A Novel Electrochemical Biosensor Based on 2D/2D g-C_3N_4/MXene Nanohybrid for Trichlorfon Detection

A DISSERTATION

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IN

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Submitted by

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We, Diksha (2K23/MSCCHE/63) and Nishul Khanna (2K23/MSCCHE/58), hereby certify that the work which is being presented in the dissertation entitled **"A Novel Electrochemical Biosensor Based on 2D/2D g-C_3N_4/MXene Nanohybrid for Trichlorfon Detection"** in partial fulfillment of the requirements for the award of the Degree of Master of Science in Chemistry, submitted in the Department of Applied Chemistry, Delhi Technological University is an authentic record of our own work carried out during the period from 2024 to 2025 under the supervision of Prof. D. Kumar.

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

Place: Delhi **Diksha** **Nishul Khanna**

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CERTIFICATE

Certified that Diksha (2K23/MSCCHE/63) and Nishul Khanna (2K23/MSCCHE/58) have carried out their research work presented in this thesis entitled **"A Novel Electrochemical Biosensor Based on 2D/2D g-C_3N_4/MXene Nanohybrid for Trichlorfon Detection"** for the award of Degree of Master of Science in Chemistry from Department of Applied Chemistry, Delhi Technological University, Delhi, under my supervision. The dissertation embodies the results of original work, and studies are carried out by the students themselves, and the content of the thesis does not form the basis of the award of any other degree to the candidate or to any other body else from this or any other University/Institution.

Prof. D. Kumar
(Supervisor)

Place: Delhi

Date: 20/06/2025

ABSTRACT

Organophosphate pesticides have been widely used to preserve agricultural produce from pest damage during growth and to prevent subsequent quality degradation**.** However, while doing this, the pesticides and their decomposition products permeate through soil and water, leading to their accumulation in food items. They exhibit significant specificity for the enzyme acetylcholinesterase (AChE) and can produce irreversible damage to the nervous system. In order to deal with this difficulty, we suggest developing an electrochemical biosensor based on acetylcholinesterase (AChE) for the detection of Organophosphate pesticide, Trichlorfon (TF), by employing (g-C_3N_4/MXene) graphitic carbon nitride and MXene-based nanocomposite. The electrostatic interactions of g-C_3N_4/MXene nanocomposite enhance electron migration throughout the electrode surface, assisting in the immobilization of the acetylcholinesterase (AChE) enzyme with glutaraldehyde as a crosslinking agent, which inhibits the enzyme from leaching out of the electrode surface. The prepared biosensor (AChE/g-C_3N_4/MXene/ITO) for trichlorfon demonstrates an ultrasensitive response within the linear range 0.1 pM- 1 μM, featuring a low detection limit (LOD) of 0.16 pM. The constructed biosensor exhibited robust stability, anti-interference ability, satisfactory reproducibility, and enhanced sensitivity (7.79 μA $(pM)^{-1}$ cm^{-2}), which allowed the determination of trichlorfon in three real samples: carrot, guava, and fenugreek.

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ABBREVIATIONS

OPP	Organophosphorus Pesticide
g-C_3N_4	Graphitic Carbon Nitride
$Ti_3C_2T_X$	MXene
AChE	Acetylcholinesterase
ATCl	Acetyl thiocholine chloride
TF	Trichlorfon
ITO	Indium tin oxide
EPD	Electrophoretic Deposition
XRD	X-Ray Diffraction
FT-IR	Fourier Transform Infrared
SEM	Scanning Electron Microscope
EDX	Energy Dispersive X-Ray Spectroscopy
PBS	Phosphate Buffer Saline
CV	Cyclic Voltammetry
DPV	Differential Pulse Voltammetry
EIS	Electrochemical impedance spectroscopy
RSD	Relative Standard Deviation
LOD	Limit of detection

CHAPTER – 1

INTRODUCTION

1.1 Introduction to pesticides

Any material or combination of materials used to stop, eliminate, or manage pests such as insects, fungi, rodents, or undesirable plant species that harm crops during production and storage is referred to as a pesticide [1]. They stand out due to their diverse physical characteristics and chemical makeup. A lot of people confuse pesticide with insecticide. Insects are not the target pest for many pesticides, though. Insecticides, fungicides, rodenticides, herbicides, bactericides, etc., are a few examples. Additionally, pesticides contain repellents, attractants, and growth regulators in addition to toxicants [2]. Some common types of pesticides mentioned above are:

1. Insecticides: Insecticides are made to eradicate or manage insects. They may have a wider range of activity or target particular kinds of insects.

2. Herbicides: These are used to suppress or get rid of weeds or undesired plants. They may target particular plant species with selectivity.

3. Fungicides: Fungicides help plants avoid or cure fungal illnesses. To prevent fungal infections, they can be sprayed on seeds and the leaves of plants.

4. Rodenticides: Rats, mice, and other rodents can be prevented with the use of these pesticides. Usually, they are made as poisons to kill rats.

5. Bactericides: These are used to protect plants and animals from germs that might cause disease. They are frequently employed in the medical and agricultural domains.

1.2 Classification of pesticides

Majorly, pesticides are classified as chemical pesticides and bio-pesticides, based on their source of origin.

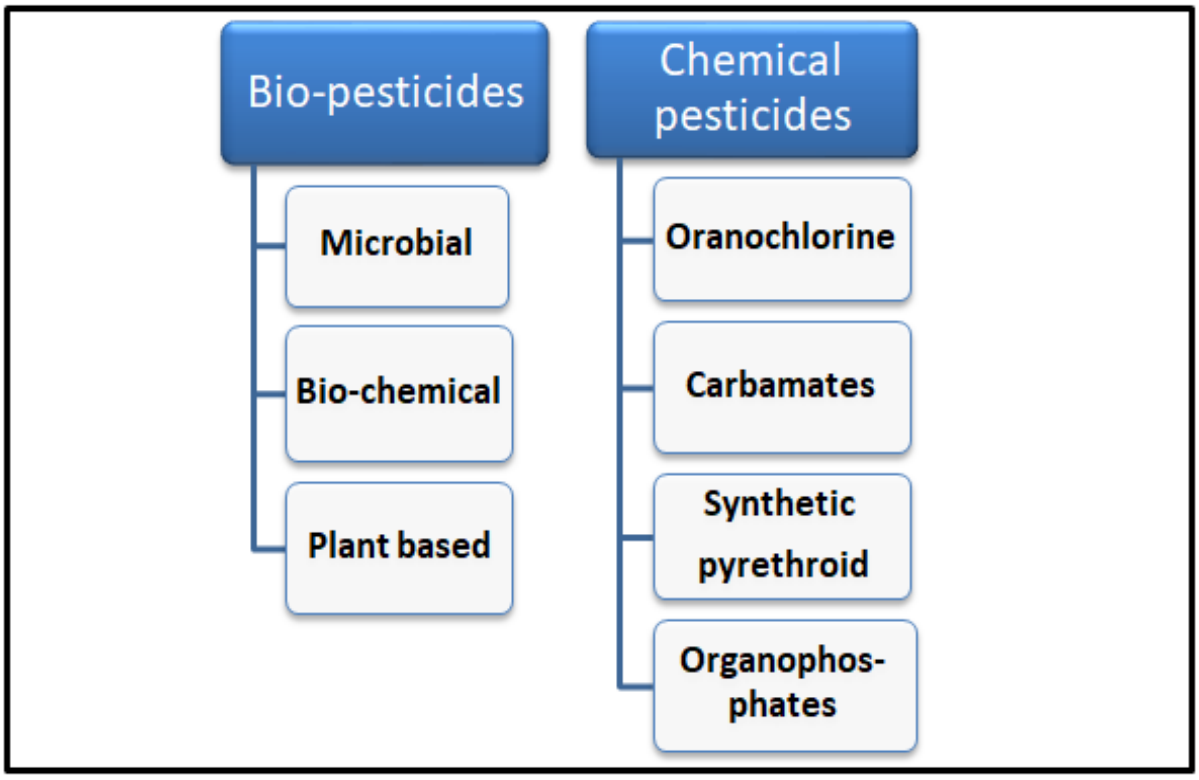


Fig. 1.1 Classification of pesticides

1.2.1 Bio-pesticides

Pesticides that are derived from natural resources, including animals, plants, and microorganisms (bacteria, viruses, fungi, and nematodes) are known as Bio-pesticides. Pesticides may be microbiological, plant-based, or biochemical [3]. Bio-pesticides offer a significant benefit as they are less sensitive to genetic change in plant populations. Bio-pesticides are significantly more advantageous than their counterparts, conventional chemical pesticides, because they are environmentally benign and specific for the host [4].

