

Electrochemical Biosensing Platform Utilizing Graphitic Carbon Nitride-Copper Sulfide Nanohybrid for Sensitive Trichlorfon Detection

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MASTERS OF SCIENCE

**IN
(CHEMISTRY)**

**Submitted by
Kritika Jaiswal
(2K24/MSCCHE/39)**

Under the Supervision of

Prof. D. Kumar

&

Prof. R.K. Gupta



**DEPARTMENT OF APPLIED CHEMISTRY
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daultpur Bawana Road, Delhi -110042**

JUNE, 2026



DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi -110042

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I, Kritika Jaiswal (2K24/MSCCHE/39), hereby certify that the work which is being presented in the dissertation entitled “**Electrochemical Biosensing Platform Utilizing Graphitic Carbon Nitride-Copper Sulfide Nanohybrid for Sensitive Trichlorfon Detection**” in partial fulfilment of the requirements for the award of the Degree of Master of Science in Chemistry, submitted in the Department of Applied Chemistry, Delhi Technological University is an authentic record of my own work carried out during the period from 2025 to 2026 under the supervision of Prof. D. Kumar and Prof. R.K. Gupta.

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

Place: Delhi

Kritika Jaiswal
(2K24/MSCCHE/39)

Date:22/06/2026



Department of Applied Chemistry
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daultpur, Main Bawana Road,
Delhi -110042

CERTIFICATE

Certified that Kritika Jaiswal (2K24/MSCCHE/39) have carried out her research work presented in this thesis entitled **“Electrochemical Biosensing Platform Utilizing Graphitic Carbon Nitride-Copper Sulfide Nanohybrid for Sensitive Trichlorfon Detection”** for the award of Degree of Master of Science in Chemistry from the Department of Applied Chemistry, Delhi Technological University, Delhi, under our supervision. The dissertation embodies the results of original work, and studies are carried out by the students herself, and the content of the thesis does not form the basis of the award of any other degree to the candidate or to any other body else from this or any other University/Institution.

Prof. R.K. Gupta
(Co-Supervisor)

Prof. D. Kumar
(Supervisor)

Place: Delhi

Date: 22/06/2026

ABSTRACT

Trichlorfon (TF) is considered the most frequently used organophosphorus compound among all compounds used for agricultural purposes. As such, TF can be quite effective in controlling insects that damage crops in several different ways. However, TF is toxic, and when individuals consume food contaminated with TF, it inhibits acetylcholinesterase, leading to a massive buildup of neurotransmitters at nerve synapses, ultimately disrupting normal nerve signal transmission and potentially causing severe neurological disorders. To address this problem, we have created a rapid, low-cost electrochemical biosensor using acetylcholinesterase (AChE) to detect the organophosphate pesticide (TF) via a g-C₃N₄/CuS-based nanocomposite. Electrostatic interactions between the g-C₃N₄/CuS nanocomposite enhance electron transfer to the electrode surface, thereby facilitating the covalent immobilization of AChE onto the electrode, with glutaraldehyde as a cross-linker to prevent enzyme leaching. Biosensor (AChE/g-C₃N₄/CuS/ITO) demonstrated an ultrahigh sensitivity toward TF over the linear range 0.1 pM - 1 μ M. The developed biosensor showed stability, interference capability, good reproducibility and improved sensitivity. These characteristics enabled us to successfully detect TF in two real samples: apple and cucumber.



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Shahbad Daulatpur, Main Bawana Road,
Delhi -110042

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Prof. R.K. Gupta
(Co-Supervisor)

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ABBREVIATIONS

OP	Organophosphorus Pesticide
TF	Trichlorfon
g-C ₃ N ₄	Graphitic Carbon Nitride
CuS	Copper Sulphide
ATCl	Acetyl thiocholine chloride
PBS	Phosphate Buffer Saline
ITO	Indium tin oxide
XRD	X-Ray Diffraction
FT-IR	Fourier Transform Infrared
CV	Cyclic Voltammetry
DPV	Differential Pulse Voltammetry
EPD	Electrophoretic Deposition
RSD	Relative Standard Deviation
EDAX	Energy Dispersive X-Ray Spectroscopy
FESEM	Field Emission Scanning Electron Microscope
TEM	Transmission electron Microscope
AChE	Acetylcholinesterase

CHAPTER 1

Introduction

1.1 Introduction to pesticides

Agriculture is an essential part of humanity and will remain so for years to come. In order to ensure the World's increasing number of people can eat, farmers are using a lot of chemicals to keep their crops from being destroyed by insects, fungi, weeds and other pests. Pesticides originated from the Latin words *pestis* (plague) and *caedere* (to kill). A pesticide is generally defined as any substance that may be applied either directly to plants or sprayed into the environment around them for preventing, destroying, repelling, or controlling any pest, including insects, mites, nematodes, slugs, snails, rodents, birds, bacteria, viruses, weed seedlings, etc., but excluding organisms present in or on commodity crops at the time of harvest [1].

Synthetic pesticides have been used in modern agriculture since World War II (1939–1945). During this time, scientists identified the insecticidal properties of DDT (dichlorobiphenyl-trichloroethane). Following World War II, many new synthetic pesticides were developed and introduced commercially. As stated earlier by the FAO (Food and Agriculture Organization), the number of pesticides used globally increased by over 100 per cent since 1990. The total amount of active pesticide ingredients was estimated at approximately 3.73 million tons in 2023 [2].

Although pesticides have greatly increased food production in many parts of the World, their overuse and inadequate regulation have caused a wide range of serious problems for the environment and public health. Pesticides will not remain at the location where they were applied; they move through the soil, into our rivers, lakes, and groundwater, and into the foods we eat. Many non-target animals are harmed by pesticides, including fish, birds, pollinating insects (bees), and the microorganisms that live in soils. Human exposure to pesticides has also been linked to several serious diseases, including cancer, Parkinson's disease, endocrine disruption and nerve disorders. The people who are at the highest risk from pesticide exposure include farmers, young children and rural residents [3].

1.2 Classification of pesticides

The main classifications of pesticides, based on the organism targeted as a pest, are Insecticides, Herbicides, Fungicides, Bactericides, Ovicides, Rodenticides, Acaricides, and Nematicides. Within each category, there are many different chemical subcategories of pesticides which vary greatly from one another in terms of how long they last in the environment, how toxic they are and how they act on living organisms [1].

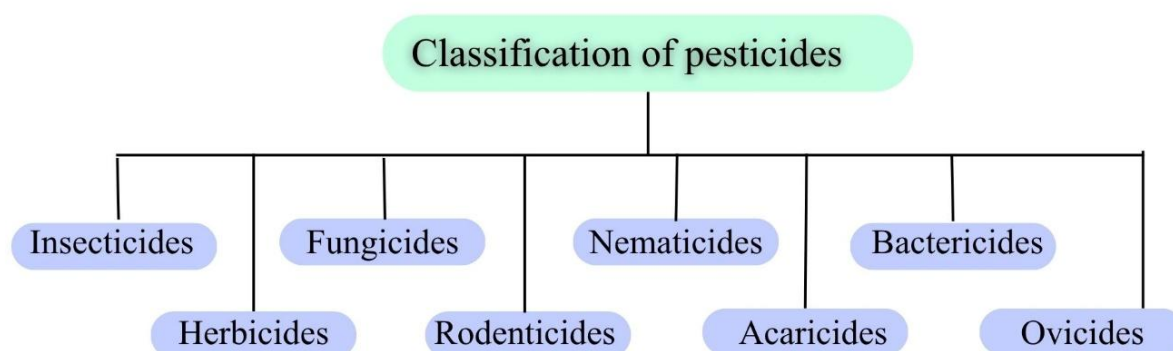


Fig. 1.1 Classification of pesticides

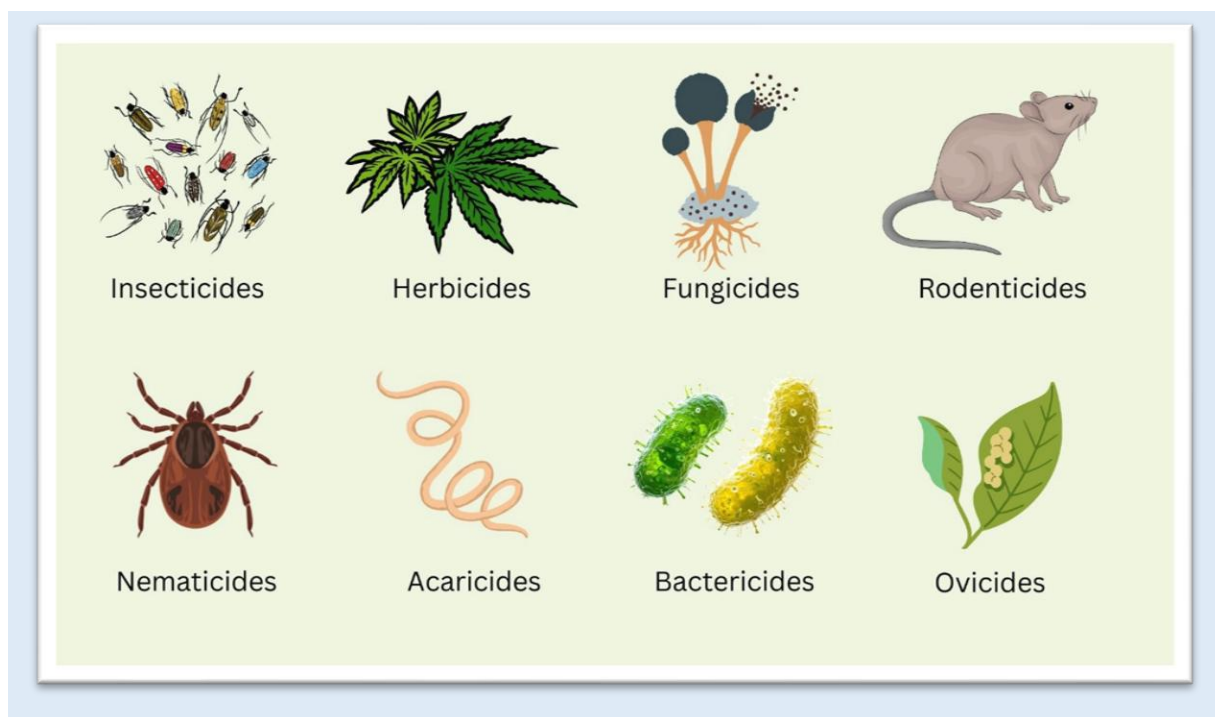


Fig. 1.2 Types of pesticides and their target pests

a) Insecticides: Pesticides that have been designed to kill or repel insects are called insecticides. Their action can be based on disrupting a pest's metabolism or developmental cycle. The major chemical classes among these insecticides include organochlorine (e.g., DDT, aldrin), organophosphate (e.g., trichlorfon, chlorpyrifos, malathion) and carbamate (e.g., carbofuran) compounds, as well as pyrethroid (e.g., permethrin) and neonicotinoid (e.g., imidacloprid) compounds [4].

b) Herbicides: Herbicides kill undesired plants like weeds by interrupting a necessary function of all plants, such as growth through photosynthesis or amino acid production, or hormone activity. Some of the most common types of herbicide chemicals include chlorophenyl compounds (such as 2,4-D), triazines (like atrazine), glycine derivatives (glyphosate) and urea derivatives (diuron) [4].

c) Fungicides: Fungicides are chemicals that have the ability to either destroy fungal spores or stop them from growing. They are also important in preventing plant diseases, including rust, blight, and mildew. There are several types of fungicides that fall into the categories of dithiocarbamates, phthalimides, benzimidazoles, and triazoles [5].

d) Rodenticides: Rodenticides are a class of pesticide products developed to manage and regulate rodent populations, such as rat and mouse populations. Rodenticides can be highly toxic and are used for agricultural or urban purposes. Rodenticides fall into three main categories: First Generation Anticoagulants (e.g. Warfarin), Second Generation Anticoagulants (e.g. Brodifacoum) and Non-anticoagulant Rodenticides (e.g. Zinc Phosphide) [6].

e) Acaricides: Acaricides are specifically designed to target and kill mites and ticks (Acari subclass) that exist as pests of both agricultural crops and livestock. They also have a role in controlling ectoparasites in humans. Most acaricides fall into one or another of the many common insecticide families, such as the organophosphate family (trichlorfon), the pyrethroid family (fenpropidin), and the carbamate family [6].

f) Nematicides: Nematicides are chemical compounds intended for use as pesticides, which target and destroy nematode organisms (which are also known as roundworms), which prey on the roots of plants. Most nematicide products are formulated as either solid granules or gases. The most commonly used nematicides are carbamate pesticides (such as carbofuran and

aldicarb) and organophosphate pesticides. Due to their high toxicity, many nematicides exhibit moderate to long-term persistence in soil [6].

g) Bactericides: Bactericides can be defined as a group of pesticides that are produced for killing or limiting the reproduction of bacteria. The use of these is most common in agricultural applications to manage bacterial infections in crops, such as fire blight in apple trees and bacterial leaf spot in tomato and pepper plants, and as disinfectants and antiseptic agents in public health and industrial environments. There are several main categories of chemically classified bactericides, including, but not limited to, copper-based formulations (copper hydroxide, copper oxychloride), antibiotics (streptomycin, oxytetracycline), phenols (triclosan), and sulpha-based formulations [6].

h) Ovicides: Ovicides are a specific type of pesticide that will destroy egg stages of all insect pests, as well as mite and arachnid eggs. Most pesticides kill at the adult stage, while ovicides kill the early embryo before it has a chance to hatch. Therefore, ovicides disrupt the pest's reproductive cycle. Some common ovicides include benzoylurea derivatives (e.g., diflubenzuron), which inhibit chitin synthesis in developing embryos [6].

1.3 Classification by Chemical Structure

There are many different classifications for pesticides based on their chemical composition and groupings. These include how a pesticide's structure influences its environmental fate and biological activity [7]. Each of the three major classes listed below:

a) Organochlorine: Organochlorines represent a class of man-made pesticides that contain one or more carbon-chlorine (C-Cl) linkages bonded to either an aliphatic or aromatic backbone. Due to the high electronegativity of chlorine atoms and the relatively strong covalent bonding energies in C-Cl linkages, organochlorines exhibit strong resistance to hydrolysis, photodegradation, and microbial biodegradation. Organochlorines also possess very low water solubility, as indicated by high octanol-water partition coefficients ($\log K_{ow}$), generally greater than 4, resulting in significant accumulation in lipid-rich tissues [7].

b) Organophosphate: Organophosphate (OP) compounds are typically derived from either phosphorus (III) containing acids, such as phosphoric acid. These have side chains that can bear

a variety of functional groups, such as alkyl, aryl, alkoxy, and aryloxy. However, regardless of the composition of these side groups, all OPs have one thing in common: they are all esterified forms of phosphoric acid and therefore contain a phosphoryl (P=O) or a thio-phosphoryl (P=S) linkage [7]. OPs act by causing irreversible inhibition of acetylcholinesterase (AChE), the enzyme responsible for the breakdown of the neurotransmitter acetylcholine. AChE uses the amino acid serine to catalyse this reaction.

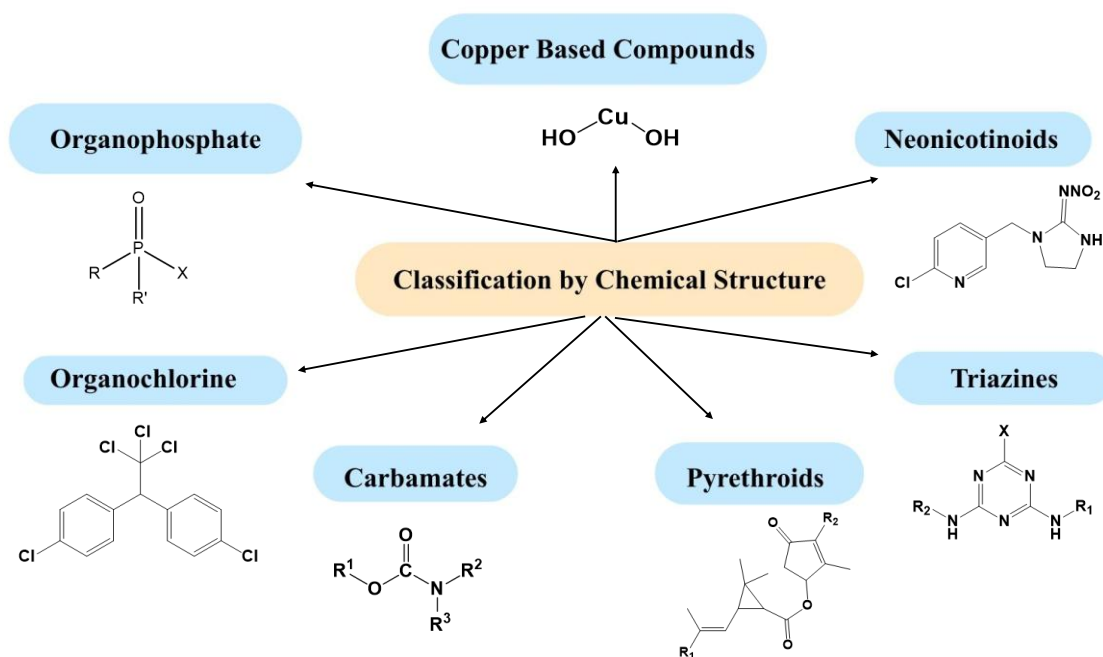


Fig. 1.3 Classification by Chemical Structure

c) Carbamates: Carbamate compounds are esters of carbohydric acid (NH_2COOH). The carbamate functional group has been attached to an aromatic ring (naphthyl or phenyl) via a labile bond.

d) Ester linkage: As with the organophosphates, carbamates inactivate AChE by forming a covalent link to the serine hydroxyl; however, this inactivation can be reversed. The carbamoyl group will bind to the serine hydroxyl group and then be rapidly removed (mins to hrs) via decarboxylation. Although reversible inhibition may make carbamates less acutely toxic to mammals than organophosphates on a mole-for-mole basis, they still pose a significant hazard.

e) Pyrethroids: Pyrethroids are artificial versions of the natural compounds pyrethrins, those being naturally occurring insecticides produced by the Chrysanthemum flower (*Chrysanthemum cineraria folium*). The fundamental structure is that of a cyclopropane carboxylic acid linked as an ester to an alcohol with a phenoxy benzyl or similar grouping. Pyrethroids cause neuronal action potentials to repeatedly fire by prolonging sodium channel opening. Eventually, this will lead to the death of the neuron, or at least render it unable to function [8].

f) Neonicotinoids: Neonicotinoids represent a recent structural class of compounds, with their chemical backbone based on the nitroguanidine or nitro methylene groups attached to an aromatic or heteroaromatic cycle (i.e., thiazole, tetrahydrofuran, etc.) or pyridine. A common structural representation of neonicotinoids describes them as "pharmacophores" that interact with high affinity with the nicotinic acetylcholine receptors (nAChRs) found in insects. Neonicotinoids cause excessive activation of the nAChR due to agonism, and this results in continuous stimulation of neurons, leading to paralysis and eventual death [8].

g) Triazines: Triazines are heterocyclic compounds that consist of a six-membered ring system including three nitrogen atoms (a 1,3,5-triazine). Some chloro-substituted triazines contain one or more chlorine atoms on their rings (i.e. atrazine, simazine), while others can be substituted at their ring positions by either a methyl group or an oxygen atom. The triazines interfere with photosynthesis in plants, specifically the PSII apparatus. They bind directly to the D1 protein, a critical component of this mechanism, and block electron transfer, thereby causing oxidative damage and killing the plant [8].

h) Copper-Based Compounds: Copper is an inorganic pesticide because it releases a chemical called Cu^{2+} , which is the active compound. The most common form of copper pesticide is copper hydroxide. When proteins are denatured, copper will interfere with the enzyme site, especially iron-sulphur cluster enzymes. Also, when bacteria and fungi are subjected to oxidative stress from reactive oxygen species (ROS), they die due to nonspecific cellular damage [8].

