CuS@rGO GRAFTED-PEDOT:PSS PAPER BASED ELECTROCHEMICAL BIOSENSOR FOR FENITROTHION DETECTION

A Project Work Submitted
In Partial Fulfillment of the Requirements for the
Degree of

MASTER OF SCIENCE

in

CHEMISTRY

by

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CANDIDATE DECLARATION

I/We Poornima Bohra (2k22/MSCCHE/24) and Priya (2k22/MSCCHE/27) hereby certify that the work which is being presented in the thesis entitled **“CuS@rGO grafted-PEDOT:PSS paper based electrochemical biosensor for the Fenitrothion detection”** in partial fulfillment of the requirements for the award of the Degree of Master in Science, submitted in the Department of Applied Chemistry, Delhi Technological University is an authentic record of my own work carried out during the period from Aug 2023 to Mar 2024 under the supervision of Prof. D Kumar.

The matter presented in the thesis has not been submitted by us for the award of any other degree of this or any other institute.

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CERTIFICATE

Certified that Poornima Bohra (2k22/MSCCHE/24) and Priya (2k22/MSCCHE/27) has carried out their research work presented in this thesis entitled **“CuS@GO grafted-PEDOT:PSS paper based electrochemical biosensor for Fenitrothion detection”** for the award of Master of Science from the Department of Applied Chemistry, Delhi Technological University, Delhi, under my supervision. The thesis embodies results of original work, and studies are carried out by the students themself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

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ABSTRACT

This study described a novel paper-based biosensor that can quickly and accurately detect fenitrothion pesticides. This work uses CuS@rGO grafted-PEDOT:PSS based paper as a transducer. Strong non-covalent interactions between PEDOT:PSS and the paper's cellulose molecules cause the polymer to alter its conformation, which significantly increases conductivity. To improve the electrochemical characteristics, CuS@rGO/PEDOT:PSS/WP electrodes were further doped with a variety of organic solvents, including methanol, ethylene glycol (EG), dimethyl sulfoxide (DMSO), N, N-dimethyl formamide (DMF), and N-methyl pyrrolidone (NMP). The conducting paper doped with DMSO is determined to have the highest conductivity out of all of these solvents. Then, using glutaraldehyde as a cross-linking agent, the AChE enzyme is attached onto the modified conducting paper strips to speed up the specific identification of fenitrothion. Under a physiological range of 1-80 pM for fenitrothion, electrochemical tests have demonstrated the high sensitivity and low detection limit of this conducting paper-based electrode, which are 0.505 mA/pM and 0.28 pM, respectively. The ITO, gold, and glassy carbon electrodes are known to have limited applications due to their high cost, fragility, limited flexibility, and environmental problems. This paper electrode, on the other hand, may be a very promising substitute. The accuracy of the suggested biosensor was thoroughly assessed in two genuine samples rice and tomato improving its effectiveness for fenitrothion (FNT) detection in real-world applications.

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LIST OF ABBREVIATIONS AND SYMBOLS

FNT- Fenitrothion

ATCl- Acetylcholine Chloride

AChE- Acetylcholinesterase

DMSO- Dimethyl Sulfoxide

DMF- Dimethyl Formamide

EG- Ethylene Glycol

WP- Whatman Paper

NMP- N-Methyl Pyrrolidone

PEDOT:PSS- Poly(3,4-ethylene dioxythiophene):polystyrensulphonate

PANI- Polyaniline

DIW- Deionized Water

PNT- Peptide nanotubes

PGE- Pencil graphite electrode

GCE- Glassy carbon electrode

SPCE- Screen-printed carbon electrode

MWCNT- Multiwall carbon nanotubes

NbC- Niobium carbide

rGO-WC NPHs- reduced graphene oxide-tungsten carbide nano-polyhedrons

$CoFe_2O_4$NPs- Cobalt ferrite nanoparticles

ITO- Indium tin oxide

IL- Ionic liquid([Emim][BF_4]), RuPc-Ruthenium phthalocyanine

SiO_2- Silicon dioxide

CuS- Copper sulfide

CeO_2- Cerium dioxide

CuO- Copper oxide

GdM- Gadolinium molybdate

NiO- Nickel oxide

MoS_2- Molybdenum sulphide

Co_3O_4- Cobalt oxide

ZnS- Zinc sulfide

TiO_2- Titanium dioxide

GO- Graphene oxide

Ops- Organophosphates

CHAPTER 1

INTRODUCTION AND LITERATURE SURVEY

1.1 Pesticide and its type

Pesticides are the chemical substances used to control pests and disease-causing organisms that harm crops, humans, and animals. They are essential for agriculture and pest management. Here are some common types:

1. Insecticides: These pesticides are originated to kill or repel insects. They may work by disrupting the insect's nervous system, interfering with its metabolism, or inhibiting its growth and development. Common insecticides include organophosphates, carbamates, pyrethroids, and neonicotinoids.

2. Herbicides: Herbicides are used to control or eliminate unwanted plants (weeds). They may target specific metabolic pathways in plants, inhibiting their growth, or they may act as contact or systemic toxins. Examples include glyphosate, 2,4-D, atrazine, and paraquat.

3. Fungicides: Fungicides are applied to crops, ornamental plants, and turf to prevent or treat fungal disease. They work by inhibiting fungal growth, disrupting fungal cell membranes, or interfering with fungal enzymes. Common fungicides include azoles, strobilurins, copper-based compounds, and dithiocarbonates.

4. Rodenticides: These pesticides are used to manage rodents, including mice and rats. Rodenticides may work by causing internal bleeding, disrupting blood clotting or affecting the rodent's nervous system. Common rodenticides include anticoagulants like warfarin and bromadiolone as well as acute toxins like zinc phosphide and bromethalin.

5. Bactericides: These pesticides are used to control bacterial infections in plants and animals preventing disease and enhancing yields.

Each type of pesticide serves a specific purpose and requires careful applications to minimize environmental impacts and ensure effectiveness [1].

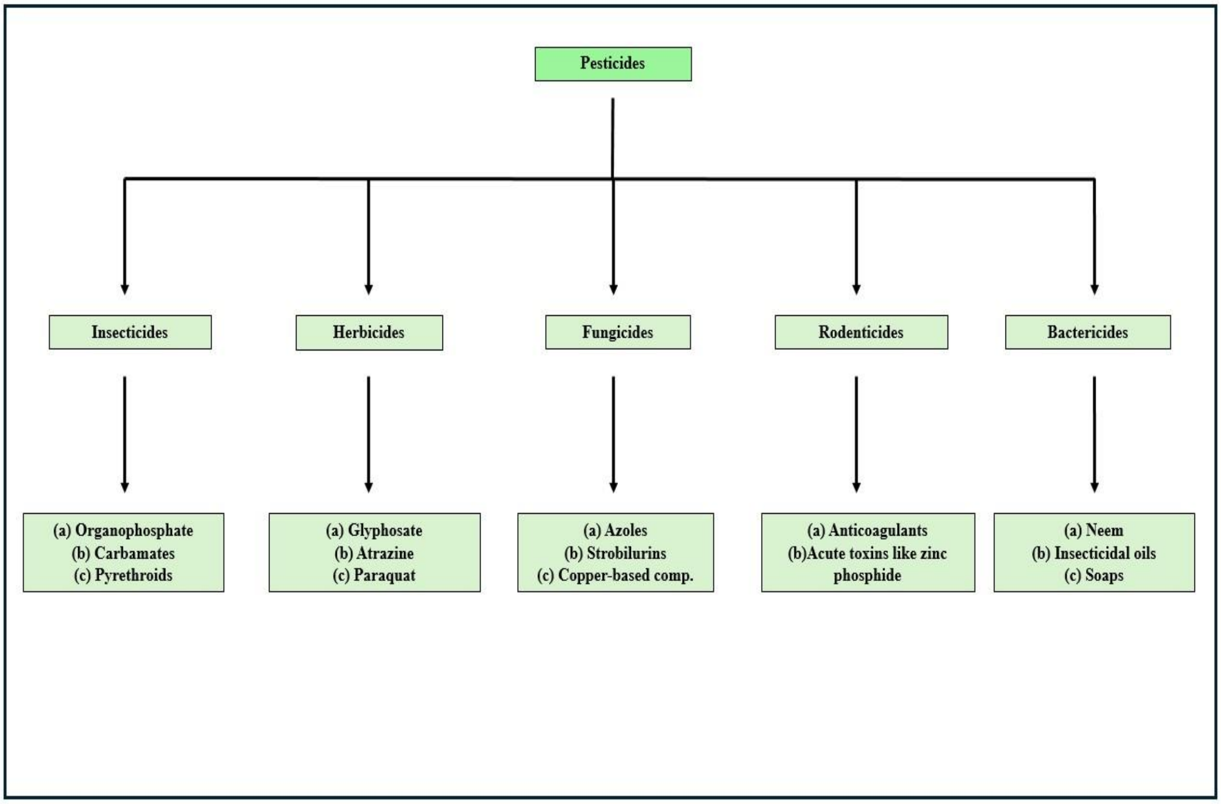


Figure 1.1 Schematic diagram of types of pesticides.

1.1.1 Advantages and disadvantages of pesticides

While pesticides are associated with negative environmental and health impacts, there are also some advantages when used responsibly.

1. Increased Agricultural Activity: Farmer can protect their crops from pests, weeds, and disease by using pesticides. By controlling these threats, pesticides can increase crop yield, ensuring food security for a growing population.

2. Protection of Public Health: Pesticides help in controlling vectors of diseases like malaria, dengue fever, Lyme disease by targeting insects that carry these diseases. This helps reduces the incidence of these illnesses among humans.

3. Preservation of Natural Habitats: By preventing crop losses due to pests, pesticides can help reduce the need for agricultural expansion into natural habitats. This can help preserve biodiversity and protect ecosystems from destruction.

4. Economic Benefits: Pesticides contribute to the economic viability of agriculture by protecting

crops and ensuring higher yields. This is especially important for farmers in developing countries who rely on agriculture for their livelihoods [2].

5. Efficient Resource Use: Pesticides can help reduce the amount of water, land, and energy needed to produce food by preventing losses due to pests. This can lead to more efficient use of resources in agriculture.

It's significant to remember that these benefits have important limitations. Pesticide misuse can have detrimental effects on human health as well as the environment, non-target creatures, and the development of pesticide resistance. Therefore, careful regulation, monitoring, and integrated pest management strategies are essential to mitigate the risks associated with pesticides [3].

Certainly, the use of pesticides comes with several disadvantages for both the environment and human health:

1. Harm to Non-Target Organisms: Pesticides have the potential to destroy beneficial insects, including earthworms, butterflies, and bees, upsetting ecosystems and lowering biodiversity. This can have cascading effects on food chains and ecosystem functions.

2. Water Pollution: Pesticides can leach into groundwater or be washed into rivers and streams, contaminating water sources. This pollution can harm aquatic organisms and even make water unsafe for human consumption.

3. Air Pollution: Pesticides can volatilize into the air and contribute to air pollution. Some pesticides contain volatile organic compounds (VOCs) that can react with other air pollutants to form smog or contribute to the formation of greenhouse gases.

4. Pesticide Resistance: Long-term pesticide use can cause the bug population to become resistant to the chemicals, making the pesticides useless. This may require the use of more hazardous pesticides or greater quantities, which increases the dangers to the environment and human health.

5. Health Risks for Humans: pesticide exposure can be harmful to the health of agriculture laborers people living nearby, and people who consume tainted food. Skin irritation, respiratory difficulties, and nausea are the examples of acute impacts and long-term exposure has been connected to major health concerns such as cancer, reproductive disorders, and neurological impairment [4].

6. Environmental Persistence: Some pesticides can persist in the environment for long periods, accumulating in soil, water, and living organisms. This persistence can result in long-term environmental damage and exposure risks.

7. Disruption of Ecosystem Services: Pesticides can disrupt essential ecosystem services such as

pollination and natural pest control. For example, the decline in bee populations due to pesticide exposure threatens the pollination of many crops, impacting food production.

8. Secondary Pest Outbreaks: Using pesticides to kill target pests might upset the natural predator-prey dynamic, which can cause subsequent pest outbreaks that were previously under control. This can create a cycle of pesticide dependence and further environmental harm.

These disadvantages underscore the importance of adopting alternative pest management strategies, such as integrated pest management (IPM), that prioritize minimizing pesticide use while promoting sustainable agricultural practices [5].

1.2 Methods for detecting pesticides and their limitations

Pesticides can be found using a variety of techniques, each with advantages and disadvantages. Typical techniques include the following:

1. Chromatography Techniques:

(a) Gas Chromatography (GC): This method separates and analyzes volatile compounds, including many pesticides. It's highly sensitive and can detect a wide range of pesticides.

(b) Liquid Chromatography (LC): LC separates compounds dissolved in a liquid solvent. It's suitable for analyzing non-volatile and thermally unstable pesticides.

2. Mass Spectrometry (MS)

(a) Gas Chromatography-Mass Spectrometry (GC-MS): Gas chromatography and mass spectrometry are used in GC-MS to provide separation and identification capabilities. Because of its high sensitivity and specificity, it is frequently utilized in pesticide analysis.

(b) Liquid Chromatography-Mass Spectrometry (LC-MS): LC-MS is similar to GC-MS but uses liquid chromatography. It's suitable for analyzing polar compounds and thermally labile pesticides [6].

3. Immunoassays:

(a) Enzyme-Linked Immunosorbent Assay (ELISA): Antibodies are used by ELISA to identify particular herbicides. It is quick, affordable, and useful for screening a lot of samples. However, it may lack specificity compared to chromatography-based methods.

(b) Fluorescence Polarization Immunoassay (FPIA): FPIA is another immunoassay technique that measures changes in the polarization of fluorescent molecules to detect pesticides.

4. Nuclear Magnetic Resonance (NMR):

NMR can provide structural information about molecules, including pesticides. While not as commonly used for pesticide analysis as chromatography and mass spectrometry, it's valuable for confirming chemical structures.

