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ABSTRACT

A widespread utilization of organophosphate pesticides in the agriculture sector has led to serious environmental and public health issues due to the toxicity and non-biodegradability. One of the most commonly used organophosphate pesticides, fenitrothion, can enter water, soil and food products causing serious health effects to people and ecosystems. So, it is highly needful to evolve a rapid, sensitive and cost-effective detection method for fenitrothion monitoring. In the present study, a nanocomposite of graphitic carbon nitride (g-C₃N₄) and zinc–nickel oxide (ZnO–NiO) has been successfully fabricated to develop an electrochemical biosensor for sensitive detection of fenitrothion. Graphitic carbon nitride was synthesized by thermal polymerization of melamine and the zinc–nickel oxide nanostructures were synthesized by a hydrothermal synthesis method. The synthesized nanomaterials were further used to make a hybrid nanocomposite that exhibited improved conductivity, high surface area and better electrocatalytic activity. The fabricated nanocomposite was then coated on an indium tin oxide (ITO) electrode to fabricate an electrochemical biosensor. X-ray diffraction, field-emission scanning electron microscopy and Fourier transform infrared spectroscopy were used to characterize the synthesized materials structurally and morphologically. Cyclic voltammetry and differential pulse voltammetry were also used for the electrochemical studies. The developed biosensor was highly sensitive, rapid, highly selective and had a low detection limit for fenitrothion. The synergistic effect between g-C₃N₄ and Zn–Ni oxide was believed to be responsible for the improved sensing performance by providing efficient electron transfer and making a more active sensing.

CHAPTER 1

Introduction

1.1 Introduction to pesticides

Detection of pesticides is of great importance for protecting the environment and safeguarding public health. Chemicals or organisms that will help to prevent, kill, repel or control the harmful organisms that may be involved in agricultural products, stored food products and public health [1]. The harmful organisms are insects, fungi, weeds, bacteria, rodents and other pests that decrease crop yields and quality. The use of pesticides has become an integral part of modern agriculture as an effective way to boost the crop production, enhance food quality and minimize post-harvest losses [2]. The term “pesticide” comes from two Latin words – “pestis” for plague and “cida” for killer. Therefore, pesticides are the chemicals that are being used to kill or manage the pests. Amount of pesticides consumed has grown rapidly since the beginning of the green revolution to meet the food demand of the growing global population [3]. They come in a variety of formulations including powders, granules, liquids, aerosols and fumigants. Depending on the pest, they can be applied directly to crops, soil, water bodies or storage [4]. Pesticides offer tremendous benefits to agriculture but overuse and mismanagement have raised serious concerns about the environment and human health. Severe environmental effects of pesticides including pollution and contamination of water resources, soil degradation and toxic effects on humans and animals may result from continued exposure [5].

1.2 Toxicological classification of pesticides

Toxicology is the science of harmful effects of the chemicals on living things. The toxicological classification of pesticides is based on the toxicity of the pesticide and its harmful effects to humans, animals and the environment [6]. Pesticides are classified by the

World Health Organization (WHO) based on their toxicity. Generally, pesticides are categorized into the following groups: an extremely hazardous, highly hazardous, moderately hazardous, slightly hazardous pesticides and those that do not pose an immediate danger [7]. Toxicity is normally estimated as the amount of pesticide required to kill 50% of the experimental animals called the LD50 [8]. Pesticides are absorbed by the human body through breathing, eating and through the skin. Chronic exposure can cause neurological disorders, respiratory diseases, hormonal imbalance, cancer and reproductive issues [9].

1.2.1 Classification of pesticides based on the mode of entry

Pesticides are grouped based on how they are used or how they affect the pest organism.

(a) Contact pesticides: These pesticides act against pests when they come in direct contact with their body surface and do not require to penetrate pest's body systems. Examples: Malathion, Chlorpyrifos [10].

(b) Systemic pesticides: Systemic pesticides are taken up by the plants and move through the plant tissues. Pests ingest the pesticide when they feed on treated plants which kills them. Examples: Dimethoate, Imidacloprid [11].

(c) Stomach poisons: These pesticides work in the digestive tract of pests. They are primarily effective as a control for chewing insects. Examples: Arsenic compounds, Carbaryl [12].

(d) Fumigants: The fumigants penetrate the pest by means of its respiratory system in the gaseous or vaporous state. Examples: Methyl bromide, Phosphine [13].

1.2.2 Classification of pesticides based on the function

Pesticides are usually grouped by the type of pest they kill. Agricultural crops, stored food materials, animals and human health are affected differently by various pests [14]. Thus, specific pesticides are developed that target specific groups of organisms. This classification helps farmers and researchers choose the appropriate pesticide for effective pest control [15].

(a) Insecticides: Chemicals used to kill or control insects that harm crops and stored food products. The mechanism of action of these pesticides targets the insect nervous system, digestive system or growth processes. Crop protection products are commonly applied in agriculture to protect crops from harmful pests such as aphids, beetles, mosquitoes and caterpillars. Examples: Malathion, Fenitrothion, Chlorpyrifos [16].

(b) Herbicides: The herbicides are the pesticides that are used to kill undesirable plants or weeds which interfere with the crops for nutrients, water, sunlight and space. Use of herbicides can help boost crop productivity by controlling weeds in fields. Examples: Glyphosate, Atrazine, Paraquat [17].

(c) Fungicides: Fungicides are chemicals used for the control of fungal diseases in plants. Fungi can cause severe damage to several crops affecting leaves, stems, roots, fruits and seeds. Fungicides inhibit fungal growth and protect against infection. Examples: Mancozeb, Carbendazim, Copper oxychloride [18].

(d) Rodenticides: Rodenticides are the chemicals used to kill rodents like rats and mice that inflict damage on crops, stored grains and buildings. Chemicals that are found in households, farms and warehouses. Examples: Zinc phosphide, Warfarin [19].

(e) Bactericides: Chemicals used to control pathogens (disease-causing bacteria) that cause plant diseases and reduce crop yields. Examples: Streptomycin, Copper compounds [20].

(f) Nematicides: Nematicides are medicines that eliminate nematodes which are tiny worms that injure plant roots and lower crop yields. Examples: Aldicarb, Carbofuran [21].

(g) Acaricides: Acaricides are chemicals effective against mites and ticks that infest crops, animals and stored products. Examples: Dicofol, Sulfur compounds [22].

(h) Molluscicides: Pesticides that are used to kill snails and slugs that harm crops on farms, particularly in wet areas; called molluscicides. Examples: Metaldehyde, Copper sulfate. So, pesticides are divided into various categories to achieve effective pest control and improved agricultural productivity [23].

1.2.3 Classification of pesticides based on chemical composition

A pesticide **classification** system that groups **pesticides** by virtue of chemical structure, chemical composition and molecular structure. The physical, chemical and toxicological characteristics vary from one chemical group to another. The classification is important because the effectiveness, persistence, toxicity and mode of action of pesticides are primarily dependent on their chemical nature [24]. The main groups of pesticides according to chemical structure are listed below.

(a) Organophosphate pesticides: Organophosphate pesticides are organic compounds that have phosphorus in their chemical composition. These pesticides work by blocking the enzyme acetylcholinesterase which is involved in the nervous systems of insects and other organisms [25]. Organophosphates are highly effective against pests and quickly break down in the environment making them popular. Examples: Fenitrothion, Malathion, Parathion, Chlorpyrifos.

Advantages: A very good insecticide, less persistent in the environment than organochlorines.

Disadvantages: Toxic to humans and animals and it can cause neurological disorders upon prolonged exposure [26].

(b) Organochlorine pesticides: Organochlorines have chlorine in their chemical makeup. Pesticides are highly persistent and tend to persist in the environment for longer periods. Many organochlorines have been banned or restricted in some countries due to their long-lasting and bio-accumulative effects. Examples: DDT, Aldrin, Endrin, Lindane. Characteristics: Long environmental persistence, high lipid solubility, bioaccumulation in living organisms [27].

(c) Carbamate pesticides: The carbamic acid is the basis of the carbamate pesticides. These pesticides have a similar mode of action but are less toxic than organophosphates in inhibiting acetylcholinesterase enzymes. Examples: Carbaryl, Carbofuran, Aldicarb.

Advantages: Effective insect control and lower environmental persistence. Disadvantages: May be toxic to some aquatic organisms and can affect the nervous system [28].

(d) Pyrethroid pesticides: Pyrethroids are synthetic chemicals that are closely related to natural pyrethrins found in chrysanthemum flowers. They were widely utilised due to the high efficacy and relatively low mammalian toxic nature and are effective on the insect nervous system. Examples: Cypermethrin, Permethrin, Deltamethrin.

Advantages: Rapid action, low mammalian toxicity. When effective, it is done at low concentrations. Disadvantages: Harmful to aquatic organisms. Some insects can become resistant to the pesticide.

(e) Neonicotinoid pesticides: Neonicotinoids are synthetic pesticides that work by disrupting the nervous system of insects by binding to nicotinic acetylcholine receptors in insects. These pesticides are usually applied as systemic insecticides. Examples: Imidacloprid, Thiamethoxam, Acetamiprid.

Advantages: Effective against sucking insects; long-lasting protection. Disadvantages: Glyphosate is not recommended for use near bees and other pollinators, and it has persistent environmental issues.

(f) Biopesticides: These are natural products derived from plants, microorganisms or other natural sources. They are believed to be safe for the environment and can be used as alternatives to synthetic pesticides. Examples: Neem oil, *Bacillus thuringiensis* (Bt), Pyrethrin.

Advantages: Eco-friendly, biodegradable, low toxicity. Disadvantages: Shorter shelf life, Slower action [29].

1.3 Other minor classes of pesticides

Besides the major classes of pesticides (insecticides, herbicides and fungicides), several minor classes are used for specific applications in agriculture, public health, forestry and environmental management. These are normally used to control special pests and unwanted organisms that do not fall within the major pesticide categories [30]. Several types of pesticides are briefly discussed below. Acaricides are pesticides that kill mites and ticks that injure crops, animals and stored products. Mites are minute animals that feed on plant tissues and decrease plant productivity. Examples: Dicofol, Sulfur compounds. Algicides are chemicals that suppress the growth of algae in water systems such as lakes, ponds, reservoirs and irrigation systems [31]. Increased algal growth can degrade water quality and reduce oxygen levels. Examples: Copper sulfate, Simazine, Avicides. Avicides are chemicals to kill undesirable birds that harm crops and stored grain. These are usually applied in farmland and in airports. Examples: 4-Aminopyridine Molluscicides. Molluscicides are pesticides that kill snails and slugs which cause damage to crops particularly in wet agricultural areas. Examples: Metaldehyde, Copper sulfate, Piscicides. Chemicals used to kill unwanted fish species in

ponds, lakes, fish farms to control unwanted species are called piscicides. Examples: Rotenone, Larvicides, Chemicals used to kill insect larvae before they become adult insects. They are often employed for mosquito control. Examples: Temephos, Silvicides. Chemicals used to manage unwanted trees and woody vegetation in forests and industrial areas are called silvicides. Examples: Sodium chlorate. These are minor classes of pesticides that are important in special pest management programs and environmental control practices [32].

1.3.1 Classification according to sources of origin

Pesticides can also be classified by their source. Pesticides can be broadly classified as natural pesticides and synthetic pesticides depending on their source. This classification is used to assess the product's environmental impact, biodegradability, toxicity and production methods.

Natural pesticides: Natural pesticides (also called biopesticides) are being created by the natural resources like plants, microorganisms, minerals and animals. These pesticides are considered environmentally friendly, biodegradable and less toxic than synthetic pesticides [33]. Natural pesticides are derived from plant sources such as extracts and essential oils. Examples: Neem oil, Pyrethrum, Nicotine, Microbial pesticides produced by microorganisms such as bacteria, fungi and viruses. Examples: *Bacillus thuringiensis* (Bt), *Trichoderma* species [34]. Mineral-based pesticides are made from natural minerals. Examples: Sulfur Copper compounds. Natural pesticides have some benefits as they are eco-friendly, biodegradable, safe to use on humans and animals. Reduced environmental pollution. Possible negative effects of natural pesticides include slower action, short shelf life, a greater number of defects and less stability in environmental conditions.

Synthetic pesticides are chemically manufactured compounds (industrial or laboratory) used in pest control [35]. These pesticides are a popular choice because they quickly and effectively manage pests. Examples: DDT, Chlorpyrifos, Fenitrothion, Carbaryl. The benefits of

chemical pesticides. Pros of synthetic pesticides: Highly effective, Fast action, Long-lasting effect, Easy large-scale production. Disadvantages of Synthetic Pesticides: environmental persistence, toxicity to humans and animals, bioaccumulation in the food chain, development of pesticide resistance [36].

Classification based on the source of origin is shown in Fig 1.1.

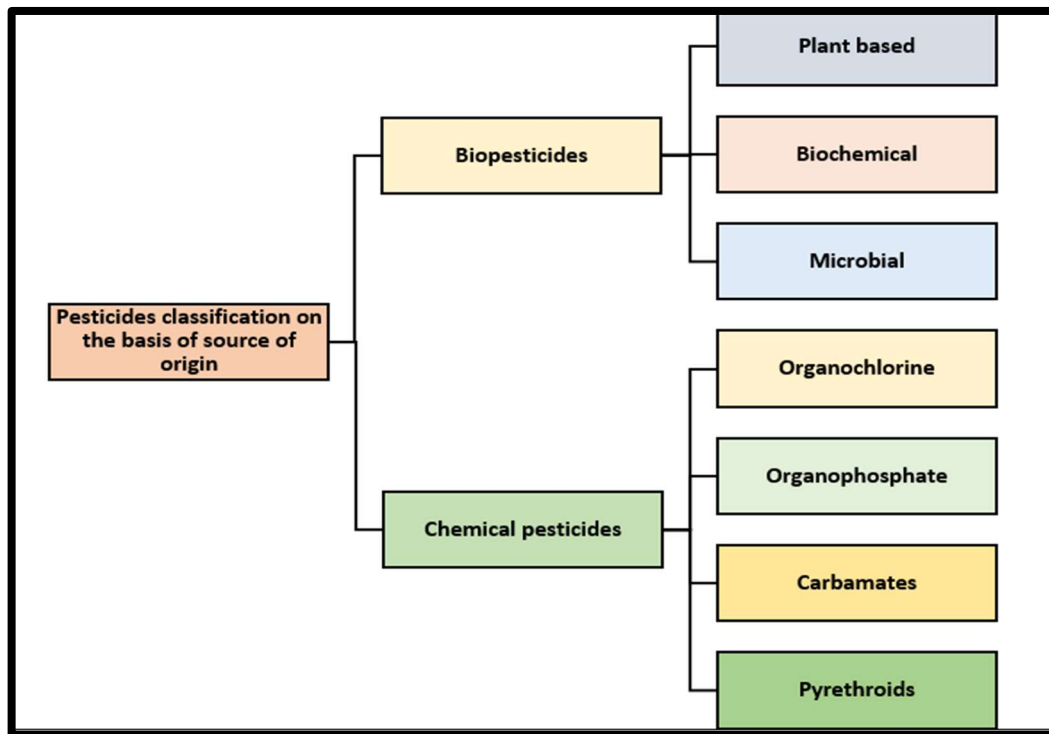


Fig 1.1: Classification based on the source of origin

1.3.2 Classification based on toxicity

There are four levels of pesticide toxicity classifications based on the detrimental effects that pesticides have on humans, animals and other living organisms. Toxicity is the toxic properties of a pesticide that can cause injury, illness or death upon exposure. The toxicity of pesticides varies depending on the chemical structure, amount, exposure duration and entry route to the body [37]. Pesticides can be assigned to toxicity

categories by regulatory bodies such as the World Health Organisation (WHO) based on their acute toxicity usually measured as LD50 values. LD50 (Lethal Dose 50) dose of pesticide is necessary to destroy the 50% of the experimental animals in controlled conditions [38]. The lower the LD50, the more toxic the product. Pesticides may be inhaled, ingested or absorbed through the skin. Careful handling, storage and application of highly poisonous pesticides will help to reduce the risks. A major classification of pesticides based on toxicity is discussed below. The highly hazardous pesticides are known as **Extremely Toxic Pesticides (ETPs)**. These pesticides can be very toxic, even at low levels [39]. Even exposure to low levels of high concentrations can lead to severe poisoning, nervous system injury, respiratory failure or death. Characteristics: very low LD50 values, immediate toxic effects, and must use serious precautions. Examples: Parathion, Aldicarb. Signal Word: "Danger" or "Poison".