Table 1.1 Major classes of pesticides by target organism, with target pests and chemical subclasses

S.No.	Target Class	Target Pests	Chemical Subclass	Example
1.	Insecticides	Insects like beetles, moths, and mosquitoes	Organochlorines	DDT, Aldrin
			Organophosphates	Chlorpyrifos
			Carbamates	Carbofuran
			Pyrethroids	Permethrin
			Neonicotinoids	Imidacloprid
2.	Herbicides	Weeds	Triazines	Atrazine
			Glyphosate-based	Glyphosate
			Urea derivatives	Diuron
3.	Fungicides	Fungi like rusts and blights	Dithiocarbamates	Mancozeb
			Phthalimides	Captan
			Benzimidazoles	Carbendazim
			Triazoles	Tebuconazole
4.	Rodenticides	Rodents like rats, mice, etc	Anticoagulants	Warfarin
			Non-anticoagulant	Zinc phosphide
5.	Acaricides	Mites and ticks	Organophosphates	Trichlorfon
			Pyrethroids	Fenpropathrin
6.	Nematicides	Nematodes like roundworms	Carbamates	Carbofuran
7.	Bactericides	Bacteria	Copper-based compounds	Copper hydroxide
			Antibiotic bactericides	Streptomycin
			Sulfone bactericides	Fubianezuofeng
8.	Ovicides	Eggs of insects	Benzoylureas	Diflubenzuron
			Natural products like neem	Azadirachtin

1.4 Useful impacts of Pesticides

Increasing crop yields and protecting global food security: Crops are protected from being destroyed by various types of pests, including insects, weeds, fungi, etc., through the use of pesticides [9]. These same pesticides enable farmers to produce significantly higher yields on their existing land. If we were to eliminate pesticide use, it is estimated that there could be an additional 50-80% loss in total crop yield, creating another serious problem in providing enough food globally [10].

a) Protecting human health: There are several types of pesticides (especially insecticides) that will reduce populations of vectors that carry diseases such as malaria (mosquitoes), Lyme disease (ticks), typhoid fever (flies), etc. Historically, programs utilizing DDT and many other insecticides have saved millions of people in less-developed areas of the world from dying of vector-borne diseases [10].

b) Reducing populations of non-native or aggressive weed species and other plant species: Herbicides allow farmers to control non-native or aggressive plant species that can outcompete crops for resources such as sunlight, water and nutrients. If herbicide use were eliminated, then crop production rates would dramatically decrease, and native ecosystems could become overrun by non-native or aggressive plant species.

c) Controlling household pests (both domestic and urban): Pesticides are commonly used within residential settings, commercial/office settings, and public spaces to control household pests such as cockroaches, termites, bed bugs, rats, etc. The use of pesticides helps prevent property damage and reduces the risk of disease transmission in urban communities [9].

d) Providing veterinary or livestock-related benefits: The agricultural industry utilizes pesticides (specifically acaricides such as trichlorfon) to control tick or mite, fly and parasitic infestation problems in animals. These pest control measures improve the health status and productivity of animals, resulting in improved quality meat and milk products [10].

1.5 Harmful impacts of pesticides

Pesticides are a serious environmental hazard of the modern age. As effective as they may be in increasing crop yields and managing plant diseases, pesticides also carry many environmental and health risks. Pesticides can persist in the air for years, contaminating our water, food, and soil, and have caused numerous health problems, including both short- and long-term toxic effects. There are three major interconnected areas of concern: the impact on human health (from acute poisonings to long-term health issues, including cancer), contamination of the environment (long-lasting presence in soils and waters), and the destruction of beneficial organisms and harm to ecosystem services [11].

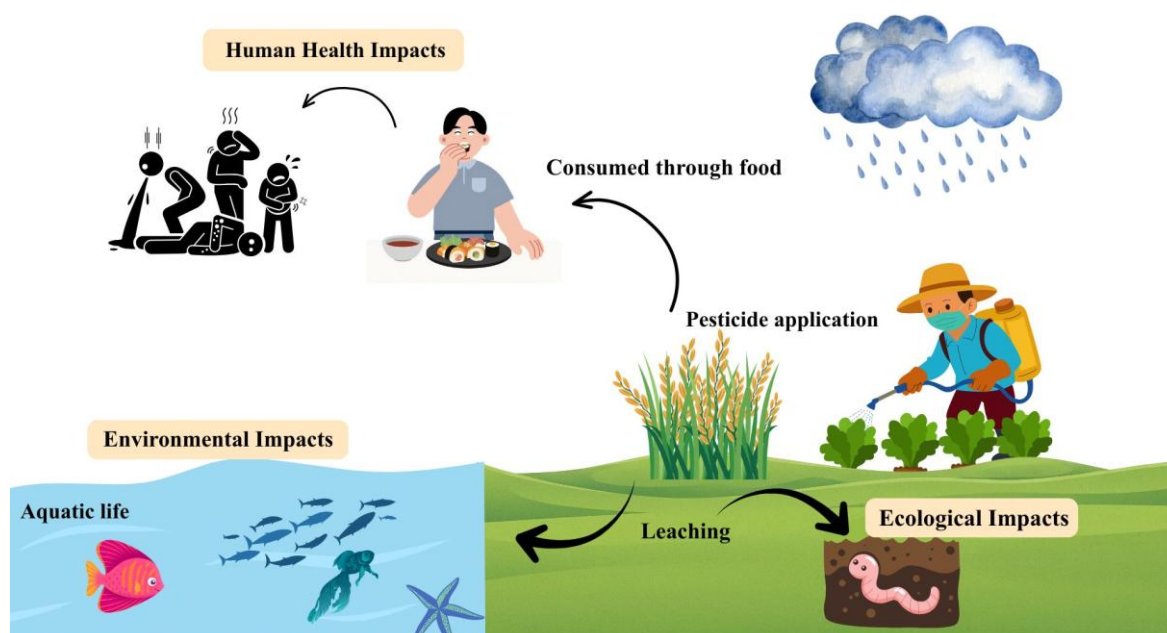


Fig. 1.4 Harmful impacts of pesticides

a) Human Health Impacts: The toxicology of pesticides has created a multitude of harmful health impacts in people, both acute and chronic. Acute poisoning occurs when a person ingests, inhales or comes into contact with pesticides at high doses, which can lead to symptoms such as dizziness, tremors, convulsions, and respiratory arrest. Additionally, some chemicals, specifically the organophosphate and carbamate families, block an enzyme called acetylcholinesterase, which is used by nerve cells to send signals. Chronic toxicity develops over time from low-dose pesticide exposure. The chronic toxicities include neurotoxicity, immunotoxicity, hormonal imbalance (endocrine disruption), developmental disorders (e.g., reproductive issues), and several forms of cancer [12]. Many pesticides have demonstrated

carcinogenic properties: DDT and other related products are listed as "probable human carcinogens" (cancer-causing agents) and are associated with liver cancer. Atrazine is likely to be carcinogenic to humans according to the World Health Organization (WHO) and may be associated with developing prostate and lung cancer earlier in life than normal. Hormone disruption is one of the largest concerns regarding pesticide usage. DDT, atrazine, and neonicotinoids all disrupt hormone homeostasis, which contributes to birth defects, infertility, thyroid disease, and increases the risk of developing type II diabetes, obesity, and breast cancer. Chemical categories of pesticides demonstrate unique patterns of toxicity. For example, organochlorine pesticides are persistent, bioaccumulate, and affect neural development; neonicotinoids affect multiple body systems and form covalent bonds with DNA, thereby creating genetic mutations; and atrazine contaminates the drinking water supplies of millions of people, contributing to reproductive and metabolic diseases [10].

b) Environmental Impacts: Pesticide contamination affects virtually every part of our planet's environment and can last anywhere from a few days to hundreds of years. Pesticides that persist for more than 10 years (e.g., organochlorines) continue to accumulate in the soil each year they are applied, resulting in declines in soil biota populations and fertility. Contamination of surface waters occurs primarily through direct runoff from treated fields, seepage of contaminants from treated fields into groundwater aquifers (leachate), and indirect aerial drift. While most pesticides do not adhere to sediment particles, organochlorines strongly bind to them [13]. Neonicotinoids are highly soluble in water and have been detected at extremely low concentrations (parts per trillion to parts per billion) in surface waters across much of North America. Surface water contamination will occur if agricultural practices involve spraying pesticides near bodies of water. Persistence and bioaccumulation are key characteristics of POPs.

c) Ecological Impacts: Agricultural practices involving pesticides result in significant detrimental effects to non-target species as well as disruptions in fundamental ecosystem processes. One of the most notable examples of the negative effects of pesticides on non-target species is the impact on pollinator populations, particularly honeybee (*Apis mellifera*) colonies and wild bee populations. Neonicotinoids and fipronil inhibit reproductive behaviour (female honeybees stop laying eggs), navigation behaviour (orientation within the hive), memory formation (learning floral scents), and immune response (ability to fight off infection), thereby contributing to colony collapse disorder (CCD). Field-realistic concentrations of neonicotinoid

pesticides have been shown to suppress the immune system of adult honeybees, making them more susceptible to parasitic infections and infestations by the varroa mite. Aquatic organisms are also significantly impacted by pesticide applications [14]. Runoff containing pesticides entering freshwater stream channels can kill fish and other aquatic animals. Organochlorines produce reproductive failure in fish, whereas neonicotinoids and pyrethroids are lethal to aquatic insects and thus disrupt entire aquatic food webs. The persistence of pesticide residues in soils can negatively affect soil organisms, including earthworms, microorganisms, and arthropods, thereby reducing both biological and functional diversity. The death of earthworms reduces nutrient mixing and movement in soils and impairs soil structure. Reduced microbial populations diminish decomposition rates and nutrient availability. Biodiversity losses are occurring rapidly and are largely driven by increased agricultural intensity and the widespread use of pesticides. Non-target plant contamination, surface water contamination, and contamination of food chains create continuous exposure pathways that compromise ecological resilience [13].

1.6 Techniques for Detection of Pesticides

Over the years, numerous analytical techniques have been developed to detect and quantify pesticide residues in food, water, soil, and biological samples for public health and environmental protection. In general terms, these many different types of analytical techniques can be categorized as either traditional or conventional, and advanced or modern [15].

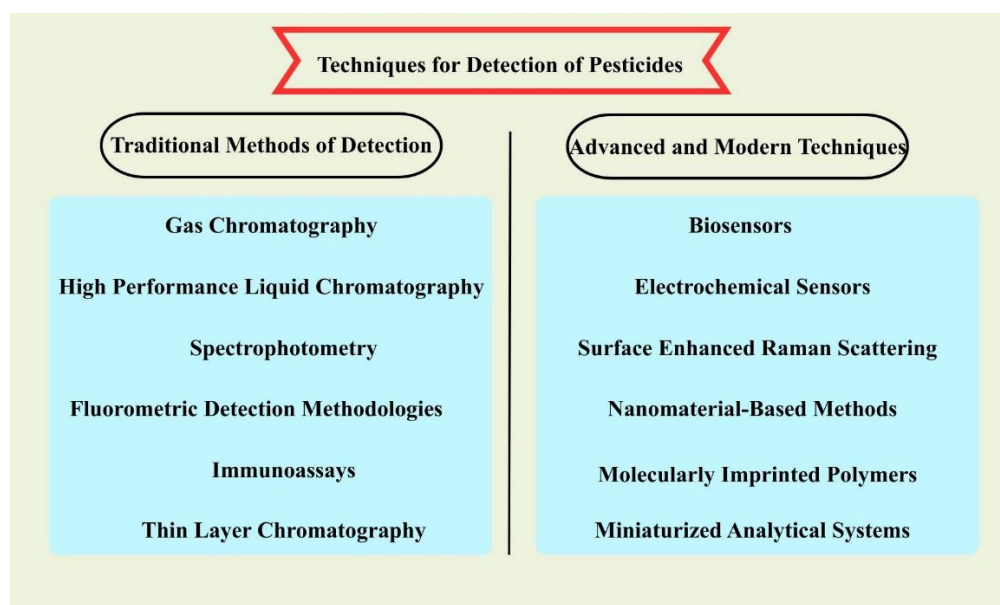


Fig. 1.5 Different Techniques for Detection of Pesticides

1.6.1 Traditional Methods of Detection

a) Gas Chromatography: Gas chromatography (GC) is a well-established, conventional laboratory technique that can be utilized to analyze many types of pesticide residues. The basic principle behind this technique is that the sample must first be vaporized, and then the vapour will move through a column of stationary-phase material using an inert carrier gas. Pesticide compounds will separate from each other along the length of the column based upon their volatility and ability to interact with the stationary phase. GC is typically coupled with various types of detectors, including Flame Photometric Detectors (FPDs), Electron Capture Detectors (ECDs), and Mass Spectrometers. FPDs are particularly sensitive to organophosphorus and organosulfur-containing pesticides. ECDs are especially sensitive to chlorinated pesticides. GC-MS offers both accurate quantitative information and unequivocal identification of pesticide residues. As such, GC-MS is the "gold standard" for pesticide residue analysis. However, this technology is quite expensive and requires a significant amount of technical expertise to operate. Sample preparation prior to analysis is also often labour-intensive [16].

b) High Performance Liquid Chromatography: HPLC is utilized when the pesticide compound being analyzed is either too thermally unstable or does not volatilize sufficiently under normal conditions. This limits the use of GC. When separated by their retention times, these compounds interact with the stationary phase within the HPLC column as they move through the liquid mobile phase. HPLC is frequently combined with UV-VIS spectrophotometers, fluorescence detectors, or mass spectrometers (LC-MS/MS). HPLC has become a common tool in food safety laboratories for detecting pesticide residues in fruit, vegetable, and water samples [17].

c) Spectrophotometry: Spectrophotometric detection involves measuring the absorption of light at specific wavelengths by pesticide residues or their chemical transformation products. These methodologies are generally less expensive than chromatographic detection methods; however, they tend to lack the higher sensitivity and selectivity found in some chromatographic methods [18].

d) Fluorometric Detection Methodologies: Some pesticide residues or their metabolic byproducts naturally fluoresce or can be chemically modified into fluorescent substances. By measuring the fluorescence emitted during excitation, fluorometric analyses can achieve very

low limits of detection. Matrix effects can still negatively affect the precision of this methodology [18].

e) Immunoassays (ELISA): Immunoassays involve using monoclonal antibodies designed to selectively bind to target pesticide residues. Due to their simplicity and speed, immunoassays are generally used to screen large numbers of samples. They are widely applied in food safety monitoring programs. Since ELISA can yield positive results that do not accurately reflect the actual levels of pesticide residues in samples, its reliability can vary by application. Furthermore, ELISA's sensitivity to a single pesticide residue makes it impractical for simultaneous multiresidue determination [19].

f) Thin Layer Chromatography: TLC is a relatively easy-to-use, low-cost technique used primarily for qualitative screening of pesticide residues. TLC employs a solid-coated plate, with separations occurring via capillary action using a solvent-based eluent. Although TLC is much less sensitive than many chromatographic techniques, it can provide quick-turnaround data for initial field screenings.

1.6.2 Advanced and Modern Techniques

a) Biosensors: Biosensors represent a new generation of detection tools for pesticides. Biosensors combine a biological sensor element (enzymes, antibodies, aptamers, DNA) with a transducer that converts the biological reaction into a quantifiable signal. There are several reasons why biosensors are superior to older technologies for pesticide detection: rapid response time, high specificity and sensitivity, portability and field applicability, cost-effectiveness, and minimal need for sample preparation.

Among all the types of biosensors that exist, electrochemical biosensors are by far the most common for pesticide detection [20]. The type of biosensor works by measuring changes in current (amperometric), voltage (potentiometric) or impedance (impedimetric) when the pesticide interacts with the biosensor. Enzyme-based biosensors using Acetylcholinesterase (AChE) are particularly effective for detecting organophosphate and carbamate pesticides because both classes of compounds inhibit AChE activity.

b) Electrochemical Sensors: Electrochemical sensors work by detecting the presence of pesticides based on an oxidation or reduction reaction occurring at an electrode. In addition to being very sensitive, they can also be made very small and constructed from nanostructured materials such as carbon nanotubes, graphene, MXene ($\text{Ti}_3\text{C}_2\text{Tx}$), metal particles (such as Au NPs), and metal oxides (such as CeO_2). Some examples of electrochemical sensor data generation methods include Cyclic Voltammetry (CV), Differential Pulse Voltammetry (DPV), Square Wave Voltammetry (SWV), and Electrochemical Impedance Spectrometry (EIS). All methods offer very low detection limits, ranging from picomoles (pM) to nanomoles (nM) [21].

c) Surface-Enhanced Raman Scattering: SERS is an optical method that uses nanostructured metallic surfaces (most often gold or silver nanoparticles) to increase the intensity of the Raman scattering signal produced when molecules adsorb onto the surface. SERS can achieve extremely high sensitivities, enabling the detection of pesticides at very low contamination levels without extensive sample preparation. As a result, SERS is now being examined for its ability to rapidly screen for pesticides in remote locations [21].

d) Nanomaterial-Based Methods: The incorporation of nanomaterials such as gold nanoparticles (AuNPs), quantum dots, carbon nanotubes, graphene oxide and MXenes has greatly improved pesticide detection. These materials offer a large surface area, enhanced electrical conductivity, and increased molecular adsorption, resulting in higher sensitivity and selectivity and lower detection limits than traditional sensor methods.

e) Molecularly Imprinted Polymers: MIPs are synthetic polymer receptor molecules engineered to specifically bind to particular pesticide molecules with greater affinity than natural biological receptors. MIPs are used as recognition elements in sensors and have been shown to be long-term stable, reusable, and inexpensive compared to their biological counterparts [22].

f) Miniaturized Analytical Systems: They consist of microfluidic devices or lab-on-chip devices. These miniaturized devices contain all the components required to perform chemical analyses, including sampling, preparation, separation, and detection. They require minimal amounts of sample volume and yield fast results, making them well-suited for applications.