There are several forms of bio-pesticides, which are classed according to their sources from which they are extracted and the kind of molecule or compound employed in their synthesis [5].

1. Microbial pesticides: As the name suggests**,** these pesticides are derived from microbes like bacteria, fungi, and viruses. Bio-insecticides target insects that harm crops, whereas bio-herbicides use microorganisms like fungi to suppress weeds. Pseudomonas, Yersinia, and Chromobacterium are examples of bacterial entomopathogens, whereas fungi include Beauveria, Metarhizium, Verticillium, Lecanicillium, Hirsutella, and Paecilomyces [4].

2. Biochemical pesticides: The naturally occurring compounds used to control pests via harmless techniques are Biochemical pesticides. Biochemical pesticides are further categorized based on whether they regulate insect pest infestations via pheromones (semiochemicals) called Insect Pheromones, plant extracts/oils, or natural insect development regulators [5].

3. Plant-based pesticides: Plant extracts or oils include biological microorganisms, such as antimicrobials, bacteria, and viruses, that prevent pests from feeding or reproducing [6].

1.2.2 Chemical pesticides

Chemical pesticides originate through several chemical techniques to manage or remove pests. Chemical pesticides are poisonous and non-biodegradable, leading to significant environmental damage [1]. They have a broad range of effects on non-target creatures. They can pollute water, harm beneficial creatures like bees, birds, and fish, and disturb ecosystems [7].

These are further classified as

1. Organochlorine: Pesticides containing organochlorines, also called chlorinated hydrocarbons. These pesticides were among the first to be produced and employed for agricultural and public health. They damage the neurological system of insects, causing

convulsions, paralysis, and eventual death. Common organochlorine pesticides include DDT, lindane, endosulfan, aldrin, dieldrin, and chlordane [1] [8].

2. Carbamates: Carbamates are structurally similar to organophosphates; however, they have different origins. Organophosphates are generated from phosphoric acid, whereas carbamates originate from carbamic acid. They decay naturally with minimal environmental impact. Common pesticides in this category include carbaryl, carbofuran, propoxur, and aminocarb.

3. Synthetic pyrethroids: Synthetic pyrethroid pesticides are organic insecticides that mimic natural pyrethrin structures. They are less harmful and degrade faster. They are thought to be among the safest pesticides for use in food. Pyrethroid pesticides include deltamethrin, cyfluthrin, lambda cyhalothrin, and permethrin [9].

4. Organophosphates (Ops): Organophosphate pesticides have numerous functions and can effectively control a wide range of pests. These pesticides are disposable, generate minimal environmental contamination, and exhibit gradual pest resistance [10]. Ops are widely utilized in agriculture, public health, and veterinary applications. Common organophosphate pesticides include parathion, malathion, diazinon, and glyphosate.

1.3 Properties of organophosphate pesticides

1. Mode of action: Their work involves inhibiting the enzyme acetylcholinesterase (AChE), causing acetylcholine accumulation and nervous system malfunction in pests [11].

2. Broad spectrum of activity: They are efficient against a variety of insects and pests, such as mosquitoes, mites, and crop pests. Their broad-spectrum activity makes them useful for pest control across multiple fields.

3. Persistence: Some OPs are moderately to strongly persistent in the environment. These organisms can accumulate in soil, water, and agricultural resources due to their long-term activity. Their accumulation pollutes the environment and harms non-target organisms [12].

4. Health effects: OPs can harm the nervous system and are considered neurotoxic. They can bind to the serine hydroxyl group of the acetylcholinesterase (AChE) enzyme, inhibiting neurotransmission activity. Acetylthiocholine chloride (ATCl) is hydrolyzed by the AChE enzyme, which helps transfer nerve impulses. OPs include paraoxon ethyl, malathion, fenitrothion, chlorpyrifos, and trichlorfon [13].

5. Toxicity: Although very poisonous to insects, they can also pose dangers to humans, wildlife, and non-target creatures.

1.4 Positive outcomes from pesticide use

Pesticide use has several good benefits –

- Controlling agricultural pests, illnesses, weeds, and plant disease vectors.
- Controlling disease vectors and nuisance species can benefit humans and cattle.
- Controlling organisms that cause harm to human activities and structures [14].
- Removing unwanted vegetation and preserving endangered species from destructive pests [15].
- Pesticides used throughout the packaging and manufacturing process help to keep packaged goods and raw materials clean.

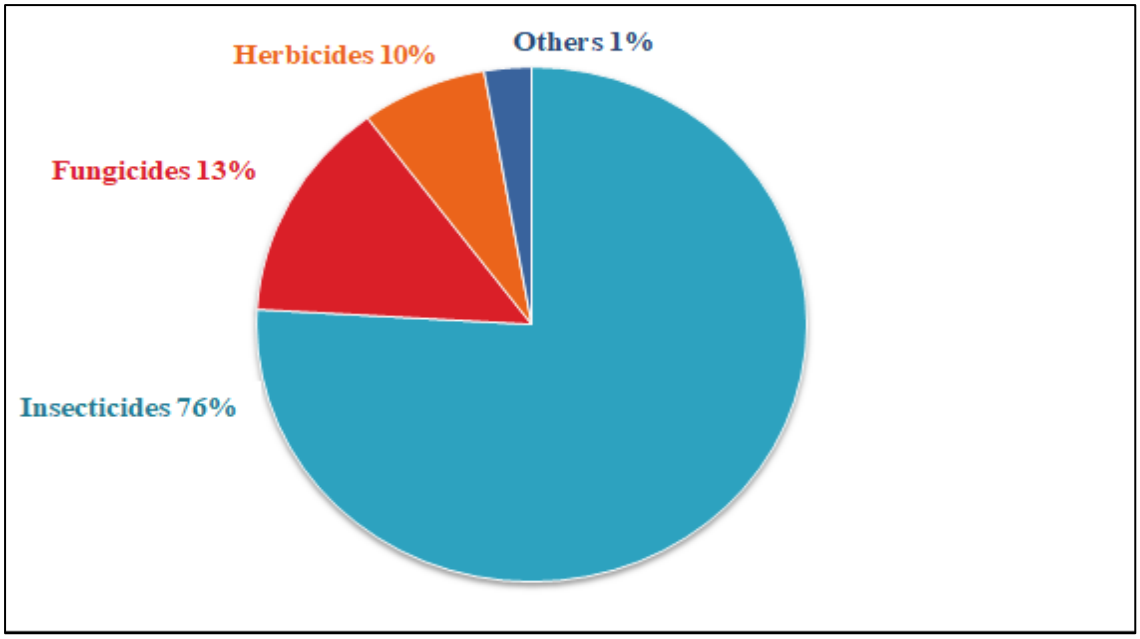


Fig. 1.2 Consumption pattern of pesticides in India

1.5 Impacts of pesticides on environment and human health

1.5.1 Impact of pesticides on environment

Excessive pesticide use can contaminate soil, water, turf, and vegetation. Pesticides can harm non-target creatures, including birds, fish, bees, beneficial insects, and plants [16].