5. High-Performance Thin-Layer Chromatography (HPTLC): Compounds are separated and identified by HPTLC according to how well they bind to a stationary phase. It's less sensitive than GC or LC-MS but can be useful for rapid screening of pesticides in samples.

6. Multiresidue Analysis: Some methods allow the simultaneous detection of multiple pesticide residues in a single sample, offering efficiency and cost-effectiveness in pesticide monitoring program [7].

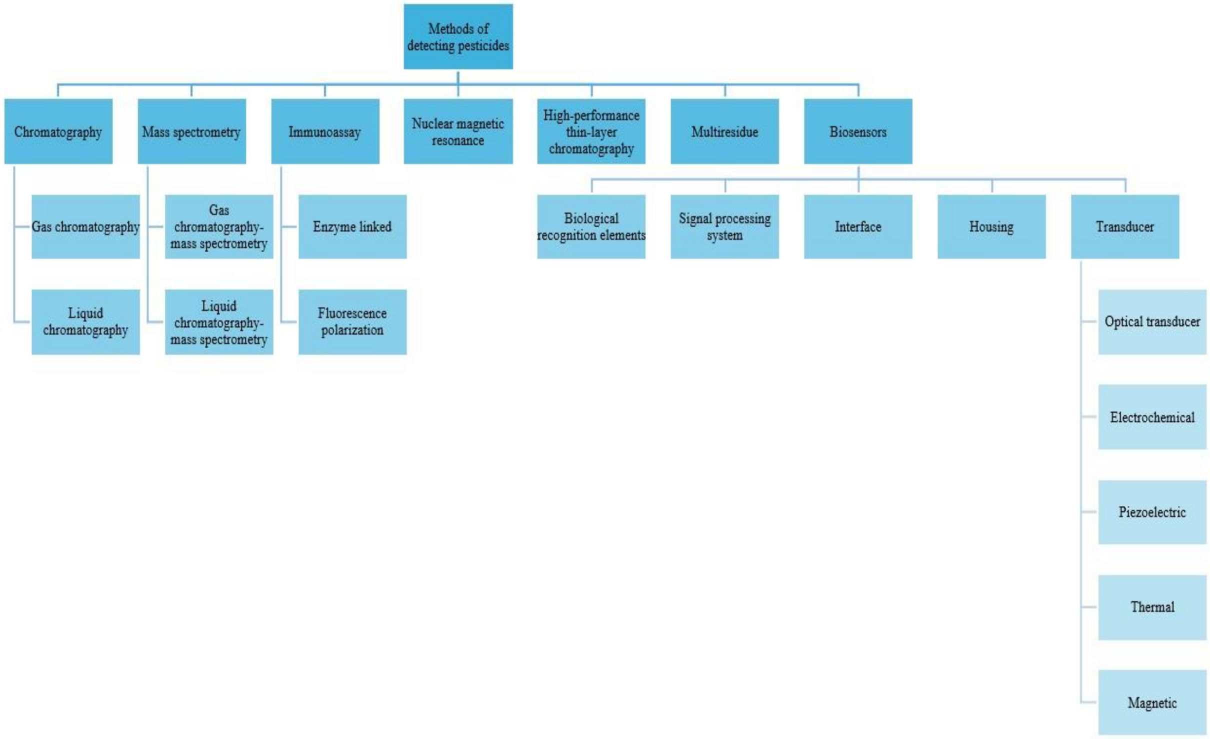


Figure 1.2 Schematic diagram of methods of detecting pesticides.

LIMITATIONS

1. Time-consuming: Traditional methods can be time-consuming, requiring several steps from sample preparation to analysis, which can hinder rapid detection, especially in urgent situations.

2. Labor-intensive: These methods often require skilled personnel to perform the analysis and interpret the results accurately.

3. Expensive: The equipment and the regents required for traditional pesticide analysis can be costly, making routine monitoring expensive, particularly for resource-limited settings.

4. Limited Scope: Some traditional methods may only detect a specific group of pesticides, and traditional methods may be needed to detect a broader range of compounds, increasing complexity and cost.

5. Environmental Impact: The use of organic solvents in extraction and analysis can have environmental impacts, requiring proper disposal measures [8].

1.3 Biosensor and its type

Biosensors are analytical tools that use a physicochemical transducer and a biological sensing element to determine the concentration or existence of a particular biological analyte. They are designed to provide rapid, sensitive, and selective detection of target molecules, often in complex samples such as blood, urine, environmental samples, or food matrices. Biosensors have diverse applications several of industries, including bioprocess control, environmental monitoring, food safety testing, and medical diagnostics [9].

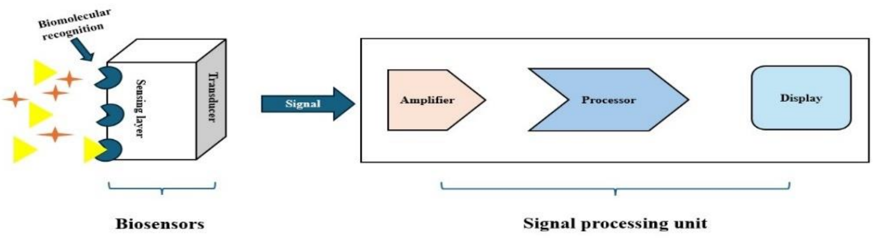


Figure 1.3 Schematic representation of biosensor.

Key components of a biosensor typically include:

(a) Biological Recognition Element: In response to the presence or concentration of the target analyte, this component selectively interacts with it to produce a quantifiable signal. Elements of biological recognition can include nucleic acids (DNA/RNA), enzymes, and antibodies entire cells, aptamers, or biomimetic substances.

(b) Signal Processing System: Thid component amplifies, processes, and analyzes the signal generated by the transducer to quantify the concentration of the target analyte. Signal processing may involve electronic circuits, data acquisition systems, microprocessors, or software algorithms.

(c) Interface: The interface facilitates communication between the biological recognition element and the transducer, ensuring efficient transfer of the biological response to the transducer. It may consist of immobilization matrices, membranes, both materials that stabilize and support the recognition element while allowing interaction with the analyte.

(d) Housing: The housing encloses and protects the biosensor components from environmental interference, contamination, and mechanical damage. It may also include features such as sample inlet/outlet ports, fluidic channels, and electrodes for interfacing with external devices [10].

(e) Transducer: The transducer converts the biological response generated by the recognition element into a quantifiable signal, such as electrical, optical, thermal, or mechanical. Different types of transducers are used based on the nature of the biological response and the desired sensitivity and specificity of the biosensor.

Some common types of transducers used in biosensors include:

1. Optical Transducers: These transducers convert changes in optical properties, such as absorbance, fluorescence, or refractive index, into measurable signals. Optical biosensors often use light sources, detectors, and optical elements to monitor changes in the intensity, wavelength, or polarization of light resulting from the interaction between the recognition element and the analyte.

2. Electrochemical Transducers: Electrochemical transducers are utilized to quantify alterations in electrical characteristics, such voltage, current, or impedance, which arise from redox reactions or shifts in the distribution of charge at the electrode surface. Electrochemical biosensors commonly employ electrodes made of materials like gold, platinum, or carbon to facilitate electron transfer between the recognition element and the analyte.

3. Piezoelectric Transducers: Piezoelectric transducers generate electrical signals in response to mechanical stress or changes in mass. When an analyte binds to the recognition element that is immobilized on the surface, piezoelectric biosensors usually use quartz crystal microbalances

(QCMs) or surface acoustic wave (SAW) devices to detect variations in the resonance frequency surface of the sensor [11].

4. Thermal Transducers: Thermal transducers measure changes in temperature resulting from exothermic or endothermic reactions occurring upon the binding of the analyte to the recognition element. Thermal biosensors may use temperature sensors, such as thermistors or thermocouples, to monitor changes in heat flow associated with the biological response.

5. Magnetic Transducers: Magnetic transducers detect changes in magnetic properties, such as magnetization or susceptibility, resulting from the interaction between magnetic nanoparticles conjugated to the recognition element and the analyte. Magnetic biosensors often utilize magneto-resistive sensors or superconducting quantum interference devices (SQUIDs) to measure changes in magnetic flux density.

These transducers play a vital role in converting the biological information obtained from the recognition element into a quantifiable signal that can be measured and analyzed to determine the concentration or presence of the target analyte. The choice of transducer depends on factors such as the nature of the analyte, the required sensitivity and specificity, and the desired application of the biosensor [12].

1.4 Electrochemical biosensors and their applications

An electrochemical biosensor for fenitrothion pesticides would be designed to detect and quantify the presence of fenitrothion molecules in a sample. Here's a general outline of how such a biosensor might work:

1. Recognition Element: The biosensor would contain a recognition element, such as enzymes or antibodies, that specifically bind to fenitrothion molecules. Enzymes like acetylcholinesterase (AChE) could be used due to their ability to interact with fenitrothion.

2. Transducer: An electrochemical transducer converts the recognition event (binding of fenitrothion to the recognition element) into a measurable electrical signal. This transducer could be based on techniques like amperometry, voltammetry, or impedimentary [13].

3. Electrode: The transducer is typically connected to an electrode, which is where the electrochemical reactions take place. This could be a working electrode made of materials like gold, carbon, or graphene, which facilitates electron transfer during the electrochemical process.

4. Signal Processing: The electrical signal produced by the transducer is then processed and analyzed. This could involve measuring changes in current, voltage, or impedance, depending on the specific electrochemical technique used.

5. Calibration: Before use, the biosensor would likely need to be calibrated using known concentrations of fenitrothion to establish a relationship between the measured signal and the concentration of the pesticide in the sample.

6. Detection: Once calibrated, the biosensor can be used to detect fenitrothion in unknown samples. The concentration of fenitrothion in the sample can be determined by comparing the measured signal to the calibration curve [14].

In biosensor applications, AChE serves as a sensitive recognition element for detecting organophosphate and carbamate pesticides. The inhibition of AChE activity by these pesticides can be monitored in real-time using various transduction methods, allowing for the rapid and selective detection of pesticide residues in environmental samples, food products, and biological fluids. AChE-based biosensors offer advantages such as high specificity, sensitivity, and rapid response, making them valuable tools for pesticide monitoring and environmental protection efforts [13].

The biosensor's performance would need to be validated to ensure its accuracy, sensitivity, selectivity, and reliability for detecting fenitrothion in real-world samples. Many biosensors offer advantages like rapid detection, high sensitivity, and portability, making them valuable tools for monitoring pesticide contamination in environmental samples, agricultural products, and food. However, developing a specific biosensor for fenitrothion would require careful consideration of the properties of the pesticide and optimization of the biosensor components for efficient detection.

1.4.1 Enzyme based electrochemical biosensor

An enzyme-based electrochemical biosensor for fenitrothion pesticides would leverage the catalytic properties of enzymes to specifically detect and quantify fenitrothion molecules in a sample. Here's how such a biosensor might be designed:

1. Enzyme Selection: The biosensor would utilize an enzyme that interacts with fenitrothion in a specific and sensitive manner. Acetylcholinesterase (AChE) is commonly used for detecting organophosphate pesticides like fenitrothion because it can hydrolyze acetylcholine, and pesticides like fenitrothion inhibit this enzymatic activity.

2. Immobilization of Enzyme: The selected enzyme (e.g., AChE) would be immobilized onto the surface of an electrode. Immobilization techniques could include physical adsorption, cross-linking, or covalent attachment to ensure stability and retention of enzymatic activity.

3. Electrochemical Transducer: The immobilized enzyme-modified electrode serves as the transducer in the biosensor setup. When fenitrothion molecules are present in the sample, they inhibit the enzymatic activity of AChE, leading to a decrease in the electrochemical signal generated by the enzymatic reaction.

4. Signal Measurement: The electrochemical signal, typically changes in current or voltage resulting from the enzymatic reaction, is measured using techniques like amperometry or cyclic voltammetry. The magnitude of the signal change is proportional to the concentration of fenitrothion in the sample.

5. Calibration and Quantification: The biosensor would need to be calibrated using known concentrations of fenitrothion to establish a calibration curve relating the measured signal to the concentration of the pesticide. This allows for the quantification of fenitrothion in unknown samples based on their electrochemical response.

6. Selectivity Enhancement: To enhance selectivity and minimize interference from other compounds in complex samples, additional strategies such as selective membranes or molecularly imprinted polymer can be employed to improve the specificity of the biosensor towards FNT.

7. Validation and Optimization: The biosensor would undergo validation to assess its performance characteristics, including sensitivity, selectivity, detection limit, and reproducibility. Optimization of experimental conditions and biosensor components may be necessary to improve its analytical performance.

Overall, enzyme-based electrochemical biosensors offer several advantages, including high specificity, sensitivity, and rapid response, making them promising tools for on-site detection and monitoring of fenitrothion pesticide contamination in environmental and agricultural samples [15].

1.4.2 Acetylcholinesterase based-biosensor

Acetylcholinesterase (AChE) is an enzyme found in the nervous system of animals, including humans. Its primary role is to catalyze the hydrolysis of the neurotransmitter acetylcholine (AChE) into choline and acetate. This enzymatic reaction is crucial for terminating nerve impulses at cholinergic synapses, where acetylcholine is the neurotransmitter responsible for transmitting signals across nerve junctions.

The catalytic activity of AChE plays a vital role in maintaining proper nervous system function by ensuring the timely termination of nerve impulses. Inhibition of AChE activity can lead to the accumulation of acetylcholine in the synaptic cleft, resulting in overstimulation of cholinergic receptors and disruption of normal neurotransmission [16].

Organophosphate and carbamate compounds including pesticides like fenitrothion, exert their toxic effects by inhibiting AChE activity. These compounds bind irreversibly or reversibly to the active site of AChE, thereby interfering with its ability to hydrolyze acetylcholine. This inhibition can lead to a range of symptoms and health effects, including neurotoxicity, muscle paralysis, respiratory failure, and even death in severe cases.

In biosensor applications, AChE serves as a sensitive recognition element for detecting organophosphate and carbamate pesticides. The inhibition of AChE activity by these pesticides can be monitored in real-time using various transduction methods, allowing for the rapid and selective detection of pesticide residues in environmental samples, food products, and biological fluids. AChE-based biosensors offer advantages such as high specificity, sensitivity, and rapid response, making them valuable tools for pesticide monitoring and environmental protection efforts [17].