1.7 Biosensors

1.7.1 Definition and History of Biosensing

Biosensors are devices used to measure the presence of biochemicals (analytes) by combining a biological component with an electronic or physical component. They produce a measurable signal related to the amount of biochemical present. The term biosensor was coined by Prof. Leland C. Clark Jr., often referred to as the "father of biosensors". In 1962, he invented the first enzyme electrode (glucose biosensor). The technology became commercially available in 1975 and was used in the first such product [20]. Over the last few decades, there have been tremendous advancements in many fields, including biotechnology, nanotechnology, materials science and microelectronics, resulting in exponential growth of biosensor development.

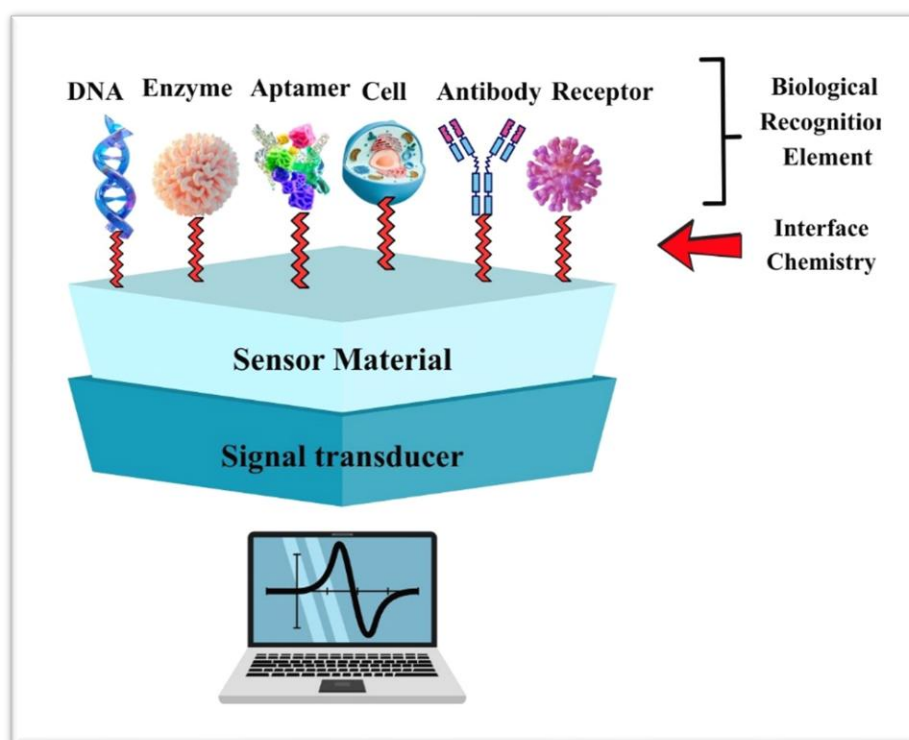


Fig. 1.6 An overview of Biosensor

1.7.2 Principles of Operation for a Biosensor

The basic premise of a biosensor is the high degree of specificity inherent in the interaction between a biochemical receptor and its corresponding analyte. Upon interaction between the receptor and analyte, a biological event occurs, i.e. a chemical reaction takes place, the receptor

undergoes a shape change, or another type of interaction occurs, which is then transformed into an electrical, optical, thermal or mass-related signal by the transducer [23].

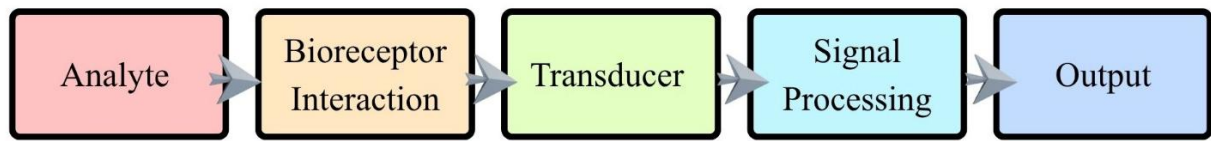


Fig. 1.7 The basic working principle of biosensors

1.7.3 Components of a Biosensor

The basic components in making up a biosensor are comprised of five main components:

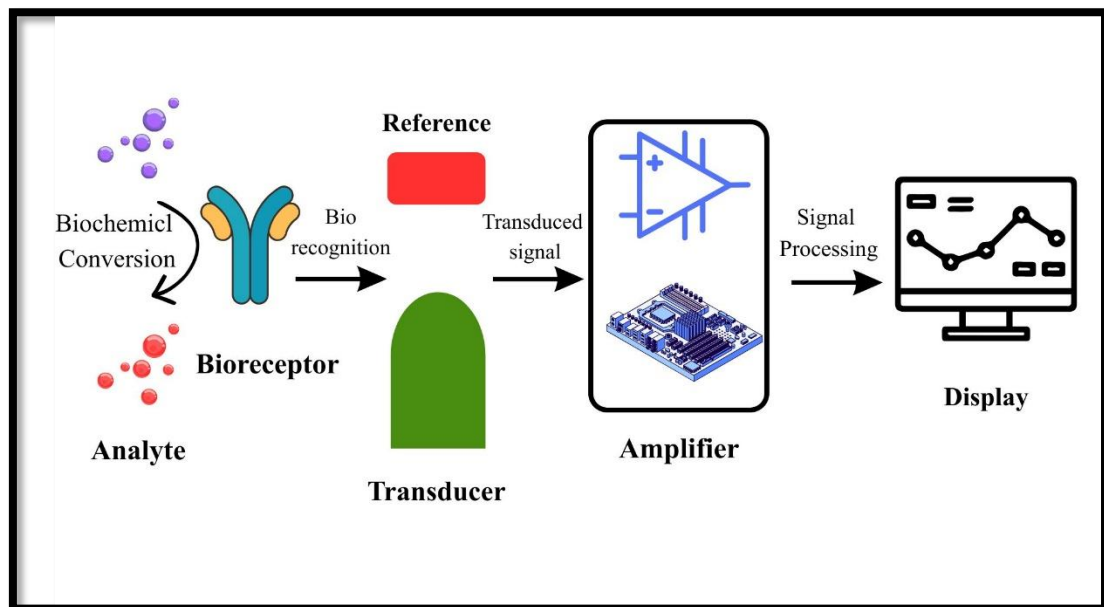


Fig. 1.8 The components of biosensors

a) Analyte: The analyte is the target substance being identified and quantified by the biosensor. This could be any type of molecule that has biological, chemical, or environmental importance. For instance, this could be a glucose molecule (to monitor for Diabetes), pesticide molecules (for use in Food Safety), heavy metal ions (for use in Environmental Monitoring), pathogenic organisms (for use in Clinical Diagnostics), or tumour biomarkers (for use in Oncology) [24].

b) Biorecognition Unit or Bioreceptor: The biorecognition unit/bioreceptor is the biological element that recognizes and binds to the analyte [25]. The biorecognition unit/bioreceptor provides specificity and sensitivity that determine the biosensor's overall performance. Examples of biorecognition units/bioreceptors include:

- Enzymes: They have catalytic action on their substrate.
- Antibodies or Antigen: Highly specific immunological reactions occur when antibodies bind to antigens.
- Nucleic Acids (DNA/RNA/Aptamer): Hybridization-based binding occurs between nucleic acid strands.
- Cells/Microorganisms: Whole cells are used as biorecognition elements in some types of biosensors.
- Molecularly Imprinted Polymers (MIPs): Synthetic polymers that mimic the binding properties of biological systems.
- Lectins: Carbohydrate-binding proteins.

c) Transducer: The transducer transforms the biochemical interaction between the analyte and the biorecognition unit/bioreceptor into a detectable physical or chemical signal. The transducer serves as an intermediary between the biosensor's biochemical component and its electronic component. The type of transducer chosen determines how you measure and classify your biosensor.

d) Signal Processor or Amplifier: Once the transducer generates a raw signal, it is sent to a signal amplifier that will amplify and clean the signal from background noise. The amplified and cleaned signal is then converted from analogue to digital, ready for final processing and analysis [26].

e) Display or Output Device: Once the data has been processed, it is shown through one or multiple display/output devices such as computers, smartphones, or even a stand-alone read-out device.

1.7.4 Classification Based on Bioreceptor

There are seven classifications of biosensors based on bioreceptors:

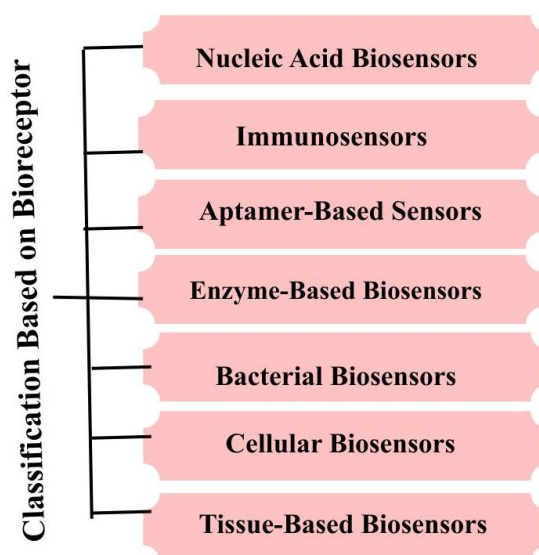


Fig. 1.9 The Classification Based on bioreceptor

a) Enzyme-Based Biosensors: Although enzyme-based biosensors contain an enzymatic reaction that leads to the formation of electrochemically active substances like O_2 , H_2O_2 , or NADH, the transducer then measures those substances; this is the largest category of biosensors in terms of commercial success. Although there are many different enzymes that have been utilized for various types of biosensors including glucose oxidase (for glucose determination), acetylcholinesterase (to determine organophosphate pesticides), horseradish peroxidase (for H_2O_2 quantitation), urease (urea determination), and lactate oxidase (lactate determination), the advantages of using enzyme-based biosensors include a large number of possible targets, very rapid response time, and a high degree of sensitivity. Disadvantages of enzyme-based biosensors include unstable enzymes, potential interference from other molecules that can inhibit enzyme activity, and high cost due to the need to produce and purify enzymes [26].

b) Immunosensors: These are based on the highly specific interaction between antigens and antibodies. The immobilized antibody on the surface of the transducer binds to its corresponding antigen, resulting in a detectable signal. As such, immunosensors are commonly used to detect pathogens, hormones, drugs, toxins, and cancer biomarkers. Immunosensors are

generally classified into two subcategories: labelled (sandwich ELISA format) and unlabelled (direct binding detection). Although one of the major advantages of immunosensor is the exceptional specificity that enables them to function reliably in samples containing multiple components, they are often expensive because antibodies can be costly to produce [27]. Additionally, the shelf life of antibodies is typically short, and regenerating the sensing surface after each use is difficult.

c) Nucleic Acid Biosensors: Genosensors have ss-DNA or ss-RNA probes attached directly to the surface of a transducer. Hybridization occurs when a complementary nucleic acid sequence is present in the sample and generates a detectable signal. Genosensors can be utilized for pathogen detection, genetic disease diagnosis and food safety analysis. There are several types of genosensors, including DNA biosensors, RNA biosensors and aptamer-based sensors (aptasensor). One advantage of genosensors is that they are highly specific and stable, and can perform multiple assays at once. However, one disadvantage is that some formats require amplification of the target sequence prior to assay performance [28].

d) Aptamer-Based Sensors: An aptamer is a small (usually less than 30 bases), single-stranded DNA or RNA molecule that has a distinct 3-dimensional structure allowing it to selectively bind to a specific molecular target much as an antibody does. Aptamers were developed using an in vitro method called SELEX (Systematic Evolution of Ligands by Exponential Enrichment). Compared with immunosensors, aptasensor have higher stability, greater reproducibility, and lower production costs.

e) Bacterial Biosensors: They contain whole living organisms (bacteria, yeast, etc.) as the biorecognition elements. When the biorecognition element detects a target analyte, it modifies its metabolic response, which results in a measurable signal such as altered oxygen utilization rates, bioluminescence or changes in electrical properties. Bacterial biosensors can be used to monitor environmental factors (e.g., biochemical oxygen demand and toxicity) and to assess food safety.

f) Cellular Biosensors: In cellular biosensors, the biorecognition elements are entire living cells or subcellular structures. These biosensors are particularly useful for identifying chemical substances that affect cellular processes, i.e. toxins, drugs, pollutants and other hazardous chemicals [29]. These biosensors measure cellular responses to identify how the target analyte affects cell function.

g) Tissue-Based Biosensors: They consist of thin slices or extracts of various types of biological material (animal or plant) containing the necessary enzymes. Because tissue-based biosensors utilize whole enzyme systems within their normal cellular matrix and protective cofactors, they tend to be more stable than purified enzyme systems and generally cheaper.

1.7.5 Classification Based on Transducer Type

a) Electrochemical Biosensors: The best-researched and commercialized type of biosensor is the electrochemical biosensor. These sensors work by measuring changes in the electrical properties of a system (such as current, potential, impedance, etc.) that occur during biological recognition events at the electrode surface. There are several types of electrochemical biosensors:

- Amperometric biosensors measure the amount of electrically active species being oxidized/reduced at a constant voltage (potential). This type of sensor is extremely popular and has been used in glucose meters.
- Potentiometric biosensors measure the difference in voltage between a reference and a working electrode. Potentiometric sensors follow the Nernst Equation and are also known as ion-selective electrodes.
- Voltametric biosensors are able to measure how many electrically active species are being oxidized/reduced as a function of changing voltage.

Voltametric sensors have three main configurations: cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV).

- Impedimetric biosensors measure how much the resistance and/or capacitance at the electrode-electrolyte interface changes due to the presence of an analyte. Impedimetric sensors offer very high sensitivity and allow for label-free detection.
- Conductometric biosensors simply measure how much the conductivity of a solution has changed due to binding events.

The biggest advantages of an electrochemical biosensor include high sensitivity, low detection limits, miniaturization, low cost, and the ability to provide real-time data. Electrochemical biosensors have many uses, including glucose monitoring, pesticide detection, heavy metal analysis, and pathogen identification [30].

b) Optical Biosensors: Optical biosensors detect changes in the physical characteristics of light - absorbance, fluorescence, luminescence, refractive index or surface plasmon resonance - caused

by interactions between an analyte and biorecognition molecules. Of all the types of optical biosensors,

- Surface plasmon resonance biosensors are among the most interesting; they measure changes in refractive index when an analyte binds to a biorecognition molecule attached to a thin layer of metal (usually gold) at its surface, allowing them to detect analytes in real-time and without using labels.
- Fluorescence-based biosensors measure either fluorescent labelling or intrinsic fluorescence changes.
- Chemiluminescent biosensors measure light produced through a chemical reaction without requiring an outside energy source.
- Colorimetric biosensors create visible colour changes that may be seen with the unaided eye or measured with a spectrophotometer.
- Fibre-optic biosensors use optical fibres to transmit light signals from the sensing element to a remote detector.

The primary advantages of optical biosensors include their high sensitivity, lack of electrical noise and suitability for remote sensing [31]. Optical biosensors have numerous applications in environmental monitoring, food safety, medical diagnostics and drug discovery.

c) Piezoelectric Biosensors: Piezoelectric biosensors measure the change in resonant frequency of a piezoelectric crystal, most often a Quartz Crystal Microbalance (QCM), as a result of small changes in the mass on its surface due to analytes' attachment. These are among the most sensitive devices available, capable of detecting mass changes as small as nanograms. Due to the fact that they do not need labels and respond in "real-time," piezoelectric biosensors are widely utilized for detecting proteins, DNA/RNA, viruses, and bacteria. They are primarily used to identify pathogens, study protein-protein interactions, and screen drugs [32].

d) Thermal (Calorimetric) Biosensors: Thermal biosensors (also referred to as enzyme thermistors or calorimetric biosensors) measure the amount of heat produced or consumed through biological reactions, including enzyme-substrate conversions or antibody-antigen binding. Nearly all biological reactions generate a change in enthalpy. Thus, these sensors will work with nearly all biological assays. The limitations of this technology are its relatively poor sensitivity and susceptibility to interference from temperature variations. As a consequence of both limitations, thermal biosensors are far less common than electrochemical or optical

technologies. Nevertheless, they remain useful in a variety of research-based environments [32].

e) Magnetic Biosensors: Magnetic biosensors represent an evolving category that utilises magnetic nanoparticles or detects variations in magnetic field strength to quantify the analyte. Using bioreceptor-functionalized magnetic labels, target molecules may be captured, and the resulting magnetic signal detected with magnetoresistance sensors, SQUIDs (Superconducting Quantum Interference Devices), or MRWS (Magnetic Relaxation Switches). Biological samples rarely contain inherent magnetization. Thus, background noise is virtually non-existent, providing high sensitivity. Interest in magnetic biosensors for clinical diagnostic applications and point-of-care testing is expected to grow significantly over time and may also extend to environmental monitoring and food safety [33].

1.7.6 Characteristics of an Ideal Biosensor

The table given below shows the characteristics of an ideal biosensor,

Table 1.2 The characteristics of an ideal biosensor

S.No.	Characteristic	Description
1	Sensitivity	The ability of an assay to measure extremely low levels of an analyte in a sample (ng/mL to fg/mL LOD).
2	Selectivity	The ability of an assay to specifically measure the presence or quantity of one analyte while excluding all others.
3	Reproducibility	The results obtained through an assay are consistent when it is performed on different days, at different times, using different personnel, etc.
4	Stability	The resistance of the measurement system to changes in temperature, pH, humidity, etc., over time.
5	Linearity	A linear response exists over a broad concentration range between the signal generated by the measurement technique and the amount of analyte present.