1. Surface and ground water contamination: Water contamination is a worldwide issue. Pesticides can enter water sources through runoff and leaching from agricultural areas.

2. Soil contamination: The Organic matter level in soil is crucial for pesticide absorption. It was found that organic matter content directly correlates with pesticide. Pesticides, including Endrin, Endosulfan, Heptachlor, Lindane, Organochlorine DDT, and TPs, are banned in agriculture, yet residues can still be discovered in soil.

3. Contamination of air and impacts on non-target vegetation: Aerial pesticide application causes spray drift to enter the atmosphere, generating an imbalance in the air ecosystem [17].

1.5.2 Impact of pesticides on human health

Pesticides can enter the body through several methods, including inhaling polluted aerosols, dust, and vapours, ingesting contaminated food/water, and coming into contact with the skin. **Fig. 1.3** shows the adverse effects of pesticides on human health. Pesticides are doused on food, particularly fruits and vegetables, and they leach into groundwater and soil, causing both acute and chronic consequences on human health [18].

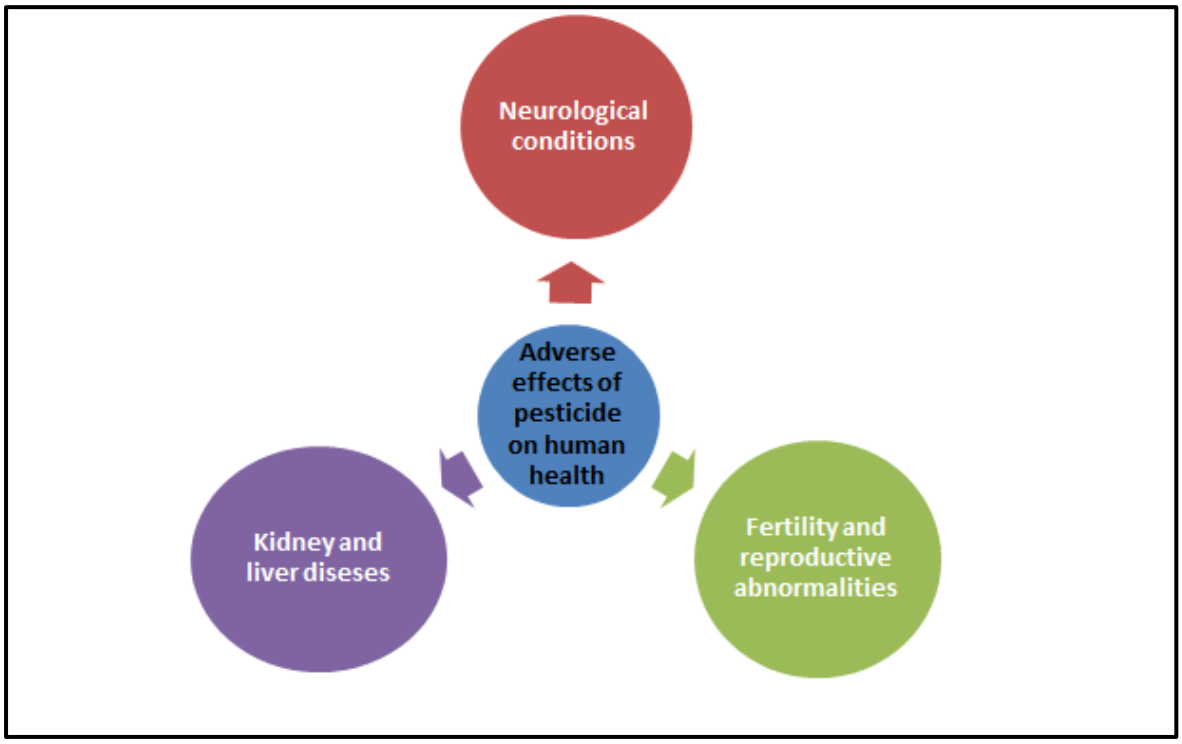


Fig. 1.3 Adverse effects of pesticides on human health

1. Acute effect: A single touch through any of the entrance sites, such as the mouth, eyes, lungs, or skin, causes acute consequences. Pesticide-related acute sickness symptoms include headaches, nausea, body aches, panic attacks, and excessive perspiration. Large doses of pesticides can lead to death [19].

2. Chronic effect: Chronic effects relate to any negative consequences that occur when pesticides are consumed regularly (even in very small amounts) over time, may impair the

immune system, and lead to a variety of chronic disorders [20]. Its principal symptoms, which occurred later, include genetic alterations, birth abnormalities, and deadly cancers [21]. **Fig. 1.4** shows the acute and chronic toxicity caused by pesticides [22].

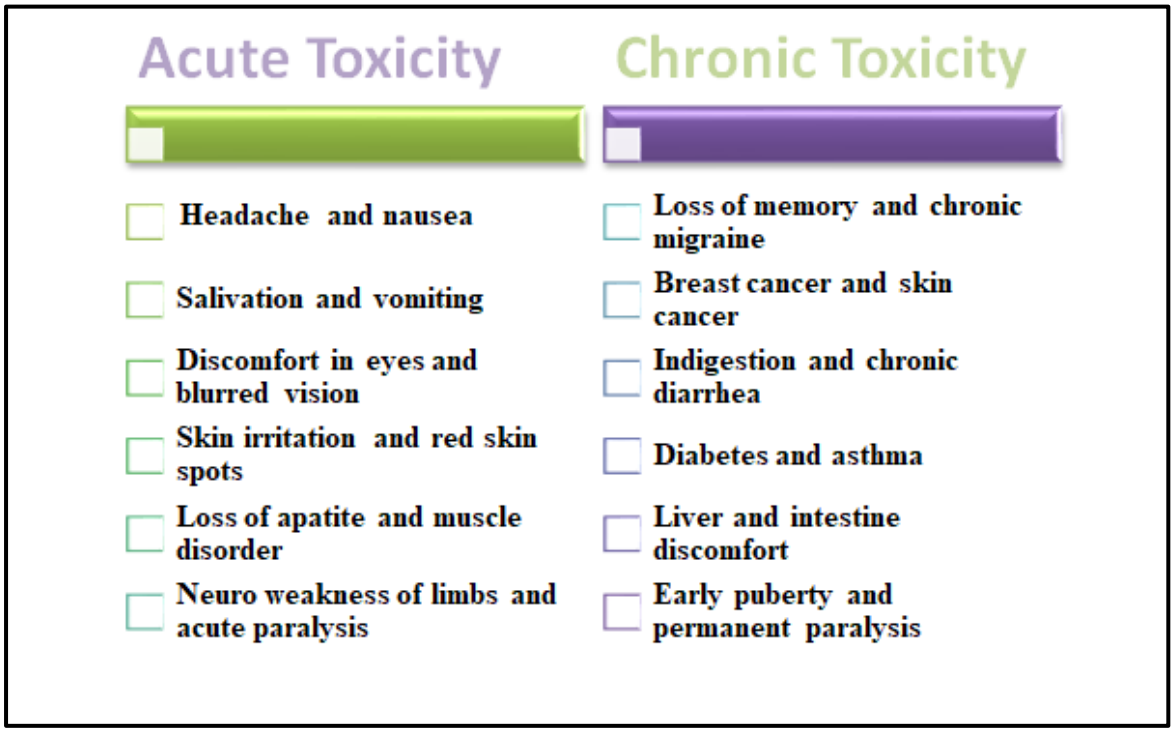


Fig.1.4 Acute and Chronic toxicity caused by pesticides

1.6. Techniques for the detection of pesticides

A technique for detecting pesticides is crucial for maximizing their advantages and minimizing associated health risks. A variety of ways have been devised to do this.