1.4.3 Paper-based electrochemical biosensors and their applications

A paper-based electrochemical biosensor is a device that integrates paper as a substrate with electrochemical sensors to detect specific biological molecules. These biosensors are cost-effective, portable, and easy to use, making them ideal for point-of-care diagnostics and environmental monitoring. A paper-based electrochemical biosensor combines the advantages of paper-based substrates with the sensitivity and selectivity of electrochemical sensors, making them a powerful tool for various applications, including health care and environmental monitoring.

A detailed explanation of how they work:

1. Paper Substrate: The substrate is typically made of cellulose paper, which is inexpensive, biodegradable, and readily available. The paper serves as a solid support for the sensor components and allows for easy handling [18].

2. Electrodes: The paper substrate is impregnated with conductive materials to create electrodes. Common materials used are graphite, carbon nanotubes, or conductive polymers like PANI, PEDOT: PSS, polypyrrole, etc. These materials facilitate the transfer of electrons, which is essential for the electrochemical reactions [19].

3. Biological Recognition Elements: The paper is then coated with a biological recognition element, such as enzymes, DNA strands, or antibodies. This element specifically interacts with the target analyte to generate a measurable signal. For example, an enzyme can catalyze reaction with the analyte to produce a detectable product.

4. Sample Applications: The sample like blood, saliva, or environmental sample is applied to the paper-based sensor. The biological recognition element interacts with the target analyte in the sample.

5. Electrochemical Detection: When the target analyte interacts with the biological recognition element, it generates a measurable electrochemical signal. The signal can be detected using techniques such as amperometry, potentiometry, or cyclic voltammetry [20].

6. Signal processing: The electrochemical signal is processed to quantify the concentration of the target analyte in the sample. This can be done using calibration curves or by comparing the signals to known standards.

7. Results: The concentration of the target analyte is displayed or transmitted to a data collection device for further analysis and interpretation [21].

Applications of paper-based electrochemical biosensors are:

1. Medical Diagnostics: These biosensors can be used for the rapid and low-cost detection of various biomarkers, such as glucose, organophosphate pesticides, cholesterol, etc. They are particularly useful in resource-limited settings where access to traditional laboratory equipment is limited.

2. Food Safety: These biosensors can detect pathogens, toxins, and other contaminants in food products. Helping to ensure food safety and prevent outbreaks of foodborne illnesses.

3. Biomedical Research: Paper-based biosensors are valuable tools for researchers studying biological process and developing new diagnostic techniques.

4. Water Quality Monitoring: These biosensors are used to detect various parameters in water, such as pH, heavy metals, and microbial contaminants.

5. Smart Packaging: Paper-based biosensor can integrate into packaging materials to monitor the freshness and quality of food products. They can detect gases released by spoilage microorganisms, helping to reduce food waste.

6. Point-of-Care Testing: Due to portability and rapid response time, these biosensors are ideal for point-of-care testing in clinics, pharmacies, and remote locations. They can be used for rapid diagnosis of diseases such as malaria, HIV, and COVID-19.

7. Veterinary Diagnostics: These biosensors can also be used in veterinary medicine for the rapid and low-cost detection of disease in animals, helping to improve animal health and prevent the spread of diseases.

1.4.4 Types of Paper

There are several types of paper commonly used in making paper-based electrochemical biosensors, each with its characteristics and suitability for different applications. Here are some of the main types:

1. Cellulose Paper: Cellulose paper is the most common substrate for paper-based biosensors. It is inexpensive biodegradable, and provides solid support for the sensor components. Cellulose paper can be modified to enhance its properties, such as by adding conductive materials for electrode fabrication or by incorporating chemical functionalities for biomolecule immobilization.

2. Filter Paper: Filter paper is often used in paper-based biosensors due to its porous structure, which allows for rapid fluid transport and analyte diffusion

3. Nitrocellulose Paper: This paper has a high binding capacity for biomolecule and can be easily functionalized with antibodies, enzymes or other recognition elements. Nitrocellulose paper is often used in lateral flow assays, where the analyte moves along the paper strip by capillary action.

4. Whatman Paper: Whatman is a brand of filter paper that is widely used in laboratory applications. Whatman paper comes in various grades and pore sizes, making it suitable for various ranges of biosensor applications. It is known for its high quality and consistency.

5. Paper-based Microfluidic Devices: In addition to traditional paper substrate, Paper-based microfluidic devices also are used in biosensors. These devices integrate paper with microfluidic channels, allowing of precise control of sample flow and reaction conditions.

6. Printing Paper: Printing paper is also used in paper-based biosensor. Due to its high porosity and smoothness, this type of paper used in various applications of biosensors.

Each type of paper has its advantages and limitations but the best result comes out with Whatman paper because of its high porosity, strength and durability, chemical compatibility, and consistent quality [22].

1.4.5 Fabrication Techniques

Fabrication techniques for biosensors vary depending on the type and application of the biosensor but generally involve several common steps and techniques. Here are some key methods used in the fabrication of biosensors:

1. Photolithography: This is the common method used to create patterns or substrate, which is essential in microfabrication. It involves coating a substrate with a photoresist, exposing it to light, through a mask, and then developing the pattern to create the desired microstructures.

2. Electrochemical deposition: This technique deposits a thin layer of material onto an electrode surface through the reduction of metal ions in solution. It is often used to create conductive paths or to immobilize biomolecules on the sensor surface.

3. Spin Coating: This involves depositing a uniform thin film of materials onto a flat substrate by spinning it at high speed. It is commonly used to apply photoresists or other coatings in the sensor fabrication process.

4. Screen Printing: This technique is used to print thick-film layers of conductive, dielectric, or biological inks onto substrates. It is widely used in the fabrication of disposable biosensors, such as glucose test strips [23].

5. Self-Assembly: This method leverages the natural tendency of molecules to organize into structured arrangements. It is used to form monolayers or multilayers of biological molecules on the sensor surface through specific interactions.

6. Dip Coating: This technique is the easiest technique among all known fabrication techniques. In this technique, the paper electrode is dipped in conductive ink solution after the stabilization of the paper in order to form a uniform conductive layer onto the surface of the paper electrodes. The dip coating technique is done twice and thrice depending on the application of the biosensor.

7. 3D Printing: Additives manufacturing techniques are increasingly used for creating biosensor components. 3D printing allows for the fabrication of complex geometries and the integration of multiple materials in a single device.

8. Plasma Treatment: This method is used to modify the surface properties of materials, such as increasing surface energy to enhance the adhesion of biomolecules or creating functional groups for further chemical attachment [24].

Among all the fabrication techniques dip coating technique produces best result in FNT detection.

1.5 Conducting Polymer

Conducting polymers are a fascinating class of materials that exhibit both electrical conductivity and the typical properties of polymers, such as flexibility and ease of processing. Unlike conventional semiconductors or metals, which derive their conductivity from the movement of electrons or holes, the conductivity in conducting polymers arises from the movement of charges carriers called polarons or solitons along the polymer chain.

These materials' prospective uses in a variety of industries, including electronics, optoelectronics, sensors, batteries, and biomedical devices, have generated a lot of attention. Some well-known conducting polymers include polyaniline, polypyrrole, and polythiophene.

Conducting polymers can be synthesized through chemical oxidation or reduction of monomers, resulting in the formation of conjugated polymer chains. The doping process, where charge carriers are introduced into the polymer, greatly influences their electrical properties. Doping can be achieved through chemical doping with dopant molecules or electrochemical doping, where the polymer is exposed to an electrolyte.

One of the remarkable features of conducting polymers is their ability to undergo doping and de-doping processes, which makes them suitable for applications such as electrochromic displays, batteries, and supercapacitors.

While conducting polymers offer many advantages, including low cost, lightweight, and easy processing, challenges such as low conductivity compared to metals and stability issues in ambient conditions still need to be addressed for widespread commercialization and practical applications. Nonetheless, ongoing research continues to push the boundaries of what these versatile materials can achieve [25].

1.5.1 Conducting polymer-based composites

Conducting polymer-based composites are materials that combine conducting polymers with other substances, typically inorganic or organic fillers, to achieve enhanced electrical, mechanical, or functional properties. These composites are engineered to capitalize on the unique characteristics of conducting polymers while addressing limitations such as low mechanical strength or processability [26].

Here are some common types of conducting polymer-based composites:

1. Carbon-based fillers: Carbon nanotubes (CNTs), graphene, and carbon black are frequently used as fillers in conducting polymer composites due to their excellent electrical conductivity. These fillers can be dispersed within the conducting polymer matrix to improve electrical conductivity while maintaining flexibility and processability.

2. Metal nanoparticles: Metals like gold, silver, or copper nanoparticles can be incorporated into conducting polymer matrices to enhance electrical conductivity and provide specific functionalities. These composites are used in applications such as flexible electronics, catalysis electromagnetic shielding.

3. Inorganic fillers: Inorganic materials such as metal oxides (e.g., titanium dioxide), metal sulfides (e.g., zinc sulfide), or ceramics (e.g., silica) can be combined with conducting polymers to impart additional properties such as improved mechanical strength, thermal stability, or optical properties.

4. Polymer blends: Conducting polymers can be blended with other polymers to create composite materials with tailored properties. For example, blending a conducting polymer with a thermoplastic polymer can improve processability and mechanical properties while maintaining electrical conductivity.

5. Functional additives: Conducting polymer composites may also incorporate functional additives such as dopants, plasticizers, or stabilizers to further enhance performance or stability.

Applications of conducting polymers-based composites span various fields including electronics, energy storage and conversion, sensors, actuators, and biomedical devices. For instance, they are used in flexible and stretchable electronics, electrochemical capacitors (supercapacitors), electrodes for batteries and fuel cells, and biosensors for detecting analytes such as glucose or DNA.

The design and optimization of conducting polymer-based composites involves careful selection of components, filler dispersion techniques, and processing methods to achieve the desired properties for specific applications. Ongoing research in this area aims to further expand the range of applications and improve the performance of these versatile materials [27].

1.5.2 Synthetic Techniques of Conducting Polymer-based composites

Several synthetic techniques are employed to fabricate conducting polymer-based composites, each offering advantages depending on the desired properties and application requirements. Here are some common techniques:

1. Solution mixing or blending: This technique involves dispersing conducting polymer and filler materials in a solvent, followed by mixing to achieve a homogeneous mixture. The solution is then cast or deposited onto a substrate, and the solvent is evaporated to form the composite film. Solution mixing is suitable for thermally sensitive polymers and allows precise control over filler dispersion and composite composition.

2. In situ polymerization: In this method, the conducting polymer is synthesized directly within a mixture containing filler materials. Monomers and dopants are dissolved or dispersed in a solvent along with the filler, and polymerization is initiated either chemically or electrochemically. In situ polymerization ensures intimate contact between the conducting polymer and filler, leading to improved compatibility and conductivity.

3. Melt blending: Melt blending involves mixing conducting polymer pellets or powder with filler materials using heat and mechanical shear forces. The mixture is processed using techniques such as extrusion, compression molding, or injection molding to form the composite. Melt blending is suitable for thermoplastic conducting polymers and offers high throughput and scalability.

4. Electrospinning: Electrospinning is a technique used to fabricate conducting polymer-based nanocomposites with fibrous structures. A solution containing conducting polymer, filler materials, and a solvent is electrostatically charged and ejected through a spinneret to form nanofibers. The fibers are collected on a substrate, and the solvent is evaporated to obtain the composite nanofibers. Electrospinning enables the production of high surface area materials for applications such as sensors, filtration, and tissue engineering.

5. Layer-by-layer assembly: This technique involves alternately depositing layers of conducting polymer and filler materials onto a substrate through sequential immersion or spraying processes. Each layer is adsorbed onto the substrate via electrostatic interactions or chemical bonding. Layer-by-layer assembly is appropriate for applications like thin-film electronics and coatings because it provides exact control over film thickness and composition.

6. Template-directed synthesis: In this approach, conducting polymers are synthesized within the pores of a template material, which is subsequently removed to obtain the composite. Templates can be sacrificial materials such as nanoparticles or porous substrates, or they can be structured surfaces with predefined patterns. Template-directed synthesis allows the fabrication of conducting polymer-based composites with controlled morphology and porosity.

These synthetic techniques can be tailored and combined to achieve specific properties and functionalities in conducting polymer-based composites, making them highly versatile for a wide range of applications [28].

1.5.3 Applications of Conducting Polymer-based Composite for Fenitrothion Detection

Conducting polymers has shown promise in various applications, including environmental sensing and remediation. While specific applications for fenitrothion pesticides may not be as extensively explored as other areas, there are potential ways conducting polymers could be utilized in this context:

1. **Sensor Development:** Conducting polymers can be used to develop sensitive and selective sensors for detecting fenitrothion pesticides in the environment. These sensors could be based on changes in electrical conductivity, optical properties, or electrochemical behavior of the conducting polymer upon exposure to fenitrothion. Such sensors could enable real-time monitoring of pesticide levels in soil, water, or air, aiding in environmental monitoring and management.
2. **Adsorption and Removal:** Conducting polymer-based materials could be designed to selectively adsorb fenitrothion from contaminated water sources or soil. Functionalized conducting polymers with tailored surface properties could enhance adsorption capacity and selectivity towards fenitrothion, offering a potential method for pesticide removal and remediation.
3. **Electrochemical Degradation:** Electrochemical methods employing conducting polymer electrodes could be explored for the degradation of fenitrothion pesticides. Conducting polymers with appropriate redox properties could facilitate electrochemical oxidation or reduction of fenitrothion molecules, leading to their degradation into less harmful products. This approach could offer a sustainable and efficient means of pesticide remediation.
4. **Pesticide Detection in Food and Agriculture:** Conducting polymer-based sensors could be integrated into agricultural systems for detecting fenitrothion residues in crops, fruits, or vegetables. By providing rapid and on-site detection capabilities, these sensors could help ensure food safety and compliance with regulatory standards.