Highly toxic pesticides: Serious health effects can occur from short-term exposure to highly toxic pesticides [40]. Good safety equipment is required for handling and application. Characteristics: High acute toxicity, harmful through inhalation and skin contact. Examples: Monocrotophos, Endosulfan. Signal Word: "Warning".

Moderately toxic pesticides: These pesticides are less dangerous than highly toxic pesticides, but may be harmful if not handled properly [41]. Characteristics: Moderate poisoning risk, safer when used according to recommended guidelines. Examples: Carbaryl, Malathion. Signal Word: Caution.

Slightly Toxic Pesticides: These pesticides are relatively low in toxicity and may be safer than other chemical pesticides. Exposure may also have adverse effects on human health and the environment, particularly with prolonged exposure. Characteristics: Low acute toxicity, Lower risk of poisoning. Examples: Pyrethrin-based pesticides, some biopesticides. Signal Word: Caution or no signal word.

Non-toxic or Practically Non-toxic Pesticides: These

pesticides are extremely low in toxicity and safe for use when used as intended. Characteristics: minimal harmful effects, environmentally safer alternatives. Examples: Neem-based pesticides, biological control agents [42]. Toxicological classification of pesticides is presented in the following chart diagram (Fig 1.2). Classification of pesticides recommended by WHO is presented in Table 1.1.

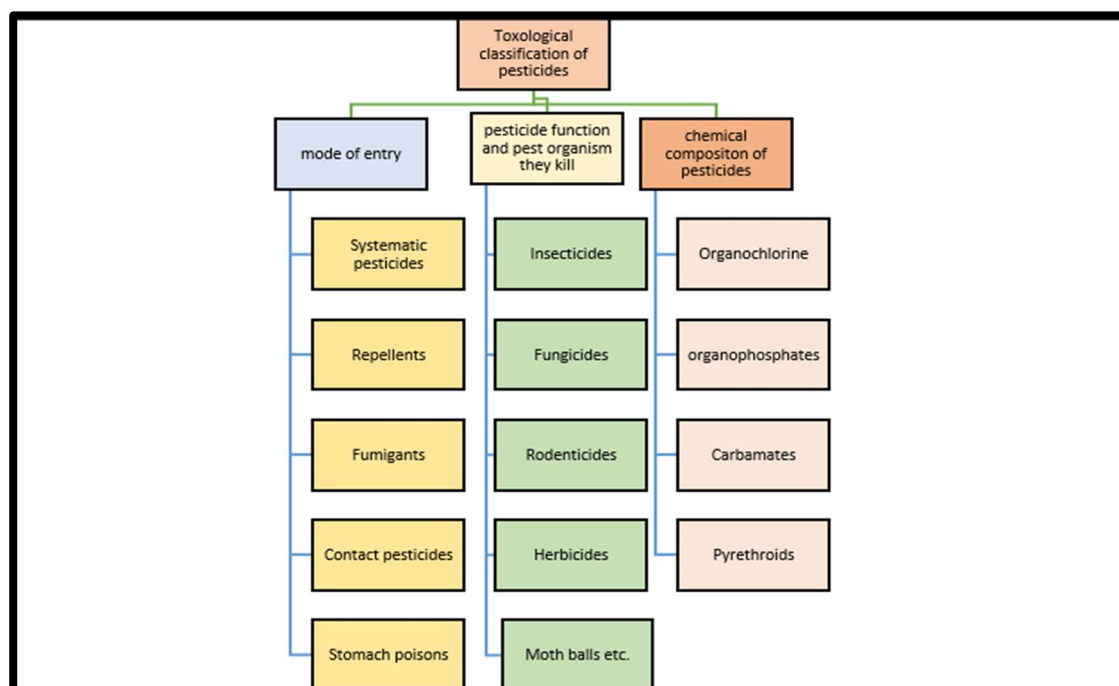


Fig 1.2: Toxicological classification of pesticides

TABLE 1.1: Classification of pesticides recommended by WHO

Classification as per WHO	LD ₅₀ (mg/kg) for rats body weight)		Example
	Oral	Skin	
Extremely hazardous (class Ia)	Less than 5	Less than 50	Parathion
Highly hazardous (class Ib)	5 to 50	50 to 200	Dichlorvos
Moderately hazardous (class ii)	50 to 2000	200 to 2000	DDT
Slightly hazardous (class iii)	more than 2000	More than 2000	Malathion
Unlikely to present acute hazard (class iv)	5000 or more	5000 or more	Cycloprothrin

1.4 Advantages of pesticides

(a) Pesticides are a significant part of modern agriculture and public health management: They are well-known and commonly used as tools to manage harmful pests, insects, weeds fungi and other organisms that negatively impact crop productivity and quality. The proper use of pesticides contributes to enhanced agricultural production and safer stored food materials from damage. Those advantages have led to the use of pesticides as an integral part of modern farming [43]. Some of the key benefits of pesticides are outlined here:

- The rise in agricultural productivity
- Pesticides are useful for controlling harmful insects, weeds, fungi and diseases, thereby increasing crop yield and productivity and farmers can feed their expanding population by minimizing crop losses [44].

(b) Safeguarding crops against pests and diseases: Cultivation and storage of crops can be severely affected by various pests and plant diseases. These harmful organisms can be controlled through pesticide applications, thereby protecting crops throughout the growing season and reduction in post-harvest losses [45].

(c) Insects, rodents and fungi may attack stored grains and food products after harvest: Pesticides are used to minimize losses after harvest by keeping harvested agricultural commodities safe from spoilage and contamination.

(d) Food quality improvement: Pesticides prevent pests and diseases and promote quality and healthy plant products. Improved crop quality results in higher market value and acceptance, and farmers' economic benefits [46].

(e) Pesticide use enhances crop production and minimises financial losses from pest attack: Farmers have higher income and greater economic stability due to improved crop yields and quality [47].

(f) Disease control/Vector-borne diseases: Some pesticides are applied in public health programs to control disease vectors like mosquitoes, flies and rodents. This will help curb the transmission of diseases such as malaria, dengue and plague [48].

(g) Use of agricultural resources efficiently: Pesticides enable farmers to manage their land, water, fertilizers and labour resources efficiently and reduce crop damage.

(h) Saves time and enables action quickly: Chemical pesticides are typically effective in rapidly controlling undesirable organisms and pests. This assists in managing larger agricultural fields in a short time.

(i) Support for large-scale farming: Pesticides are essential tools in modern intensive agriculture for controlling pests and ensuring high crop productivity in extensive cultivation areas.

(j) Food security contribution: Food security is important in ensuring the supply of foodstuffs to the population in adequate quantity but pesticides also contribute to food security by ensuring that the food production is not lost due to pests and diseases [49].

1.5 Effects of pesticides

While pesticides are beneficial in agriculture and public health, their overuse and improper application can have detrimental effects on people, animals, plants and the environment. Pesticides are chemicals that are toxic to harmful organisms and can also harm non-target organisms affecting the ecological balance. Pesticides regularly added to the environment can cause significant environmental contamination and health issues [50].

The negative effects of pesticides are mentioned below:

(a) Human health effects: When pesticides are sprayed, they may contact skin and cause contact with the skin during handling and they can be inhaled or ingested by people during spraying or when eating contaminated food and water. Pesticides can cause a range of health issues, both short and long-term. Acute health effects exposure to pesticides can cause: skin irritation, eye irritation, headache, nausea and vomiting, dizziness, respiratory problems. Chronic health effects exposure over a long period of time can result in neurological disorders, hormonal imbalance, liver and kidney damage, reproductive disorders, cancer, weak immune system. Organophosphate pesticides act on the nervous system, blocking the activity of acetylcholinesterase enzymes [51]. Acute effects of pesticides on human health is presented in Fig. 1.3.

(b) Environmental effects: When pesticides are found in soil, water and air, their impact is significant to the environment. Soil contamination: Overuse of pesticides can lead to loss of soil fertility and kill soil microorganisms involved in nutrient cycling. Water pollution: Agricultural runoff and leaching can contaminate drinking water and aquatic ecosystems by adding pesticides to rivers, lakes and groundwater [52].

(c) Air pollution: When spraying is carried out, pesticides may be released to the air and inhaled by nearby living organisms. This will directly affect water organisms. This will directly impact aquatic organisms. Fish, algae and aquatic plants are very sensitive to pesticides. Pesticides that enter water bodies can cause fish mortality and reduced oxygen levels when the water balance of the aquatic habitat is disrupted. Interruption of aquatic food chains causes the harmful impact on aquatic life [53].

(d) Impacts on beneficial organisms: Pesticides not only kill bad pests but can kill beneficial organisms as well including bees, butterflies, earthworms and pesticide-resistant varieties of

plants. Pollinator declines negatively impact pollinator-dependent crops and agricultural productivity [54].

(e) Bioaccumulation and biomagnification: Some pesticides, particularly organochlorine pesticides, bioaccumulate in living organisms. This is called bioaccumulation. These chemicals are biomagnified as they pass through the food chain to high trophic levels. This can lead to high toxicity in animals, birds and humans who eat contaminated food [55].

(f) Pesticide resistance: A regular and repeated utilization of the same pesticides may impart the development of resistance in pests. Pesticide resistance occurs when pests survive spraying and grow back into a problem. This results in increased pesticide use, higher production costs, and reduced pesticide effectiveness [56].

(g) Misuse of pesticides can also result in loss of crop and non-target plants due to leaf burn, reduced growth, delayed flowering and decreased crop productivity.

(h) Ecological imbalance: Pesticides have a negative impact on natural ecosystems through the disruption of a range of organisms in the food chain. The destruction of beneficial insects and microorganisms may disrupt the ecological balance and biodiversity.

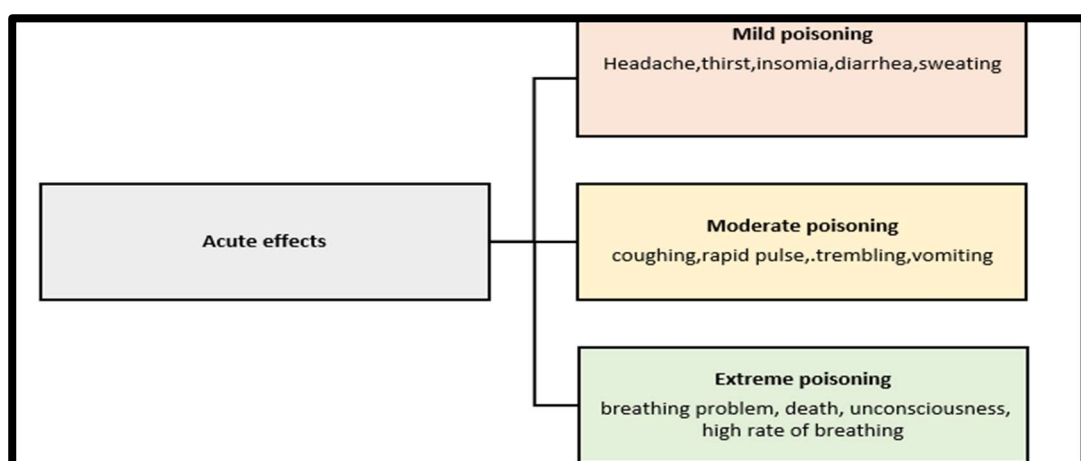


Fig 1.3 Effects of pesticides on human health

1.6 Chronic diseases caused by pesticides

It is noticed that a long-term exposure to pesticides can result to the several chronic diseases and serious health complications. Continuous exposure to low concentrations of harmful chemicals through food, water, air or occupational activities may gradually affect human health. Individuals such as farmers, agricultural workers, pesticide manufacturers and people living near agricultural areas are at a higher risk of long-term pesticide exposure [57]. Pesticides can accumulate in body tissues and negatively affect various organs and biological systems by disturbing hormones, enzymes and normal cellular functions.

One of the major health problems associated with pesticides is cancer, as many pesticides are considered carcinogenic and may increase the risk of cancers such as lymphoma, brain tumors, lung cancer, cervical cancer and prostate cancer. Long-term pesticide exposure can cause damage to the nervous system by disrupting neurotransmission, which is associated with various neuro diseases like Alzheimer's disease, Parkinson's disease, loss of memory, anxiety, decreased cognitive functioning, and behavioural abnormalities. Continuous exposure to pesticides through inhalation can cause respiratory issues, including asthma, bronchitis, airway irritation and breathing problems, especially among agricultural workers [58]. Endocrine disruption caused by certain pesticides may lead to hormonal imbalances, thyroid dysfunction, diabetes and metabolic disorders. Pesticides can also affect one's reproductive health in terms of infertility, low sperm count, deformities and poor fetal development. Moreover, pesticides can harm the body's detoxifying system by damaging the liver and kidneys, thus resulting in their inflammation and malfunctioning as well as toxic hepatitis. Pesticides may also impair the immune system making individuals more prone to infections, allergic reactions and autoimmunity. Additionally, exposure to pesticides might affect cardiovascular health by raising blood pressure inducing heart disease and producing

arrhythmias. A schematic representation of chronic diseases caused by pesticides is given in Fig. 1.4.