1.6.1 Conventional methodological approaches

Several classical approaches have been proposed in the literature, including gas chromatography [23], fluorimetry [24], immunoassay [25], liquid chromatography coupled mass spectroscopy [26], colorimetry [27], capillary electrophoresis [28], and spectrophotometry [23] for the detection of pesticides. These techniques are precise and selective, but require experienced, trained operators, are time-consuming, and require

expensive machinery. Because of these faults, these approaches can be replaced by exceedingly sensitive pesticide detection methods [29].

1.6.2 Advanced sensing technologies

To address the inadequacies in old techniques, Biosensors are used to detect biological molecules because they combine their specificity and sensitivity with the speed, mobility, and cost-effectiveness of sophisticated sensing technologies [30].

1.7 Biosensors

A biosensor is a specific form of bioelectronic device that is often used in biological analysis. A sensor is the principal part of a measurement chain, quantifying chemical and biological interactions by creating signals that correlate with analyte concentrations [31]. Modern biosensors have greatly improved in sensitivity, selectivity, and multiplexing capacity since the introduction of the Clark oxygen electrode sensor, demonstrated by Prof. Leland C. Clark, called the father of biosensors [32]. Any biological material, including enzymes, tissues, microorganisms, antibodies, and cell receptors, can form complexes with immobilized physiologically active compounds and targeted analytes. This specific and transient interaction generates secondary chemical or physical signals that can be detected by an electrochemical, optical, calorimetric, conductometric, or piezoelectric sensor [33]. They are widely used for environmental, food, and soil quality monitoring, disease detection, and veterinary applications [34]. A biosensor works on two core principles: "biological recognition" and "sensing." Thus, a bio-receptor, a transducer, and a signal processing system are the three primary parts of a biosensor. **Fig. 1.5** represents the basic principle of a biosensor.

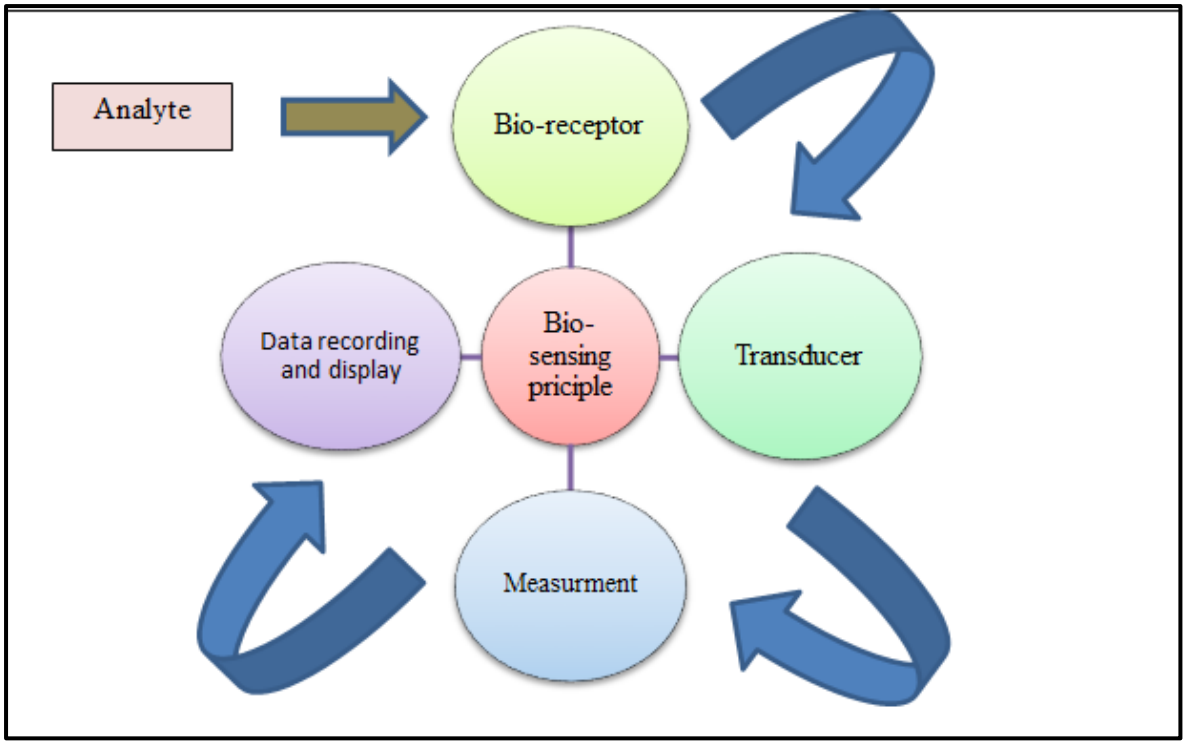


Fig.1.5 Basic principle of Biosensor

1.7.1 Components of Biosensor

A biosensor requires three main components to function properly. These include biomolecular recognition, biomolecule immobilization, and transducers. These Components are represented in **Fig. 1.6**.

1. Bio-receptor

Receptors are appealing for biosensing due to their versatile "receiving" and "sending" functions [35]. Bioreceptors are molecules like enzymes, antibodies, cells, and DNA that interact with analytes to produce a signal in the form of light, heat, etc. Bioreceptors commonly utilized in biosensors include enzymes, antibodies, tissue, organisms, and nucleic acids.

- **Enzyme-based biosensor:** In enzyme-based biosensors, enzymes are used as biocomponents, which are first exploited as a biorecognition element. Enzymes, which

are composed of protein biomolecules, catalyze processes to produce signals that are indirectly detected by biosensors via the production of enzyme-substrate complexes [36]. Enzyme biosensors are frequently employed because of their numerous potential uses, high sensitivity [37], high selectivity and rapid response. In enzyme biosensors, an enzyme is kept close to the sensor surface, and the enzymatic reaction determines the substrate concentration. It occurs through two distinct reaction processes: diffusion in the product enzyme layer and enzymatic conversion in the substrate. The analyte concentration is measured by detecting changes such as proton concentration, gas release and absorption, and so on [38]. The transducer transforms these changes into quantifiable impulses. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are commonly utilized as enzymes to detect pesticides [39].

- **Immunosensors:** Immunosensors work by interacting with certain target molecules (antigens) and antibodies. When an antibody interacts with antigens, a detectable signal is produced; this signal takes the form of a change in conductivity, mass, etc. Immunosensors have numerous applications, including the detection of cancer biomarkers, environmental surveillance, and food safety [40]. Immunosensors are sensitive and selective, but their limited shelf life and high cost make them less effective for detecting OPs.
- **Nucleic acid biosensor:** Nucleic acid biosensors are used to identify nucleic acid sequences (DNA and RNA). The interaction between analyte and DNA alters DNA's redox characteristics, specifically guanine base oxidation [41]. These biosensors are sensitive, but require extensive sample preparation and may be susceptible to deterioration.
- **Microbial biosensor:** These techniques include immobilizing microbial cells on a conducting surface. It is based on microbes' ability to recognize various chemicals in their

environment. These biosensors are preferred over chemical biosensors due to their affordability, sensitivity, and capacity to detect a wide range of target molecules in the form of light, color, electrical signals, etc. It has several disadvantages also- The reaction time of microbial biosensors, as well as the time necessary to return to the basic signal points, is long after use. One of the major difficulties during immobilization is contamination and diminished activity.

- **Lactate biosensor:** Lactate biosensor assesses the amount of lactate present in biological materials such as blood, sweat, or saliva, to show anaerobic metabolic activity. These biosensors typically incorporate a biorecognition component attached to a transducer substrate, such as lactate oxidase or lactate dehydrogenase. The recognition element and lactate combine to initiate a metabolic process that causes detectable changes in electrical conductivity, pH modulation, or fluorescence emission.