5. Smart Packaging: Conducting polymer-based materials could be incorporated into packaging films or coatings for food products to detect and signal the presence of fenitrothion vapors, thereby providing consumers with information about pesticide exposure and freshness of the packaged food [29].

While these applications show promise, further research and development are needed to optimize the performance and scalability of conducting polymer-based technologies for fenitrothion pesticide detection and remediation. Additionally, considerations such as stability, selectivity, and cost-effective needed to be addressed to enable practical implementation in real-world scenarios.

1.6 Applications of PEDOT:PSS

PEDOT:PSS is a conducting polymer with various applications, primarily in electronics and optoelectronics. Some of the key applications include:

1. Organic Electronics: PEDOT:PSS is used in organic light-emitting diodes, and organic field-effect transistors. Its high conductivity and transparency make it ideal for use as transparent electrodes.

2. Sensors: PEDOT:PSS can be used as a sensing material in various types of sensors, including chemical sensors and biosensors. Its conductivity changes in response to external stimuli, enabling the detection of analytes.

3. Antistatic Coatings: Due to its high conductivity, PEDOT:PSS is used in antistatic coatings for materials such as textiles, films, and electronic devices to prevent the buildup of static electricity.

4. Electrochromic Devices: PEDOT:PSS is used in electrochromic devices, which can change their optical properties in response to an electrical stimulus. These devices are used in smart windows, displays and mirrors.

5. Supercapacitors: PEDOT:PSS can be used as electrode materials in supercapacitors, which are energy storage devices that can deliver high power density. Its high conductivity and electrochemical stability are advantageous for this application.

6. Biomedical Applications: PEDOT:PSS has been explored for various biomedical applications, including tissue engineering, neural interfaces, and drug delivery systems [30].

1.7 PEDOT:PSS based nanocomposites

PEDOT:PSS (Poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate)) is a conductive polymer widely used in various electronic applications due to its excellent conductivity, flexibility, and transparency. When combined with metal oxides or metal sulfides, it can lead to the formation of nanocomposites with enhanced properties for applications in fields such as sensors, energy storage devices, and optoelectronics [31].

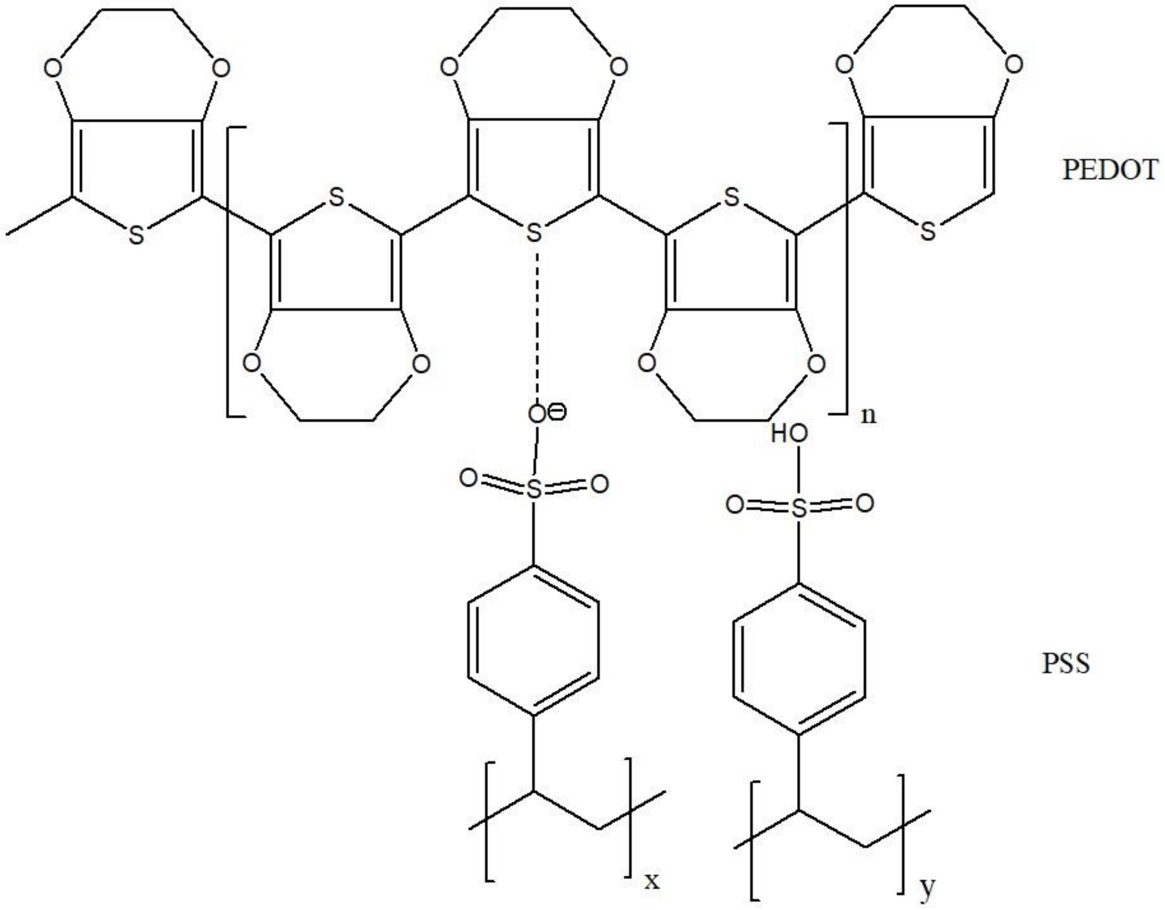


Figure 1.4 Structural arrangement of PEDOT:PSS conducting polymer

Metal oxide and metal sulfide nanocomposites with PEDOT:PSS can exhibit synergistic effects that combine the conductivity of PEDOT:PSS with the unique properties of the metal oxides or metal sulfides [32]. Here's a brief overview of each:

1. NiO@PEDOT:PSS

Nickel oxide (NiO) is a transition metal oxide with interesting properties such as high stability, good electrochemical activity, and environmental friendliness. When combined with PEDOT:PSS, it can create nanocomposites with enhanced functionalities, particularly in energy storage and conversion devices. Here's a breakdown of the potential characteristics and applications of nickel oxide-based PEDOT:PSS nanocomposites:

(a) Improved Electrochemical Performance:

NiO is known for its electrochemical activity, which makes it suitable for applications in supercapacitors, batteries, and electrochromic devices. PEDOT:PSS, on the other hand, enhances the electrical conductivity of the composite, facilitating charge transport within the materials.

(b) Enhanced Capacitive Behavior:

The combination of NiO nanoparticles with PEDOT:PSS can lead to an increase in the effective surface area, promoting charge storage in supercapacitor applications. PEDOT:PSS provides a conductive network that improves the electron transport kinetics, leading to enhanced charge/discharge rates.

(c) Compatibility with Solution Processing Techniques:

Solution-based methods such as spin coating, inkjet printing, or doctor-blade coating can be employed to fabricate NiO-based PEDOT:PSS nanocomposite thin films, making them suitable for large-scale production and flexible device applications[33].

2. MoS_2@PEDOT:PSS

Molybdenum disulfide (MoS_2) is a two-dimensional transition metal dichalcogenide (TMD) with remarkable electronic, optical, and mechanical properties. When combined with PEDOT:PSS, it can lead to nanocomposites with enhanced functionalities suitable for various electronic and optoelectronic applications. Here's an exploration of the characteristics and potential applications of MoS_2-based PEDOT:PSS nanocomposites:

(a) Enhanced Conductivity:

MoS_2 is a semiconductor with a direct bandgap, exhibiting excellent electronic properties such as high carrier mobility and on/off ratio. PEDOT:PSS, as a conductive polymer, can improve the overall conductivity of the nanocomposite, facilitating charge transport through the material.

(b) Improved Electrochemical Performance:

MoS_2 has shown promise in energy storage and conversion applications, including lithium-ion batteries, supercapacitors, and hydrogen evolution reactions. PEDOT:PSS can enhance the electrochemical performance of MoS_2-based electrodes by providing a conductive network and improving the kinetics of charge transfer processes.

(**c) Solution Processability:**

Both MoS_2 and PEDOT:PSS are compatible with solution processing techniques, allowing for the fabrication of thin films and coatings via methods such as spin coating, inkjet printing, or spray deposition. Solution processing enables the scalable production of MoS_2-based PEDOT:PSS nanocomposites for large-area electronics and device integration[34].

3. Co_3O_4@PEDOT:PSS nanocomposite

Cobalt oxide (Co_3O_4) is a versatile transition metal oxide known for its interesting properties, including high catalytic activity, good electrical conductivity, and potential for energy storage applications. When combined with PEDOT:PSS, it can form nanocomposites with enhanced functionalities suitable for various electronic, energy storage, and sensing applications[35]. Here's an overview of the characteristics and potential applications of Co_3O_4-based PEDOT:PSS nanocomposites:

(a) Enhanced Electrical Conductivity:

Co_3O_4 is a semiconductor with moderate electrical conductivity. When integrated with PEDOT:PSS, which is a conductive polymer, the nanocomposite can exhibit improved electrical conductivity. This enhanced conductivity is crucial for applications such as electrodes in energy storage devices and electronic sensors.

(b) Electrochemical Performance:

Co_3O_4 is promising for energy storage applications due to its high theoretical specific capacity and good cycling stability. PEDOT:PSS can further enhance the electrochemical performance of Co_3O_4-based electrodes by providing a conductive matrix that facilitates ion and electron transport during charge and discharge processes.

(c) Catalytic Activity:

Co_3O_4 is known for its catalytic properties, particularly in oxygen evolution reactions (OER) and oxygen reduction reactions (ORR), which are important processes in energy conversion and storage devices such as fuel cells and rechargeable batteries. Incorporating Co_3O_4 into PEDOT:PSS can enhance its catalytic activity and stability, making it a promising catalyst for various electrochemical reactions.

4. ZnS@PEDOT nanocomposite

Zinc sulfide (ZnS) is a wide-bandgap semiconductor material with notable optical, electrical, and chemical properties. When combined with PEDOT:PSS, it can yield nanocomposites with enhanced functionalities suitable for various applications. Here's an exploration of the potential application and characteristics of ZnS-based PEDOT:PSS nanocomposites:

(a) Optoelectronic Properties:

ZnS exhibits interesting optical properties, including a wide bandgap and strong photoluminescence, making it suitable for optoelectronic applications such as light-emitting diodes (LEDs), photodetectors, and solar cells. Integration with PEDOT:PSS can enhance the charge transport properties and stability of ZnS-based optoelectronics devices, leading to the improved device performance.

(b) Improved Electrical Conductivity:

ZnS is a semiconductor with relatively low electrical conductivity. Incorporating ZnS nanoparticles into PEDOT:PSS can enhance the overall conductivity of the nanocomposite, facilitating charge transport and improving the performance of electronic devices. The conductive network provided by PEDOT:PSS can improve the electron transport kinetics within the nanocomposite, leading to enhanced device efficiency.

(c) Chemical Stability:

ZnS is chemically stable under ambient conditions and exhibits good resistance to oxidation and corrosion, which is advantageous for device fabrication and long-term stability. Integration with PEDOT:PSS can further enhance the stability of ZnS-based nanocomposites, ensuring their performance and functionality in various environmental conditions[36].

5. TiO_2@PEDOT:PSS nanocomposite

TiO_2 is an insulator in its pure form; however, doping or surface modification can introduce charge carriers and improve its electrical conductivity. PEDOT:PSS, being a conductive polymer, can further enhance the overall conductivity of the nanocomposite[37]. This is crucial for applications such as electrodes in solar cells, sensors, and electronic devices.

(a) Photocatalytic Activity:

TiO_2 is widely known for its photocatalytic properties, which find applications in environmental remediation, water purification, and solar energy conversion. Integrating TiO_2 nanoparticles into PEDOT:PSS can create nanocomposites with improved photocatalytic activity, as PEDOT:PSS can provide a conductive matrix that facilitates charge separation and transport during photocatalysis.

(b) Optical Properties:

TiO_2 exhibits high transparency in the visible region of the spectrum and strong UV absorption due to its wide bandgap. The integration of TiO_2 with PEDOT:PSS may allow for the tuning of the nanocomposite's optical properties, making it suitable for applications such as UV filters, photovoltaics, and optoelectronic devices.

(c) Surface Area and Porosity:

TiO_2 nanoparticles have a high surface area and porosity, which are beneficial for applications such as gas sensing, dye-sensitized solar cells (DSSCs), and energy storage devices. PEDOT:PSS can stabilize TiO_2 nanoparticles and prevent their aggregation, ensuring maximum exposure of active sites and enhancing the performance of the nanocomposite in various applications.

Limitations of above PEDOT:PSS composites:

TiO_2 can cause environmental pollution during production and health risks if inhaled. Cobalt (II,III) oxide (Co_3O_4) possess toxicity risks, including respiratory issues and it is also expensive. MoS_2 has environmental impacts from mining and potential hazards if inhaled, and it tends to restack, reducing effectiveness. ZnS is unstable under UV light and moisture, possess environmental toxicity risks, and is costly to produce in high purity. NiO is carcinogenic, can cause respiratory and skin issues, and is chemically reactive with acids and bases, limiting its applications.

1.8 CuS@rGO/PEDOT:PSS nanocomposite

Copper sulfide (CuS) nanoparticles combined with reduced graphene oxide (rGO) and PEDOT:PSS can form a nanocomposite with unique properties suitable for various applications. The characteristics and potential applications of CuS@rGO/PEDOT:PSS nanocomposites:

(a) Enhanced Conductivity:

Reduced graphene oxide (rGO) is highly conductive due to its sp^2-hybridized carbon structure, providing efficient pathways for charge transport within the nanocomposite. CuS nanoparticles, with their semiconducting properties, can further enhance the conductivity of the nanocomposite when integrated with PEDOT:PSS, a conductive polymer. This synergy results in improved electrical conductivity crucial for electronic and energy storage applications.