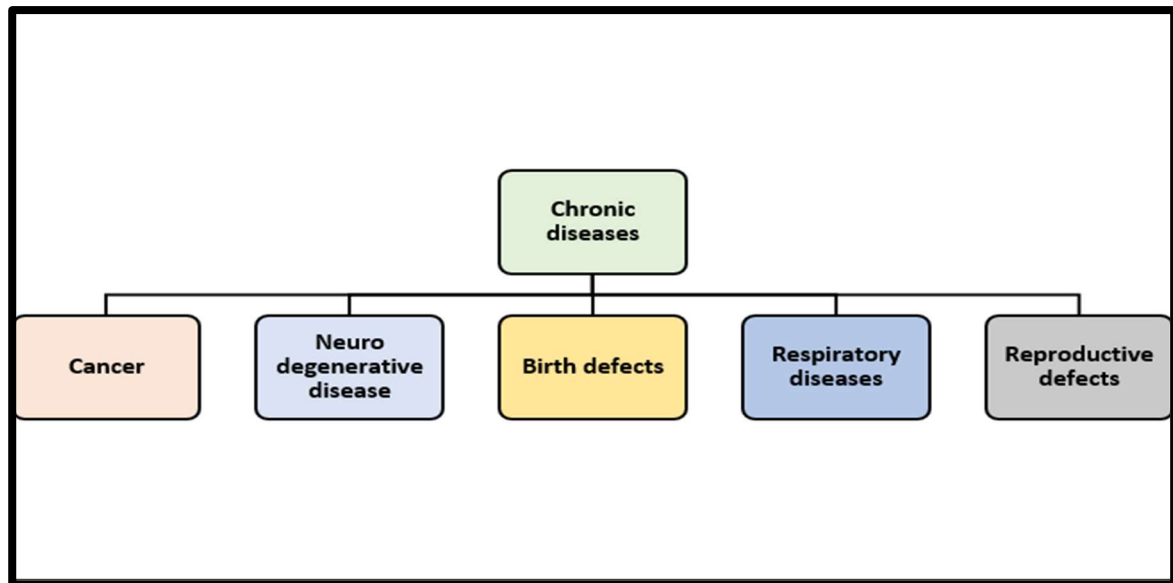


Fig 1.4: Chronic diseases caused by pesticides

1.7 Methods of pesticides detection

Excessive use of pesticides in agricultural fields contaminates food, water, soil and the environment. Thus, detecting pesticides is crucial to safeguard human health and the environment. Methods used in pesticide detection must be sensitive, selective, accurate, fast and cost-effective. GC, HPLC and MS are common analytical techniques that yield accurate and reliable results. Nevertheless, these techniques require sophisticated equipment, trained personnel and complex sample preparation procedures. This problem has been addressed through the introduction of new advanced techniques of pesticide detection. For instance, biosensors that rely on biological interactions are frequently used for rapid, sensitive detection of pesticides. Electrochemical sensors which enable the detection of electrical signals arising from chemical reactions are favored for their portability, simplicity and ease of use. Optical sensors detect pesticides via fluorescence, color changes and light absorption whereas

nanosensors which employ nanomaterials such as graphene, metal oxides and metal nitrides, enhance sensitivity and detection capabilities [59].

1.8 Introduction of biosensors

An analytical tool which uses a transducer and biological recognition element to identify and quantify specific chemical or biological molecules is known as biosensor. Biological reactions are transformed by biosensors into quantifiable signals such as mechanical, thermal, optical or electrical signals. Since their introduction in the early 1960s, biosensors have become increasingly important in industrial applications, agriculture, food safety, environmental monitoring and medical diagnostics [60].

The three key parts of a biosensor are as follows:

1. Bioreceptor
2. Transducer
3. Signal analyzer

The biologically active substance is known as the bioreceptor. The transducer produces measurable electrical or optical signals from the biological interaction which are then amplified and displayed by the signal processing system.

The unique interaction between the analyte and the biological recognition element underlies biosensor operation. A biochemical reaction occurs when the target analyte interacts with the bioreceptor. The transducer transforms the signal produced by this interaction into quantifiable information [61]. Schematic representation of components of biosensor is shown in Fig. 1.5.

Biosensors are superior to traditional analytical methods in several ways: they are highly sensitive, provide rapid responses are adaptable, support real-time monitoring and have low operating costs. Because of these benefits, biosensors are widely utilized in:

- Monitoring glucose, pathogen detection, analysis of environmental pollutants

- Monitoring of food quality, detecting pesticide residues

Because of their ease of use, downsizing capability, low power consumption and superior analytical performance, electrochemical biosensors are among the most popular biosensors.

Recent advances in nanotechnology have significantly improved the performance of biosensors. Electron transport, catalytic activity and sensing sensitivity are all enhanced by nanomaterials such as graphene, graphitic carbon nitride (g-C₃N₄) and metal oxides as well as quantum dots.

As a result, biosensors have become potent analytical tools for the rapid and accurate detection of multiple analytes across industrial, agricultural, medical and environmental domains [62].

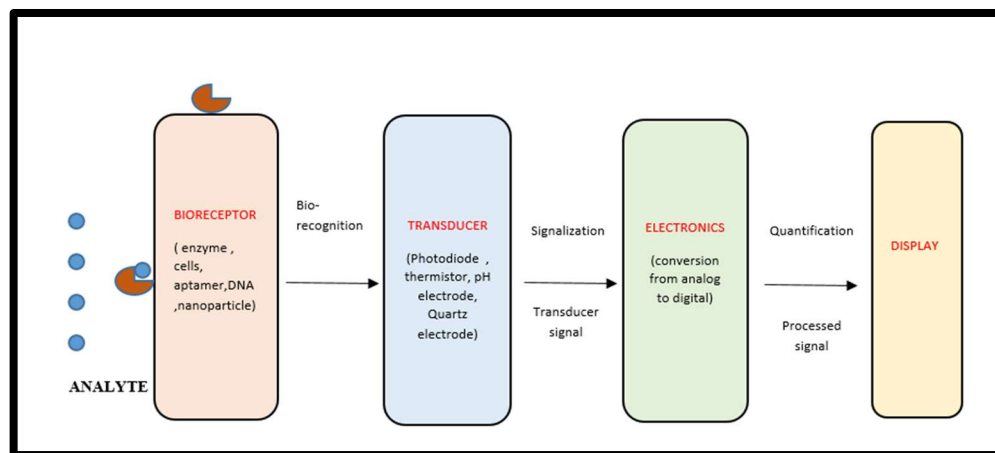


Fig.1.5 Schematic representation of components of biosensor

1.8.1 Biosensor characteristics

(1) Selectivity- It is a characteristics of biosensor which can identify and measure a specific analyte in a sample specimen that may also contain other substances such as contaminants and impurities is known as selectivity. Selectivity in biosensors is best illustrated by the interaction between an antigen and an antibody. Traditionally, antibodies are attached to the transducer surface and act as bioreceptors. After that, the transducer is exposed to a solution, usually a

buffer containing a salt and the antigen in which antibodies bind to the antigen selectively. Selectivity must be prioritized when creating biosensors and choosing bioreceptors [63].

(2) Reproducibility- Reproducibility in biosensors is their capability to produce consistent responses when experiments are performed many times. It is defined by the precision and accuracy of biosensors. Consistent signals result in the reliability and resilience of conclusions drawn from the biosensor's responses.

(3) Stability- It describes the biosensing system's sensitivity to outside disturbances in its environment, as these disturbances alter the output signal measurement and alter the suggested biosensor's accuracy and precision. A significant additional factor influencing stability is the bioreceptors' affinity. The degree of interaction between bioreceptors and the analyte is known as affinity. When affinity is high, strong electrostatic or covalent bonds form between bioreceptors and analytes.

(4) Sensitivity- In medical and environmental monitoring uses, biosensors detect a minimum amount of analyte concentration, i.e., the limit of detection or sensitivity, as low as fg/mL. It validates the existence of an analyte.

(5) Linearity –The ability of a measured response to a straight line at a different analyte concentration is known as linearity. The straight line is represented mathematically by $y = mc$. Here, m is the sensitivity, c represents the conc of analyte and y gives the output signal. Linear range, another term related to linearity is the extent of analyte concentration where biosensor responding linearly with conc [64].

Characteristics of biosensors is summarized in the pictorial view (Fig. 1.6).

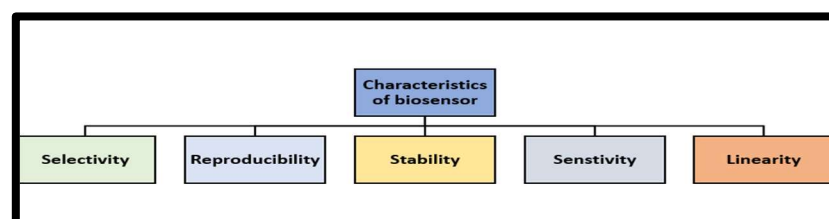


Fig. 1.6 Characteristics of biosensors

1.8.2 Biosensor's types

The classification of biosensors is based on:

1. The biological recognition,
2. The transducer type

Depending on the target analyte and sensing method, biosensors are classified in several ways.

1.8.2.1 Biosensor based on biological recognition

- **Enzyme biosensor:**

In enzyme-based biosensors, enzymes serve as biocomponents. Made up of protein biomolecules, enzymes catalyze reactions to produce signals that are measured indirectly by biosensors through the formation of enzyme-substrate complexes [65]. Enzyme biosensors are widely used due to their numerous potential applications. In enzyme biosensors, an enzyme is kept near the sensor surface, and the enzymatic reaction detects the substrate concentration. It occurs via two distinct processes: diffusion in the product enzyme layer and enzymatic conversion in the substrate. Moreover, four enzyme-based biosensors are commercially available: urea, lactate, glucose and glutamate. Enzymes are immobilized in the matrix on the transducer surface to sustain enzyme activity. The analyte concentration is detected by measuring changes in proton concentration, gas release and uptake, etc. [66]. The transducer converts these changes into measurable signals. Generally, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are used as enzymes for detecting pesticides [67].

Enzyme-based biosensors are very popular due to the following reasons-

Sensitivity-They can detect the analyte even at very low concentrations. Hence, shows high sensitivity [68].

Selectivity- They are highly selective, recognizing specific analytes in complex samples.

Rapid response - They detect the analyte in a short period of time.

- **Immunosensors**

The immunosensor uses antibodies, glycoproteins produced by the immune system, as a biocomponent. It functions by interacting with antigens and antibodies. When an antibody interacts with an antigen, a detectable signal is produced; this signal manifests as a change in conductivity, mass, or other measurable properties. Immunosensors are widely used in many potential fields including the detection of cancer biomarkers, environmental monitoring and food safety [69].

- **Nucleic acid biosensors**

Nucleic acids are utilized as bio-components in these biosensors. Such biosensors are being used to determine the nucleic acid sequences like DNA and RNA. It operates on the basis of complementary base pairing between the probe molecule (DNA or RNA) immobilized on a surface and the target nucleic acid sequence [70].

- **Microbial biosensors**

Microorganisms are utilized in microbial biosensors as biocomponents [71]. It is based on microorganisms' ability to identify different substances in their surroundings. When certain target molecules interact with these microorganisms, measurable responses, such as changes in light or electrical conductivity, occur. Due to their affordability, sensitivity and ability to detect numerous target molecules in the form of light, color, electrical signals, etc., these biosensors are more favored than chemical biosensors. These biosensors are used in several industries including the detection of contaminants in water and ensuring food safety. It has several disadvantages: appropriate biosensors for molecules and macromolecules that cannot cross the membrane cannot be created, as the cell membrane acts as a diffusion barrier. The response time of microbial biosensors and the time required to return to baseline after use are long. Among the most significant issues during immobilization are contamination and decreased activity.

• Lactate biosensors

Anaerobic metabolic activity is reflected by the amount of lactate present in biological specimens such as blood, sweat, or saliva which is measured by lactate biosensors. These biosensors usually include a biorecognition component immobilized on a transducer substrate such as lactate oxidase or lactate dehydrogenase. The recognition element and lactate initiate a biochemical reaction that results in observable changes in electrical conductivity, pH modulation or fluorescence.

1.8.2.2 Biosensor classification based on transducer

A transducer is a device for analysis that yields an output quantity that is correlated with the input quantity. Based on their transduction mechanism, biosensors are categorized into the following classes [72].

- **Amperometric biosensors** - Amperometric biosensors work by transferring electrons to an electrode surface through the action of an oxidoreductase enzyme. The majority of these biosensors work with an enzyme system that catalytically converts electrochemically inactive target analytes into the products which gets reduced or oxidized at working electrode. Also, the potential of this electrode is monitored in relation to a reference electrode. Using an amperometric transducer, an electrochemical reaction, either reduction or oxidation, can take place at electrode surface which produce a current proportional to bulk substrate conc.
- **Potentiometric biosensors** - Potentiometric biosensors measure the potential in equilibrium at the electrode [73]. They work on the principle of charge formation in the sample via the Nernst equation (Eq. 1) in relation to the analyte activity a_1 .

$$E = E_0 \pm (RT/nF) \ln a_1 \quad (1)$$

When the standard electrode potential (E_0) is 1 mol/L, then the activity(a_1) is 1. Here, T, R, F and n have their usual meanings. In potentiometric biosensors, an inert electrode (the reference electrode) and a working electrode are used to measure potential. Various analyte

concentrations are detected as **the potential** calculated **is proportional to the logarithm of the concentration** of electro-active species

- **Optical biosensors** - The oldest techniques are optical techniques which are also thought to be the most sensitive and effective. The creation of superior optical fibres intended for biosensors has been extended to the field of communication systems. Due to a number of factors including **(a)** the optical signal is not affected by any electrical or magnetic field, **(b)** the optical sensors do not require a reference signal and **(c)** they are simple to fabricate with high efficiency hence optical fibre biosensors are advantageous in biochemical and clinical analysis [74].
- **Piezoelectric biosensors**- These transducers are thought to be exceptionally sensitive for use in biosensor applications. These biosensors' fundamental idea is based on the binding of a molecular species to the surface of the crystal, causing a mass change that ultimately causes the crystal's oscillation frequency to change [75]. It can also measure changes in viscosity and density on the sensor surface. The piezoelectric crystal is coated with high-selectivity compounds containing biological materials such as enzymes or antibodies to harness the piezoelectric effect. Practical applications of immunology and immunodetection use piezoelectric transducers. Real-time tracking, tag-free identification and ease of use are among the benefits of using this type of converter.
- **Thermal biosensor**- It functions by measuring the amount of heat that is absorbed during a biological reaction [76]. A physical transducer and biomaterial (a calorimetric biosensor) are combined to form it. It is utilized for clinical detection, environmental control and the determination of antigen-antibody interaction (thermometric ELISA test). However, one drawback of the method is the high cost of the instruments.

Schematic representation of types of biosensors is shown in Fig. 1.7.