6	Limit of Detection (LOD)	The lowest concentration of an analyte that can be accurately measured.
7	Biocompatibility	The material properties of a device or instrument that are non-toxic to living organisms and compatible with biological materials.
8	Cost-effectiveness	An affordable cost per unit for devices such that they can be deployed in sufficient quantities to provide adequate coverage.
9	Reusability	An assay's ability to make repeated measurements without loss of analytical performance.
10	Response Time	The speed at which a measurable signal is produced by the measurement system upon introduction of the analyte into the system.

1.7.7 Immobilization Techniques for Biosensors

Fabrication of the biosensor relies heavily on the immobilization of the bioreceptor on the surface of the transducer. To ensure that the bioreceptor maintains its biological activity throughout the immobilization process and provides stable contact with the sensor, it is essential to utilize an immobilization technique that fulfils these two requirements. Some of the key techniques utilized in the immobilization of bioreceptors include:

a) Adsorption (Physical): Adsorption is a physical binding method which utilizes weak van der Waals, hydrophobic, and electrostatic forces to bind the bioreceptor to the surface of the electrode. This approach is simple and does not disrupt the bioreceptor's enzymatic activity; however, because it relies on weak bonds, the bioreceptor can be removed from the surface by elution or degradation over time [34].

b) Chemical Coupling (Covalent Attachment): Chemical coupling involves covalently attaching the bioreceptor to the electrode surface through chemical linkages formed between the bioreceptor and functional groups present on the electrode. Since this type of linkage is much stronger than those involved in adsorption-based immobilizations, chemical coupling provides a strong and relatively permanent attachment of the bioreceptor to the electrode. This

is the preferred immobilization method in commercial biosensors. The use of glutaraldehyde as a crosslinking agent is particularly prevalent in biosensor construction [34].

c) Encapsulation: Entanglement occurs when the bioreceptor is physically entrapped within a polymeric matrix (such as acrylamide gel, sol-gel derived matrices, etc.) at the electrode surface. Biocatalysts such as enzymes and even intact cells have been effectively immobilized using entrapment techniques. However, because diffusion pathways are limited within an entrapment matrix, enzymatic activity may also be affected.

d) Cross Linking: Cross-linking refers to the formation of links between bioreceptor molecules themselves and/or between bioreceptor molecules and a solid support matrix using a bifunctional cross-linker (for example, glutaraldehyde). While cross-linking provides a highly effective means of immobilising biocatalysts, it often alters the structure of the immobilized enzyme, thereby affecting its catalytic activity [35].

e) Affinity-Based Immobilization: Affinity-based immobilization uses specific biological interactions (streptavidin-biotin, nickel-histidine tags, protein A/G-antibody) for oriented immobilization of biocatalysts. As this method is extremely selectivity-based immobilization minimizes disruption to the bioreactor's natural biological functions.

1.7.8 Nanostructures in Biosensors

The application of nanostructured materials in biosensing platforms significantly enhances the analytical capabilities of biosensors. In particular, several types of nanostructured materials are being investigated, including:

a) Carbon Nanostructures:

- Carbon nanotubes (CNTS): They exhibit high electrical conductance and very high surface areas.
- Graphene and reduced graphene oxide (rGO): These structures display high electron mobility and high surface areas.
- Carbon quantum dots: They possess fluorescence characteristics and are biocompatible.

b) Metallic Nanoparticles:

- Gold nanoparticles (AuNPs): They exhibit good biocompatibility along with surface plasmon resonance characteristics.
- Silver nanoparticles (AgNPs): They show anti-microbial effects along with optical characteristics.
- Platinum nanoparticles: They display good electro-catalytic behaviour.

c) Metal Oxide Nanoparticles:

- Zinc oxide (ZnO): It displays semiconductor characteristics and piezoelectricity.
- Titanium dioxide (TiO₂): It shows photo-catalysis and good biocompatibility.
- Cerium oxide (CeO₂): It exhibits redox cycle properties (Ce³⁺/Ce⁴⁺), it has good electro-catalytic properties and has been found useful for constructing non-enzymatic sensors.

d) Two-Dimensional Materials:

- Ti₃C₂T_x MXene: Excellent electrical conductivity (6000-8000 S/cm) and very large surface area. Its surface chemistry is tunable, and it is excellent for supporting the growth of biological components. Currently, Ti₃C₂T_x is emerging as one of the most promising platforms for the development of electrochemical biosensors [36].

e) Quantum Dots:

- Quantum dots represent a class of semiconductor nanocrystals whose size-dependent fluorescence can be engineered to allow multiple analytes to be detected simultaneously.

1.7.9 Applications of Biosensors

Biosensors are used in many areas of medicine, environmental monitoring, agriculture, and pharma.

a) Medical Diagnostics

- Blood glucose monitoring: The use of glucose sensors has become widespread as they monitor diabetes patients by continuously measuring their blood glucose levels.
- Cardiac biomarkers: They detect cardiac biomarkers, which allow clinicians to rapidly identify acute myocardial infarction.
- Cancer biomarkers: Specific proteins (PSA, CEA, AFP) are detected in order to screen for cancer.
- Infectious diseases: Biosensors are used to rapidly diagnose infections (HIV, COVID-19, Hepatitis, Malaria), and also to detect blood-borne pathogens.
- Therapeutic drug monitoring: Concentration of therapeutic drugs is measured in patients' bloodstreams to determine if dosage is correct and if there are side effects.
- Hormone & vitamin levels: Biosensors can measure the exact number of important vitamins and hormones in a person's blood [37].

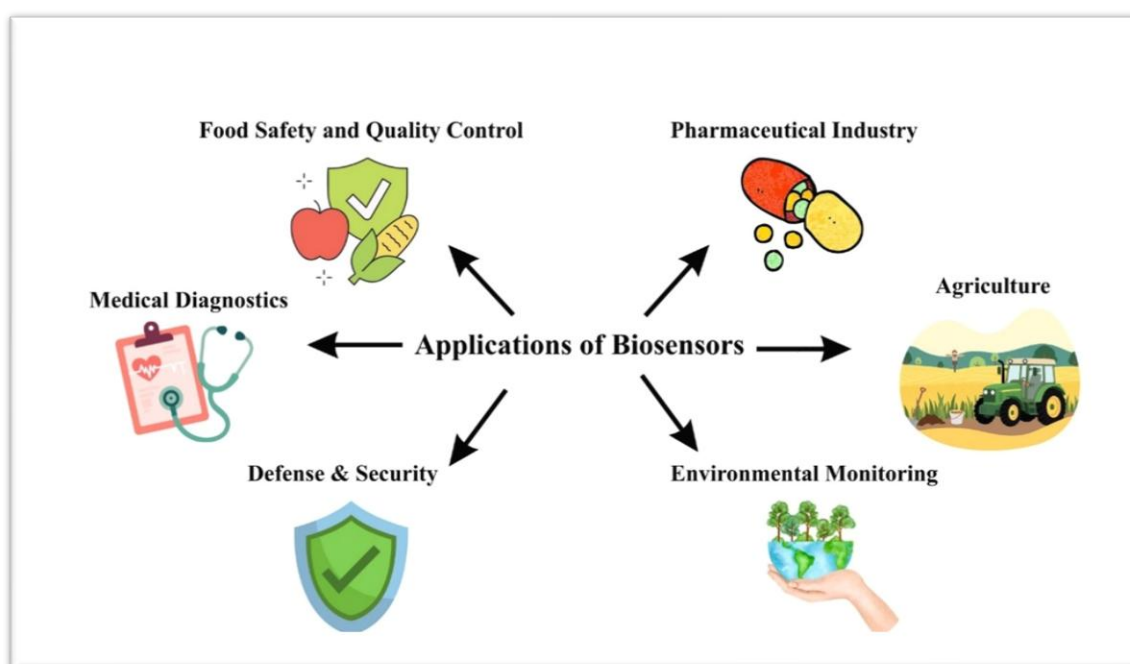


Fig. 1.10 The application of biosensors

b) Environmental Monitoring

- Pesticide residues: Biosensors can find residues of pesticides from contaminated soil, groundwater, or food.

- Heavy metal testing: Water samples can be tested using biosensors for toxic heavy metals (Lead, Mercury, Cadmium, Arsenic).
- Pollutant detection: Industrial pollutants and endocrine disruptors in an ecosystem can be found using biosensors.
- Wastewater management: BOD can be monitored at the wastewater treatment facility using biosensors.
- Toxic algae: Biosensors can detect the presence of toxic algae in waterways [37].

c) Food Safety and Quality Control

- Pesticide detection: Fresh produce can have residues of pesticides from farming practices, while other types of produce may contain residues from processing. These residues can be detected using biosensors.
- Pathogen screening: Foodborne pathogens (Salmonella, E. coli, Listeria) can be identified in food using biosensors.
- Freshness indicators: Chemical signals that occur when food spoils or rots can be detected by biosensors.
- Nutritional analysis: Amounts of nutrients in certain food items (glucose, lactate, cholesterol) can be determined through biosensor technology [38].

d) Defence & Security

- Chemical warfare agents: Trace amounts of chemicals such as nerve agents or mustard gas can be identified rapidly using biosensors.
- Biological warfare agents: Anthrax and Botulinum Toxin can be screened for as biothreats using biosensors.
- Explosive detection: Small quantities of explosives can be found using biosensors in security applications.
- Radiation measurement: Biosensors can be used to track high levels of radiation in an area.

e) Pharmaceutical Industry

- Drug discovery: In addition to finding new medicines, biosensors assist researchers in identifying potential compounds to test further.

- **Quality control:** Many manufacturers utilize biosensors to check the quality and purity of manufactured pharmaceuticals.
- **Fermentation monitoring:** Biosensors help companies to track and optimize the results of large-scale fermentation.
- **Drug interactions:** Researchers study how medications react with biological targets by using biosensors.

f) Agriculture

- **Soil health:** Conditions of the soil (nutrient content etc.), can be monitored by tracking pH level and nutrient availability.
- **Plant pathogen detection:** Early detection of diseases/plant pathogens allows farmers to treat problems early to avoid losses.
- **Plant hormone levels:** By tracking hormone levels, farmers can determine if plants are stressed or growing properly.
- **Crop monitoring:** Some biosensors can detect pesticide residue on farm-grown crops before they are harvested [39].

1.7.10 Future Scope and Emerging Trends in Biosensors

The future of biosensors is incredibly bright due to the integration of many emerging technology trends:

a) Wearable Biosensors: Future biosensors are anticipated to be wearable biosensors that include flexible skin-worn devices such as biosensor patches, tattoo biosensors, or smart watch devices that provide continuous and real-time health parameter tracking (i.e., glucose, lactate, cortisol, etc.) without drawing a drop of blood.

b) Implantable Biosensors: In addition to wearable biosensors there will also be miniaturized implantable biosensors used to continuously monitor glucose levels in diabetic patients, drug levels, and physiological parameters in severely ill patients.

c) Internet of Things (IoT) Connected Biosensors: As part of the Internet of Things (IoT), biosensors will have the capability of transmitting real-time data to healthcare providers using a variety of methods, i.e., via smartphones, cloud computing platforms, and/or artificial

intelligence systems. This will enable remote monitoring, data analysis, and telemedicine services.

d) Artificial Intelligence & Machine Learning: Additionally, artificial intelligence and machine learning systems are becoming more prevalent in analyzing biosensor-generated data. The ability of these advanced analytical systems to identify patterns in biosensor-generated datasets will enable early disease diagnosis, prediction of future disease based on trends in data collected over time, and the elimination of false-positive readings, which may occur when analysing large amounts of complex multi-analyte data [40].

e) CRISPR-Based Biosensors: Another area where significant advancements are being made involves integrating CRISPR-Cas systems into biosensors. These advanced systems use RNA guides to target very specific nucleic acid sequences within samples. Two examples of these types of systems are SHERLOCK and DETECTR. Both systems exhibit an unprecedented level of sensitivity and specificity in terms of detecting target nucleic acids [40].

f) Point-of-Care (POC) and Lab-On-A-Chip Devices: Point-of-care and lab-on-a-chip microfluidic biosensors are another example of how technology continues to advance in this field. These tiny chips contain all of the necessary components required to analyse samples and produce results in just mins. As a result, they can be used at the patient's bedside or even in remote locations. They eliminate the need for traditional laboratory equipment.

g) Nanomaterial Enhanced Next Generation Biosensors: Researchers are currently exploring various forms of nanomaterials as potential materials for next generation biosensors. Some examples of materials being studied include MXene, metal-organic frameworks (MOFs), covalent organic frameworks (COFs), black phosphorous, and boron nitride. Each type of material has the potential to improve the performance characteristics of biosensors and allow them to detect smaller quantities of analytes than current biosensors [41].

h) Multiplexed Biosensors: An additional emerging trend is multiplexed biosensing. Multiplexed biosensing refers to the use of arrays of biosensors that can measure multiple analytes simultaneously. This allows researchers and clinicians to obtain detailed information about diseases and assess environmental conditions more quickly and efficiently than would otherwise be possible.

i) Self-Powered Biosensors: Lastly, self-powered biosensors represent yet another exciting area of development. Biofuel cells are one type of self-powering device. Unlike most other biosensors, biofuel cells do not require an external power source to operate. Instead, they generate their own electrical energy through biochemical reactions occurring inside the device. This makes them well-suited for long-term deployment at remote sites or for use in implanted medical devices [42].

CHAPTER 2

Literature Review

2.1 Introduction to Organophosphate

Organophosphate (OP) pesticides are a wide-ranging and structurally diverse group of man-made compounds derived from phosphoric acid and related phosphorus acids. They represent the most widely used class of insecticides globally, accounting for an estimated 40-50% of total global use and 30% of global pesticide sales. The majority of OP compounds were developed in the early 1930's and mid-1940's. These developments occurred in part through advances in organophosphorus nerve agents made during wartime efforts in WWII. OP compounds have since become essential tools in agricultural applications, public health vector control, veterinary medicine and aquaculture [43]. The large-scale use of OP compounds was due largely to their relatively low environmental persistence compared with the organochlorine pesticides they displaced. The half-lives of OP compounds generally range from days to weeks when subjected to hydrolysis in soils and aquatic environments. Although OP compounds may degrade rapidly in the environment, the potential for acute toxicity remains high, and some of the more common OP compounds remain highly toxic relative to other man-made substances. As such, the misuse or overuse of OP compounds has raised increasing concerns for scientists, regulators and public health officials around the World [44].

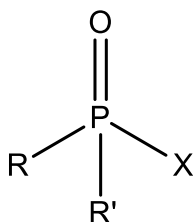


Fig. 2.1 Structure of OP

In terms of chemical structure, most OP compounds contain a central phosphorus atom with bonds to four substituent groups, often two alkoxy groups (e.g., methoxy or ethoxy), one double-bonded oxygen or sulphur atom, and one leaving group that will be displaced once the compound is exposed to water via hydrolysis or enzymes is shown in **Fig. 2.1**. Due to the

various possible combinations of these four functional groups, OP compounds can be grouped into several different chemical classes including phosphate, phosphorothioates, phosphonodithioates, phosphonates and phosphonamidites. The variety of structures exhibited by members of the OP family explains the differences in physical characteristics, environmental behaviour and biological effects observed between individual compounds within this family [43].

2.2 Health Consequences of Organophosphate Pesticide Exposure

a) Acute Toxicity: The vast majority of all acute poisonings from pesticides throughout the World result directly from the use of OP pesticides. According to the WHO, it is estimated that each year there are approximately three million people who suffer from acute pesticide poisoning. This number represents the largest portion of such poisonings involving OP pesticides. Intentional self-poisoning using very high toxicity levels of OP pesticides has become a major public health issue within many developing countries, especially within South and Southeast Asia, resulting in an average annual death toll of hundreds of thousands of people [45].

b) Chronic Exposure: There is substantial evidence that exposure to low-dose levels of OP pesticides over long periods of time via occupational routes (such as farm workers, or pesticide applicators) and diet from consuming food products contaminated with these pesticides may lead to a wide range of negative health consequences among individuals participating in various epidemiologic studies:

- **Neurotoxicity:** Various epidemiologic studies have indicated that there exists a positive relationship between chronic exposure to low-dose levels of OP pesticides and the risk of suffering from Parkinson's disease, Alzheimer's disease, and additional neurodegenerative conditions. The California-based CHAMACOS study and the FARM study provide considerable evidence of a positive association between organophosphate exposure, as measured by biomarkers, and the risk of Parkinson's disease [45].
- **Developmental Neurotoxicity:** Prenatal and early postnatal exposure to low-dose amounts of OP pesticides has been positively correlated with neurodevelopmentally-related developmental problems, including decreased IQ, diminished ability to perform working memory tasks, increased occurrence of ADHD, and increased occurrences of autism-

spectrum disorders. The CCCEH birth cohort study showed significant negative relationships between prenatal chlorpyrifos exposure (as measured by cord blood biomarkers) and cognitive performance measures at age 7.

- **Carcinogenicity:** The IARC has identified several OP compounds as being either “probable” (Group 2A) or “possible” (Group 2B) human carcinogens. Studies assessing the relationship between exposure to OP pesticides and human cancer have shown statistically significant associations. These associations include non-Hodgkin lymphoma, brain cancer, and breast cancer [46].
- **Endocrine Disruption:** Many different biochemical processes are capable of being negatively impacted by OP compounds, including interference with endocrine function through direct actions on the nucleus, which are involved in the breakdown of both OP compounds and oxidized lipids, interaction with nuclear hormone receptors and disruption of thyroid hormone biosynthesis. Adverse effects on reproductive function have also been observed in individuals who work in environments where they are exposed to OP compounds, including reductions in male fertility (reduced sperm counts, reduced sperm motility), alterations in the female menstrual cycle, etc.
- **Immunotoxicity:** Suppression of cell-mediated immunity and humoral immunity, increased susceptibility to infection, and increased incidence rates for autoimmune disorders have been documented as potential immunologically related adverse health effects associated with prolonged exposures to OP pesticides [46].

c) Vulnerable Populations: Children are much more susceptible to the adverse neurological effects associated with exposures to OP pesticides due to several factors related to their physiology: a) their blood-brain barriers are less well-developed, b) their brains are experiencing rapid growth and synaptogenesis, c) their metabolic capabilities to detoxify OP compounds are lower than those in adult humans, d) their food intake relative to body mass is greater than that experienced by adults [47]. Urinary OP metabolites were significantly higher in children residing in agricultural areas compared to urban children, as determined by a landmark study published by Eskenazi et al. (2007). Therefore, the pathways through which children are exposed to OP pesticides include dietary and environmental residential pathways.