(b) Improved Electrochemical Performance:

CuS nanoparticles possess high theoretical specific capacities, making them promising materials for energy storage devices like batteries and supercapacitors. The combination of CuS with rGO and PEDOT:PSS can enhance the electrochemical performance by providing additional active sites for redox reactions, improved charge transfer kinetics, and enhanced stability during cycling.

(c) High Surface Area and Porosity:

CuS nanoparticles can provide a high surface area and porosity, which are beneficial for applications such as gas sensing and catalysis. Integration with rGO and PEDOT:PSS can stabilize the structure of CuS nanoparticles and prevent aggregation, ensuring maximum exposure of active sites for gas molecules or catalytic reactions.

(d) Tunable Optical Properties:

CuS nanoparticles exhibits tunable optical properties depending on their size and morphology, with absorption in the visible and near-infrared regions. The incorporation of CuS into the nanocomposite can allow for the engineering of optical properties, making it suitable for optoelectronic applications such as photodetectors and photovoltaic devices.

(e) Solution Processability and Scalability:

PEDOT:PSS, rGO, and CuS nanoparticles are compatible with solution processing techniques, enabling the fabrication of thin films or coatings on various substrates. Solution processing facilitates large-scale production and the integration of CuS@rGO/PEDOT:PSS nanocomposites into flexible and lightweight electronic devices.

Applications:

(i) Energy storage devices:

CuS@rGO/PEDOT:PSS nanocomposites can be used in lithium-ion batteries, supercapacitors, and hybrid capacitors to enhance energy storage capacity and cycling stability.

(ii) Gas sensors: The high surface area and semiconducting properties of CuS@rGO/PEDOT:PSS nanocomposites make them suitable for detecting gases such as hydrogen sulfide (H_2S) or ammonia (NH_3) with high sensitivity and selectivity.

(iii) Catalysis: The nanocomposite can serve as an efficient catalyst for various reactions, including hydrogen evolution, oxygen reduction, and pollutant degradation.

CuS@rGO/PEDOT:PSS nanocomposites offer a synergistic combination of electrical, electrochemical, optical, and structural properties, making them promising materials in sensing applications for FNT detection [38].

CHAPTER 2

MATERIALS AND SYNTHESIS

2.1 Materials Required

Graphite powder, Sulphuric acid (H_2SO_4), potassium permanganate, butanol, copper acetate, hydrogen peroxide (H_2O_2), hydrochloric acid (HCl), thiourea, poly(3,4-ethylene dioxythiophene):polystyrensulphonate (PEDOT:PSS), phosphoric acid (H_3PO_4), potassium ferrocyanide, sodium chloride, potassium ferricyanide, acetyl cholinesterase, acetyl thiocholine chloride, fenitrothion, ethylene glycol, dimethyl formamide, dimethyl sulfoxide, N-methyl pyrrolidone, methanol, Whatman paper no.1, glutaraldehyde, monobasic potassium phosphate, dibasic potassium phosphate, sodium hydroxide and all the solvents were purchased from Sigma-Aldrich.

2.2 Synthesis of GO

In a typical process, pure graphite powder was converted into graphene oxide (GO) using a modified hummers method. This procedure involves mixing and stirring 27ml of sulfuric acid (H_2SO_4) and 3ml of phosphoric acid (H_3PO_4 for several minutes). Next, 0.225g of powered graphite was added to the mixture while it was being stirred. Next, 1.32g of potassium permanganate (KMnO4) was gradually added to the mixture. The solution turned dark green after six hours of vigorous stirring of this mixture. 0.675ml of hydrogen peroxide (H_2O_2) was added gradually and agitated for ten minutes to eliminate the extra $KMnO_4$. After the exothermic reaction took place, allow it to cool. After adding 10ml of hydrochloric acid (HCl) and 30ml of deionized water (DIW), the mixture was centrifuged for 10 mins at 5000rpm using a centrifuge. After that, the supernatant was poured off and the leftover material was thoroughly cleaned five more times using DIW and HCl. To make the GO powder, the cleaned GO solution was dried in an oven for one day at 100°C [39].

2.3 Synthesis of CuS@GO

A suitable quantity of copper acetate was dissolved in water butanol solution for 30 min to make CuS compounds. Thiourea was added to the water butanol solution after copper acetate was originally mixed with it, and the mixture was then stirred for one hour. After that, the finished solution was put into a stainless-steel autoclave lined with Teflon and heated to 200°C for 18h. After utilizing a centrifuge to extract the CuS precipitate, deionized water (DIW) was used to wash it, and air was allowed to dry it for a whole day. For the manufacture of CuS@GO composites, an equivalent procedure was used, the only modification was to add 30 mg of graphene oxide (GO) to the butanol solution before adding the copper acetate. The pure CuS sample and those with 30mg GO are referred to as CuS and CuS@GO respectively [40].

CHAPTER 3

CHARACTERISATION TECHNIQUES AND FABRICATION METHOD

3.1 X-Ray Diffraction (XRD)

X-ray diffraction (XRD) is a powerful analytical technique used to investigate the structure, composition, and properties of crystalline materials. Here's how XRD works and its applications:

Principle: When X-rays interact with a crystalline material, they undergo constructive and destructive interference due to the periodic arrangement of atoms within the crystal lattice. This results in the scattering of X-rays in specific directions, producing a diffraction pattern. By analyzing the angles and intensities of the diffracted X-rays, one can determine the crystal structure and lattice parameters of the material.

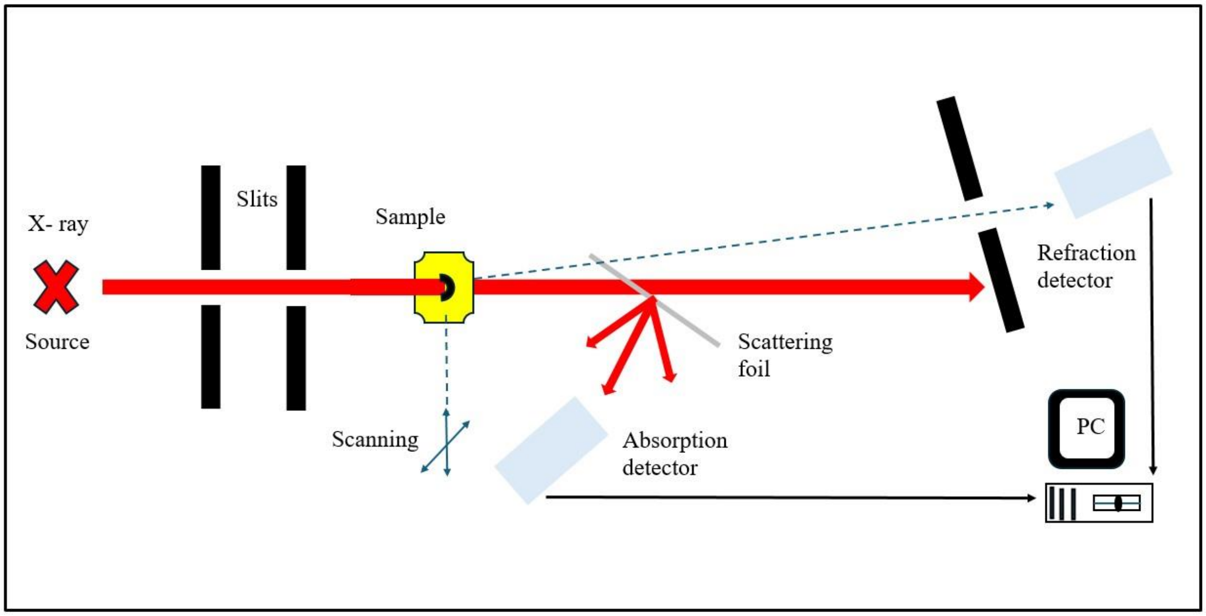


Figure 3.1 Schematic diagram of X-Ray diffraction

Instrumentation: XRD instruments typically consist of an X-ray source, a sample holder, and a detector. The X-ray source emits monochromatic X-rays, usually copper Kα radiation (λ = 1.5406 Å) or cobalt Kα radiation (λ = 1.78897 Å). The sample is mounted on a goniometer, which allows precise control of the angle of incidence and measurement of the diffraction angles. The detector records the intensity of diffracted X-rays as a function of angle.

Data Analysis: The collected diffraction data are analyzed to identify the crystalline phases present in the sample and to determine their crystal structure and orientation. This is typically done by comparing the experimental diffraction pattern with reference patterns from databases such as the International Centre for Diffraction Data for Diffraction Data (ICDD) or the Powder Diffraction File (PDF) [41].

3.2 Fourier Transform Infrared (FTIR) Spectroscopy

Fourier-transform infrared spectroscopy (FTIR) is a widely used analytical technique for identifying chemical bonds, functional groups, and molecular structures in organic and inorganic materials. Here's a detailed explanation of how FTIR works:

Principle: In FTIR spectroscopy, infrared (IR) radiation is passed through a sample, and the intensity of the transmitted light is measured as a function of wavelength. The sample interacts with the IR radiation, causing specific chemical bonds to absorb energy at characteristic frequencies by analyzing the absorbance spectrum, which represents the amount of light absorbed by the sample at each wavelength, one can identify the functional groups and chemical bonds present in the material.

Instrumentation:

An FTIR spectrometer consists of several key components: a broadband IR source (e.g., a global or a Nernst glower), an interferometer, a sample compartment, and a detector.

The IR source emits radiation spanning a broad range of wavelengths. This radiation is directed onto an interferometer, where it is split into two beams.

One beam passes through the sample, while the other serves as a reference. The two beams recombine, creating an interference pattern that contains information about the sample's absorption spectrum.

The interference pattern is detected by a sensitive detector (e.g., a mercury cadmium telluride (MCT) detector), and a computer analyzes the resulting interferogram to generate the sample's absorbance spectrum.

Data Collection and Analysis:

FTIR spectroscopy typically covers the mid-infrared (MIR) region of the electromagnetic spectrum, which ranges from approximately 4000 to 400 cm^{-1} (wavenumbers).

The resulting absorbance spectrum displays peaks corresponding to the absorption of IR radiation by specific functional groups or chemical bonds in the sample.

Each peak in the spectrum represents a vibrational mode of the molecular structure, with the position (wavenumber) and intensity of the peak providing information about the type and abundance of functional groups present.

Modes of Operation:

FTIR spectroscopy can be performed in various modes, including transmission, reflection, attenuated total reflection (ATR), and diffuse reflectance.

Transmission mode involves passing IR radiation through a thin sample, while reflection mode measures the IR reflectance of the sample's surface.

ATR spectroscopy probes the sample's surface by measuring the evanescent wave generated when IR radiation interacts with a high-refractive-index crystal.

Diffuse reflectance spectroscopy is used for analyzing powdered samples or materials with irregular surfaces by measuring the reflectance of IR radiation from the sample [42].

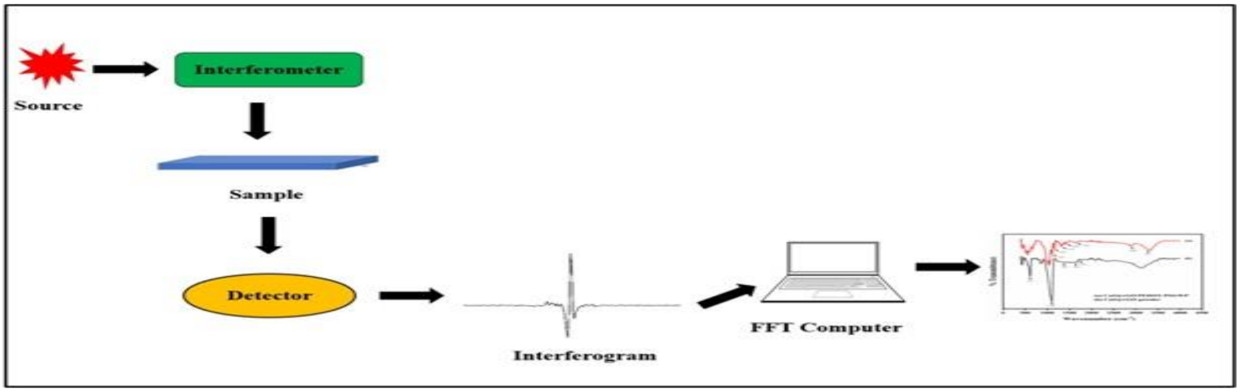


Figure 3.2 Schematic diagram of FTIR Spectrophotometer

3.3 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is a powerful imaging technique used to visualize the surface morphology and structure of materials at high magnification and resolution. Here's a detailed explanation of how SEM works:

Principle of SEM: SEM works on the principle of scanning a focused electron beam over the surface of a sample. When the electron beam interacts with the atoms in the sample, various signals are emitted, including secondary electrons, backscattered electrons, and characteristic X-rays. These signals carry information about the topography, composition, and crystal structure of the sample's surface.

Instrumentation:

A SEM consists of several main components: an electron gun, electromagnetic lenses, a specimen chamber, detectors, and a computer system for image processing.

The electron gun generates a finely focused beam of electrons, typically accelerated to energies ranging from a few kiloelectron volts (keV) to tens of kiloelectron volts.

Electromagnetic lenses focus and scan the electron beam across the surface of the sample with high precision.

Detectors collect various signals emitted by the sample, including secondary electrons (SE), backscattered electrons (BSE), and characteristic X-rays (EDS/EDX).

The computer system processes the collected signals to generate high-resolution images and elemental maps of the sample's surface.