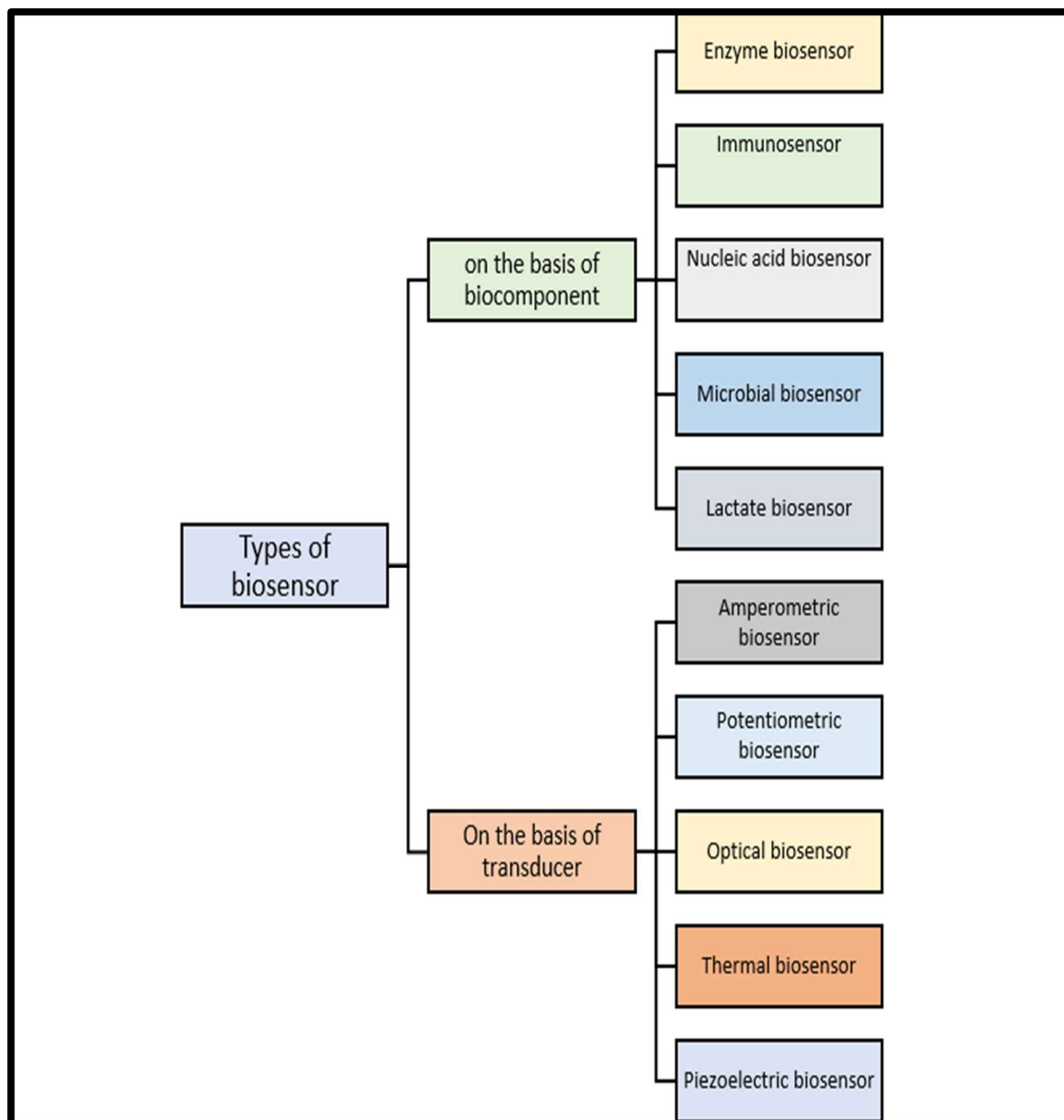


Fig. 1.7 Schematic representation of types of biosensors

1.9 Immobilization of biomolecules

The method of attaching biomolecules to solid supports so they can stay active for extended periods of time is known as immobilization [76]. Matrix refers to the stable solid surface to which an enzyme can bind. The immobilization process is determined by the matrix's mechanical and chemical properties. As a matrix, materials such as glass, screen-printed electrodes, conducting and non-conductive polymer films, etc. Immobilization improves stability by limiting the mobility of biomolecules and fixing them in a specific location. When selecting an immobilization technique, it should be ensured that a reactive group of enzyme's binding site remains unchanged and that no enzyme activity is lost. Physical entrapment, physical immobilization, covalent binding and cross-linking are frequently employed immobilization techniques [77].

1.10 Methods of immobilization

• Cross-linking

In the cross-linking technique, compounds having two or more functional groups that can bind two different materials under distinct conditions are used [78]. Compounds with two or more functional groups, like 4-azido-1-fluoro-2-nitrobenzene that can bind two different materials under distinct circumstances are used in the cross-linking technique. Bifunctional and multifunctional reagents are generally used. Glutaraldehyde is most commonly used.

Advantages

1. Cost-effective
2. Less loss of biocatalyst

Disadvantages

1. Chemical reagents can alter enzymes and biomolecules' active sites.
2. It requires specific conditions, so it is not suitable for every biomolecule and enzyme.

- **Physical adsorption**

Using this technique, a biomolecule is physisorbed onto a matrix surface. As a result, the technique does not modify the enzyme's conformation or cause only a slight change. In this technique, hydrogen bonding and van der Waals forces are required to bind the biomolecule thereby altering factors such as temperature and ionic strength.

Advantages

1. No alteration of the biocatalyst
2. Regeneration of the matrix is possible

Disadvantages

1. Due to the weak binding forces, there is a significant chance that the adsorbed molecules will separate from the support.
2. The loading capacity of adsorbed molecules is lower than that of other immobilization techniques.

- **Entrapment**

The enzyme layer constrains the biomolecules in the matrix, allowing the intended biomolecules to remain within it. The mixture is allowed to gel or polymerize on the sensor surface after combining the polymer solution or gel precursor (polyacrylamide) with the biorecognition element. Because the entrapment conditions are harsh and lead to inactivity, caution must be used when selecting them.

Advantages

1. This method preserves enzyme activity.
2. Suitable for different types of matrices

Disadvantages

1. Large barrier to diffusion
2. Significantly large response time.

- **Covalent binding**

In this technique, the biomolecule and the support matrix form a covalent bond. Most situations use matrix functional groups that can either be activated or covalently coupled to produce such groups. Typically, the nucleophilic functional groups like amino, carboxylic, indole, etc. found in the side chains of amino acids of proteins, are employed for covalent coupling. The conditions employed are harsher and more complex than those used with other techniques because they shift the active centre, leading to a significant decrease in substrate activity.

Advantages

1. There is no enzyme loss because of the strong binding force between the enzyme and carrier.
2. Steady even in challenging conditions

Disadvantages

1. Regeneration of the matrix is not possible
2. Change in conformation of substrate

1.11 Applications of biosensors

- **In the medical field:** Biosensors are extensively employed in the medical sector. A glucose biosensor is a well-known example for the diagnosis of diabetes. Infectious disease diagnosis is another application for them [79]. They accurately detect biomarkers at low concentrations. It is frequently used to identify biomarkers and detect cancer. They are able to recognize dangerous pathogens as well as other biological elements like enzymes and antibodies. These days, biosensors are built into mobile units and can detect cardiac troponin, a protein complex that senses cardiac injury and can indicate acute coronary syndrome. These biosensors leverage concepts such as machine learning, disk-based readers and fluorescent microfluidics.

COVID-19 was identified in the present circumstances using biosensors that were made of nanomaterials.

- **In food authenticity and processing:** Foodborne pathogen detection is accomplished by means of biosensors. Biosensors are used to measure glucose and lactate levels in foods such as milk and yoghurt [80]. Biosensors detect lipids, alcohol, cholesterol, glutamate, glucose and lactate. They also measure and identify obesity, a disease-causing condition. Biosensors are used to assess chemical constituents, evaluate residual levels of agricultural drugs, assess food quality and monitor factors such as freshness and aroma.
- **For military defence purposes:** Biosensors identify threat-causing organisms (biowarfare agents), primarily bacteria and viruses, aiding in the detection of biological attacks. Since diseases are spreading and bioterrorism is on the rise, it is critical to identify and prevent both because they have the potential to wreak massive havoc. Biosensors look for these biowarfare agents, such as viruses, fungi and bacteria.
- **In environmental protection** - It finds the causes of soil disease as well as the concentrations of pesticides, fertilizers and heavy metals in the soil and water [81]. Biosensors detect harmful algal blooms, algal RNA and halogenated compounds in water bodies. They help monitor pollution by detecting concentrations of harmful pollutants.
- **Nanotechnology** - By leveraging the unique characteristics of nanomaterials, biosensors that employ nanotechnology can greatly improve their sensitivity, specificity and overall performance when it comes to identifying biological materials. A **high surface area-to-volume ratio of nanomaterials improve the** interaction between sensors **and** target molecules. It reduces the time needed to analyze small molecules with intricate structures. Its selectivity increases as the value found increases and the surface area is proportional to the volume. There is no diffusion issue and very little energy is needed for the research. Thus, the biosensors are guaranteed a long life. It guarantees the study's completion without endangering the cells.

1.12 Future scope of biosensors

The biomarker approaches of the future will investigate the development of several options for producing precision diagnostics, medications and equipment [82]. Implantable biosensors have the potential to greatly speed up the manufacturing of tailored drugs. It will enable researchers to precisely monitor how potential drugs interact with the body and assess whether they could advance medicine. Moreover, biomolecular chip might be implanted in the body to detect subtle blood DNA alterations before the onset of illness during the initial growth phases. Biosensor technology can be applied to low-cost, reversible care point equipment. It's also applicable for ongoing implanted equipment tracking. Numerous sensors that use nucleic acid hybridization detection are making exciting progress in this field. One such sensor is the "Environmental Sample Processor," which is being established at the Monterey Bay Aquarium Research Institute. Its objective is to automate the in-situ detection of toxic algae from moorings using ribosomal RNA probes. One of the main aims is also the monitoring of pollutants, toxic metals and pesticides.

The demand for biosensors is expanding due to their widespread application in medical and healthcare procedures. Furthermore, biosensors have advanced in several fields including diagnosis, disease identification, patient wellness monitoring and human health management.

1.13 2D-based nanocomposite

Progress in materials science has been driven by the formation of nanocomposites. Nanocomposites are special materials made up of combining two or more particles of nano-size [83]. They have wide, unique and physical and chemical properties due to the combination of their constituent materials' properties [84]. They are used in wide applications. From the perspective of their unique designs and the combination of properties not found in traditional composites, they are said to be the materials of the twenty-first century [85]. 2-D

materials are composed of thin layers held together by van der Waals forces [60]. The layers are a few nm thick. Electrons easily move in the layers of 2-D materials. Materials such as g-C₃N₄, graphene, hexagonal boron nitride and transition-metal dichalcogenides are examples of 2-D materials [86]. They show excellent mechanical strength, electrical conductivity, rigidity and flexibility. Various 2D nanomaterials being used in the fabrication of biosensors are presented in Fig. 1.7.

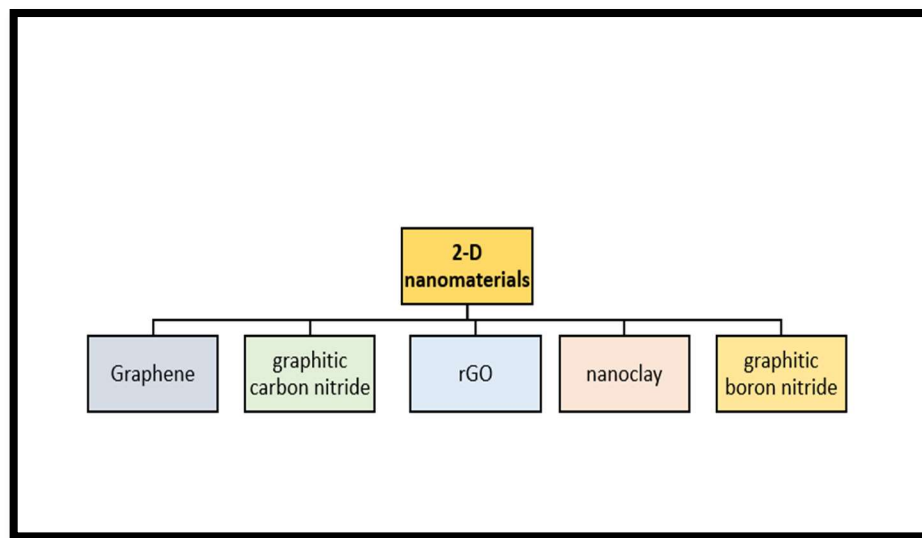


Fig.1.8 Numerous 2D nanomaterials

1.13.1 Properties of 2D nanocomposite

They possess unique properties, such as

1. **Improved mechanical property-** They show improved strength and toughness when compared to other conventional materials due to their large surface area of the nanocomposite which reinforced in to the matrix [87].
2. **Enhanced thermal stability-** By adding nanoparticles, composite materials can become more thermally stable and resistant to heat-related damage. This feature is especially handy for uses that involve high temperatures [88].

3. **Improved electrical conductivity-** Nanocomposites can have either improved electrical conductivity or insulation properties depending on the kind of nanoparticles used and how dispersed they are within the matrix. Therefore, they can be used in a variety of electronic applications [89].
4. **Enhanced optical properties-** Nanocomposites can exhibit special optical properties such as increased transparency, fluorescence or light scattering depending on the nanoparticles mixed into the matrix [90].
5. **Barrier properties-** Because of the high surface area and fragility of the areas within the matrix that are filled with nanoparticles, nanocomposites can offer better protection against gases, moisture and other environmental variables.

1.13.2 Applications of 2D nanocomposite

1. **Structural materials:** Metals, ceramics and polymers are materials whose mechanical qualities can be improved using nanocomposites. Integrating nanoparticles increases the durability, rigidity and strength of these materials, making them appropriate for use as building materials, automobile parts and aerospace components [91].
2. **Packaging:** By strengthening the barrier qualities of food packaging and preventing oxygen, moisture and other impurities from passing through the material, nanocomposites help prolong the shelf life of perishable items [92].
3. **Electronics:** To create conductive substances with better electrical and thermal conductivity, nanocomposites are used.
4. **Energy conversion and storage:** Batteries and supercapacitors are two examples of devices that extensively use nanocomposites for energy storage.

CHAPTER 2

Literature Review

2.1 Introduction to organophosphate pesticides

Organophosphate pesticides are widely used class of pesticides in modern agriculture. These pesticides contain phosphorus in their chemical structure and are mainly used to control insects and agricultural pests. Organophosphate compounds were initially developed as chemical warfare agents but later became widely used in agricultural and public health applications due to their high insecticidal activity [93].

Common organophosphate pesticides include: Fenitrothion, Malathion, Chlorpyrifos, Parathion, and Diazinon. Organophosphate pesticides are highly effective and their excessive exposure can affect humans by causing paralysis and death in insects and animals [94].

Long-term exposure may cause neurological disorders, respiratory problems, hormonal imbalance and cancer. Environmental pollution and agricultural runoff may also occur and improper handling of these pesticides can contaminate soil, water, food products and air. Hence, monitoring and detection of organophosphate pesticide residues are necessary for environmental safety and public health protection. Organophosphate pesticide detection is crucial due to their widespread use and importance. General use of organophosphate pesticides has led to concerns about environmental contamination and human health risks. All pesticides, even in trace amounts, can be harmful if in food or water. Thus, rapid and accurate diagnosis of organophosphate pesticides is of great importance [95]. Importance of detection:

(a) Food safety: Pesticide residues can end up in fruits and vegetables, milk and grains and be consumed by humans. Monitoring for pesticide levels is essential to ensure food safety and quality.

(b) Environmental protection: Organophosphate pesticides pollute soil, groundwater, rivers and lakes. Continuous monitoring is essential to avoid damage to the ecology and water pollution.

(c) Human health protection: Long-term exposure to organophosphate pesticides may cause severe health disorders such as nervous system damage, respiratory diseases, reproductive disorders, cancer. Early detection will minimize exposure risks and safeguard public health.

(d) Agricultural monitoring: Detection methods are useful for the farmer to use in order to make the most of pesticide use and avoid over-application.

(e) Regulatory compliance: Maximum residue limits (MRLs) are set by regulatory agencies for pesticides in environmental and food samples. It is crucial to have accurate detection methods to ensure compliance with safety regulations.

Need for advanced detection techniques:

Unless otherwise stated, the following are conventional analytical tools: Gas chromatography (GC), high-performance liquid chromatography (HPLC) and mass spectrometry (MS). They have very high accuracy but have several drawbacks: expensive instrumentation, time-consuming analysis, complex sample preparation and the need for specialized operators. Electrochemical biosensors are proposed as alternatives to overcome these limitations, offering high sensitivity, rapid response, low cost, portability and real-time monitoring. So, research on the development of efficient organophosphate pesticide biosensors has become an important field [96].

2.2 Effects of organophosphate pesticides

The detection of organophosphorus pesticides is necessary for the following reasons:

1. Posing a threat to the environment- Organophosphate pesticides pose a threat to the environment as they are intentionally toxic to non-target organisms like mammals, birds, aquatic animals, etc.