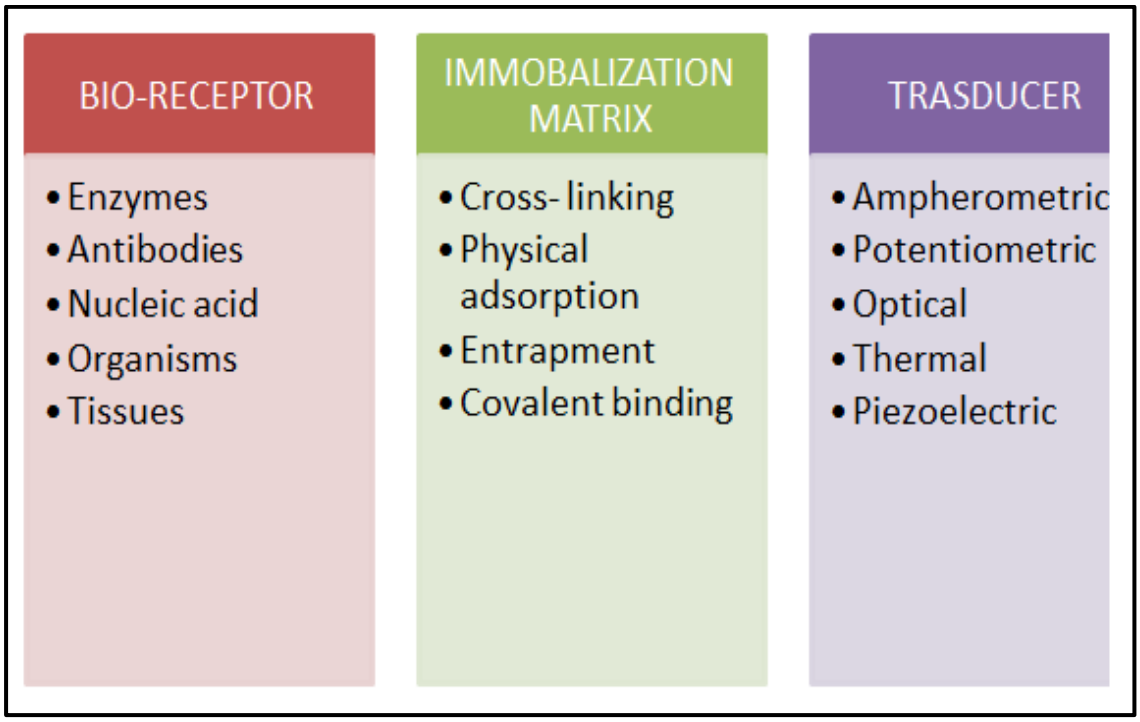


Fig. 1.6 Components of biosensor

2. Immobilization matrix

Matrix is a stable solid surface, such as glass, screen-printed electrodes, conducting and non-conductive polymer films, etc., to which biomolecules can bind. The process of connecting biomolecules to solid substrates in order for them to remain active for an extended period is referred to as immobilization [42]. It enhances biomolecule functioning and facilitates access to analytes. The ideal solid support for biosensors should have qualities such as inertness, bio-affinity, hydrophilicity, and low cost to improve electron kinetics and performance [43]. The chemical properties of bio-receptors and the intended solid surface determine the type of immobilization (e.g., covalent bonding, adsorption, entrapment, cross-linking) [44]. When choosing the type of immobilization, attention should be given to making sure that the reactive group of the enzyme's binding site remains unaltered and no enzyme activity is lost.

- **Cross - linking:** Compounds with two or more functional groups capable of binding two different materials under unique conditions are employed in the cross-linking process [42]. This approach results in less loss of biocatalyst and is cost effective. However, Reagents with bifunctional or multifunctional properties are commonly employed. The most widely utilized substance is glutaraldehyde. Reagents used can modify enzymes and biomolecules' active sites. It requires specific conditions, making them not appropriate for all biomolecules and enzymes.
- **Physical adsorption:** This method involves physisorption a biomolecule onto a matrix surface. As a result, the approach makes no or only minor changes to the enzyme's structure. Adsorbed molecules have lesser loading capacity compared to other immobilization approaches. The binding of the biomolecule requires weak forces such as hydrogen bonding and van der Waal force therefore the adsorbed molecules have a high possibility of separating from the support.

- **Entrapment:** In entrapment (encapsulation), enzyme is trapped in the porous polymer matrix or sol-gel matrix [45]. The enzyme layer restricts the biomolecules in the matrix, allowing only the desired biomolecules to remain within it. This approach has a huge diffusion barrier and a significantly long response time.
- **Covalent binding:** Enzyme covalent binding to polymeric supports is widely used for developing biosensors. Biocatalysts bind to surfaces using non-essential functional groups such as amino, carboxylic, indole, etc. Enzyme binding to a solid support involves activating the surface with multifunctional reagents (e.g. glutaraldehyde or carbodiimide), coupling the enzyme, and removing superfluous biomolecules [46]. This approach results in no enzyme loss due to the strong binding force between the enzyme and carrier. Even in difficult circumstances, it remains stable.

3. Transducer

The transducer recognizes and translates the interaction between analyte and biological sensing element into an electrical signal that can be measured. Electrical and optical signals are proportional to interactions between the biorecognition element and the analyte. Depending on the transduction mechanism, the biosensors are classified as follows [23]:-

- **Amperometric biosensors:** Amperometric biosensors measure the electric current or potential on a transducer caused by electroactive materials' chemical reactions. Amperometric biosensors use an oxidoreductase enzyme to transport electrons to the surface of an electrode. This reaction results in an electrochemical current. The current generated by a faradic reaction in amperometry is directly proportional to the concentration of the analyte. Amperometric enzyme electrodes are commonly used for medical diagnosis, drug discovery, food safety, and environmental monitoring due to their excellent sensitivity and selectivity [47].

- **Potentiometric biosensor:** Potentiometric biosensors assess the potential difference between electrodes due to the redox reaction between analyte and bioreceptor. The essential component of a potentiometric measurement is an indicator electrode, whose potential is a function of the activity of the target analyte [48] shown by equation below-

$$\mathbf{E = E_0 \pm (RT/nF) \ln a_1}$$

When the standard electrode potential (E_0) is 1 mol/L, the activity (a_1) is one. Here, T -: Kelvin temperature, R -: gas constant, F -: Faraday constant, and n -: total number of ions' charges. Anions and cations are represented by the symbols ±, -, and +. Their uses include pH, gas, and ion sensing.

- **Optical biosensors:** They rely on basic optical concepts like reflectance, fluorescence, interference, and polarization. Sensitive layers, made of chemical or biological ('receptor') material, are an important component of sensor systems [49]. Because optical sensors do not require a reference signal and are easy to build with high efficiency, optical fiber biosensors are beneficial in biochemical and clinical analysis [50].
- **Piezoelectric biosensors:** Piezoelectric biosensors detect analytes by affine interactions, requiring no additional reagents. Piezoelectric biosensors can operate in multiple modes, but direct, label-free interaction with analytes maximizes the platform's advantages [51]. The biosensor detected chemicals that block the enzyme acetylcholinesterase. Piezoelectric biosensors are gaining prominence in modern bioanalysis, particularly for diagnosing macromolecules.
- **Thermal biosensor:** Thermometry is essentially the measuring of temperature. These biosensors use biological responses to detect heat absorption or evolution. These devices measure temperature changes in circulating fluids when a substrate reacts with immobilized enzyme molecules [52]. It is used in clinical diagnostics, environmental

management, and antigen-antibody interaction determination (thermometric Elisa test). However, one disadvantage of the procedure is that the instruments are expensive.