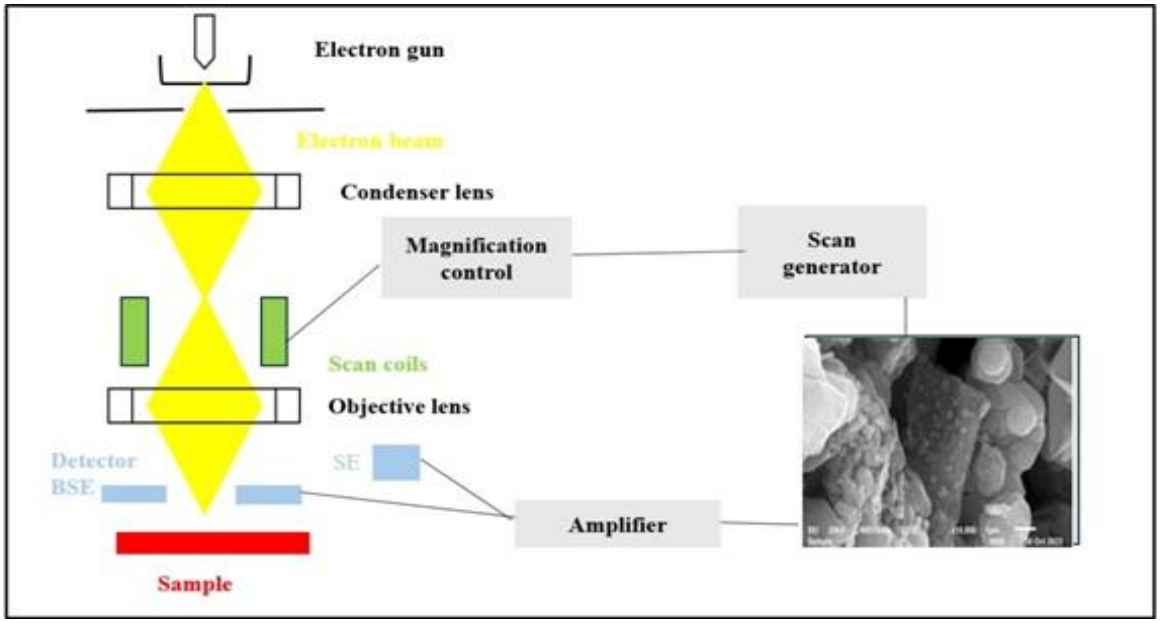


Figure 3.3 Schematic diagram of Scanning Electron Microscope (SEM)

Imaging Modes:

Secondary Electron Imaging (SEI): SEI is the most commonly used imaging mode in SEM. It provides detailed topographical information about the sample's surface by detecting low-energy secondary electrons emitted from the top few nanometers of the specimen.

Backscattered Electron Imaging (BEI): BEI provides compositional contrast based on differences in atomic number (Z) between regions of the sample. Higher Z materials produce more backscattered electrons, resulting in brighter areas in the image.

Elemental Mapping (EDS/EDX): Energy-dispersive X-ray spectroscopy (EDS or EDX) is often integrated with SEM to analyze the elemental composition of the sample. Elemental maps can be generated to visualize the distribution of specific elements across the sample's surface.

Sample Preparation:

Sample preparation is crucial for SEM analysis and typically involves cleaning, drying, and coating the sample with a conductive layer (e.g., gold, palladium) to prevent charging effects and improve image quality. Non-conductive samples may require special treatment, such as sputter coating or carbon coating, to enhance conductivity and imaging quality [43].

3.4 Fabrication method

The dipping method of fabrication was adopted for fabricating paper electrodes. Before dip coating, the Whatman paper was stabilized to gain more surface area and high porosity. For stabilizing the paper, Whatman paper was washed with surfactant Tween 20 about 5 to 6 times and then sonicated in ethanol for 30 min and dried at 100℃.

Take GO (10 g) and water (10 ml) and stirred with the help of a stirrer for about 30 min and then add PEDOT:PSS (2.5 ml). Similarly, CuS@rGO solution was prepared, and both solutions at the stirrer overnight. After stabilizing, paper was cut into (1 cm × 1 cm) strips and dipped in both solutions thrice for 30 min. The GO electrodes and CuS@rGO electrodes were prepared [44].

3.5 Immobilization of Acetyl cholinesterase (AChE) enzyme

20 µL of enzyme solution was drop casted onto the electrode surface, and the biosensor (AChE/CuS@GO/PEDOT:PSS/WP) was formed and incubated for 12 h at 4°C in a humid environment.

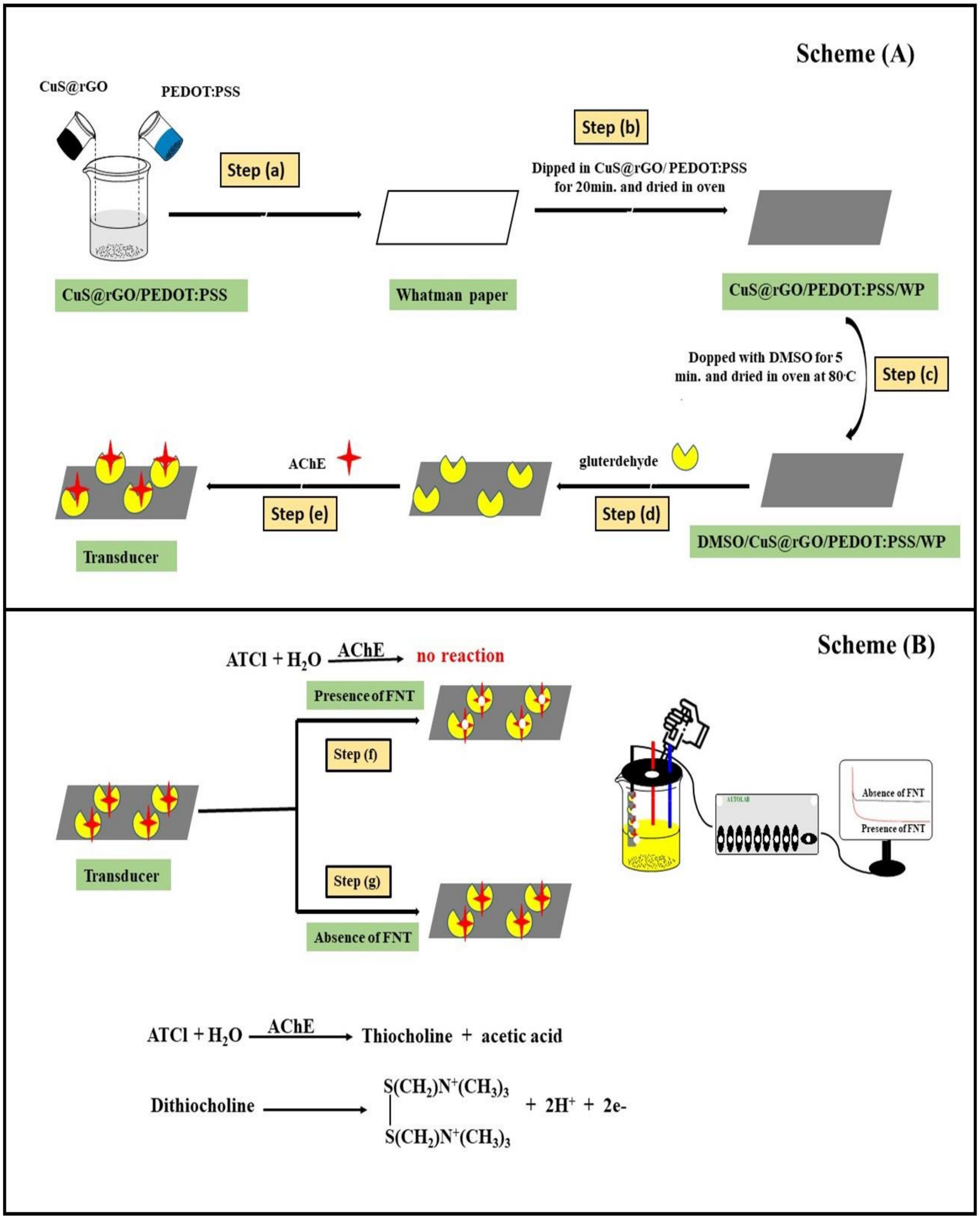


Figure 3.4 **Schematic representation of electrochemical conducting paper-based biosensor for FNT detection**

CHAPTER 4

EXPERIMENTAL SECTION

4.1 Development of Biosensor

This work used CuS@GO/PEDOT:PSS as a sensing platform to produce a flexible, affordable, and biodegradable biosensor. The suggested biosensor's sensitivity is increased by the combined action of PEDOT:PSS and CuS@rGO. WP was first dipped into the aqueous dispersions of CuS@GO and PEDOT:PSS in order to fabricate biosensors, and it was subsequently dried in an oven. Before the immobilization of the enzyme, glutaraldehyde was drop casted onto the surface of electrodes, acts as an organic linker, and combines with an amino group of the AChE enzyme to prevent leaching [45]. After one hour, immobilization of the enzyme takes place, and it is left overnight in a humid chamber at 4˚C. The biosensor that was developed underwent additional incubation with FNT and acetylthiocholine chloride (ATCl). The research shows that when FNT is not present, an electrocatalytic process between the enzyme AChE and ATCl takes place, producing an additional two electrons. The oxidation peak current is brought on by these electrons. By obstructing the active serine residues of the AChE enzyme, FNT reduces the strength of the oxidation peak and thus lowers the peak current.

4.2 Chronoamperometry study of proposed biosensor for FNT detection

Chronoamperometry is a technique used in electrochemistry to study the behavior of electrochemical systems by measuring the current at fixed applied potential overtime. This method is particularly useful for investigating reaction kinetics, electrode processes, and the behavior of electroactive species in solution.

Galvanostat is an instrument commonly used in chronoamperometry to control the current passing through the electrochemical cell. In chronoamperometry, the galvanostat applies a constant current to the cell, and the resulting changes in current over time are measured. This allows for the determination of parameters such as reaction rates, diffusion coefficients, and the concentration of electroactive species.

Chronoamperometry studies are used to evaluate the efficacy of the designed biosensor (AChE/CuS@GO/PEDOT:PSS/WP) for FNT detection. For this analysis, different concentrations (1–80 pM) of FNT are incubated in 7.4 pH PBS with 3 mM ATCl and 5 mM $[Fe(CN)_6]^{3-/4-}$. As the concentration of FNT rises, its ability to react with ATCl consistently diminishes, as the figure makes evident. The constant decrease in current is brought on by AChE activity inhibition when FNT is present.

4.3 Electrochemical Studies

Electrochemical studies of the developed biosensor were assisted by chronoamperometry. When compared to GO/PEDOT:PSS (0.55mA) and PEDOT:PSS/WP (0.22mA), the peak current of the CuS@rGO/PEDOT:PSS/WP electrode (0.67mA) indicates higher conductivity. Additionally, the conductivity changes of the CuS@GO/PEDOT: PSS/WP electrode were investigated in the presence of various organic solvents, including methanol, dimethylformamide (DMF), ethylene glycol (EG), and 1-methyl-2-pyrrolidone (NMP), dimethyl sulfoxide (DMSO). The electrical current data of the CuS@GO/PEDOT:PSS/WP electrode in the presence of all organic solvents under consideration was shown in Fig. 4B. The highest current is shown in this figure for the CuS@GO/PEDOT:PSS/WP electrode that has been treated with DMSO. Additionally, CuS@GO catalyzes the AChE biosensor's electrocatalytic process, increasing its suitability for FNT detection. Consequently, DMSO-treated CuS@GO/PEDOT:PSS/WP was employed as a sensing platform for additional analyses.

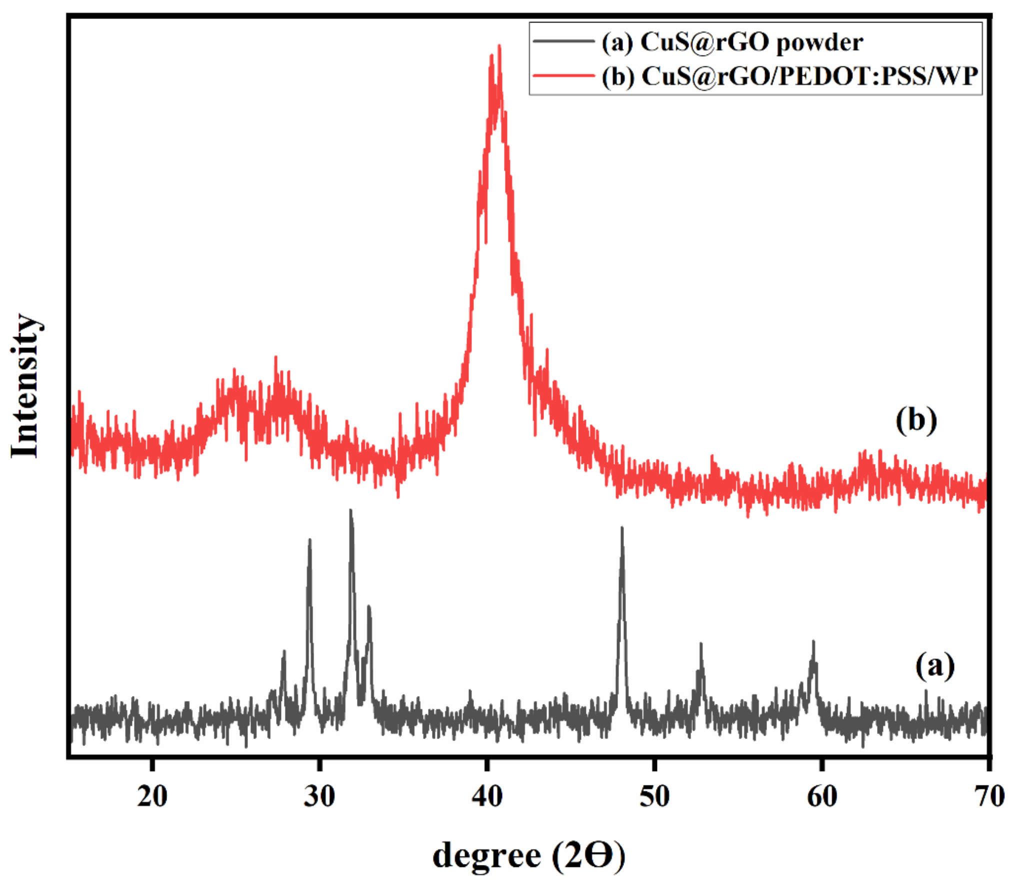
CHAPTER 5

RESULTS AND DISCUSSION

5.1 X-Ray Diffraction (XRD)

The X-ray crystallographic patterns of (a) CuS@rGO powder and (b) CuS@rGO/PEDOT:PSS/WP electrode are shown in Figure 2B. CuS hexagonal covellite structure is indicated by the characteristic peak in curve (a) at particular 2θ angles of 22.9˚, 29.3˚, 31.9˚, 32.8¯, 48˚, 52.9˚, and 59.6˚which correspond to different crystallographic planes (100), (101), (102), (006), (110), (107), (202), and (116)[46]. The lack of additional peaks in the CuS@rGO composite XRD pattern, relates to the material's purity during synthesis. The undoped PEDOT:PSS film displays two broad peaks in the curve (b). The first peak, located at 2θ = 17.57°, is ascribed to the amorphous halo PSS, while the second peak, located at 2θ = 25.45°, is linked to the Π-Π stacking of the PEDOT thiophene ring [47]. A second, highly intense peak, located at about 2θ = 40.7°, is associated with the lattice planes of (107) in the CuS covellite structure [48].