2. Adverse effects on humans, plants, and ecosystems - It can negatively impact humans, plants and animals even at minimal concentrations. OPPs typically enter the body by ingestion, inhalation, cutaneous absorption and injection. Contamination to OPPs might occur in workers manufacturing pesticides, floriculturists, pesticide applicators and farmworkers. Prolonged exposure to OPPs is associated with a range of issues, including memory and verbal difficulties, visual-spatial performance, coordination, etc. Moreover, OPPs are observed to affect the health of humans during embryonic as well as adult stage which raise morbidity and mortality cases in patients with chronic poisoning. The presence of residues in agricultural products can cause chronic toxicity to human health.

3. Causes harmful diseases in the human body - Some studies reveal the connection between OPPs and diseases such as leukemia and lymphoma. Though many reports have shown that OPPs such as chlorpyrifos and diazinon increased the probability of Parkinson's disease. Furthermore, OPPs have a negative impact on the human reproductive system and reduce male fertility. They also cause effects that are non-neurological like cardiac arrest, infertility, chronic disruption, etc. [89]. Sex hormones, such as testosterone, are reduced due to the presence of OPP metabolites in the body. Thus, it is clear that quantitatively determining OPPs **has a significant impact on** the ecosystem. **Therefore, it is necessary to** design simple, rapid **and** inexpensive techniques for **the** detection of organophosphate pesticides, given the need to consider environmental health, toxicity and safety.

Recent developments in analytical capability **of electrochemical biosensors for the** determination **of fenitrothion are** reported **in** the following **Table 2.1**.

Recent Advances in the Analytical Capability of Electrochemical Biosensors for Fenitrothion Detection

Year	Biosensor / Electrode Material	Biorecognition Element	Detection Technique	Linear Range (μM)	Limit of Detection (LOD)	Major Analytical Advancement
2020	AuNPs/Graphene modified electrode	AChE	DPV	0.01 – 100	3.5 nM	Enhanced conductivity and fast electron transfer due to graphene–AuNP hybrid
2020	CNT-based biosensor	AChE	CV / DPV	0.05 – 50	10 nM	Improved enzyme immobilization and sensitivity
2021	g-C ₃ N ₄ nanosheet modified electrode	AChE	DPV	0.001 – 80	0.5 nM	High catalytic activity and larger active surface area
2021	ZnO nanorod/Au electrode	OPH	Amperometry	0.01 – 60	2.0 nM	Rapid response and improved stability
2022	MXene nanocomposite electrode	AChE	EIS / DPV	0.0005 – 100	0.2 nM	Superior conductivity and anti-fouling characteristics
2022	NiS/g-C ₃ N ₄ hybrid biosensor	AChE	DPV	0.001 – 120	0.12 nM	Synergistic electrocatalytic performance
2023	Zn–NiS decorated g-C ₃ N ₄ /ITO electrode	AChE	DPV	0.0001 – 150	0.05 nM	Excellent repeatability and ultra-high sensitivity
2023	rGO/PtNPs modified electrode	AChE	SWV	0.001 – 90	0.09 nM	Fast response and high selectivity
2024	MOF-derived porous carbon electrode	AChE	DPV	0.00005 – 200	0.02 nM	Ultra-high surface area and enhanced charge transfer
2025	MXene/g-C ₃ N ₄ hybrid biosensor	AChE	EIS / DPV	0.00001 – 250	0.008 nM	Next-generation ultrasensitive sensing platform

Abbreviations: AChE – Acetylcholinesterase; OPH – Organophosphorus Hydrolase; DPV – Differential Pulse Voltammetry; CV – Cyclic Voltammetry; SWV – Square Wave Voltammetry; EIS – Electrochemical Impedance Spectroscopy; g-C₃N₄ – Graphitic Carbon Nitride; AuNPs – Gold Nanoparticles; CNT – Carbon Nanotubes; MXene – Two-dimensional transition metal carbides/nitrides.

2.3 Literature review

There are many analytical and electrochemical techniques for detecting organophosphate pesticides. The most common ones are GC, HPLC and spectrophotometry. These methods are quite accurate but rather costly and complicated. Currently, worldwide interest is increasing in the electrochemical biosensors field owing to their rapidness, simplicity and cost-effectiveness. Nanomaterials have been incorporated into biosensor design to enhance sensitivity, conductivity and catalytic activity [97]. Graphitic carbon nitride (g-C₃N₄) is one of the promising 2D materials due to its high specific surface area, stability and catalytic activity. Research shows that g-C₃N₄ enhances electron transfer and improves the efficiency of electrochemical sensors. Another important group of materials considered recently is transition metal oxides including nickel oxide, zinc oxide and molybdenum oxide, due to their:

- High conductivity
- Electrochemical activity
- Excellent catalytic properties

It was found that combining g-C₃N₄ with a metal oxide enables the creation of novel, efficient sensing devices.

Numerous studies indicate that:

- g-C₃N₄/metal oxide composites
- nanostructured electrochemical electrodes are successfully applied in the analysis of pesticides.

The use of graphitic carbon nitride-decorated zinc–nickel oxide nanocomposites as an electrode material for detecting fenitrothion requires additional investigations.

2.4 Characteristics of the material

The choice of materials in electrochemical biosensors plays a crucial role in affecting the sensing characteristics such as stability, conductivity and sensitivity. Various **nanomaterials have been extensively used owing to their remarkable electrical, catalytic and structural properties** [98].

The ideal biosensor material must have all the following characteristics: Excellent electrical conductivity, large surface area, high catalytic activity, chemical stability and biocompatibility. Various two-dimensional nanomaterials such as carbon nitride and metal oxides are widely utilised owing to their superior physicochemical properties.

Such materials offer the following advantages in sensing systems:

Enhanced rate of electron transfer, increased analyte adsorption capacity, increased sensing efficiency and improved electrochemical stability. For this reason, composite-based materials are widely used in the fabrication of contemporary electrochemical biosensors.

2.4.1 Graphitic carbon nitride (g-C₃N₄)

Graphitic carbon nitride represents a metal-free polymeric semiconductor made up of mainly C and N atoms. The material has a graphite-like layered structure and stands out among other two-dimensional nanomaterials owing to its remarkable electrochemical and photochemical properties [99]. Some of the outstanding properties of the material include high thermal and chemical stability, a large surface area, good catalytic activity and biocompatibility. Due to its layered nature, the compound has been shown to offer an effective rate of electron transfer and increased adsorption sites. g-C₃N₄ is synthesized from the nitrogen-rich precursors like melamine, thiourea, urea and cyanamide. As stated earlier, g-C₃N₄ has a wide range of applications due to its remarkable physicochemical properties including but not limited to electrochemical biosensors, photocatalysis, energy storage devices and environmental remediation. Unfortunately, the material lacks sufficient electrical conductivity, necessitating its combination with other conductive nanomaterials and transition-metal compounds. The representation of g-C₃N₄ and its uses is given in the Fig. 2.1.

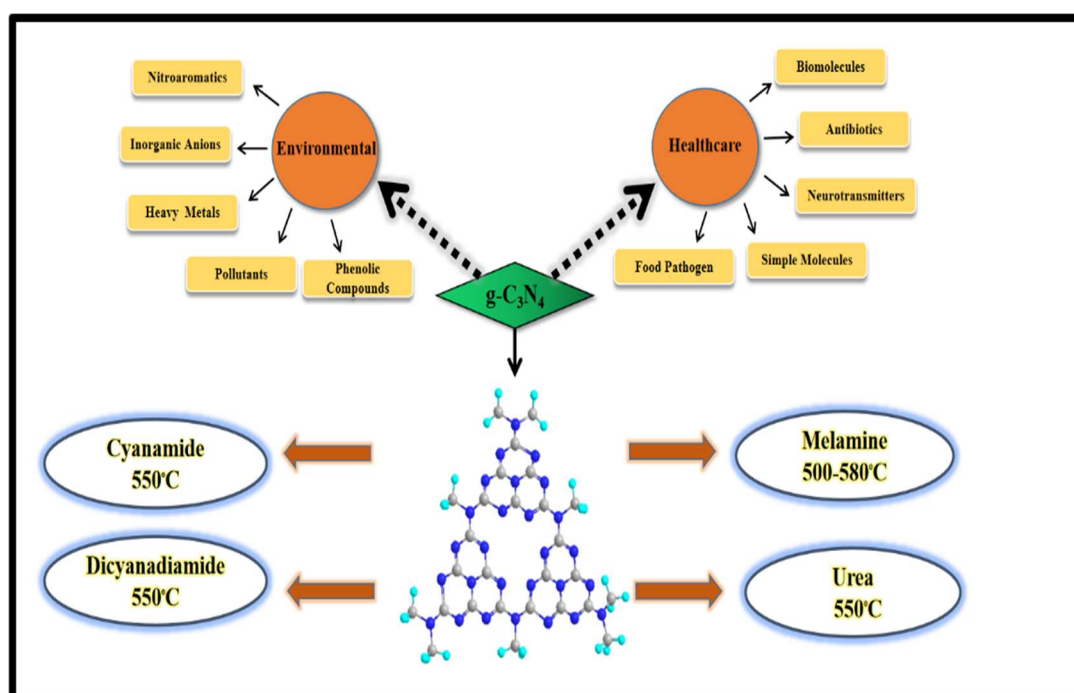


Fig. 2.1 Diagrammatic representation of g-C₃N₄ and its uses

2.4.2 Applications of g-C₃N₄

Graphitic carbon nitride has numerous applications in science and technology due to its unique structural and electronic properties.

(a) Electrochemical biosensors: g-C₃N₄ is widely used in biosensors for the detection of pesticides, glucose, heavy metals and biomolecules. Its large surface area and catalytic activity improve sensor sensitivity and electron transfer.

(b) Photocatalysis: g-C₃N₄ is an efficient photocatalyst used for the degradation of organic pollutants, wastewater treatment and hydrogen production. Its semiconductor properties enable light-induced catalytic reactions.

(c) Energy storage devices: g-C₃N₄ is used in supercapacitors, lithium-ion batteries and fuel cells because of its good electrochemical stability and conductivity.

(d) Environmental remediation: g-C₃N₄-based materials help remove toxic dyes, heavy metals and organic pollutants from environmental samples.

(e) Biomedical applications: g-C₃N₄ is used in drug delivery, bioimaging and antibacterial applications due to its biocompatibility and low toxicity.

(f) Sensor applications: g-C₃N₄-based nanocomposites are used in gas sensors, humidity sensors and electrochemical sensors because of their excellent sensing performance. Thus, graphitic carbon nitride has become an important material in advanced nanotechnology and biosensor research due to its outstanding physicochemical and electrochemical properties [100].

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CHAPTER 3

Materials and Methods

3.1 Introduction

The experimental protocols and characterization techniques used to fabricate an electrochemical biosensor for fenitrothion detection which is functionalized with zinc-nickel oxide and graphitic carbon nitride are presented in this chapter. It also outlines the synthesis of carbon nitride (g-C₃N₄), zinc–nickel oxide nanocomposite, the modification of electrodes, and the electrochemical methods to characterize the biosensor. All the details on characterization techniques used for the structural, morphological, elemental and electrochemical evaluation of the produced composites and sensor performance are presented in this chapter. In addition, some techniques used for the immobilization of biomolecules on the surface of electrode. The analytical techniques exploited for the detection of fenitrothion are also discussed here [101].

3.2 Materials

The details of materials utilized in the present experimental work are described below.

3.2.1 Chemicals

The following materials were used for the synthesis and electrochemical studies: zinc nitrate hexahydrate $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, nickel nitrate hexahydrate $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, melamine, sodium hydroxide [102], acetone, ethanol, Fenitrothion, potassium ferricyanide, potassium ferrocyanide, potassium chloride, phosphate buffer saline (PBS), glutaraldehyde solution, enzyme acetylcholinesterase (AChE) and purified water [103].

1

3.2.2 Solutions and buffers

- 0.2 mM Phosphate buffer saline (PBS), pH 7
- 5mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ used in PBS solution as redox initiator

3.3 Characterization methods

Several techniques including FTIR and XRD are being used for the structural analysis and thus all developed electrodes and synthesized materials were characterized. The morphological and elemental techniques of characterization like SEM and EDX were used in the current study. Electrochemical characterization was performed by using cyclic voltammetry (CV) and differential pulse voltammetry (DPV).

3.3.1 Fourier transform infrared spectroscopy

In IR spectroscopy, a sample is subjected to IR, where one part is absorbed and the other part is transmitted through the sample. The sample's molecular fingerprint is produced by the resulting spectrum which shows the absorption of IR by the molecule and transmission (Fig. 3.1) depicting IR spectroscopy [104]. Thus, IR spectroscopy is used to analyse organic, inorganic and polymeric materials. The fingerprint of the sample is shown by IR spectroscopy with the absorption peak that matches the vibrational frequency with the bonds/atoms [105][106].

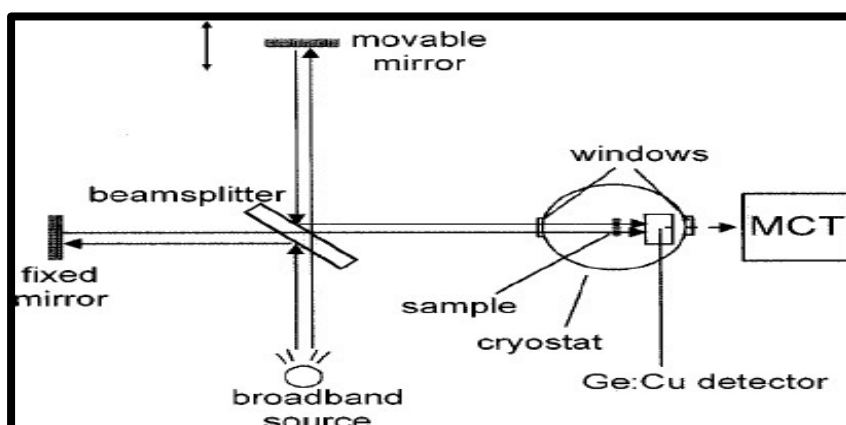


Fig. 3.1 Diagrammatic representation of Michelson interferometer

FTIR spectroscopy can be used across a broad range of frequencies including near-infrared, far-infrared and mid-infrared by using appropriate beam detectors and splitters. None of the other dispersive techniques can encompass such a broad frequency range [107]. One of the biggest advantages of FTIR is that it can serve as a quantitative tool for multicomponent studies [108]. Beer's law, the additive nature and available software form the basis for quantifying several components enabling the determination of absorbance values, the computation of the calibration factor matrix and the estimation of unknown concentrations. The majority of FTIR multicomponent analysis techniques depend on the K/P values of the matrix which uses a mixture of certain concentrations as a calibration standard and directly on the mixture of unknown concentrations. FTIR differs fundamentally from traditional dispersive IR(infrared) spectroscopy as it relies on interferometry. Spectrophotometry is the device that identifies a compound's absorption spectrum. In contrast to traditional spectrophotometers, a Fourier transform (FT) spectrophotometer gives an IR spectrum more rapidly.

The FTIR instrument emits an infrared radiation beam generated by a blackbody source. Eventually, the beam is directed to the interferometer where spectral encoding occurs. An interferogram results from the recombination of beams with different path lengths leading to constructive and destructive interference. This beam now reaches to the sample compartment where sample accumulates certain frequencies of energy that are present only in the interferogram. Moreover, the detector monitors the distinct interferogram signal in energy versus time for each and every frequency. Further, the beam is imposed to serve as a background or reference for the operation of the instrument [109].

Lastly, the desired spectrum was generated as the interferogram subtracted the spectrum's background from the spectrum of the sample by using the Fourier transformation (FT) computer software [110][111].