1.8 Features of a Biosensor: For biosensors to be used in a variety of fields, they must have certain features shown in **Fig. 1.7**

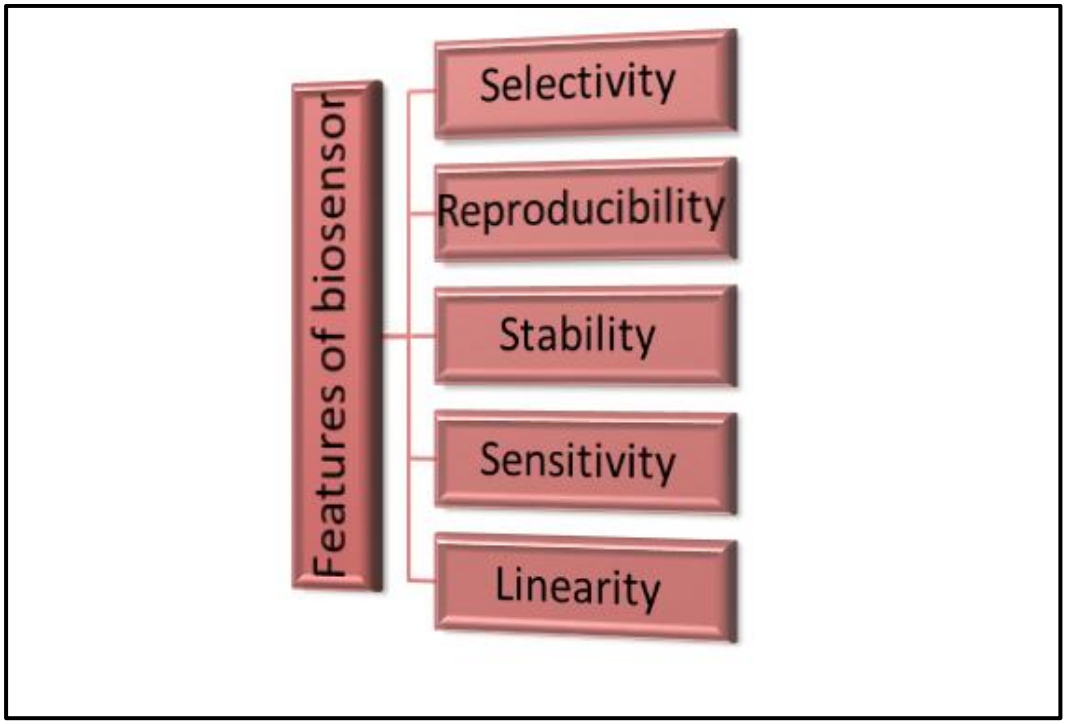


Fig. 1.7 Features of biosensors

1. Selectivity: Selectivity refers to a biosensor's ability to recognize and measure a specific analyte in a sample that may also include other chemicals, such as pollutants and impurities. The connection between an antigen and an antibody illustrates the selectivity in biosensors. Antibodies are often linked to the surface of the transducer and function as bioreceptors. Following that, the transducer is exposed to a solution, often a salt and antigen buffer, in which antibodies selectively bind to antigens. When constructing biosensors and selecting bioreceptors, selectivity should be prioritized [53].

2. Reproducibility: Reproducibility in biosensors refers to their capacity to deliver consistent results when repeated studies are done. Biosensor precision and accuracy are what define it. Consistent signals enhance the reliability and durability of inferences formed from biosensor responses.

3. Stability: It describes how sensitive the biosensing system is to external disturbances in its surroundings since these disturbances change the output signal measurement and the level of accuracy and precision of the proposed biosensor. The affinity of the bioreceptors is an important additional element affecting stability. Affinity is the degree to which bioreceptors and the analyte interact. Strong covalent or electrostatic connections are formed between bioreceptors and analytes when affinity is high.

4. Sensitivity: The ability of a biosensor to recognize and differentiate a molecule of interest from other elements in the sample is known as sensitivity [54]. Biosensors can detect the analyte in a very low range of concentrations, that is, ng/mL or fg/mL, which is known as the limit of detection or sensitivity, in both environmental and healthcare surveillance applications. It confirms that an analyte is present in a sample.

5. Linearity: Linearity is the feature that allows a measured response to a straight line at a varied analyte concentration to be linear. In mathematics, the straight line is represented by the formula y = mc, where m stands for sensitivity, c for analyte concentration, and y for output signal. The term "linear range" refers to the range of analyte concentrations for which the biosensor response varies linearly with the concentration [55].

1.9 Applications of Biosensors: Biosensors are employed in many domains [53]:-

- **In medical field:** Research on biosensors is gaining a lot of attention because of its potential applications in the pharmaceutical, clinical, biomedical, and healthcare

industries. A protein compound called cardiac troponin, which detects heart damage and potentially diagnoses acute coronary syndrome, can now be detected by biosensors integrated into mobile units. The diagnosis of diabetes is frequently made using glucose biosensors. They are also used in the diagnosis of infectious diseases [56]. Biosensors enabled by nanomaterials are employed to detect COVID-19. Numerous chances for the design and development of biosensors utilizing nanomaterial-based mechanisms will arise in order to handle the operation of numerous intricate medical issues [57].

- **In food industry:** The food sector prioritizes quality control, necessitating quick means for monitoring food quality. Conventional procedures are costly, laborious, and lengthy. Designing effective sensors will expedite the procedure and result in cost savings.[58]. Biosensors can detect lipids, alcohol, cholesterol, glutamate, glucose, and lactate. Biosensors are used in the detection of foodborne pathogens. Foods like milk and yogurt can have their glucose and lactate levels measured using biosensors [59].
- **In environment protection:** It determines the levels of pesticides, fertilizers, and heavy metals in the soil and water, as well as the causes of soil illness. In water bodies, biosensors identify halogenated chemicals, algal RNA, and toxic algal blooms [60]. By determining the concentration of dangerous contaminants, they aid in pollution monitoring.

1.10 Future scope of biosensors

With anticipated improvements in sensitivity, specificity, downsizing, and integration with other technologies such as artificial intelligence and microfluidics, the future of biosensors appears bright and expansive. Future biomarker techniques will look into the creation of multiple options for manufacturing precision diagnostics, drugs, and equipment [61]. It will allow scientists to closely track the effects of potential medications on the body and determine whether they have the potential to improve medicine. In the early stages of

development, biosensor chip technology may also be incorporated into the body to detect complex changes in blood DNA before disease manifests. In this area, a number of sensors that rely on nucleic acid hybridization detection are making fascinating advancements. Monitoring of contaminants, hazardous metals, and pesticides is another primary goal.

Due to their extensive use in medical and healthcare processes, biosensors are becoming more and more in demand. Biosensors have also improved in a number of areas, such as human health management, disease detection, patient wellness tracking, and diagnosis.

1.11 2-D based nanocomposite

Creation of nanocomposites has led to advancements in material science. Composites, often known as complex materials, are created by combining two or more basic elements that have unique physical or chemical properties. The creation of new composites based on cutting-edge 2D nanomaterials has made it possible to accomplish amazing feats with yet undiscovered applications [62].

More than one thin layer makes up two-dimensional (2D) materials. Weekly coupling between these layers occurs via van der Waals interactions. The thickness of a single-atom-thick layer is typically a few nanometres. The layers of two-dimensional materials allow electrons to travel freely. Examples of 2-D materials include transition metal dichalcogenides, graphene, hexagonal boron nitride, and g-C_3N_4 [63]. They exhibit outstanding rigidity, flexibility, electrical conductivity, and mechanical strength. 2D composites are classified into carbon, graphene, polymers, ceramic, metal, and bio-based materials.