2.3 Characteristics of the material

Appropriately chosen electrode materials are among the most important factors in the sensitivity, selectivity, and overall electroanalytical performance of an electrochemical biosensor. Over the past decade or so, due to superior physicochemical properties such as high surface area, modifiable electronic structure, numerous catalytically active sites, and better-than-typical bulk materials, considerable interest has been directed toward the use of 2D and nanostructured electrode modifiers for highly efficient electrocatalysis. This research employs a composite comprising nanoscaled particles of graphitic carbon nitride (g-C₃N₄) and copper sulphide (CuS) to construct an enzymatic electrochemical sensor [48]. The detailed descriptions in the sections below outline the structural, physical, chemical, optical, and electrochemical properties of each material and its composite.

2.3.1 Graphitic carbon nitride (g-C₃N₄)

Structure and Physical Properties:

The allotropes of carbon nitride include g-C₃N₄, the most stable allotrope of carbon nitride under normal temperature and pressure.

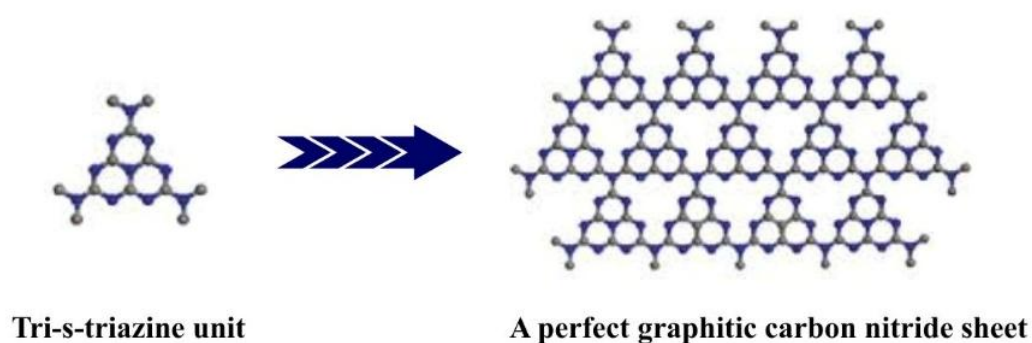


Fig.2.2 Structure of g-C₃N₄

The carbon and nitrogen atoms are arranged in a 3:4 molar ratio and include residual hydrogen as terminal amino (-NH₂) and imino (-NH-) groups along the edges of their polymeric layers. Each layer consists of heptazine (tri-s-triazine) units bonded together via planar tertiary nitrogen bridges, as shown in **Fig. 2.2**. These layers are then stacked together through Van Der Waal's

forces and pi-pi interactions similar to those found in graphite, with an interlayer distance of about 0.326nm [49].

Optical Properties:

The g-C₃N₄ is a visible light absorbing semiconductor with a very narrow bandgap of approximately 2.7eV and therefore absorbs light at wavelengths less than 460nm. Therefore, g-C₃N₄ can absorb visible light and utilize energy from the sun, unlike titanium dioxide (TiO₂), which only utilizes UV light due to its larger bandgap size of 3.2eV. The absorption of UV-VIS radiation demonstrates significant absorption bands at approximately 320nm due to π - π^* electronic transition in the aromatic heptazine ring structures. There is also a secondary absorption band centred near 460nm originating from n- π^* transitions due to the lone pair electrons of the bridged nitrogen atoms. In addition, g-C₃N₄ emits strong photoluminescence in the blue-green region from approximately 430 to 530nm when excited at wavelengths ranging from 350-380 nm [50]. Photoluminescence arises from the recombination of photogenerated electrons and holes produced by absorbed photons in the heptazine framework, a feature commonly used in fluorescence-based optical sensors.

Electrochemical Properties:

Due to its numerous surface nitrogen-rich functional groups, such as terminal amino (-NH₂) and bridged imino (-NH-) groups that act as electro-catalytically active sites facilitating analyte adsorption on the working electrode surface and promoting electron transfer between the analyte and the electrode, g-C₃N₄ makes an ideal additive material for developing electrochemical sensor electrodes. Additionally, g-C₃N₄ has a wide electrochemical operating window, thus significantly reducing background currents and greatly improving signal-to-noise ratios, ultimately decreasing detection limits [51]. Although pure bulk g-C₃N₄ has inherently low electrical conductivity, limiting its use as a sole electrode material, it is typically paired with electrically conductive nano-materials such as copper sulphide, graphene, single-walled carbon nanotubes, or metallic nanoparticles to create high-performing nanocomposites that possess enhanced electron transfer rates and improved electrochemical capabilities while maintaining the surface-functionalized nitrogen-rich characteristics of g-C₃N₄ [52].

2.3.2 Applications of g-C₃N₄

a) Electrochemical Sensors and Biosensors: g-C₃N₄ has become a very popular material used to modify electrodes in order to improve the sensitivity of detecting various analytes, including pesticides, heavy metals, pharmaceuticals, dopamine, uric acid, glucose and H₂O₂. The unique properties of g-C₃N₄, such as high surface area, numerous nitrogen active sites on the surface and ability to adsorb analytes, provide an ideal combination to increase electrode sensitivity and decrease the detection limits, which is why g-C₃N₄ has emerged as one of the most commonly studied non-enzymatic electrode materials for electrochemical sensing applications [51].

b) Photocatalysis: g-C₃N₄ has shown great potential as a metal-free, visible light-activated photocatalyst for the production of hydrogen gas from the decomposition of water via solar energy, conversion of CO₂ to solar fuels and photocatalytic degradation of organic pollutants, pharmaceutical residues, dye contaminants and heavy metals present in wastewater. As of today, g-C₃N₄ remains the most widely studied metal-free photocatalyst globally, with many thousands of studies demonstrating its photocatalytic activity under visible-light irradiation.

c) Energy Storage: g-C₃N₄ has been incorporated into lithium-ion batteries as anode materials, super-capacitors as electrode materials and in fuel cells as catalyst materials. In each case, the layered structure of g-C₃N₄ facilitates the insertion or de-insertion of ions; the large surface area of g-C₃N₄ provides ample sites for charge storage; and the nitrogen-containing functional groups within the molecular structure of g-C₃N₄ enhance both wetting characteristics and electron transfer across the electrolyte/electrode interface. Furthermore, g-C₃N₄-based electrocatalysts have exhibited good ORR (oxygen reduction reaction) activity in alkaline fuel cells [53].

d) Fluorescence or Optical Detection: Utilizing the inherent blue-green fluorescence emission at 430-530 nm of g-C₃N₄ nano-sheets, researchers have successfully developed fluorescent sensors and assays based upon g-C₃N₄ nano-sheets to detect a variety of analytes, including metal ions (Hg²⁺, Pb²⁺, Cu²⁺), pesticides, antibiotics and nucleic acids using fluorescence quenching or turn-on mechanisms. Additionally, due to their narrow band gap and small particle sizes (typically <10nm), g-C₃N₄ QDs display extremely stable and tunable fluorescence that can be utilised for biological imaging applications.

e) Biomedical Applications: The biocompatibility, intrinsic photoluminescence, large surface area and adjustable surface chemistry properties of g-C₃N₄ enable the use of g-C₃N₄ in biomedical applications, including drug delivery systems, PDT treatment of cancer cells, antimicrobial coating materials, and biosensors designed to detect disease biomarkers. Drug delivery systems incorporating g-C₃N₄ can encapsulate drugs that are then released in response to external stimuli (e.g., pH or light) [53].

f) Environmental Cleanup: Recent studies have demonstrated that g-C₃N₄-based composite nanomaterials can effectively degrade a wide range of environmental pollutants, textile dyes, endocrine disruptors and heavy metals from polluted waters using visible light for activation. The photostability of g-C₃N₄-based composites also enables these materials to remain effective during repeated use.

2.3.3 Copper Sulphide (CuS)

a) Structure and Physical Properties

A naturally occurring mineral, covellite, has been found to exhibit properties similar to those of a p-type inorganic semiconductor. Covellite has a hexagonal crystal structure. The complex structure of covellite is composed of alternating layers of trigonal-planar CuS and tetrahedral CuS units. These two types of units are connected by S-S bonds. Therefore, the same lattice contains Cu⁺ and Cu²⁺ oxidation states, resulting in mixed valency. This mixed state leads to many unusual electronic and electrochemical properties in CuS [54].

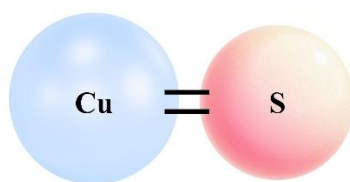


Fig. 2.3 Structure of CuS

Compared with other semiconductor materials, CuS has a relatively small direct band gap of 1.2–2.2 eV. Depending upon how the material was synthesized, what shape and size particles were produced, and how well the crystals formed, the band gap will vary. However, this range includes much of the visible and near-infrared (NIR) portion of the electromagnetic spectrum, indicating that CuS is a good broadband absorber of light. This property makes it particularly

interesting for use in various photocatalytic, photothermal, and photoelectrochemical applications.

b) Optical Properties

There are two factors that determine the optical properties of CuS: its small bandgap and its ability to produce localized surface-plasmon-resonance (LSPR). Unlike LSPR in gold and silver nanoparticles, where the resonance occurs primarily in the visible part of the electromagnetic radiation spectrum, LSPR in CuS nanoparticles produces absorption peaks in the near-infrared (NIR) region of the spectrum between approximately 900–1200 nm. This behaviour is due to collective oscillations of positive charge carriers (holes) at the surface of the CuS nanoparticles. These oscillations are similar to those observed in gold nanorods; however, they occur at much lower cost, making them potentially useful for various applications, including photothermal therapy, photoacoustic imaging, and NIR-driven photocatalysis [55].

Although there are no sharp peaks present in either the UV-visible absorption spectrum or the IR transmission spectrum of CuS, it does show broad absorption extending across the entire UV-visible-NIR spectral range. The broad absorption spectrum is responsible for its typical dark blue/black color. In addition to identification, the FTIR spectrum of CuS provides information on the presence of specific chemical bonds in the material. Bands associated with stretching vibrations involving the Cu-S bond appear at approximate wavenumber values of 620 cm^{-1} [56].

c) Electrochemical Properties

The potential uses of CuS in electrochemical sensing and energy storage applications depend largely on its electrochemical properties. Due to its ability to undergo rapid reversible oxidation-reduction processes, CuS exhibits favourable redox characteristics. The redox cycles result from the simple conversion between Cu^+ and Cu^{2+} oxidation states within the CuS lattice. By allowing CuS to take an active role in catalysing electrochemical reactions (e.g., oxidizing organic analytes, reducing dissolved O_2 and reducing pesticide compounds), it generates measurable currents during operation. The resulting electrochemical responses include both amperometric and voltametric signals [55]. The high electrical conductivity exhibited by CuS results from hole-carrier-mediated p-type conduction through the Cu-S bonding network. The

high electrical conductivity allows CuS to serve as an effective conductive scaffold when combined with electrically insulating materials such as g-C₃N₄. The low charge-transfer resistance, fast electron-transfer rates, and high electroactive surface areas observed in CuS-modified electrodes enhance their electrochemical sensitivities in sensing applications.

2.3.4 Applications of CuS

a) Electrochemical Sensing: CuS has been heavily utilized as a sensor electrode modifier for the ultra-sensitive electrochemical detection of pesticides, heavy metals (Hg²⁺, Pb²⁺, Cd²⁺, As³⁺), drugs (antibiotics, etc.), dopamine, H₂O₂, and glucose. The very good electrical conductivity of CuS, along with the excellent redox behaviour, the high electroactive surface area, and the strong interaction between the analytes and the CuS surface, allow CuS to be among the best transition-metal chalcogenide electrode materials for electrochemical sensing. This is demonstrated by the extremely low detection limit achievable in the nanomolar to picomolar range.

b) Photocatalysis: The photocatalytic activity under visible light and near-infrared light of CuS has been studied for many years in relation to the degradation of chemical contaminants in wastewater, including antibiotics and textile dyes. The narrow band gap of CuS allows for optimal use of solar radiation; additionally, the p-type character of CuS enables oxidation via holes generated during illumination [57].

c) Photothermal Therapy (PTT): The superior absorption capability of CuS nanoparticles in the near-infrared region converts this wavelength efficiently into localized heat. As a result, CuS nanoparticles are increasingly used as photothermal agents in cancer treatment via near-infrared laser-induced thermal destruction. The selective heating of cancerous tissues upon exposure to NIR laser light is mediated by CuS nanoparticles accumulated in these regions, thereby protecting nearby normal cells [57].

d) Energy Storage: Supercapacitor and lithium or sodium ion battery applications of CuS have received considerable interest lately. When used as a pseudocapacitive electrode material, CuS stores electric charge via rapid, reversible faradaic redox reactions of the Cu²⁺/Cu⁺ couple.

e) Solar Cells and Photo-electrochemistry: Counter electrode in dye-sensitized solar cells (DSSCs), Hole transport layer in perovskite solar cells, photoelectrodes in

photoelectrochemical water splitting devices. High electrical conductivity, catalytic activity towards the I^-/I_3^- redox pair, and a cost-effective production process render it a substitute for expensive Pt counter electrodes in DSSC applications [[58].

f) Biomedical and Antibacterial Applications: Due to their inherent ability to exhibit antibacterial and antifungal activities resulting from the controlled release of toxic amounts of Cu^{2+} ions capable of disrupting cellular membranes of bacteria and inhibiting essential biological processes, CuS nanoparticles have been integrated into various types of antimicrobial coatings, wound dressings, and biomedical implant materials. Additionally, they possess favourable optical properties suitable for application in photoacoustic imaging (PAI) and NIR fluorescence bioimaging (NIRFBI) techniques [59].

CHAPTER 3

Material and Methods

3.1 Introduction

This chapter provides a general overview of the materials used to create graphitic carbon nitride (g-C₃N₄) and copper sulphide (CuS), and their respective nanocomposite materials, as they relate to the detection of organophosphates (OP). The chapter will cover all morphological, structural, and electrochemical characterization techniques for g-C₃N₄, CuS, and the respective nanocomposite materials derived from these two materials [60]. In addition, this chapter will discuss various methods for immobilizing acetylcholinesterase (AChE) onto the electrode surface.

3.2 Materials

The details of the materials used in the experiment are as follows:

3.2.1 Chemicals

Acetylthiocholine Chloride (ATCl), Trichlorfon (TF), Glutaraldehyde Solution, Glucose (Glu $\geq 99.5\%$), Ascorbic Acid (AA 99 %) and Uric Acid (UA $\geq 99\%$) were obtained from Sigma Aldrich Pvt Ltd., India. Chemicals such as Acetone, Ethanol, Sodium Chloride (NaCl), Melamine and Copper (II) Sulphate Pentahydrate (CuSO₄.5H₂O), Sodium Thiosulphate Pentahydrate (Na₂S₂O₃.5H₂O) were purchased from Thermo-Fisher Scientific Pvt Ltd., India. The purity of all analytical-grade chemicals is at least 98%. The chemicals have been used in their pure form [61].

3.2.2 Solutions and Buffers

- 0.1 M Phosphate buffer saline (PBS), pH 7
- 5mM [Fe(CN)₆]^{3-/4-} used in PBS solution as redox initiator.

3.3 Characterization Methods

Several methods are available to determine the structural properties of a material which include the use of Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Diffraction (XRD). Therefore, the morphology and elemental composition of all developed electrodes and synthesized materials will be characterized by various analytical techniques. Those analytical techniques used to characterize the physical and elemental characteristics of the materials studied included Transmission Electron Microscopy (TEM). Field Emission Scanning Electron Microscopy (FESEM) and Energy-Dispersive X-ray Spectroscopy (EDX) for the morphological and elemental characterization. Electrochemical analytical characterization techniques including Cyclic Voltammetry (CV) and Differential Pulse Voltammetry (DPV) were also used to perform electrochemical characterization [62].

3.3.1 Fourier transform infrared spectroscopy (FTIR) spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) is a powerful non-destructive technique that is capable of identifying the presence of different types of functional groups and molecular environments within a solid material. It works according to the principles of molecular vibrations where molecules vibrate when exposed to infrared radiation at energies equivalent to those of the vibrational modes of their chemical bonds. This vibrational energy may be absorbed by the molecule if the infrared radiation has an appropriate frequency, which reduces the amount of radiation transmitted through the sample. Each type of bond like stretching, bending, twisting or rocking, will have its own characteristic absorption frequency resulting in a unique 'fingerprint' spectrum for each compound [63].

The use of FTIR involves irradiating the sample with infrared light having a wide range of wavelengths from a broadband infrared source. A Michelson interferometer modifies the wavelength of this light into an interference pattern referred to as an interferogram. An interferogram is then mathematically transformed (Fourier transformed) to obtain the absorption spectrum of the sample as a function of wavenumber. The mid-infrared spectral region (wavelengths: 2.5-100 μm or 400-4000 cm^{-1}) is preferred because most common organic and inorganic compounds exhibit their fundamental vibrational modes here. As such, FTIR spectra provide information about the presence of different types of chemical bonds and functional groups in materials. For example, g- C_3N_4 displays two distinct absorption regions: one related to C-N stretching and C=N stretching modes in the range 1200-1650 cm^{-1} , and

another due to a very broad N-H stretch absorption at higher wave numbers ($>3000\text{ cm}^{-1}$). A diagrammatic representation of FTIR is shown in **Fig. 3.1**.