3.3.2 X-ray diffraction (XRD)

X-ray diffraction method (Fig. 3.2) is a commonly used method for analyzing atomic spacing and crystal structure [112]. The principle of X-ray diffraction relies on the constructive interference of crystalline samples and monochromatic X-rays. The X-rays are produced by a CRT and then filtered to generate monochromatic radiation aligned to focus and placed on the sample [113]. X-rays have a wavelength of 10 nm, a covalent bond length and an atomic radius. Using longer-wavelength radiation (ultraviolet) would yield low resolution in determining atomic positions. Conversely, shorter wavelength radiations (Gamma radiations) would result in the phenomenon of inelastic scattering [114]. The positions of the polycrystalline and crystalline structures are determined by this method. Bragg devised a law called Bragg's law from which relationships can be established between the incident beam's wavelength, the diffracted beam's angle and the atomic spacing that could be determined in the sample's crystalline structure.

$$n\lambda = 2d \sin \Theta$$

where integer n signifies the diffraction order, λ signifies the X-rays wavelength that are emitted, d signifies the interplanar distance of the planes of the crystal which diffract through the beam, Θ signifies an angle between the reflecting crystalline lattice surface and incident ray [115]. In this study, the material is scanned across a predefined 2θ range and then diffracted X-rays are collected. After monitoring the diffraction pattern, the d -spacings so obtained were used to carry out a qualitative analysis, i.e., identifying the chemical components of sample [116].

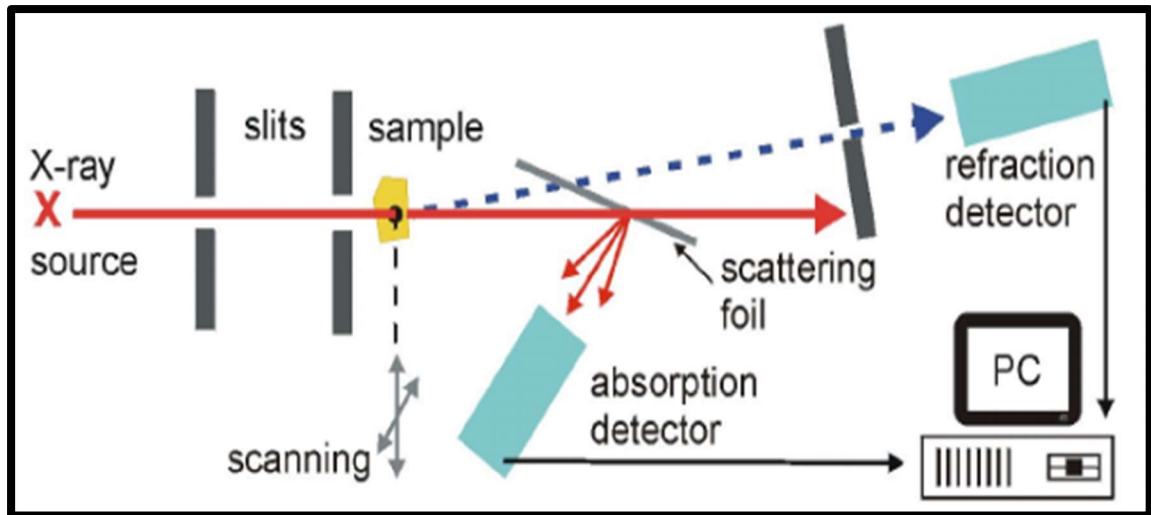


Fig. 3.2 Diagrammatic representation of XRD

3.3.3 Field emission scanning electron microscopy (FE-SEM)

This instrument is a very useful instrument for the examination of surface morphology of microstructure, crystalline structure, topography and characterizations of chemical composition [117] (Fig. 3.3). It is important to understand the fundamental principle of light optics to comprehend the basics of electron microscopy. The limit of microscopy of an optical microscope is $\sim 2000 \text{ \AA}$ which is due to the enlargement of the visual angle [118][119]. In FESEM, image formation depends on the ability to acquire signals from interactions between the specimen and the electron beam. Such interactions are broadly contributed due to the presence of elastic and inelastic components. Electrons that are scattered elastically when the incoming electron is deflected by an angle of more than 90° are known as back-scattered electrons. Inelastic scattering takes place due to multiple interactions within the incoming electrons and the atoms and electrons of the sample and leads to primary beam electrons transferring a significant amount of energy to the atom.

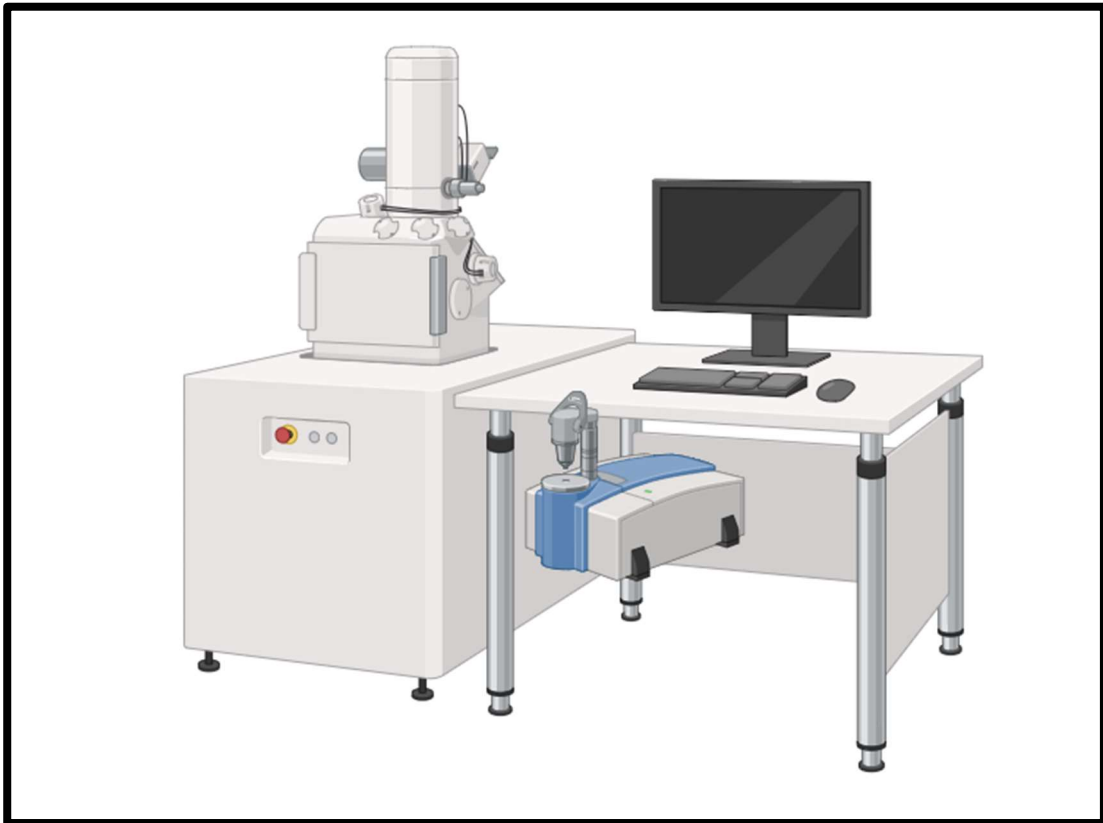


Fig. 3.3 Diagrammatic representation of FESEM machine

3.3.4 Energy dispersive X-ray spectroscopy (EDX)

An **elemental analysis of a sample** is performed by EDX spectroscopy by utilizing scanning electron microscopy. EDX has the ability to determine the elements that contain an atomic number greater than boron and thus detect at least 0.1% concentration [120][121]. EDX's numerous applications include material evaluation, analysis of spot detection in areas up to 10cm in diameter, identification, contamination analysis, etc. Upon collision with the electron beam, the sample emits X-rays. Because of the reason that neither of the elements has a similar X-ray pattern which can be measured and differentiated for the sample conc [122][123]. X-ray emission occurs when the primary beam interacts with the nucleus of an atom, ejecting an electron and releasing X-rays. The X-rays that are emitted comprise characteristic and continuum X-rays.

3.3.5 Electrochemical techniques

Electrochemical techniques are a category of analytical methods that utilize electrochemical principles to analyze and examine chemical reactions, investigate substances' properties and determine concentrations. Electrochemical techniques present more benefits than technologies of surface modification. Moreover, electrochemical dissolution gradually maintains the surface characteristics such as the composition and orientation of crystals, unchanged. Now, the electrochemical methods consist of measurements, potentiometry, cyclic voltammetry, electrochemical impedance spectroscopic (EIS) and chronoamperometry [124]. In electrochemical techniques, three electrodes such as working, reference and counter electrodes are present in an electrochemical cell. Now, these electrodes are linked via a potentiostat which monitors the associated current response. In this type of electrochemical experiment, the potential is measured at working electrode and the corresponding current is plotted against time. In this project, the electrochemical response of all the developed electrodes is examined by using an Autolab (Ecochemie, Netherlands) Galvanostat/Potentiostat and reference electrodes such as Ag/AgCl and Platinum (Pt) are used as counter electrodes as shown in Fig. 3.4.

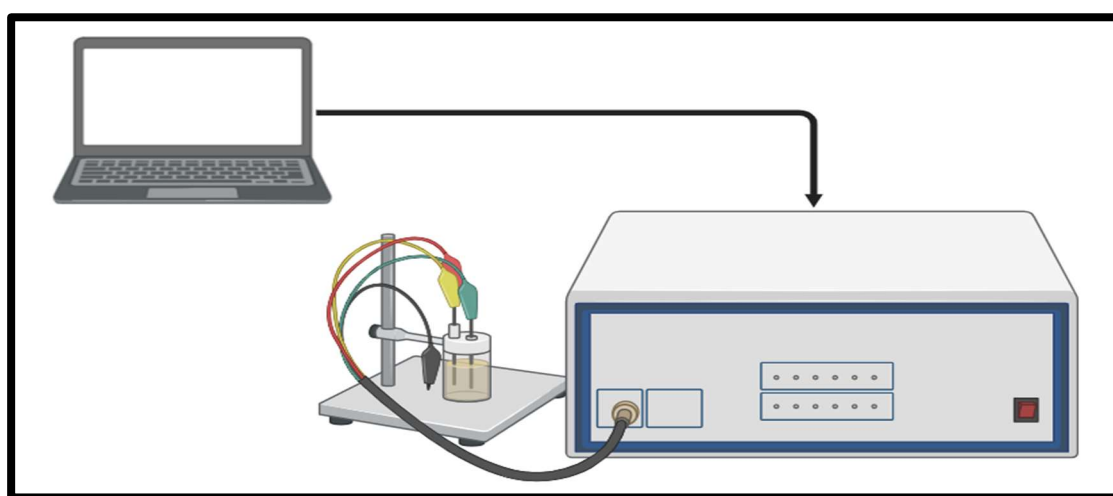


Fig. 3.4 Diagrammatic representation of potentiostat

3.3.5.1 Cyclic voltammetry (CV)

It is widely being used technique for the characterization of the electrochemical behavior of electrochemically active substances [125]. Reducible mass transport or oxidizable species that are electroactive in CV are restricted to diffusion. Thus, to avoid current migration and ensure conductivity, a 0.1 M supporting or ground electrolyte is added to the solution. Tetra alkyl ammonium salts and alkali metals are highly effective in this technique. In the CV technique, the x-axis represents the parameter applied to the system where (E) is the applied potential while the y-axis represents the resultant current (i). There are two conventions which are being widely used to report CV such as sign convention and acquiring data [126]. Throughout the reduction-oxidation process, ions are introduced to electrodes in an electrolytic solution [127].

3.2.5.2 Differential pulse voltammetry (DPV)

This technique is most accurate and commonly used voltammetric method besides anodic stripping voltammetry. This method was developed to achieve a large differential between the charging current and the analytical current in the mercury drop's half-life at the end. As the need is increasing for trace analysis for essential oxidizable materials (e.g., vitamins, drugs, and carcinogens), the method is utilized for different solid electrodes like platinum or carbon that have a greater anodic range (potential) as compared to mercury electrodes [128]. Further, a series of pulses with moderate amplitude are overlaid on voltage; then, the current is shown within pulse voltage and ramped baseline voltage. Current is recorded before applying the pulse (1st point) and the end of pulse (2nd point). The variations in the measurement of current following each pulse at those locations are recorded [129][130].

3.3.5.3 Electrochemical impedance spectroscopic (EIS)

Electrochemical impedance spectroscopic (EIS) is a powerful technique for examining the mechanism of electrochemical reactions for calculating the transport and dielectric characteristics of materials, investigating the characteristics of porous electrodes as well as for examining the passive surfaces [131]. It has been intensively utilized for charge transport characterization, explanation of the mechanisms of corrosion, battery optimization and interfaces of solution/membranes. In the application of biosensors, it is appropriate for the detection of events of binding on the surface of the transducer [132].

An electrochemical cell is supplied with alternating current (AC) voltage to measure its flow of current. Moreover, a sinusoidal potential stimulation was utilized, leading to AC current production. A short excitation pulse is widely utilized to calculate electrochemical impedance and obtain a pseudo-linear response within the electrochemical cell. Irrespective of the phase shift, the matching current has a similar frequency to that of the sinusoidal waveform. In the linear system, the response of current is sinusoidal. By describing the impedance data as charge transfer resistance (R_{ct}), solution resistance (R_s) as well as double layer capacitance (C_{dl}). Mass transport diffusion or the cell's diffusion is also illustrated by Z_w (Warburg element). Thus, the Equivalent circuit model EIS data for the electrochemical cell is shown by the Nyquist plot, where imaginary impedance represents the inductive as well as capacitive characteristics of the electrochemical cell. [133]

This work uses the EIS method to calculate R_{ct} from the obtained EIS spectra. By using the obtained data values of R_{ct} , the exchange current per geometric unit area and the apparent electron transfer rate constant (K_{app}) of the different electrodes have been computed by utilizing the equations (1) and (2).

$$i_0 = \frac{nRT}{R_{ct}F} \quad (1)$$

$$K_{app} = \frac{RT}{n^2 F^2 A R_{ct} C} \quad (2)$$

where C is concentration and F is Faraday's constant, n the electrons and A is the geometrical area of electrode, T is temp and R is a gas constant [134].

CHAPTER 4

Fabrication of Novel Electrochemical Biosensor for Fenitrothion Detection using g-C₃N₄@ZnO-NiO Nanohybrid

4.1 Introduction

The fabrication and characterization of novel acetylcholinesterase (AChE)-based electrochemical biosensor for fenitrothion detection that uses graphitic carbon nitride (g-C₃N₄) decorated zinc–nickel oxide (ZnO–NiO) nanocomposite [96] has been presented in this chapter. The sensing platform's electroactive surface area, electrocatalytic activity, charge-transfer capacity and biocompatibility are all greatly enhanced by the synergistic interaction between g-C₃N₄ nanosheets and ZnO–NiO nanoparticles. ZnO–NiO is added to g-C₃N₄ nanosheets to enhance electron mobility and provide a large number of active sites thereby improving electrochemical sensing capability [97].

For the detection of fenitrothion, the developed AChE/g-C₃N₄@ZnO–NiO/ITO biosensor demonstrated outstanding selectivity, high sensitivity, quick response and low detection limit. Additionally, the fabricated biosensor showed good applicability for the detection of fenitrothion in actual agricultural and food samples, suggesting its potential for applications in food safety and environmental monitoring [98].