Figure 5.1 X-ray diffraction pattern of (a) CuS@rGO and (b) CuS@rGO/PEDOT:PSS/WP



5.2 Fourier Transform Infrared (FT-IR) Spectroscopy

The FTIR spectra of CuS@rGO/PEDOT:PSS/WP electrode (curve a) and CuS@rGO powder (curve b) are shown in Figure 2A. The characteristic absorption band in the curve (a) at 1039 cm^{-1} represents the symmetric stretching vibrations of the PSS molecule, whereas the bands at 1116 and 1163 cm^{-1} follow the asymmetric stretching vibrations of the PSS molecule. The bands at 1066, 1316, and 675 cm^{-1} correspond to the C-O, C-C, and C-S stretching vibrations in the PEDOT thiophene ring.[49] The band at 717 cm^{-1} represents the C=C bending vibrations, whereas the C-C bending vibrations may be observed at 1438 and 1645 cm^{-1}.[50] The stretching vibrations of the C-H bond in the PEDOT:PSS ring is responsible for the peaks at 2900 and 3277 cm^{-1}. Curve (b) confirms the GO phase with COOH and C-O-C, C-OH functional groups by showing characteristic stretching vibration bands for the presence of C=O (1744 cm^{-1}), C=C (1642 cm^{-1}), and C-O (1385 cm^{-1}). Furthermore, the signal at 610 cm^{-1} is associated with Cu-S.[51]

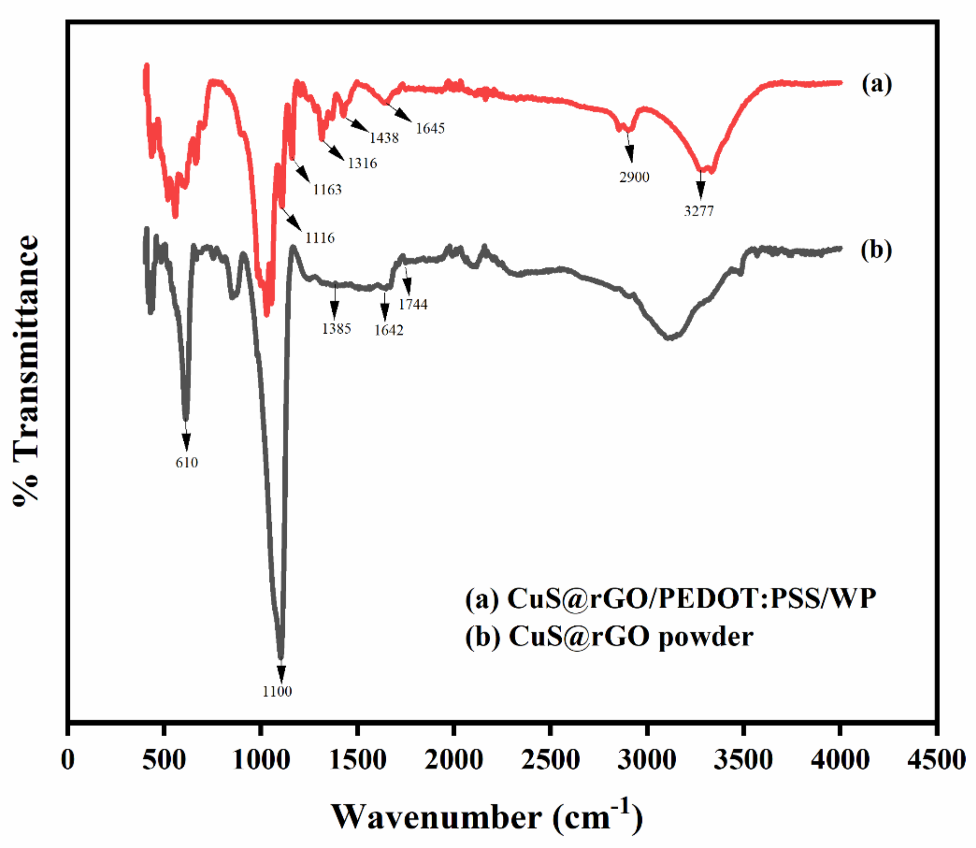


Figure 5.2 FTIR spectra of (a) CuS@rGO/PEDOT:PSS/WP, (b) CuS@rGO powder

5.3 Scanning Electron Microscopy (SEM)

The surface morphologies of powdered GO, CuS@rGO, and PEDOT:PSS are all determined via SEM examination. Figure 3A shows that the conducting polymer PEDOT:PSS has even adsorption onto the Whatman paper (WP) and that cellulose fibers are visible on the surface of the paper. CuS@rGO in powder form is shown in Figure 3B, which also shows the extensive distribution of GO sheets containing spherically embedded CuS particles. Additionally, when CuS@rGO is integrated into the stabilized WP, a consistent structure is observed, indicating that the CuS@rGO/PEDOT:PSS/WP electrode was successfully fabricated. This is demonstrated in Figure 3C. A closer look at CuS@rGO absorbing rGO sheets and CuS nanoparticles can be found in Figure 3D.

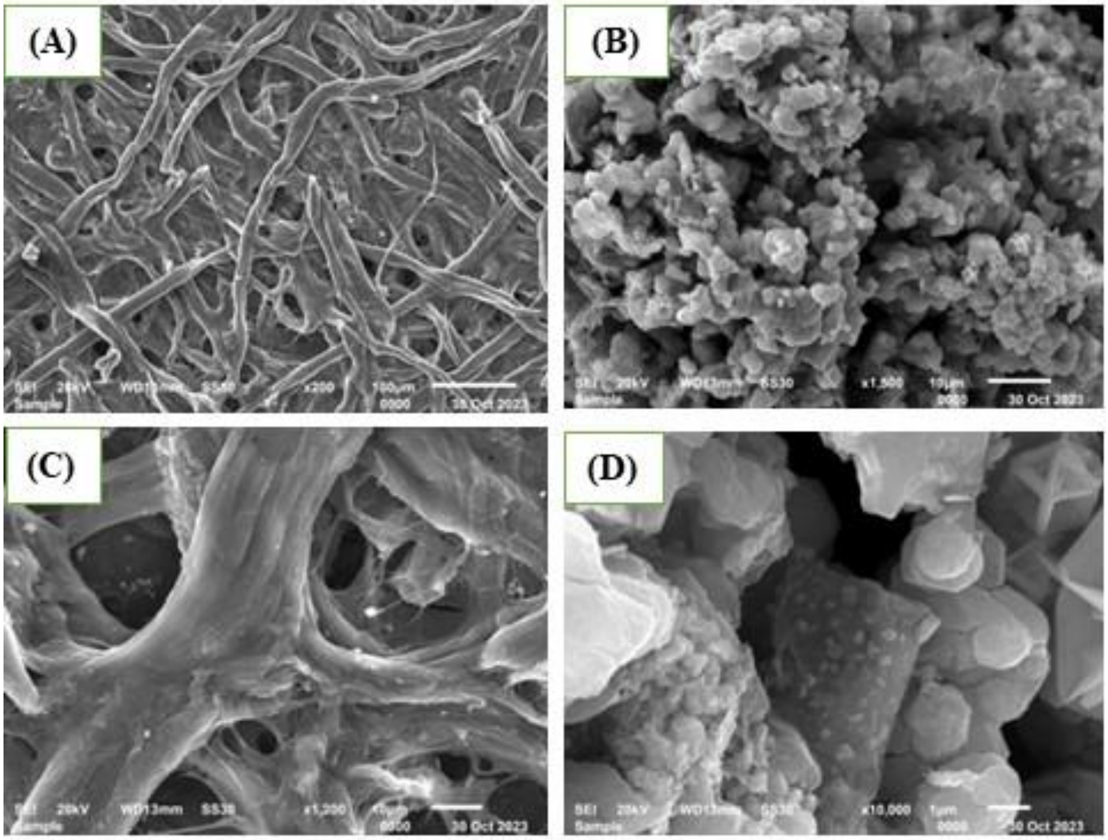


Figure 5.3 SEM image of (A) PEDOT:PSS/WP, (B) CuS@rGO powder, (C) CuS@rGO/PEDOT:PSS/WP and (D) closer view of CuS@rGO/PEDOT:PSS/WP

5.4 Chronoamperometric study of developed biosensor

5.4.1 Chronoamperometric behavior of different electrodes

In this study, PEDOT:PSS/WP, GO@PEDOT:PSS and CuS@rGO/PEDOT:PSS electrode undergoes chronoamperometric analysis. The result shows in Figure 5.4 (A) that CuS@rGO/PEDOT:PSS electrode shows the highest electric current as compared to other two electrodes.

5.4.2 Chronoamperometric response of CuS@rGO/PEDOT:PSS/WP doped with different organic solvents

In this study, CuS@rGO/PEDOT:PSS electrodes were dipped with various organic solvents like DMSO, DMF, methanol, NMP and ethylene glycol to increase the conductivity of the desired electrode. It is found that the electrode treated with DMSO was found to have the highest conductivity among all the solvent treated electrodes which is shown in Figure 5.4 (B).

5.4.3 Electrochemical response of AChE/CuS@rGO/PEDOT:PSS/WP electrode after the incubation of different concentrations of FNT (0-80 pM)

Chronoamperometry studies are used to evaluate the efficacy of the designed biosensor (AChE/CuS@GO/PEDOT:PSS/WP) for FNT detection. For this analysis, different concentrations (1–80 pM) of FNT are incubated in 7.4 pH PBS with 3 mM ATCl and 5 mM $[Fe(CN)_6]^{3-/4-}$. As the concentration of FNT rises, its ability to react with ATCl consistently diminishes, as Figure 5.4 (C) makes evident. The constant decrease in current is brought on by AChE activity inhibition when FNT is present.

5.4.4 Calibration plot of current vs. logarithm of concentration of FNT

The calibration plot of current against FNT concentration (CFNT) is shown in Figure 5.4 (D). This figure, which corresponds to the following equation, displays a linear drop in current as $C_{(FNT)}$ increases from 1 to 80 pM.

$I = 1.25\ \text{mA} – 0.505\text{mA/pM} \times C(\text{FNT}),\ R^2 = 0.9467$ (1)

Through this plot, the suggested biosensor's sensitivity and detection limit (LOD) (3σ/sensitivity) were determined to be 0.505mA/pM and 0.28 pM, respectively. In terms of linear range, LOD, and sensitivity, the suggested biosensor's performance is further contrasted with recently developed biosensors for FNT detection. The findings validate the benefit of the suggested biosensor over the other enzymatic biosensors that were examined. strength of the oxidation peak and thus lowers the peak current.

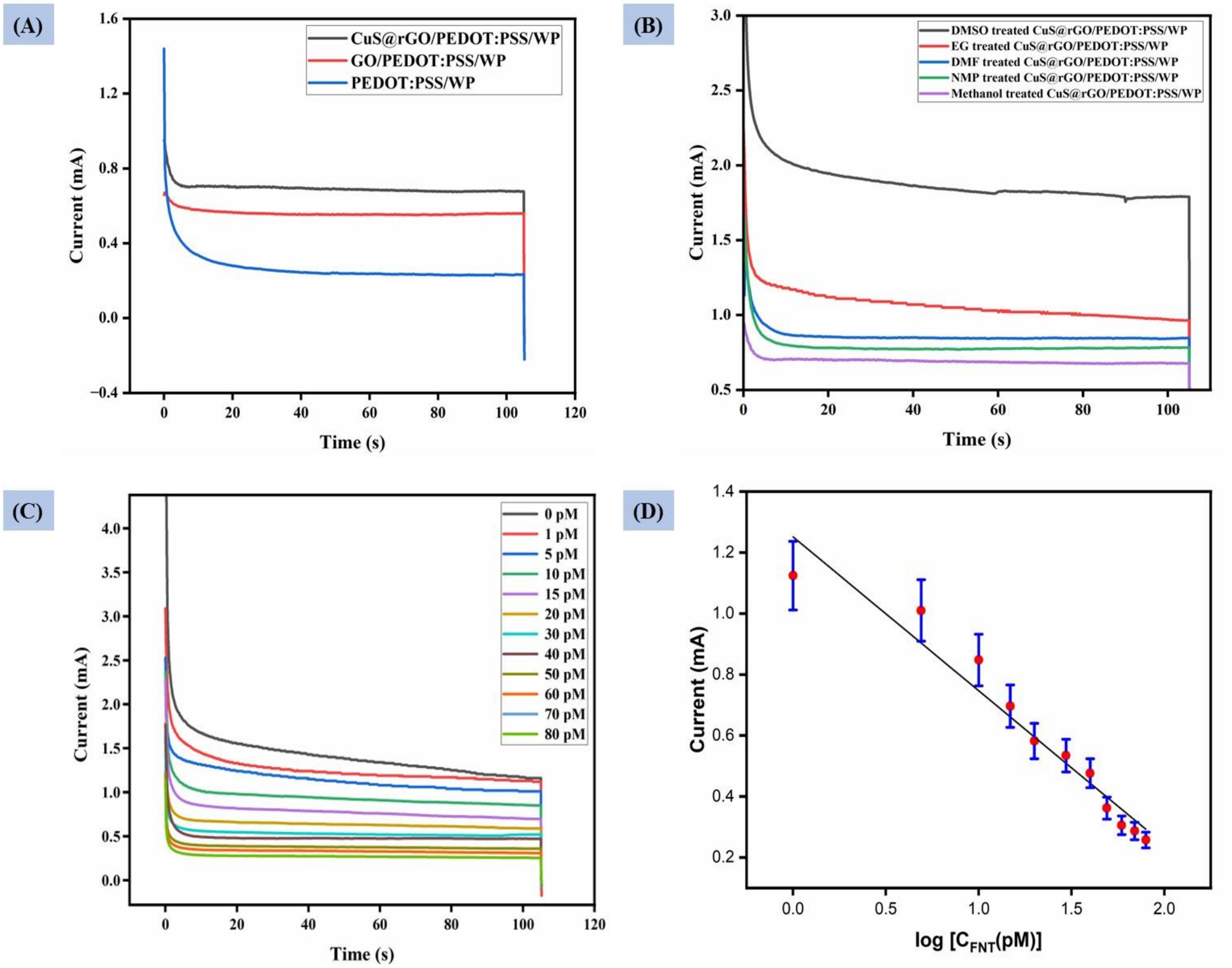


Figure 5.4 (A) Chronoamperometric behavior of different electrodes, (B) Chronoamperometric response of CuS@rGO/PEDOT:PSS/WP doped with different organic solvents, (C) Electrochemical response of AChE/CuS@rGO/PEDOT:PSS/WP electrode after the incubation of different concentration of FNT (1-80 pM), (D) Calibration plot of current vs. logarithm of concentration of FNT.