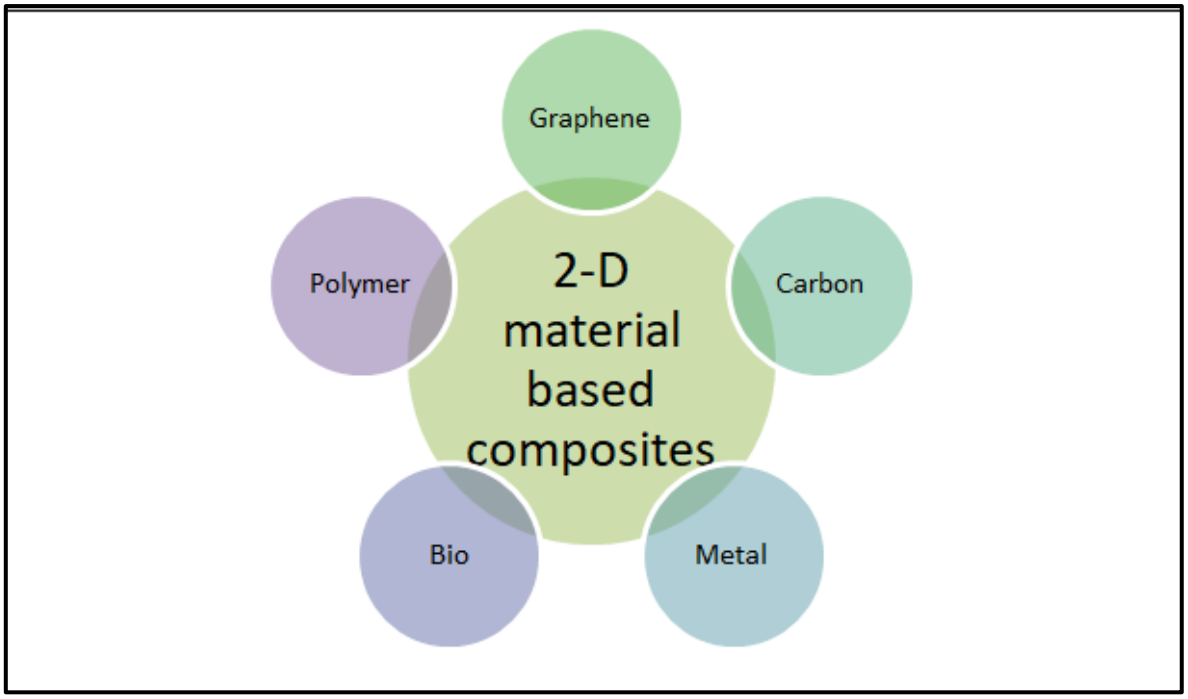


Fig. 1.8 Classification of 2-D materials-based composites

1.11.1 Properties of 2-D nanocomposite

Two-dimensional (2D) materials offer numerous options to develop multifunctional features [64]. The following section primarily discusses the mechanical, electrical, thermal, optical, and barrier characteristics of nanocomposites reinforced with two-dimensional materials.

1. Improved Mechanical properties: Because of the nanocomposite's enormous surface area, which reinforces the matrix, they exhibit better strength and toughness when compared to other conventional materials [65].

2. Enhanced thermal stability: Nanoparticles can be added to composite materials to increase their thermal stability and resistance to damage from heat. Particularly useful for applications involving high temperatures is this capability [66].

3. Improved electrical conductivity: Depending on the type of nanoparticles employed and how well distributed them are inside the matrix, nanocomposites can have better insulation or

electrical conductivity. As a result, they are suitable for a wide range of electronic applications [67].

4. Enhanced optical properties: Certain optical characteristics, including enhanced transparency, fluorescence, or light scattering, can be displayed by nanocomposites based on the nanoparticles incorporated into the matrix [68].

5. Barrier properties: Nanocomposites can provide superior protection against gases, moisture, and other environmental factors due to their high surface area and the fragility of the regions within the matrix that contain nanoparticles.

1.11.2 Applications of 2-D nanocomposites

1. Structural materials: Nanocomposites can be used to enhance the mechanical properties of metals, ceramics, and polymers. By adding nanoparticles, these materials become stronger, more resilient, and more suitable for use as building materials, vehicle parts, and aerospace components [69].

2. Packaging: Nanocomposites assist in increasing the shelf life of perishable goods by fortifying food packaging's barrier properties and blocking the passage of oxygen, moisture, and other contaminants [70].

3. Electronics: Nanocomposites are utilized to make conductive materials with improved thermal and electrical conductivity. In addition to having potential for sub-5nm technology, these materials are well-suited for use in flexible electronics, sensors, and energy storage [71].

4. Energy Conversion and Storage: Nanocomposites are widely used in energy storage devices such as batteries and supercapacitors. They are used in gasoline as well.

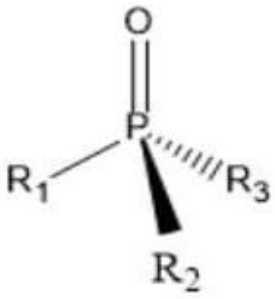
CHAPTER 2

Literature Review

2.1 Overview of Organophosphate Pesticides

Organophosphorus pesticides (OPs) are a class of (thio) phosphate compounds that are extensively used in agricultural production. They control agricultural diseases and improve the quality and yield of agricultural products. They are primarily used as pesticides, but they are also occasionally used as herbicides, fungicides, and plant growth regulators [72]. The typical OPs are trichlorfon, dichlorvos, paraoxon, glyphosate, malathion, and chlorpyrifos [73]. In addition to their positive impacts on agriculture, OPs have harmful toxicological consequences on both human and animal populations [74]. Therefore, new pesticides are required. The toxicity of pesticides varies, and they can be classified as harmful, common substances, or poisonous.

Organophosphate pesticides have the following structure:



The widespread usage of these OP chemicals leads to contamination of water supplies, fruits, vegetables, and processed foods has a negative impact on the health of many non-target creatures, including humans, fish, and birds [75]. The reason for toxicity of OPs is that they

inhibit the enzyme acetylcholinesterase (AChE), which is necessary for the central nervous systems of insects and mammals to operate. This causes acetylcholine (Ach) to build up, which disrupts muscle contractions, impairs heart and respiratory function, and can even be fatal [76]. Hence, this indicates it is now more crucial than ever to determine the trace levels of OPs quickly, effectively, and reliably for the protection of public health and safety, and related research is ongoing [77].

In humans, OPs induce four neurotoxic diseases. These syndromes include:

1. The cholinergic syndrome - Cholinergic syndrome symptoms, which are expected due to the molecular mode of action of OP pesticides and significantly correspond with levels of acetylcholinesterase (AChE) activity, can be brought on by acute poisoning with these pesticides [78]. General severe symptoms of peripheral and nicotinic poisoning are readily apparent in human poisoning scenarios. Sweating, tremors, lacrimation, abdominal cramps, and other gastrointestinal symptoms are some of these symptoms. Central effects like headache, tiredness, dizziness, and paraesthesia follow these symptoms. Furthermore, convulsions, coma, and seizures are all possible.

2. Intermediate Syndrome - The most common sign of intermediate syndrome (IS), which manifests 1-4 days following poisoning, is weakening of the cranial and respiratory muscles [79]. Despite being classified as a neuromuscular junction condition, the precise risk factors, etiology, and incidence of intermediate syndrome remain unclear. The majority of people affected by this syndrome do not have cholinergic symptoms [80]. IMS usually happened to individuals who had acute and severe AChE inhibition, which resulted in a persistent excess of AChE at the neuromuscular junctions, even not all patients were affected.

