 7%	Operational stability of about 40 days.	Two times of Spiked tap water	#NA	[61]
Electrochemical biosensors	0.24 nM	5 to 30 nm acarbosulfan	99.06–100.96%	#NA	Three replicates of rice samples	Pt/ZnO/AChE/	[62]
Amperometric biosensors	0.212 uM	1.0 to 6.0 ppb paraoxon	96.3 and 104%	Stability decrease at the end of 25th day.	Milk and tap water	AgNWs	[63]
Fluorescence biosensors	0.22 nM	Trypsin concentration of 0–16 nm	#NA	#NA	#NA	Trypsin and Su-TPE/prs platform	[64]
Voltammetric biosensors	0.45 µM	0.46-1.84 nm paraoxon	#NA	20 consecutive measurements, stability 94.13%. Storage stability was found as 70% even after 60 day	Tap and river waters	Pt/PPy/Chi/AChE	[65]
This work	0.56 pg/ml (2,04 nM)	2-100 pg/ml	10 times with %5	15 days with %85 stability	Tap water, waste water testing with %84-115 recover	More than 85% over other molecules	

CHAPTER 5

CONCLUSION

In this master's thesis, a biosensor system that can detect paraoxon, an organophosphate agent, was developed using Carbon SPE electrodes coated with CNT, graphene, and rGO nanoparticles. Carbon nanomaterials were used to provide electrode surface modification in this study due to their unique structure, physical and chemical properties, and biocompatibility.

In this thesis, CNT, graphene, and rGO nanoparticles were modified on the Carbon SPE electrode surface using drop casting and electrodeposition techniques. After each modification process, the samples were examined with EIS, CV and DPV techniques. When the results were compared, it was seen that the rGO nanoparticles were better coated on the surface. When the rGO nanoparticles were modified on the electrode surface with the drop-casting technique, it was observed that a better and more homogeneous coating was obtained compared to the electrodeposition technique. Then, the acetylcholine enzyme was added to the surface-modified electrodes.

Biosensor performance conditions were investigated under optimum conditions, and the saturation amount of acetylcholine was 800 µM when the pH was 7.5 in the system prepared with the amount of acetylcholine at different concentrations. After reaching the saturation point, Paraoxon chemical, a terrorism agent, was added to the prepared system. Changes in the amount of enzyme on the surface were recorded, and an acetylcholine-paraoxon calibration curve was created. When the amount of paraoxon increased, a linear decrease in acetylcholine electrochemical signal was observed. The limit of detection of developed enzyme inhibition based biosensor system was also calculated as equal to 0.56 pg/ml (S/N=3).

Repeatability, stability, and selectivity studies were also carried out for the experiments. The prepared system can be repeated with a standard deviation of 10%, and even if different terrorism agents come into contact with this sensor, it can be said that other terrorism agents are non-target since paraoxon is the target for this system.

For other terrorism agents (gallic acid, phenol, hypoxanthine, and catechol), this system can detect a maximum of 15%. Instability tests were performed on different days, the system's stability was examined every week, and a decrease in stability was observed after the 15th day.

Dirty water samples were used as real samples, and according to the results, it was decided that real samples could be analyzed 5% to 10% error margin and %84 to %115 recovery values in our system.

The created rGO nanoparticle-based biosensor system will therefore allow for quicker, less expensive, more sensitive, and real-time tests. Our results outperform most in terms of sensitivity, linear detection range, and stability efficiency based on recent studies for the detection of the terrorist agent paraoxon. This enhanced enzymatic biosensor system may also analyze paraoxon detection in real samples using a portable potentiostat and screen-printed electrodes. Therefore, a potential direction for the study in the future is the creation of a lightweight, user-friendly prototype product for actual sample testing.

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