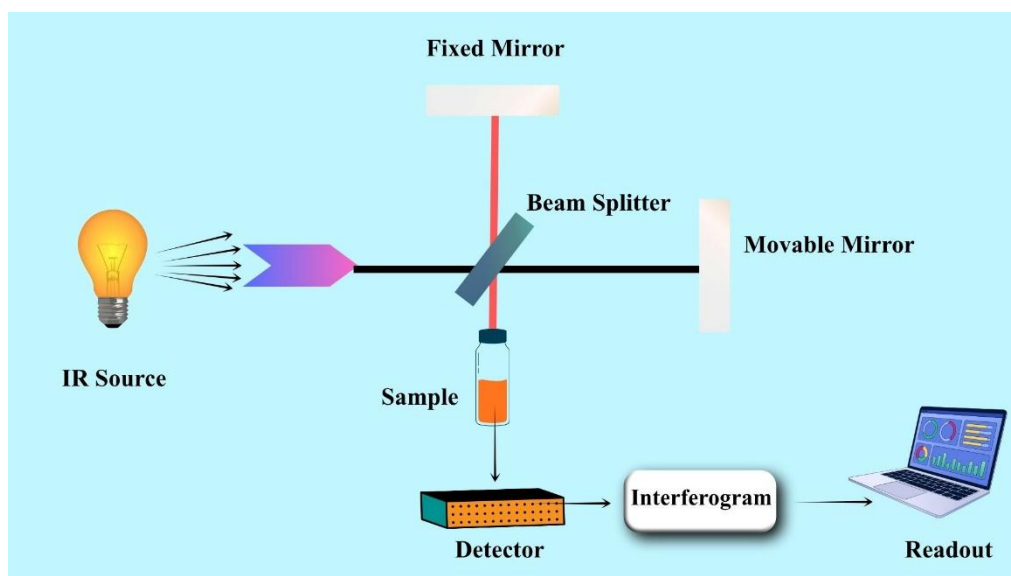


Fig. 3.1 Diagrammatic representation of FTIR

Lattice vibrations are expected to dominate the FTIR spectra of CuS samples. Therefore, FTIR studies of these nanomaterials are essential for confirming the formation of the composite, assessing the nature of bonding between components and determining whether any significant changes occur to functional groups during processing. This research uses a FTIR instrument to collect IR data for g-C₃N₄, CuS and g-C₃N₄/CuS composites in the range $400\text{-}4000\text{ cm}^{-1}$ at a resolution of 4 cm^{-1} [63].

3.3.2 X-ray diffraction (XRD)

The main method used for identifying the crystallographic structures, phases, and particle sizes of solid products through X-Ray Diffraction (XRD) uses Bragg's Law of diffraction. According to Bragg's Law, constructive interference of the rays that have been diffracted off a crystal lattice will occur whenever the wavelength of the X-ray diffraction will produce a path length difference equal to an integer number of times the X-ray wavelength. This can be represented mathematically as follows:

$$n\lambda = 2d\sin(\theta)$$

where:

- n is an integer representing the order of diffraction
- λ represents the X-ray wavelength
- d represents the distance between adjacent layers of atoms in the crystal lattice
- θ represents the angle at which the diffracted X-rays emerge from the sample

A plot of X-ray intensity versus 2θ produces a diffractogram pattern, which contains sharp peaks. The position of these peaks is indicative of both the arrangement of atoms within a unit cell and the distances between layers of atoms in the crystal lattice. Identification of the phases present in a sample is determined by comparing experimental diffractograms to known reference patterns available in databases such as those developed by the International Centre for Diffraction Data [64]. A diagrammatic representation of XRD is shown in **Fig. 3.2**.

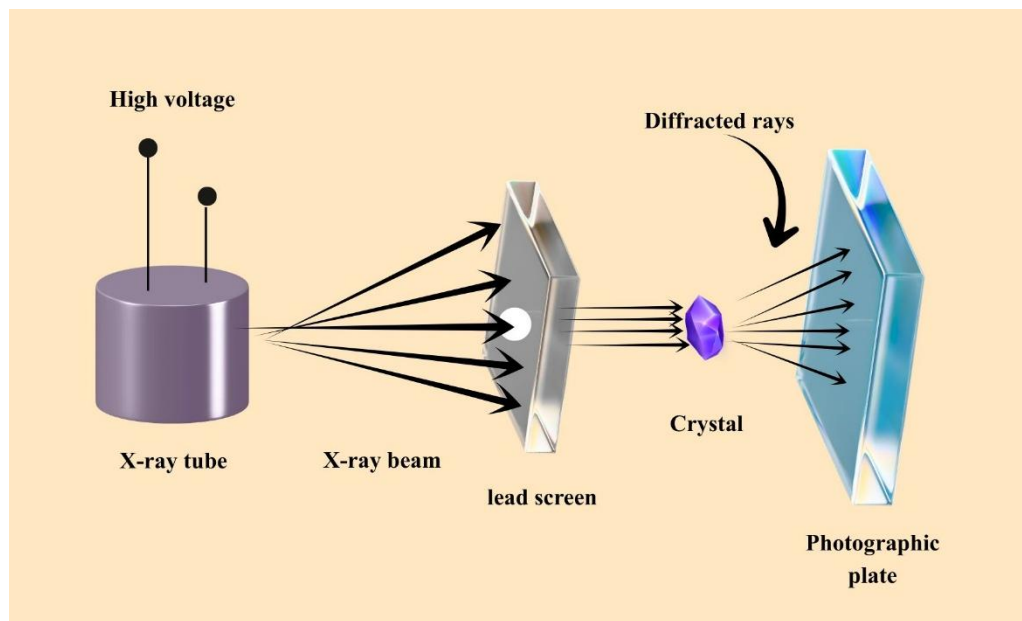


Fig. 3.2 Diagrammatic representation of XRD.

3.3.3 Field Emission Scanning Electron Microscopy (FESEM)

Field Emission Scanning Electron Microscopy (FESEM) is a highly accurate method for obtaining surface imaging data on the size, shape, and texture of nanomaterials. While FESEM provides similar information to conventional scanning electron microscopy (CSEM) regarding the surface characteristics of materials, CSEM utilizes a thermionic tungsten filament as its electron source. The FESEM system uses a field emission gun instead [65]. The metal tip used

in the field emission gun has a sharp end. Placing an extremely high electric potential difference across this metallic tip causes quantum mechanical tunnelling of electrons through the metallic barrier. As a result, the FESEM produces a very coherent, low-energy electron beam with minimal chromatic aberrations. This results in higher spatial resolution for FESEM compared to CSEM systems. Spatial resolutions can be achieved with FESEM ranging from 1-5 nm. A diagrammatic representation of FESEM is shown in **Fig. 3.3**.

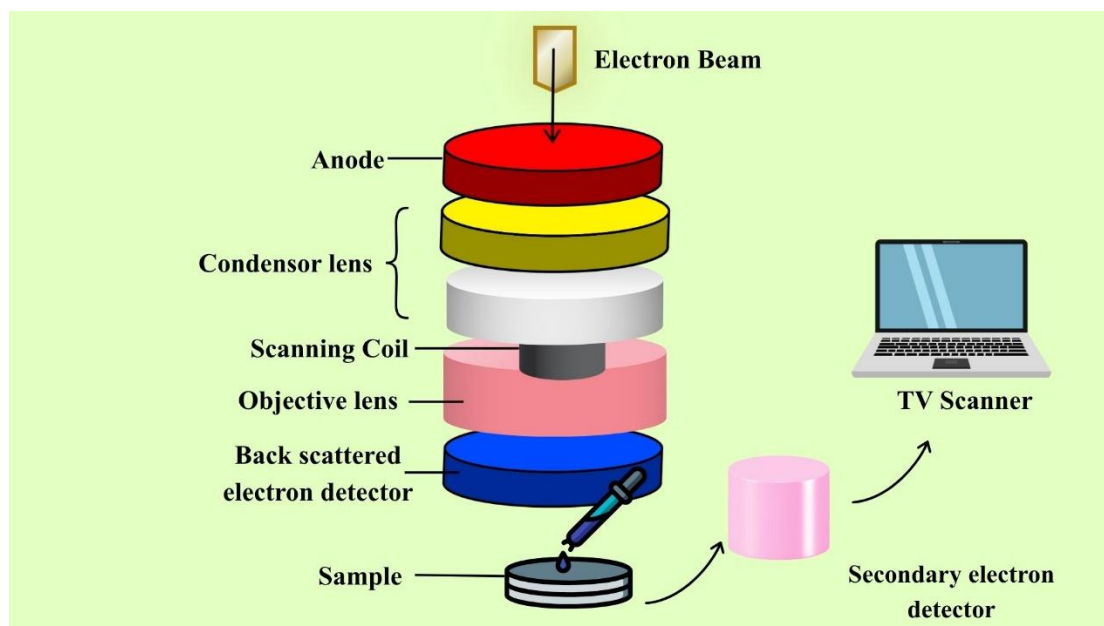


Fig. 3.3 Diagrammatic representation of FESEM machine

In FESEM, the primary electron beam produced by the field emission gun is directed onto the sample surface, raster-scanning the entire surface. When the primary electron beam interacts with near-surface atoms on the sample, secondary electrons (SE) are produced. These SE are relatively low-energy electrons produced from the first few nanometres of the surface. An Everhart-Thornley detector collects these SEs, producing high-contrast topographic images. From these topographic images, researchers can see the surface morphology of their samples, how particles have aggregated, and what types of nanostructural or microstructural features are present. When studying electrochemistry and electrochemical applications, the morphology of samples also determines the electroactive surface area and how easily analyte molecules can access the working electrode surface. Therefore, in order to determine the electrochemical behaviour of our synthesized materials, we chose to use FESEM to study the surface morphology of our g-C₃N₄, CuS and g-C₃N₄/CuS nanocomposite samples [65].

3.3.4 Energy Dispersive X-ray Spectroscopy (EDX)

EDX, also called EDS or EDXA, uses a primary electron beam that strikes the sample, ejecting inner-shell electrons from its constituent atoms. This leaves the atom with characteristic electron vacancies. The outer-shell electrons drop to fill the vacancies, creating energy-level differences, and, in doing so, emit characteristic X-ray photons. These X-rays have energies that correspond specifically to the atomic numbers of their respective emitting elements. EDX spectra show energy-dispersive detectors sorting X-rays by energy; each spectral peak corresponds to a specific element in the sample.

EDX has significant value in characterizing nanomaterials because it can simultaneously provide both morphological information and elemental composition from the same sample using a single instrument [66]. This is a particular advantage over traditional methods, in which samples would require additional processing steps to prepare them for each analytical technique. While EDX provides point-analysis information, elemental mapping can provide a visual representation of how individual elements distribute throughout the field of view, demonstrating whether there is compositional homogeneity or heterogeneity in nanocomposites. Therefore, we anticipate that EDX will show the presence of C, N, Cu, and S as the major elements in the g-C₃N₄/CuS nanocomposite, and elemental mapping will reveal the close proximity and uniform mixing of the g-C₃N₄ and CuS phases throughout the nanocomposite structure. The EDX analysis utilized a detector connected to our FESEM instrument. The elemental mapping was conducted to determine whether the various elements were spatially distributed relative to one another [64].

3.3.5 Transmission Electron Microscopy (TEM)

The atomic-scale structural and morphological information of Transmission Electron Microscopy (TEM) is provided by the high-energy electron beam (usually 80–300 kV) that passes through the thin-sectioned specimen and then collects all of the electrons that pass through and are deflected from the thin-sectioned specimen to produce a magnified image. Compared with the Field Emission Scanning Electron Microscope (FESEM), which examines the surface morphology of the specimen, TEM can examine the internal nanostructure, lattice fringes, particle size distribution, and crystallographic relationships at sub-nanometre resolution. The contrast in TEM images arises from variations in electron scattering due to

changes in sample thickness, density, and atomic number. Heavy elements scatter more strongly and therefore appear darker in bright field images [67].

High-Resolution TEM (HRTEM) extends the capabilities of conventional TEM imaging to provide atomic-plane-level resolution. Therefore, it is possible to directly observe the crystal lattice fringes, measure the d-spacings, and compare these measurements with X-ray Diffraction (XRD) results. A diagrammatic representation of TEM is shown in **Fig. 3.4**.

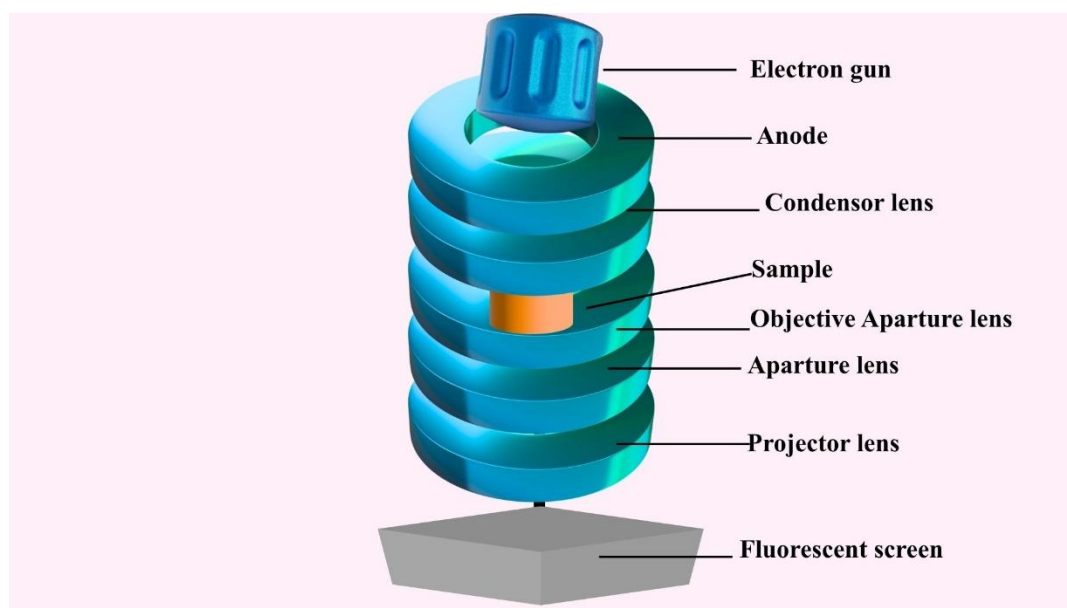


Fig. 3.4 Diagrammatic representation of a TEM machine

For nanocomposites, TEM is required to examine the interfacial relationships between phases in the composite material, determine whether particles have been anchored to other phases, and evaluate whether quantum size effects occur as dimensions approach the nanometer scale. In this research study, TEM was used to analyze the dispersion and anchorage of CuS nanoparticles on g-C₃N₄ nanosheets and to verify the lattice parameters using High-Resolution TEM (HRTEM) [68].

Sample preparation involved ultrasonic agitation to disperse a small amount of powdered material in ethanol, followed by depositing a few microliters of the resulting solution onto carbon-coated copper TEM grid support films. Following drying of the deposited liquid, samples were analyzed.

3.4 Electrochemical Characterization Techniques

Electrochemical analysis is a branch of analytical chemistry in which electrochemical concepts are applied to assess chemical reactions, identify some properties of substances, and determine their concentrations. The advantages of electrochemical analysis over other surface modification technologies include its ability to preserve the surface features during electrochemical dissolution. The crystal structure and orientation remain intact. Cyclic voltammetry and differential pulse voltammetry are among the main types of electrochemical analysis. The basic unit of an electrochemical system includes at least one working electrode, one reference electrode, and one counter electrode. These electrodes are connected with a potentiostat, which measures the resulting current. When we run this type of electrochemical test, the working electrode is held at a specific potential, and the corresponding current is plotted against time. In this work, each electrode was analyzed with respect to its electrochemical responses utilizing an Autolab, Pt or Ag/AgCl as counter-electrodes [69].

3.4.1 Cyclic Voltammetry (CV)

A variety of different voltammetric methods can be used to measure changes in charge and mass transport across an electrode interface. One of the most common and versatile methods is cyclic voltammetry (CV). CV is often utilized to analyze the thermodynamic and kinetic properties of electrode reactions, to identify electrochemically active species within a solution environment, or to evaluate how modifying the electrode surface affects its electrochemical characteristics.

During a CV experiment, the working electrode will have a triangular voltage signal applied to it. The initial potential (E_i) at which the electrode starts is first scanned linearly toward the maximum potential (E_λ) of the triangular shape at a specific scan rate (v) [70]. After reaching E_λ , the direction of the applied potential is reversed, returning the working electrode to either the starting point. As the potential changes during this scanning procedure, a current is produced by the reaction at the working electrode. This current is measured and plotted as a function of the applied potential. The resulting plot is called a cyclic voltammogram (CV) [71].

The CV obtained for a reversible redox couple contains two distinct features: an anodic peak (I_{pa}) when the working electrode is moved in the positive direction, representing oxidation, and a cathodic peak (I_{pc}) when the working electrode is moved in the negative direction,

representing reduction. If all of the electrochemistry occurring at the working electrode is controlled by diffusion (all of the reactants are diffusing through the solution to reach the working electrode), then both the anodic and cathodic peak currents should depend upon the square root of the scan rate. If there are no diffusion limitations in the electrochemistry at the working electrode, then both peak currents should depend directly on v [70].

In this research project, we also used CVs. We used CV to investigate several aspects of our system to obtain information about the chemical steps involved in each of our three sequential modifications to create the composite material that makes up our working electrodes to obtain values for the effective area of bare glassy carbon electrodes (GCE) and those which had been subjected to our modifications and to understand how increasing the scan rate would affect the ability to oxidize trichlorfon and finally to establish whether diffusion control or adsorption control mechanisms were controlling electron transfer at the surface of our modified working electrodes. All of our CV experiments were done under potentiostatic conditions over a range of scan rates [71].

3.4.2 Differential Pulse Voltammetry (DPV)

Differential Pulse Voltammetry (DPV) is an extremely sensitive electroanalytical method for analysing very low concentrations of electrochemically active species. DPV was created to address limitations of Linear Sweep Voltammetry (LSV), where the significant non-faradaic (charge-transfer) current associated with double-layer capacitance obscures the much smaller faradaic (reduction or oxidation) currents associated with the analytes. Thus, reducing overall sensitivity and increasing the noise-to-signal ratio [72].

A series of small-potential pulses (typically 10-100 mV) is applied on top of a staircase-shaped potential ramp, with the voltage increasing with each pulse. Two current values are taken during each pulse cycle: one immediately prior to applying the pulse (t_1), when primarily non-faradaic current is observed, and one closer to the end of the pulse (t_2), when a pseudo-steady-state condition exists. The two current values are then subtracted from one another to produce a difference current value (ΔI) that is proportional to the concentration of the analyte. This ΔI value is then measured as a function of the applied potential. Since the charging current is eliminated in this process, the resulting curve is symmetrical and bell-shaped, with its peak proportional to the analyte concentration [73]. Additionally, the peak potential of the DPV

curve closely approximates the formal reduction potential (E°) of the redox couple, allowing for information regarding the mechanism of electron transfer.

Limits of Detection (LOD) and Limits of Quantitation (LOQ) obtained via DPV are generally 1-3 orders of magnitude lower than those achieved via Cyclic Voltammetry (CV) or Linear Sweep Voltammetry (LSV) for the same analyte and electrode system. Due to these characteristics, DPV has become the preferred method for detecting trace amounts of pesticides in samples [72].