4.2 Experimental section

4.2.1 Synthesis of g-C₃N₄ nanosheets

The nanosheets of graphitic carbon nitride have been synthesized by using thermal condensation process. Initially, 10 g of melamine was heated at 550°C for 4 h at a heating rate

of 5°C per min in a muffle furnace. The product had naturally cooled down to normal temperature and then it was washed several times with distilled water followed by ethanol to remove impurities. The last step was to store the product for later use after it was dried at 60°C in a vacuum oven.

4.2.2 Synthesis of g-C₃N₄@ZnO-NiO nanocomposite

First of all, the zinc nitrate hexahydrate and nickel nitrate hexahydrate have been dissolved in 50 mL of distilled water with continuous stirring at suitable concentrations to get the homogeneous solution. Then, 100 mg of a synthetic g-C₃N₄ nanosheets were added. This precursor solution has been stirred for 2 h and ultrasonicated for 30 min to ensure uniform dispersion of g-C₃N₄. This solution was then heated at 180°C for 18–20 h in a 100 mL stainless-steel autoclave lined with Teflon and then autoclave was cooled to room temperature. The product was centrifuged and then washed with ethanol and distilled water and finally dried at 80°C in a vacuum oven. The resulting black powder was termed as g-C₃N₄@ZnO–NiO nanocomposite.

4.2.3 Electrophoretic deposition of g-C₃N₄@ZnO-NiO nanocomposite

The generated g-C₃N₄@ZnO-NiO nanocomposite was deposited onto pre-cleaned indium tin oxide (ITO) glass substrates using electrophoretic deposition (EPD) using a two-electrode technique. Before deposition, 0.5 mg of the g-C₃N₄@ZnO-NiO nanocomposite was ultrasonically dispersed in 15 mL of distilled water to prepare a stable, uniform solution. The working electrode was the cleaned ITO substrate while the counter electrode was platinum foil. 10 V was applied for 10 s during the deposition procedure. After deposition, the modified electrodes were cleaned with distilled water and allowed to air-dry.

4.2.4 Fabrication of g-C₃N₄@ZnO-NiO nanocomposite based biosensor

The electrochemical biosensor was made by drop-casting 20 μL of acetylcholinesterase (AChE) enzyme solution (0.08 mg mL^{-1}) onto the g-C₃N₄@ZnO-NiO/ITO electrode. To properly fix the enzyme onto the surface of the nanocomposite, the modified electrode was then kept at 4°C for 12 h. Phosphate-buffered saline (PBS) was used to gently remove loosely bound enzyme molecules from the created bioelectrode after incubation. The bioelectrode was then kept at 4°C until further electrochemical testing.

4.3 Results and discussion

XRD analysis has been performed to understand the crystalline structure and phase composition of synthesized g-C₃N₄ and g-C₃N₄@ZnO-NiO nanocomposite. Fig. 4.1 shows the XRD data of the pure g-C₃N₄ and g-C₃N₄@ZnO-NiO nanocomposite. The g-C₃N₄ shows two characteristic peaks at 2θ values of 13.1° and 27.4° , respectively which were due to diffraction from the (100) and (002) crystallographic planes. A peak at 13.1° attributes due to the in-plane structural packing of tri-s-triazine units while a peak at 27.4° attributes due to the interlayer stacking of aromatic conjugated systems in graphitic carbon nitride. The XRD pattern of the g-C₃N₄@ZnO-NiO nanocomposite exhibited several additional diffraction peaks at 21.7° , 31.4° , 38.2° , 50.3° and 55.1° . These peaks correspond to the characteristic crystal planes of zinc–nickel oxide nanostructures, confirming the successful incorporation of ZnO-NiO onto the g-C₃N₄ nanosheets. Furthermore, the relatively reduced intensity of the characteristic g-C₃N₄ peaks in the nanocomposite may be due to the uniform distribution of ZnO-NiO nanoparticles on the surface of graphitic carbon nitride. The observed diffraction peaks (Fig. 4.1) indicate good crystallinity and successful formation of the composite material.

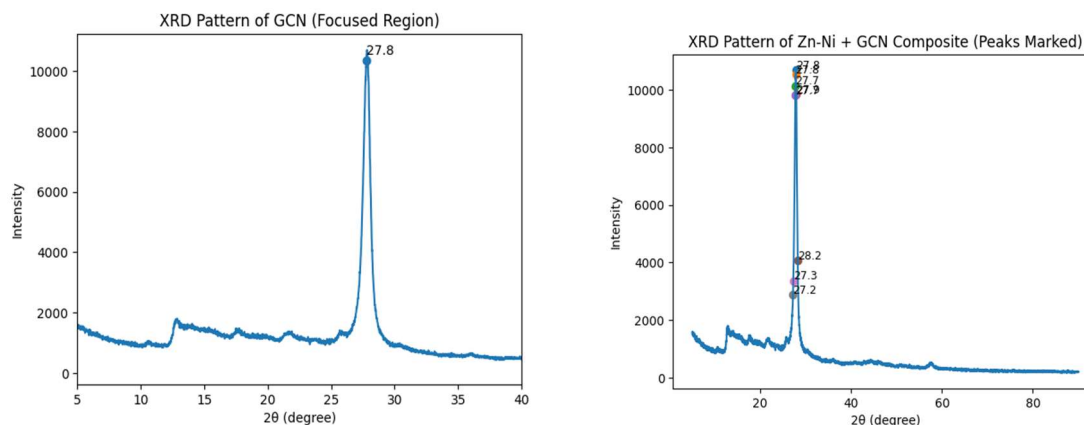


Fig. 4.1 XRD pattern of g-C₃N₄ and g-C₃N₄@ZnO-NiO

FTIR analysis was performed to identify the presence of functional groups and chemical bonding that exist in g-C₃N₄ and g-C₃N₄@ZnO-NiO nanocomposite (Fig. 4.2). A few key absorption bands in the range 1200–1650 cm⁻¹ were identified in the pure g-C₃N₄ spectrum, and these bands are associated with the C–N and C=N stretching modes of aromatic rings with N–C–N and C–N–C–N configurations. A strong absorption peak at 808 cm⁻¹ attributes due to the breathing mode of tris-triazine components which is evidence of the formation of the graphitic carbon nitride framework. Furthermore, a band between 3150–3300 cm⁻¹ range was seen due to the stretching vibrations associated with amino functional groups (–NH and –NH₂) on g-C₃N₄ surface. The FTIR spectra of the g-C₃N₄@ZnO-NiO nanocomposite showed all major characteristic peaks of g-C₃N₄, suggests that the structural framework of g-C₃N₄ is stable during the composite preparation. Moreover, a new absorption peak appeared near 620 cm⁻¹ in the nanocomposite spectrum corresponding to metal–sulfur vibrations (Zn–O and Ni–O). The presence of this peak confirms the successful formation and incorporation of zinc–nickel oxide within the g-C₃N₄ matrix. Thus, FTIR data strongly support the successful synthesis of the g-C₃N₄@ZnO-NiO nanocomposite and confirm the interaction between graphitic carbon nitride and zinc–nickel oxide components.

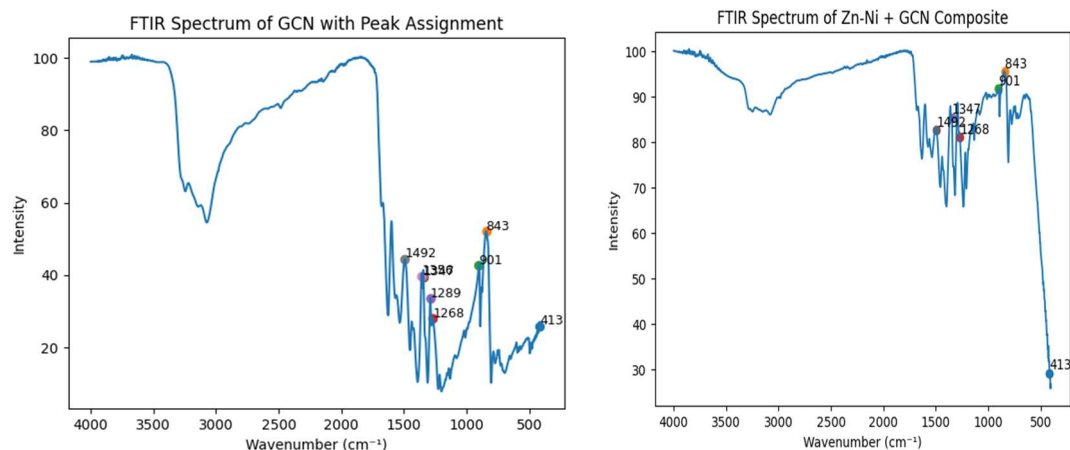


Fig. 4.2 FTIR spectra of $g\text{-C}_3\text{N}_4$ and $g\text{-C}_3\text{N}_4@\text{ZnO-NiO}$

FE-SEM technique was used to analyze the surface morphology of produced materials as shown in Fig. 4.3. A layered, sheet-like morphology with folded and wrinkled edges was observed in FESEM images of pure $g\text{-C}_3\text{N}_4$, indicating the fabrication of thin nanosheets having large surface area. The FESEM image of $g\text{-C}_3\text{N}_4@\text{ZnO-NiO}$ nanocomposite showed evenly spaced ZnO-NiO particles resembling flowers, anchored to the $g\text{-C}_3\text{N}_4$ nanosheet surface. To ascertain the elemental composition and purity of the produced nanocomposite, additional energy-dispersive X-ray (EDX) examination was performed. It is confirmed by the EDX spectra that the presence of carbon (C), nitrogen (N), zinc (Zn), nickel (Ni) and sulfur (S) demonstrating the successful fabrication of the $g\text{-C}_3\text{N}_4@\text{ZnO-NiO}$ nanocomposite without any noticeable impurity.

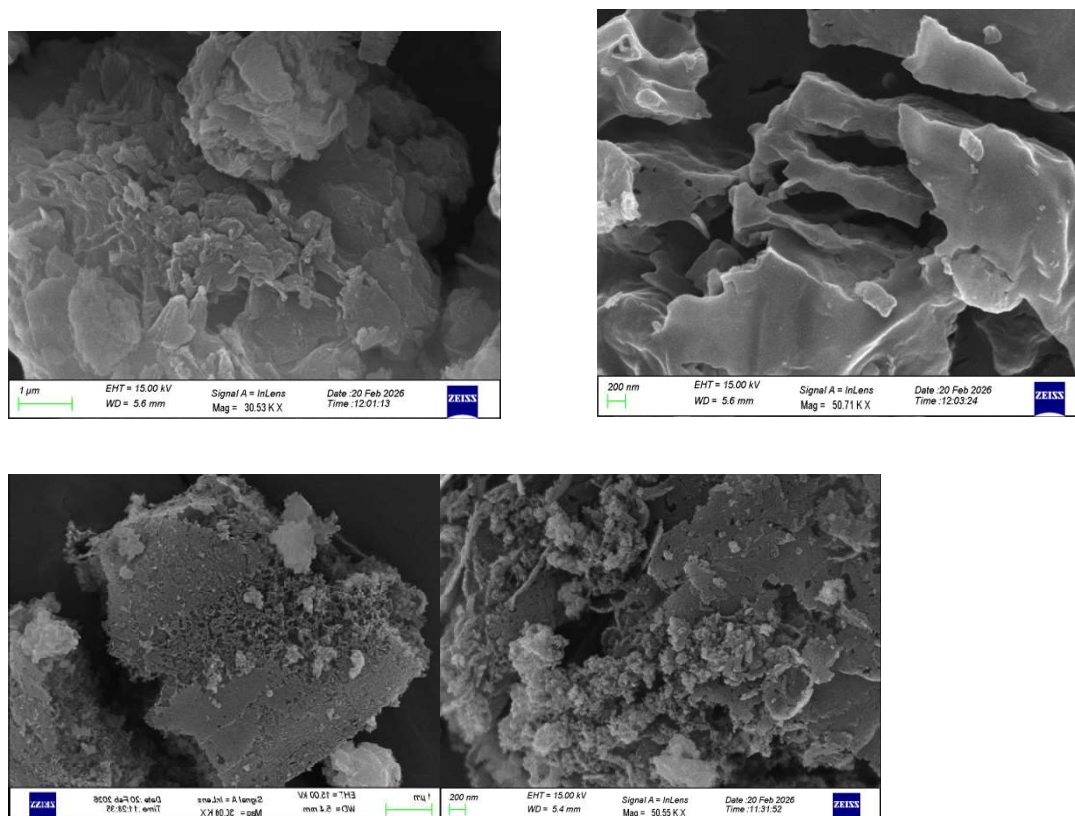


Fig. 4.3 FESEM images of (A) g-C₃N₄ (B) Zn-NiO (C) g-C₃N₄@ZnO-NiO and (D) EDX of g-C₃N₄@ZnO-NiO

4.3.1 Electrochemical characterization

Cyclic voltammetry was used for electrochemical characterization of the produced electrodes in 0.2 M phosphate-buffered saline (PBS) using a redox probe of 5 mM [Fe(CN)₄]^{3-/4-}. The cyclic voltammograms of different fabricated electrodes are shown in Fig. 4.4 (A&B). Because of its superior conductivity and greater electroactive surface area, the g-C₃N₄@ZnO-NiO/ITO electrode showed the greatest anodic and cathodic peak currents in comparison to pure g-C₃N₄ and ZnO-NiO. On the other hand, because of limited electron mobility, the AChE/g-C₃N₄@ZnO-NiO/ITO electrode demonstrated a drop in peak current following enzyme immobilization.

In the range of 10–300 mV s⁻¹, the impact of scan rate on the g-C₃N₄@ZnO-NiO/ITO electrode's electrochemical response was examined (Fig. 4.4 (C&D)). A diffusion-controlled

electrochemical process was demonstrated by linear relationship of anodic and cathodic peak currents with square root of the scan rate.