Table 1. Comparative study of AChE/CuS@rGO/PEDOT:PSS/WP biosensor with recently designed enzymatic biosensor for FNT Detection

S.No.	Fabricated electrodes	Detection limit	Linear range	Sensitivity	References
1.	PNT/PGE	0.0196 µM	0.114-1.712 µM	-	[52]
2.	Polyzincon/GCE	1.5 nM	5-8600 nM	-	[53]
3.	NbC@Mo/SPCE	0.15 nM	0.01-1889 µM	-	[54]
4.	GdM/GCE	5 nM	0.02-123 µM	1.364 µA(µM)$^{-1}$ cm^{-2}	[55]
5.	rGO-WCNPHs/SPCE	1.15 nM	10nM-471.2 µM	5.376 µA(µM)$^{-1}$ cm^{-2}	[9]
6.	IL@CoFe$_2$O$_4$NPs@MWCNTs@GCE	0.0135 µM	0.02-160 µM	-	[56]
7.	AChE/EG-doped-CeO$_2$@PEDOT:PSS/ITO	0.12 nM	0.1-200 nM	52.10 µA(nM)$^{-1}$	[57]
8.	AChE/PANI@CuO/ITO	0.096 nM	1-200 nM	49.86 µA(nM)$^{-1}$	[58]
9.	AChE/CuO@PEDOT:PSS/WP	0.41nM	10-80 nM	9.13 µA(nM)$^{-1}$	[49]
10.	SiO$_2$/MWCNTs/RuPc	0.45 ppm	3-66 µM	0.0822 µA(µM)$^{-1}$mm^{-2}	[59]
11.	**AChE/CuS@rGO/PEDOT:PSS/WP**	**0.28 pM**	**1-80 pM**	**0.505 mA(pM)$^{-1}$**	**Present work**

5.5 Stability, selectivity, and reproducibility of developed biosensor

Selectivity and stability are two of a biosensor's most important practical qualities, and they both drive its effectiveness. By monitoring the chronoamperometry response of the suggested biosensor while detecting FNT, the stability of the developed biosensor was assessed. The AChE/CuS@rGO/PEDOT:PSS/WP electrodes were kept in a refrigerator at 4˚C throughout the 7, 14, 21, and 28-day evaluation. Even after 28 days, there is a small change in the peak current value in Figure 5.5 (A), indicating that the manufactured electrodes have exceptional stability. In PBS with a pH of 7.4, which included 5 mM $[Fe(CN)_6]^{3-/4-}$ and 3 mM ATCl, the proposed biosensor under consideration was exposed to 10 pM concentrations of several interfering species (NaCl, glucose, ascorbic acid, and a mixture of analytes) to test its selectivity. Figure 5.5 (B) displays the interference analysis of the manufactured electrodes in the presence of a mixture of analytes, ascorbic acid, glucose, and 10 pM of NaCl. The diagram demonstrates that, in addition to more interfering species, FNT exhibits the largest peak current, confirming the AChE/CuS@rGO/PEDOT:PSS/WP biosensor's acceptable selectivity for FNT detection.

Using four different electrodes under the same conditions, chronoamperometric analysis was used to assess the reproducibility of the developed biosensor. The high reproducibility of the biosensor, with a low relative standard deviation (RSD) of 2.21%, is illustrated in bar diagram 5(D).

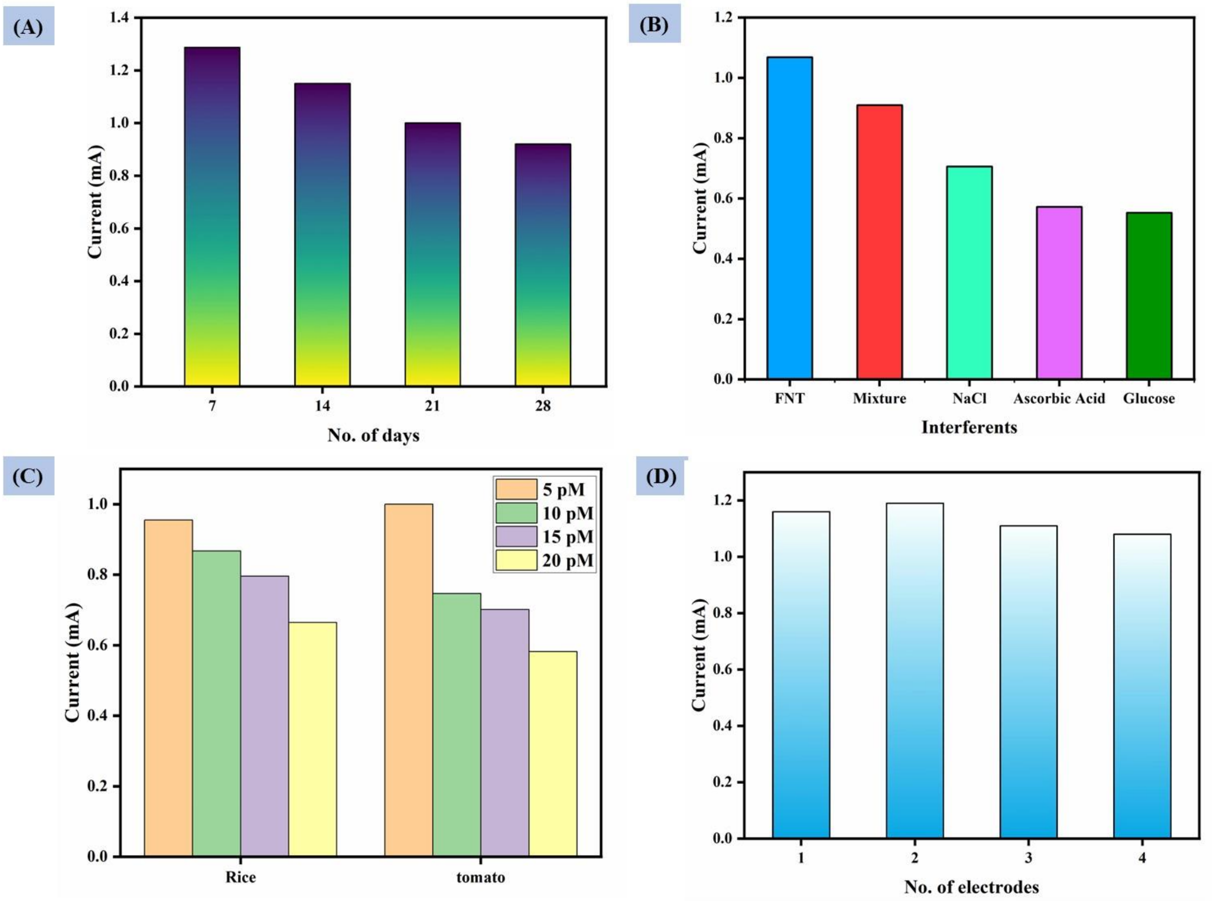


Figure 5.5 (A) Stability study of AChE/CuS@rGO/PEDOT:PSS/WP fabricated electrodes over 28 days, (B) Interference study of fabricated electrodes in the presence of 10 pM of NaCl, ascorbic acid, glucose, and mixture of analytes, (C) Detection of concentration of FNT in two natural samples (rice and tomato), (D) Reproducibility study of four electrodes

5.6 Real Sample Analysis

Rice and tomato, the two natural samples were used to further explore the proposed biosensor's potential in real-world applications. Each sample was collected, cleaned with deionized water, and allowed to air dry. 5g of rice flour, tomato pulp, and 7.4 pH PBS were combined, and the mixture was centrifuged for 20 min at 4000 rpm. Different concentrations of FNT (5, 10, 15, and 20 pM) were added to the liquid supernatant after separation. After that, chronoamperometric analysis was performed using the spiked solutions. The chronoamperometric investigation is shown as the current vs. concentration of FNT in two natural samples, such as tomato and rice, in Figure 5.5

(C). As indicated in Table 2, the results obtained indicate that the recovery rates range from 90 to 107%, and the relative standard deviation (RSD) for the detection of FNT in rice and tomato samples was determined to be less than 8%.

Table 2. Detection in two natural samples through AChE/CuS@rGO/PEDOT:PSS/WP electrodes

S.No.	**Sample**	**Added amount (pM)**	**Found amount (pM)**	**Recovery (%)**	**RSD (%)**
1.	Rice	5	4.53	90.7	6.30
		10	9.82	98.2	1.24
		15	15.79	105.3	7.00
		20	21.3	106.5	4.45
2.	Tomato	5	4.76	95.3	3.35
		10	10.15	101.5	1.11
		15	15.70	105.1	3.54
		20	21.30	106.7	4.59

CHAPTER 6

CONCLUSION

6. Conclusion

This study designs a disposable, flexible, versatile, and cost-effective biosensor for the very sensitive detection of FNT. CuS@rGO and PEDOT:PSS modified conducting paper is used in the sensing platform to increase the enzyme binding capacity, which promotes effective electron transfer rates. The combined effects of GO and CuS improve current characteristics, which improve the recommended biosensor's selectivity, repeatability, and sensitivity. The biosensor exhibits excellent selectivity, stability, low detection limit (0.28 pM), dependable repeatability, and extraordinary sensitivity of 0.505 mA/pM. The accuracy of the suggested biosensor was thoroughly assessed in two natural samples, enhancing its usefulness for fenitrothion detection in practical situations.

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LIST OF CONFERNCE ATTENDED

8. Presented our research work at the ***<u>"International Conference on Crossroads of Chemistry, Biology & Atmospheric Environment: A Modern Perspective"</u>*** organized by University of Delhi, Indian Science Congress Association (ISCA), Delhi Chapter and The Indian Society of Analytical Scientist-Delhi Chapter, held at **Department of Chemistry, University of Delhi** on 26, 27, & 28 February 2024.

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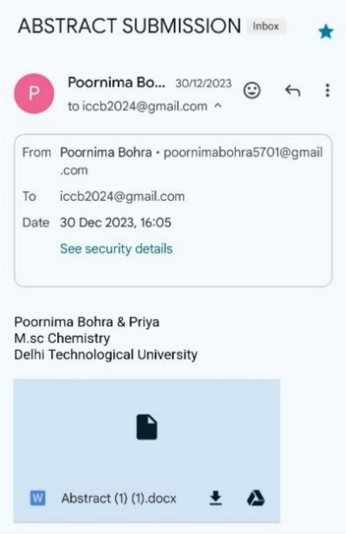
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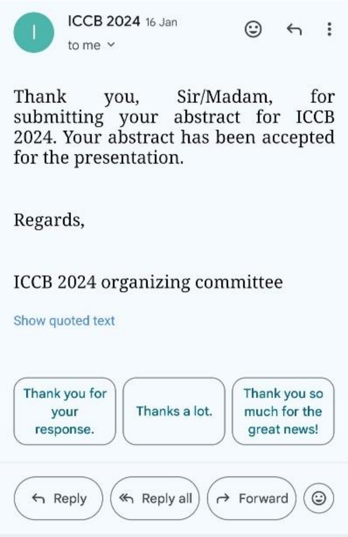
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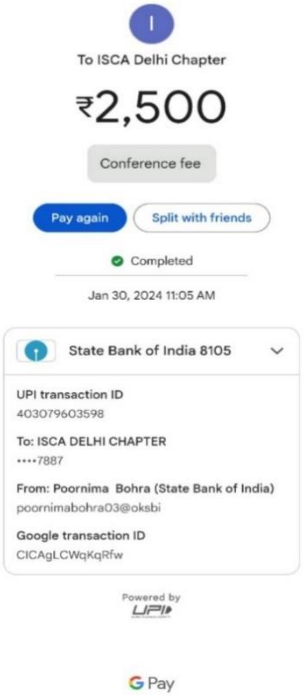
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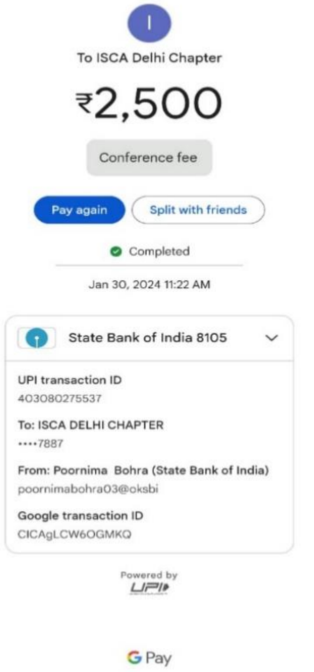
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Awasthi
Prof. Satish K. Awasthi
Convenor, ICCB-2024
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