Electrochemical Biosensing Platform Utilizing Graphitic Carbon Nitride-Copper Sulfide Nanohybrid for Sensitive Trichlorfon Detection

4.1 Introduction

This chapter covers an innovative and highly effective AChE-based electrochemical biosensor that utilizes g-C₃N₄-decorated CuS for the detection of TF. Due to synergistic effects in the synthesized nanohybrid (g-C₃N₄/CuS), there is a substantial increase in electrochemically active surface area, improved catalytic activity, fast charge transfer, and significant bio-compatibility. A diagrammatic representation of the biosensor fabrication is shown in **Fig. 4.1**.

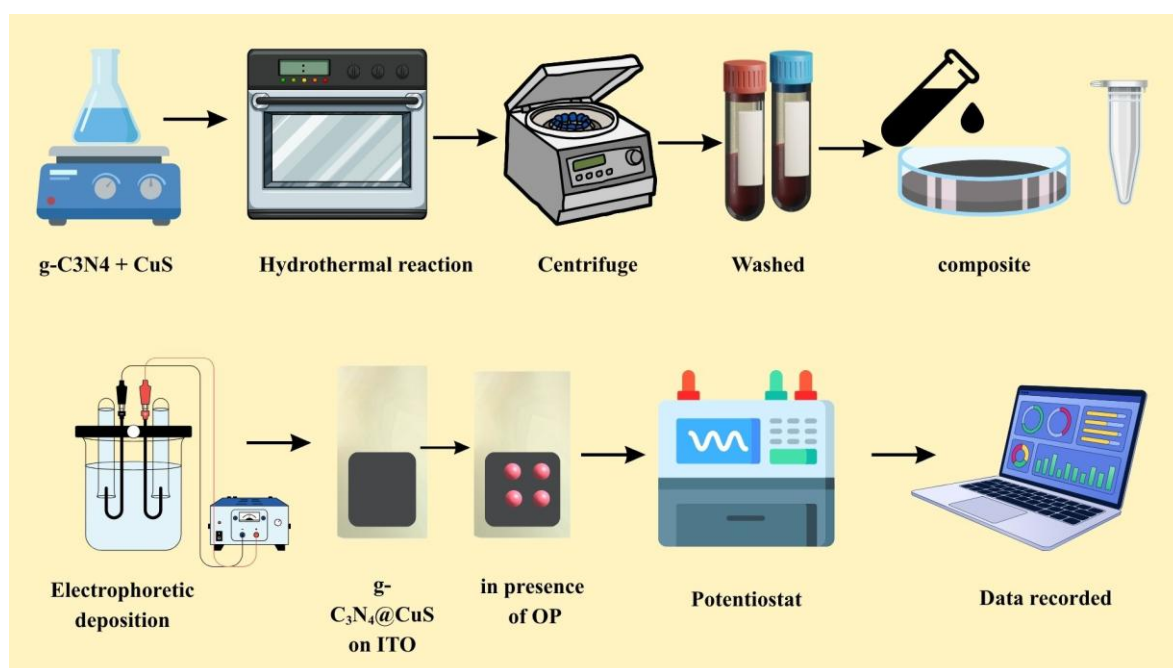


Fig. 4.1 Schematic representation of TF detection using fabricated g-C₃N₄/CuS/ITO bioelectrode.

The fabricated biosensor showed good specificity, very high sensitivity, low detection limit, good reproducibility, and long-term stability [74]. Moreover, the biosensor can be used for detecting TF with acceptable results in two different real samples.

4.2 Experimental section

4.2.1 Synthesis of g-C₃N₄ nanosheets

To produce g-C₃N₄ powder, a typical high-thermal condensation process is performed under ambient pressure. Generally, 10g of melamine is placed in a muffle furnace at 550°C for 4 hrs. After heating, the pale-yellow product will first be washed with distilled water and ethanol in equal portions. Finally, it can be dried using a vacuum oven [75].

4.2.2 Synthesis of CuS

The CuS was prepared using a method where approximately 0.8 g of sodium thiosulfate (0.125 M) and copper sulphate (0.5 M) were dissolved in 40 and 20 mL of DI Water, respectively. The two solutions were mixed after 30 mins, under agitation and then placed into an autoclave for 12 hrs at 180°C. Upon completion of the reaction, the resulting black-coloured slurry was centrifuged, first with DI Water, then with ethanol. The final precipitate was dried in a vacuum at 70°C to yield powdered CuS [76].

4.2.3 Synthesis of g-C₃N₄/CuS nanocomposite

To make the nanocomposite, 2.5g of sodium thiosulfate (Na₂S₂O₃.5H₂O), 24.8g of copper (II) sulphate (CuSO₄.5H₂O) and 50 mL of deionized water were combined in a beaker and vigorously shaken until a homogeneous solution formed. After this point, 1.9 g of g-C₃N₄ was added to the solution. This solution was then continuously agitated and ultrasonically treated for half an hr. Then the solution was transferred into a 100 mL Teflon-lined hydrothermal autoclave and heated at 180°C for 20 hrs. A brown solid was obtained after heating. The solid was centrifuged at 4000 rpm and washed several times using an equal volume ratio of distilled water to ethanol, and finally dried under a vacuum at 80°C [77].

4.2.4 Electrophoretic Deposition (EPD) of g-C₃N₄/CuS on ITO

The g-C₃N₄/CuS hybrid, synthesized, is electrochemically deposited onto the previously hydrolyzed ITO (glass) substrate. A two-electrode setup is utilized, in which a platinum wire is employed as the counter electrode and placed 1cm away from the working electrode, which is the ITO-coated glass substrate. Prior to deposition, 1 mg of g-C₃N₄/CuS is sonicated in

deionized water (15 mL) to form a colloidal solution [76]. Subsequently, electrochemical deposition of the colloidal solution is performed by applying an optimum direct current voltage of 10 V for 8 s.

4.2.5 Fabrication of g-C₃N₄/CuS nanohybrid-based biosensor

The fabrication of the biosensor began with incubation of the g-C₃N₄/CuS/ITO electrode in a glutaraldehyde solution (10 μ L) at room temperature for 60 mins. The next step was to add 20 μ L of AChE solutions at a concentration of 0.06 mg/mL, prepared in PBS at pH 7.5, onto the surface of the previously modified electrode and incubate it for another 12 hrs at 4°C. Finally, the biosensor was washed with 50 μ L of PBS before performing electrochemical measurements to remove free enzyme [77].

4.3 Results and Discussion

4.3.1 Structural Characterisation

XRD analysis for g-C₃N₄, CuS and the g-C₃N₄/CuS nanocomposite is given in **Fig. 4.2 (A)**. In the case of pure g-C₃N₄, it has been found that there are only two characteristic XRD peaks. There is a low-intensity peak observed near $2\theta = 13^\circ$ associated with the (100) plane, resulting from the in-plane structural arrangement of the tri-s-triazine unit. A very intense, narrow XRD peak at $2\theta = 27.4^\circ$ corresponds to the (002) plane arising from interlayer stacking of the conjugated aromatic sheets in g-C₃N₄. The peak width provides evidence of the typical graphitic and layered nature of g-C₃N₄. Pure CuS produces many sharp and well-defined XRD peaks near 2θ values of approximately $27 - 33^\circ$, 48° , 53° , and 59° , associated with the characteristic planes of the hexagonal covellite phase of CuS. Therefore, CuS is confirmed to be highly crystalline. The sharpness and high intensity of these peaks indicate that the synthesized CuS has a well-ordered hexagonal structure.

An examination of the XRD data for the g-C₃N₄/CuS nanocomposite shows that most of the main diffraction peaks present in the XRD spectrum correspond to those produced by CuS. Due to the higher degree of crystallinity exhibited by CuS, CuS is dominant in terms of peak production in the composite's XRD spectrum. Furthermore, there are no additional or novel peaks in the XRD pattern of the composite; therefore, there is no indication of either a structural distortion or the formation of secondary phases during synthesis [78].

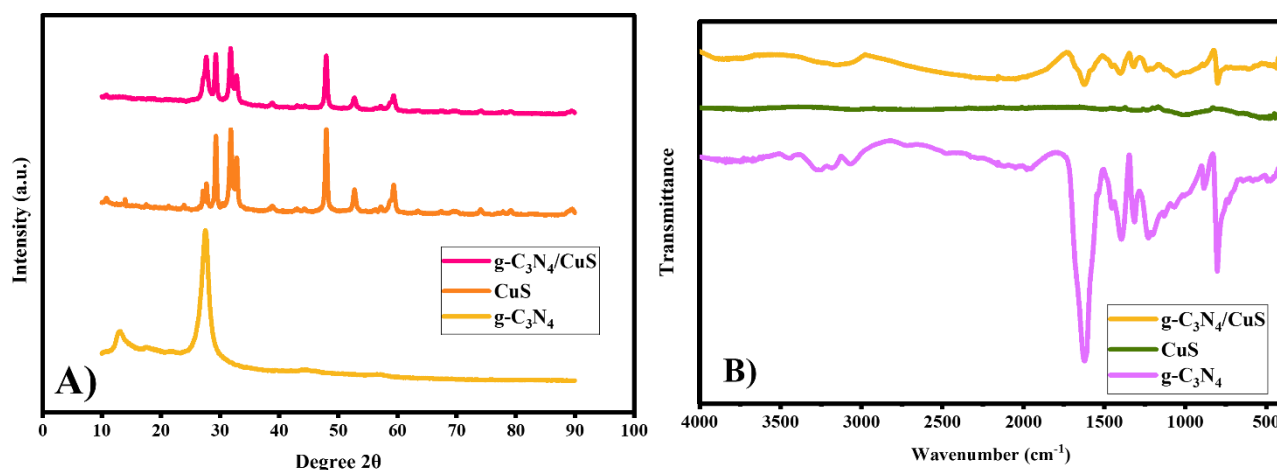


Fig. 4.2 (A) XRD and (B) FTIR of (a) g-C₃N₄/CuS, (b) CuS, and (c) g-C₃N₄

FTIR was used to examine the structural and functional group properties of the synthetic materials. The infrared spectra for g-C₃N₄, CuS and g-C₃N₄/CuS nanocomposites are depicted in **Fig. 4.2 (B)**. The infrared spectrum of pure g-C₃N₄ showed an absorption band in the range of 3000-3300 cm⁻¹ due to N-H and O-H stretching resulting from the incomplete condensation of the melamine precursor during heat treatment. The most diagnostic peak observed in the IR spectrum at about 808 cm⁻¹ corresponds to the triazine ring breathing mode through the out-of-plane bend vibration and thus confirms the existence of the heptazine-based conjugated framework.

The several bands occurring between 1200 and 1640 cm⁻¹ are assigned to the C-N and C=N stretches, respectively; these are characteristic of the aromatic heterocyclic ring structure of g-C₃N₄ and arise from sp²-hybridized C-N networks. The low-wavenumber fingerprint region between 400 and 700 cm⁻¹ shows distinct absorption bands for pure CuS due to Cu-S stretching and bending vibrations of the metal-sulphide lattice, and this verifies the formation of the covellite CuS phase [79].

Furthermore, there was a slight shift toward lower wavenumbers in certain characteristic bands of the g-C₃N₄/CuS composite compared with those of the individual components. This shift suggests a degree of electronic interaction between the g-C₃N₄ nanosheet layers and the CuS nanoparticle surface.

4.3.2 Morphological Studies

The microarchitecture of the produced nanomaterials has been studied using FESEM [89]. The FESEM image of the synthesised g-C₃N₄ shows 2D nanosheets with thin, folded, crumpled, and stacked layers, as is typical of these materials, thereby reducing the free surface area, as shown in **Fig. 4.3(A)**. On the contrary, CuS nanostructures exhibit a distinct 3D hierarchical structure consisting of rose-shaped micro-flowers composed of closely packed nanoflakes. Such an interconnected, flower-like structure is typical of CuS synthesized by different methods, as shown in **Fig. 4.3(B)**.

Finally, the FESEM image of the composite g-C₃N₄/CuS directly demonstrates that a 2D/3D heterostructure is possible. In fact, from this image, we can see that the 3D CuS micro-flowers are well attached to the 2D matrix formed by the g-C₃N₄ nanosheet, thereby preventing their aggregation [80]. We can also observe that the intimate contact at the interface between the 3D CuS structures and the 2D g-C₃N₄ substrate forms a “flowers on stalk” structure, as shown in **Fig. 4.3(C)**.

TEM was used to provide additional information on the morphology and structural integration of the different components in the samples produced [82]. As seen in **Fig. 4.3(D)**, the TEM image of g-C₃N₄ shows the typical characteristics of a 2D nanosheet, with each flake exhibiting an extremely thin, pleated shape. On the other hand, the TEM image in **Fig. 4.3(E)** shows well-formed spherical CuS particles with a relatively small size range.

Importantly, the TEM images of the CuS/g-C₃N₄ composite, shown in **Fig. 4.3(F)**, provided unequivocal evidence of the successful formation of the heterostructures. The images indicate that each spherical CuS nanoparticle is firmly attached, or "anchored," to the surface of the g-C₃N₄ sheets. Thus, they are also uniformly dispersed throughout the composite structure.

EDX analysis is conducted on a sample to identify the elements it contains and its purity [81]. The EDX test of g-C₃N₄/CuS, showing elemental peaks for carbon, copper, sulphur, and nitrogen, is shown in **Fig. 4.3(G)**.

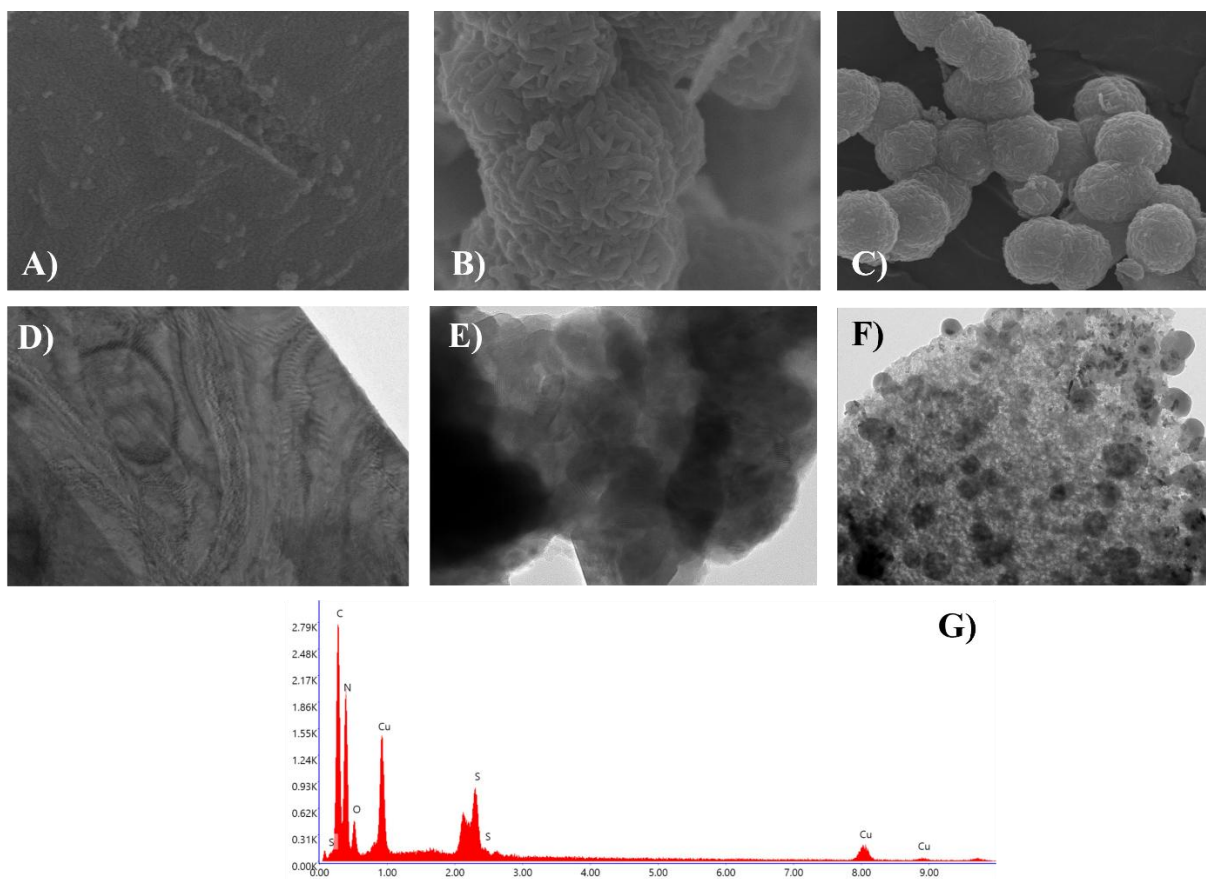


Fig. 4.3 FESEM images of (A) g-C₃N₄, (B) CuS, (C) g-C₃N₄/CuS, TEM images of (D) g-C₃N₄, (E) CuS, (F) g-C₃N₄/CuS and EDAX of (G) g-C₃N₄/CuS

4.3.3 Electrochemical Characterization

Cyclic voltammograms (CVs) are used to assess an electrode's performance. The CV plots shown in **Fig. 4.4(A)** demonstrate the cyclic voltammogram studies done for the fabricated (a)g-C₃N₄/CuS /ITO, (b)CuS/ITO, (c)g-C₃N₄/ITO and (d)AChE/g-C₃N₄/CuS/ITO using a scan rate of 50 mV/sec in 0.1M PBS solution that contained 5 mM [Fe(CN)₆]^{-3/-4}. It can be seen from **Fig. 4.4(A)** that the largest redox peak current was found for g-C₃N₄/CuS/ITO (curve a, 0.301 mA) compared to CuS/ITO (curve b, 0.233 mA), g-C₃N₄/ITO (curve c, 0.209 mA). The large redox peak current associated with g-C₃N₄/CuS/ITO is thought to arise because of both its high surface area and excellent electronic conductivity provided by the g-C₃N₄/CuS composite [83]. Conversely, it is expected that the lowest currents and least mobilities will be present for AChE/g-C₃N₄/CuS/ITO (curve d, 0.193 mA) due to the poor conductivity exhibited by the enzyme AChE.