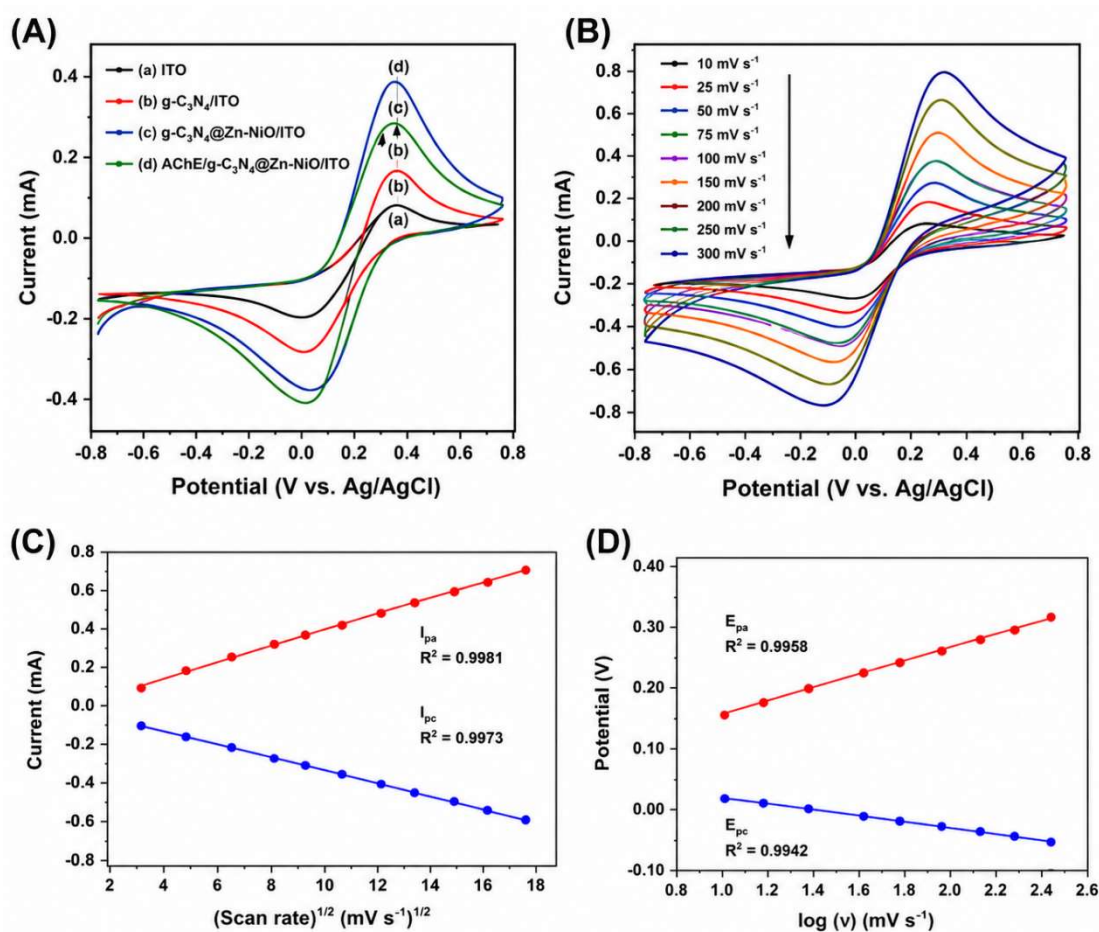


Fig. 4.4: Cyclic voltammograms and electrochemical response of fabricated electrodes

4.3.2 Optimization studies

The pH, enzyme concentration and incubation time were all adjusted using differential pulse voltammetry (DPV) to optimize the biosensor's analytical performance (Fig. 4.5). The electrochemical response was examined as a function of pH over the range 6–8. Under mildly acidic conditions, good enzymatic activity and effective electron transfer were indicated by the maximal current response at pH 6.5. As a result, the ideal pH for more research was determined to be 6.5. Acetylcholinesterase (AChE) concentrations ranging from 0.04 to 0.12 mg mL⁻¹ were investigated in order to maximize the quantity of AChE. As the enzyme concentration increased, the current response first increased and peaked at 0.06 mg mL⁻¹.

Increased diffusion resistance due to high enzyme loading caused the current to drop below this concentration. The optimal incubation period for fenitrothion interaction with the biosensor was between 2-14 min. The response rapidly decreased with increasing incubation time, reaching saturation at around 10 min indicating that fenitrothion completely inhibited AChE activity.

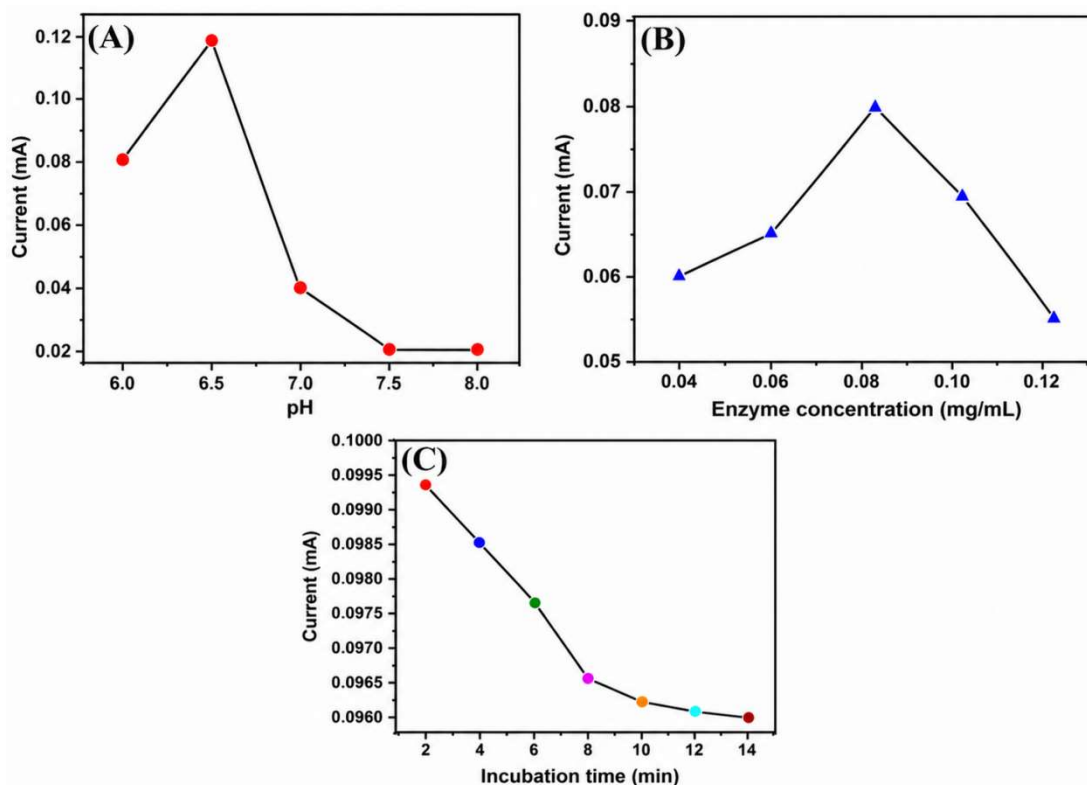


Fig. 4.5: Optimization studies of (A) pH effect (B) enzyme concentration and (C) incubation time

4.3.3 Electrochemical biosensing study

Under ideal conditions, the DPV approach was used to examine the electrochemical sensing of the manufactured AChE/g-C₃N₄@ZnO-NiO/ITO biosensor for fenitrothion detection. The DPV response was measured at fenitrothion concentrations of 5-100 nM in 0.2 M PBS (pH 6.5) containing 5 mM [Fe(CN)₄]^{3-/4-}. As fenitrothion concentration increased, a progressive

drop in anodic peak current was seen which might be explained by the suppression of acetylcholinesterase activity by fenitrothion molecules. The calibration curve showed a high regression coefficient and a linear relationship between peak current and fenitrothion concentration demonstrating the manufactured biosensor's superior analytical capability. For the detection of fenitrothion, the developed biosensor showed good selectivity, a low detection limit and high sensitivity. The synergistic effect of high-surface-area g-C₃N₄ nanosheets and conductive ZnO-NiO nanostructures which facilitate rapid electron transfer and efficient enzyme immobilization, underlies the enhanced sensing properties (Fig. 4.6).

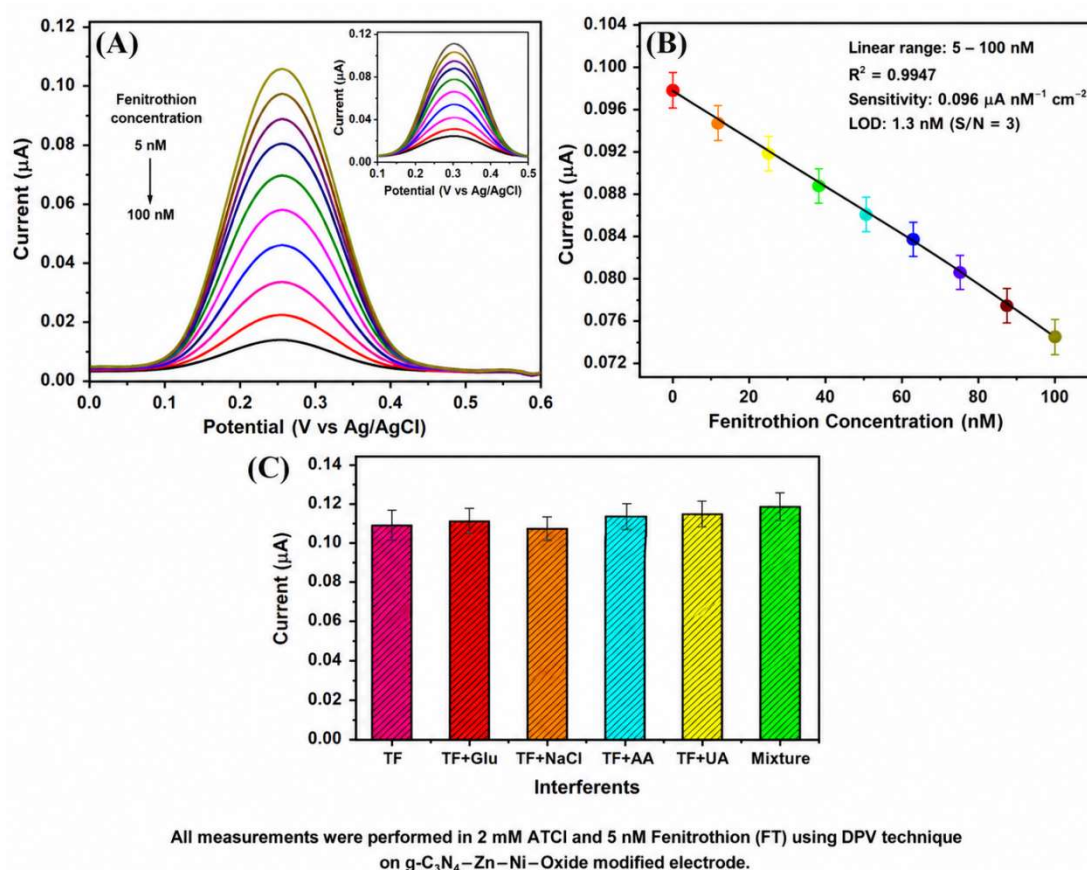


Fig. 4.6: (A) DPV responses of AChE/g-C₃N₄@ZnO-NiO/ITO electrode towards different concentrations of fenitrothion (B) Calibration curve and (C) Selectivity study

4.3.4 Interference study and real sample analysis

The contamination of common interfering species like glucose, uric acid, ascorbic acid, NaCl and fenitrothion, the selectivity of the manufactured biosensor was assessed. The designed biosensor's good selectivity for fenitrothion detection was demonstrated by the results which showed little change in current response in the presence of interfering analytes.

Fruit and vegetable extracts were used as spiked real samples to further investigate the biosensor's practical usefulness. After adding known amounts of fenitrothion to the samples, recovery tests were carried out. The acquired recovery values, ranging from 96 to 108%, demonstrated the designed sensing platform's good accuracy and reliability for actual sample analysis.

4.3.5 Reproducibility and stability studies

The constructed AChE/g-C₃N₄@ZnO-NiO/ITO biosensor was tested for repeatability using five independently generated electrodes under identical experimental circumstances. The low relative standard deviation (RSD) result demonstrated excellent production reproducibility and consistency.

The storage stability of the generated biosensor was further investigated by maintaining the electrode at 4°C and routinely evaluating its electrochemical response. The biosensor demonstrated strong long-term stability and dependability for practical applications retaining a significant portion of its original response even after storage for a few days.

Reproducibility and stability study using different fabricated electrode AChE/g-C₃N₄@ZnO-NiO/ITO electrode over time is shown in Fig. 4.7.

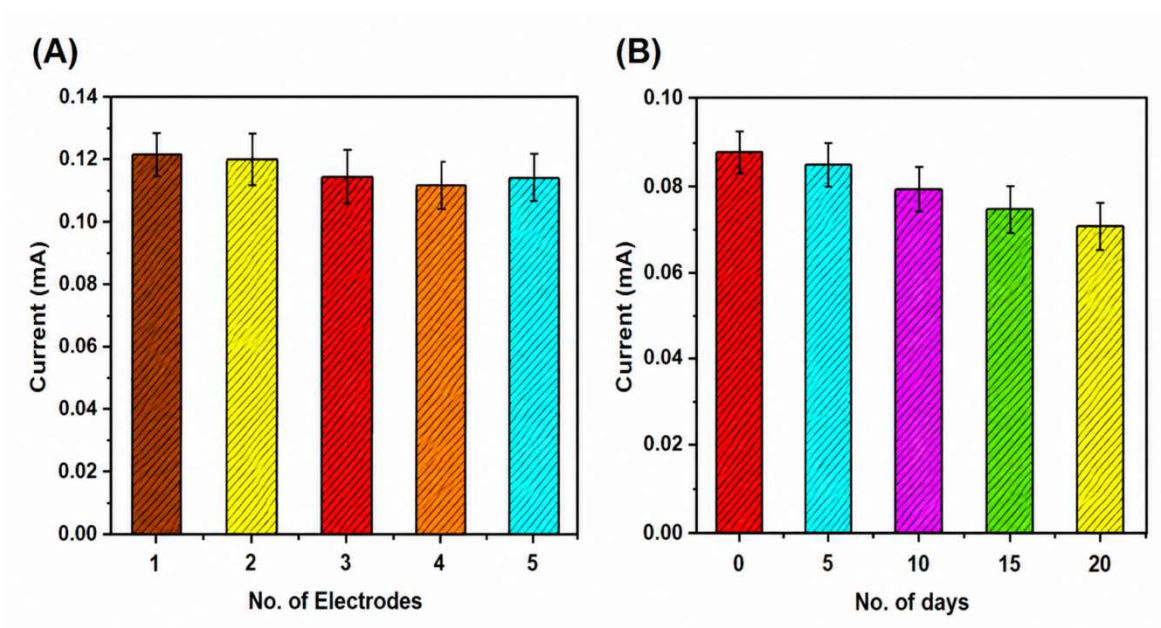


Fig. 4.7 (A) Reproducibility study using different fabricated electrodes and (B) stability study of AChE/g-C₃N₄@ZnO-NiO/ITO electrode over a period of 20 days

CHAPTER 5

CONCLUSION

The present study successfully developed and characterized a graphitic carbon nitride ($\text{g-C}_3\text{N}_4$) decorated zinc–nickel oxide (ZnO-NiO) electrochemical biosensor for the sensitive detection of fenitrothion. The synthesized $\text{g-C}_3\text{N}_4/\text{ZnO-NiO}$ nanocomposite exhibited enhanced electrochemical properties due to the synergistic interaction between graphitic carbon nitride and zinc–nickel oxide resulting in improved conductivity, catalytic activity and electron-transfer efficiency. Morphological and electrochemical characterization confirmed the successful formation of the nanocomposite and its suitability as an effective sensing platform. The fabricated biosensor demonstrated excellent analytical performance including sensitivity, a low detection limit, a wide linear response range, selectivity and satisfactory repeatability and stability. An enhanced sensing behavior was attributed due to the large surface area and the efficient charge-transfer characteristics of $\text{g-C}_3\text{N}_4/\text{ZnO-NiO}$ composite. Optimization studies revealed the significant influence of pH and electrochemical parameters on sensor performance while real sample analysis confirmed the practical utility of developed biosensor for fenitrothion determination. The recovered values showed good accuracy and reliability in complex sample matrices. Overall, the developed biosensor provides a simple, rapid, cost-effective and reliable approach for pesticide monitoring. The findings of this study demonstrate that the $\text{g-C}_3\text{N}_4/\text{ZnO-NiO}$ nanocomposite is a promising electrode material for electrochemical sensing applications and offers considerable potential for environmental monitoring, food safety assessment and agricultural quality control. Further studies focusing on long-term stability and portable sensor development may broaden its practical applications.

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