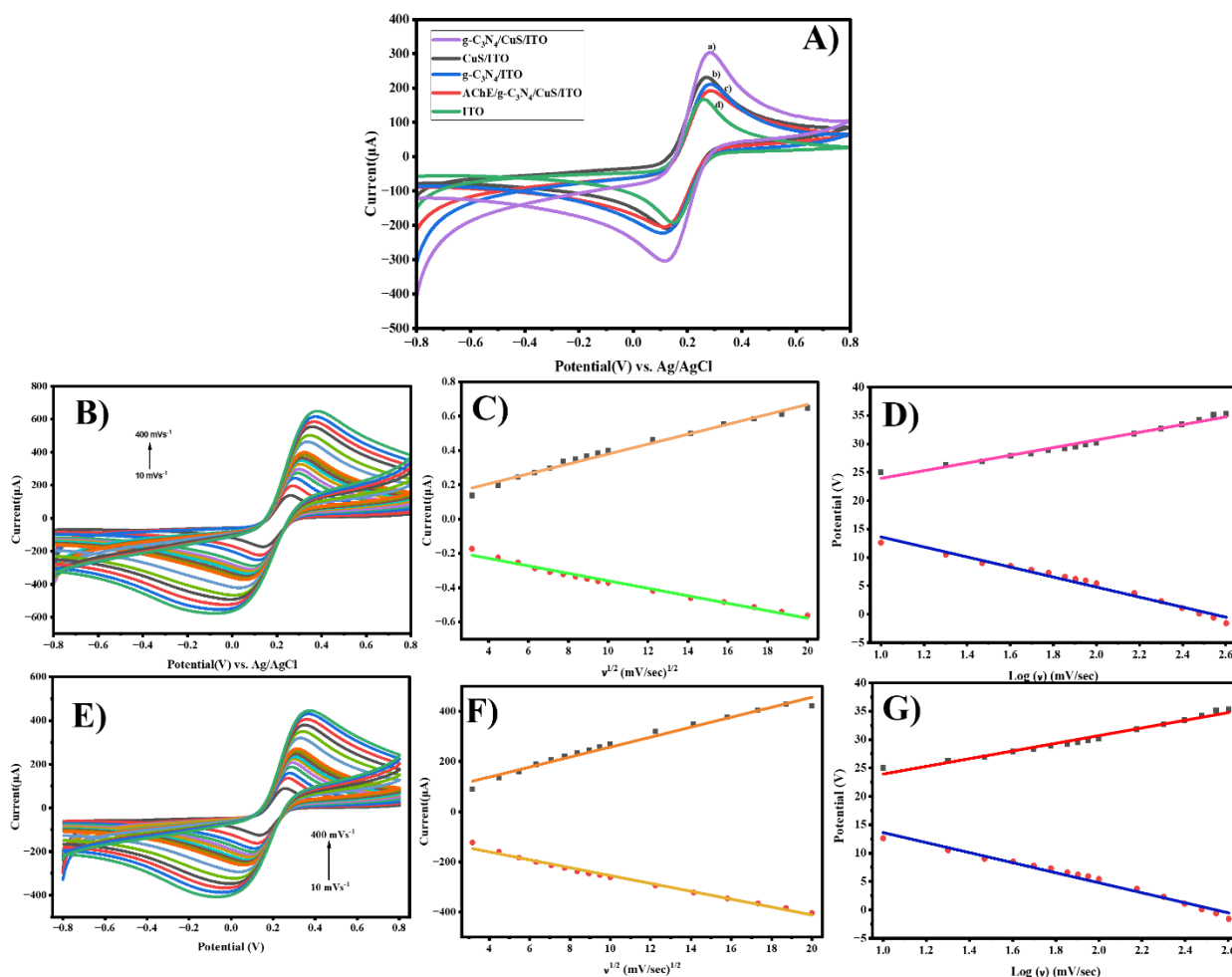


Fig. 4.4 Different scan rate of (A) g-C₃N₄/CuS/ITO and (D) g-C₃N₄ electrode (10-400 mV/sec), (D) Graph of current v/s square root of scan rate of (C) g-C₃N₄/CuS/ITO and (F) g-C₃N₄, (E) Graph for potential v/s log (scan rate) of g-C₃N₄/CuS/ITO, (G) Cyclic voltammogram for different developed electrodes (a) g-C₃N₄/CuS/ITO, (b) CuS/ITO, (c) g-C₃N₄/ITO, (d) AChE/g-C₃N₄/CuS/ITO

The CV has been used to investigate the g-C₃N₄/CuS/ITO and g-C₃N₄/ITO electrode materials over a scan rate range of 10-400 mV/s. **Fig. 4.4(C)** and **Fig. 4.4(F)** clearly show how the anodic peak current (I_{pa}) becomes larger and moves towards a more positive potential, while the cathodic peak current (I_{pc}) becomes larger and moves toward a more negative potential when increasing the scan speed from 10 to 400 mV/sec, respectively. Thus, this indicates that both the anodic and cathodic peak currents (E_{pa} and E_{pc}) are linear with the logarithm of the scan rate ($\log v$), as shown in **Fig. 4.4(D)** and **Fig. 4.4(G)**, which is supported by equations (4.1) and (4.2).

$$E_{pa} [g-C_3N_4/CuS/ITO] = 0.763 (V) \log (v) + 0.165 (V), R^2 = 0.98323 \quad (4.1)$$

$$E_{pc} [g-C_3N_4/CuS/ITO] = -0.0855 (V) \log (v) + 0.236 (V), R^2 = 0.98292 \quad (4.2)$$

We also observed an inverse-square-root relationship between I_{pa} and I_{pc} , with $v^{1/2}$ [84]. These relationships can be described using the following equations (4.3), (4.4), (4.5) and (4.6).

$$I_{pa} [g-C_3N_4/CuS/ITO] = 28.93 \mu A (s/mV)^{1/2} v^{1/2} + 90.25 \mu A, R^2 = 0.98323 \quad (4.3)$$

$$I_{pc} [g-C_3N_4/CuS/ITO] = -21.82 \mu A (s/mV)^{1/2} v^{1/2} - 142.08 \mu A, R^2 = 0.98292 \quad (4.4)$$

$$I_{pa} [g-C_3N_4/ITO] = 19.86 \mu A (s/mV)^{1/2} v^{1/2} + 58.4 \mu A, R^2 = 0.9773 \quad (4.5)$$

$$I_{pc} [g-C_3N_4/ITO] = -15.69 \mu A (s/mV)^{1/2} v^{1/2} - 97.54 \mu A, R^2 = 0.98822 \quad (4.6)$$

By using the Randles-Sevcik equation,

$$(I_p = 2.69 \times 10^5 n^{3/2} A C D^{1/2} v^{1/2})$$

We found the values of effective surface area to be 0.35 cm^2 and diffusion coefficient to be $7.77 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, where n is the number of electrons, C is the concentration of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in mole cm^{-3} , and v is the scan rate in V/s .

4.3.4 Optimization studies

The DPV method determines optimal conditions for the electrolyte solution's pH, AChE concentration, and incubation duration. To determine the optimal enzyme concentration, the DPV response of the AChE/ $g-C_3N_4/CuS/ITO$ electrode to AChE/ $g-C_3N_4/CuS/ITO$ in the presence of 2 mM ATCl and 1 nM TF was examined [85]. **Fig. 4.5 (A)** illustrates how the DPV response of the AChE/ $g-C_3N_4/CuS/ITO$ electrode varies as a function of increasing concentrations of AChE from 0.04 mg/mL to 0.1 mg/mL while keeping the amounts of ATCl and TF constant. When the concentration of AChE is increased from 0.04 mg/mL to 0.06 mg/mL, the current signal increases; beyond this concentration, it decreases due to excessive mass-transfer resistance. For these experiments, therefore, a concentration of 0.06 mg/mL AChE is used. In **Fig. 4.5(B)**, the influence of the electrolyte solution pH, ranging from 6 to 8, on the sensor's performance is demonstrated. The largest current signal was observed at pH 6, as shown in the data below. This is the pH value where all subsequent electrochemical analyses were conducted.

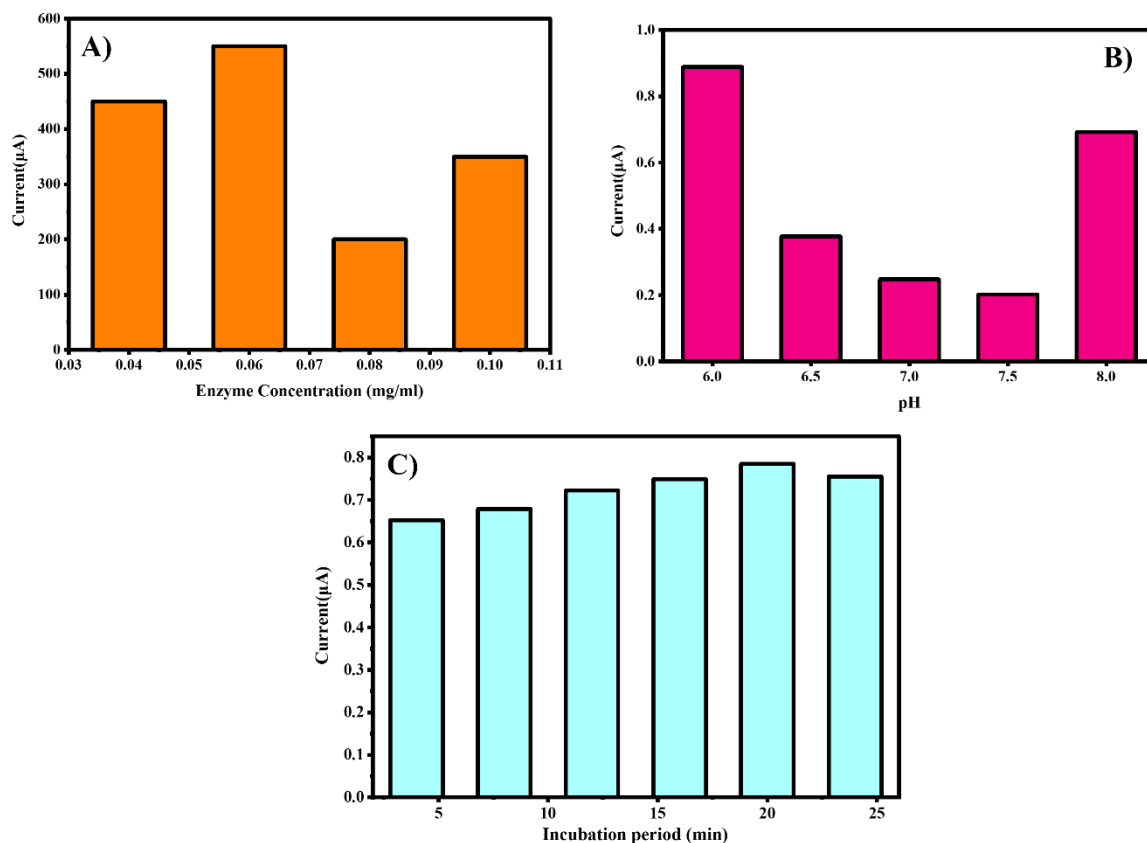


Fig 4.5 Optimization studies of (A) Concentration of an enzyme, (B) Effect of pH, (C) Incubation time

Further studies on the optimal incubation period have also been conducted. These studies involved measuring the peak current over incubation durations ranging from 4 min to 24 min, as depicted in **Fig. 4.5 (C)**. Results indicate that longer incubation times lead to decreased peak currents, with saturation occurring at 20 min.

4.3.5 Electrochemical Biosensing Study

DPV was used for the electrochemical characterization of the AChE/g-C₃N₄/CuS/ITO electrode to measure the response of the developed sensor toward TF. The peak current of the fabricated bioelectrode at various TF concentrations, from 0.1 pM to 1 μM, was measured using DPV in 0.1 M PBS at pH 6.0 and ATC1 (2mM) [86].

An increase in TF is associated with a decrease in peak current, as shown in **Fig. 4.6(A)**, indicating that TF inhibits the AChE enzymatic reaction. The calibration plot in **Fig. 4.6(B)** shows a linear relationship between I peaks and TF concentration, indicating that the peak current is proportional to the TF concentration.

$$I = 2.77375 \mu A/pM + 53.342 \mu A \times C_{TF}, R^2 = 0.93503$$

The sensitivity of the biosensor (AChE/g-C₃N₄/CuS/ITO) was 7.925 μA(pM)⁻¹cm⁻². The regression coefficient was 0.9242. The LOD is 0.757 pM, using the equation 3σ / S, in which σ represents the standard deviation of the bioelectrode, and S represents its sensitivity [90].

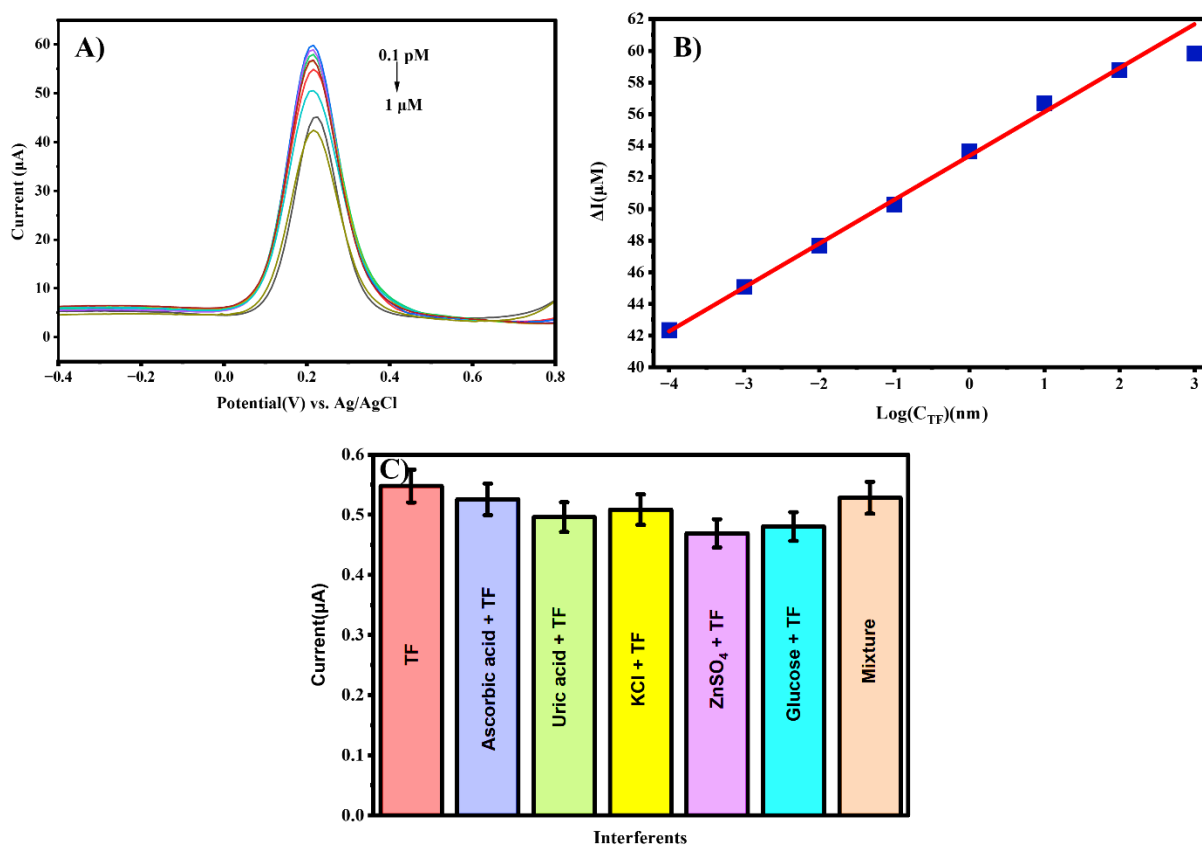


Fig. 4.6 (A) DPV response study of AChE/g-C₃N₄/CuS/ITO developed electrode at different concentrations of TF (0.1 pM - 1 μM), (B) Linearity plot for current v/s TF concentration, (C) Study of selectivity of AChE/ g-C₃N₄CuS/ITO electrode

4.3.6 Interference Study and Real Sample Analysis

The selectivity of the fabricated biosensor (AChE/g-C₃N₄/CuS/ITO) is demonstrated through an incubation of the prepared biosensor with 100 nM glucose, uric acid, ascorbic acid, ZnSO₄ and KCl in addition to 1 nM TF. The data show no change in current after exposure to interfering analyte concentrations, indicating a high degree of selectivity for TF detection [87]. The utility and practicality of the AChE/g-C₃N₄/CuS/ITO biosensor have been evaluated using spiked samples (apple & cucumber) at established concentrations of 1, 10 and 100 nM. The

recovery values from the spiked-sample experiments ranged from 90% to 103%, indicating the reliability of the AChE/g-C₃N₄/CuS/ITO biosensor. The TF detection data from the spiked samples are found in Table 4.1.

Table 4.1 Detection of TF in two real samples using AChE/g-C₃N₄/CuS/ITO electrode

Samples	Added amount (nM)	Found amount (nM)	Recovery (%)	RSDs (%)
Apple	1	0.987	98.73	0.899
	10	9.347	93.47	4.76
	100	103.18	103.18	2.21
Cucumber	1	0.932	93.26	4.92
	10	9.285	92.85	5.23
	100	90.68	90.68	6.91

4.3.7 Reproducibility and Stability Studies

The reproducibility of the AChE/g-C₃N₄/CuS/ITO electrode was evaluated using the DPV method to measure the responses of five similarly prepared electrodes in the presence of 1 nM TF. The results show an acceptable level of variability, as evidenced by the low Relative Standard Deviation (RSD) of 6.29%.

Stability testing of the AChE/g-C₃N₄/CuS/ITO electrode has been conducted by storing the bio-sensing electrode at 4°C in a refrigeration unit and measuring its electrochemical activity every 6 days over a total period of 30 days. After 30 days, the measured electrochemical activity was 69.96% of the initial value.

CHAPTER 5

Conclusion

In conclusion, an electrochemical biosensor using a g-C₃N₄/CuS composite was successfully fabricated. This new biosensor has excellent sensitivity toward TF. Factors affecting its performance have been optimized, including PBS pH at 6, AChE and ATCI concentrations, and an incubation period of approximately 20 mins. The use of g-C₃N₄/CuS modified transducers increases the rate of electron transfer between AChE and the electrode surface. These improvements increase the sensitivity of the proposed biosensor. The developed biosensors demonstrate superior performance based on sensitivity (7.925 $\mu\text{A (pM)}^{-1}\text{cm}^{-2}$), lower limits of detection (LOD) are 0.757 pM and wider dynamic ranges (0.1 pM - 1 μM). Good recoveries (between 90 % to 103%) and small relative standard deviations (RSD%) are also demonstrated for two different food matrices, demonstrating the feasibility of utilizing the fabricated biosensor.

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