3. Organophosphate Induced Delayed Polyneuropathy (OPIDP) - The rare toxicity known as organophosphate-induced delayed polyneuropathy (OPIDP), which is caused by

exposure to specific OP esters, is characterized by the distal degeneration of some peripheral and central nervous system axons that happens 1-4 weeks after single or brief exposures [81]. Distal numbness, paraesthesia, and cramping pain in the lower limbs are followed by increasing weakening and a depression of deep tendon reflexes in the lower limbs and, in more severe cases, the upper limbs. Indications include pyramidal signs, high-stepping gait accompanied by bilateral foot drop, and in extreme situations, quadriplegia with foot and wrist drop. Although there may be a notable improvement of peripheral nerve function over time, spastic ataxia may be a permanent consequence of severe OPIDP, dependent on the extent of pyramidal involvement [82].

4. Chronic organophosphate-induced neuropsychiatric disorder (COPIND) - People subjected to high concentrations of organophosphorus chemicals exhibited a risk for specific neurobehavioral alterations, which have been collectively referred to as COPIND. Sleepiness, bewilderment, lethargy, anxiety, emotional instability, melancholy, exhaustion, and irritability are some of these side effects [83]. COPIND is independent of AChE inhibition and manifests without cholinergic symptoms [84]. COPIND typically develops slowly and persists for a long time, which may be a sign of an irreversible central nervous system ailment [85].

2.2 The requirement and significance of organophosphate pesticide detection

1. Posing threat to the environment - Although organophosphates (OPs) are essential to contemporary agriculture, overuse is causing their pesticide residues to leak into the soil, build up in the groundwater, and pollute both terrestrial and aquatic food webs, posing a threat to the environment [86].

2. Adverse effects on humans, plants, and ecosystems - Excessive pesticide use not only pollutes the environment (water, rivers, soil, etc.), but it can also accumulate in people and

animals over time through sources like crop surface residues, groundwater infiltration, and surface water flow, leading to harmful effects [87]. The way the organophosphate chemical functions is by blocking the acetylcholinesterase (AChE) enzyme, which is a vital and essential enzyme for nerve impulse induction. When AChE is inhibited, acetylcholine accumulates, which further depolarizes the exposed organism permanently, causing convulsions, respiratory arrest, and eventually death [88]. Furthermore, it has been noted that OPPs impact human health in both the embryonic and adult phases, increasing morbidity and mortality in chronic poisoning patients [89].

3. Causes harmful diseases in the human body - Organophosphorus insecticides can be either chronic or acutely toxic to humans, depending on how long the symptoms are present and how long the exposure occurs. Skin conditions that might result from it include contact dermatitis, erythema multiforme, porphyria cutanea tarda, occupational acne, ashy dermatosis, nail and hair disorders, and contact urticaria. The incidence of multiple myeloma and non-Hodgkin lymphoma leukemia has been observed to be on the rise among communities exposed to pesticides, and it has also been connected to the prevalence of cancer in those populations. Solid tumors, haematological malignancies, and genotoxic consequences are also linked to them. It affects the nervous system, which can result in neurological conditions like Parkinson's disease. They have detrimental effects on the reproductive system as well. Among these impacts are reduced fertility, birth abnormalities, fetal mortality, and delayed intrauterine growth [90]. Thus, it is crucial to develop methods for quickly estimating the degree of grain contamination with pesticide residues and the threat these residues pose to human and animal health in order to safeguard the ecosystem and ensure the quality of food [91] .

2.3 Literature Review

Nanomaterials have proven to be extremely appealing. in impacting the broad domain of biological sensing [92]. Target biomolecules may interact effectively with nanomaterials because of their minuscule sizes and appropriate surface modifications. Many types of nanomaterials, such as metal, carbon, magnetic, up-conversion, and quantum dots, have been successfully used to create a variety of biosensors due to their superior physicochemical and magnetic qualities, high electronic conductivity, and increased surface area to volume ratio [93]. Therefore, a comparison has been made between the current work and the analytical performance of the created electrode reported in earlier investigations.

Table 2.1 Recent developments in the analytical efficiency of electrochemical biosensors for trichlorfon detection

S.No.	Fabricated electrode	Detection methods	Linear range	LODs	Ref.
1.	AChE/g-C_3N_4@MoS_2/ITO	DPV	5 nM–100 nM	2.1 nM	[94]
2.	CHIT/Au electrode	SGGT	300-3000 nM	10 nM	[95]
3.	Nafion-AChE/PB-DSPE	CA	0.1–5 μg/mL	0.1 μg/mL	[96]
4.	AChE/Ag@CuO/ITO	CV	5-35 nM	1.59 nM	[97]
5.	AgNPs	Colorimetric	5–100 nM	5.46 nM	[98]
6.	Poly(FBThF)/Ag-rGO-NH_2/AChE/ GCE	Amperometry	0.0206–2.06 μg L^{-1}	0.001 μg L^{-1}	[99]
7.	MWCNT@Au-g-PMAEFc/GCE	DPV	50 nM–10 μM	27 nM	[100]
8.	MHPBC/GCE	DPV	0.1 nM–10 μM	35 nM	[101]

2.4 Characteristics of the material

Finding new materials is necessary to create a biosensor with great performance. Nanomaterials comprising large surface areas, electrical conductivity, and biocompatibility are thought to be appealing for the creation of biosensors. Enzyme immobilization on nanomaterials can improve biosensor performance, stability, and functionality. As a result, different nanomaterials have been applied to the electrode in order to create enzyme biosensors, creating a novel way to boost the biosensors' signal [102]. Out of these nanomaterials, the two-dimensional (2D) layered nanomaterials show promise for enhancing biosensor performance due to their exceptional physical and chemical characteristics, including superior biocompatibility, extensive specific surface areas, and favourable electronic transport characteristics [103].

2.4.1 Graphitic carbon nitride (g-C_3N_4)

Graphitic carbon nitride (g-C_3N_4) is well known in the scientific world as the most reliable of seven carbon nitride compounds under normal scenarios [104]. Graphitic carbon nitride, or g-C_3N_4, is a synthetic polymer made up of C, N, and a trace amount of H that are joined by patterns based on tris-triazine. It has basic surface functions, H-bonding motifs, and electron-rich properties due to the presence of N and H atoms that set it apart from most carbon materials [105]. A typical g-C_3N_4 structure has π-conjugated aromatic plane layers that are kept together by weak vander Waals forces, much like graphite [106]. The 2-D π layer structure of g-C_3N_4 is made of tri-s-triazine, which is endowed with a unique electronic structure by the lone pair of nitrogen. Being an n-type semiconductor, g-C_3N_4 's tunable band gap offers a flexible channel that not only allows for the achievement of both controllable lowest unoccupied molecular orbitals (LUMO) and highest occupied molecular orbitals (HOMO), that have a significant impact on the functional layer's photoelectronic performance, but also makes modification easier, primarily through elemental doping or

heterojunction structure construction coupling with other semiconductors, thereby expanding its range of applications [107]. The inherent peroxidase activity of g-C_3N_4 nanosheets is further increased upon binding to biomolecules. This characteristic supports its application as a matrix to render bio-receptors immobile [108].

In addition to having good physicochemical stabilization, g-C_3N_4 has an attractive electronic framework with a medium band separation of 2.7 eV, which is a result of its heptazine ring structure and significant condensation extent. These special qualities make g-C_3N_4 appealing for a variety of uses, such as photocatalytic water splitting in the presence of visible light and the photodegradation of organic contaminants in the environment. Despite being widely used as a catalyst support and a metal-free catalyst, g-C_3N_4 's other possible uses have not been thoroughly investigated [109].

The incredible durability, less toxicity, great fluorescence quantum yield, impressive biocompatibility, and distinctive photoelectrochemical and electroluminescent characteristics of g-C_3N_4-based materials make them attractive options for utilization in biosensors [110].

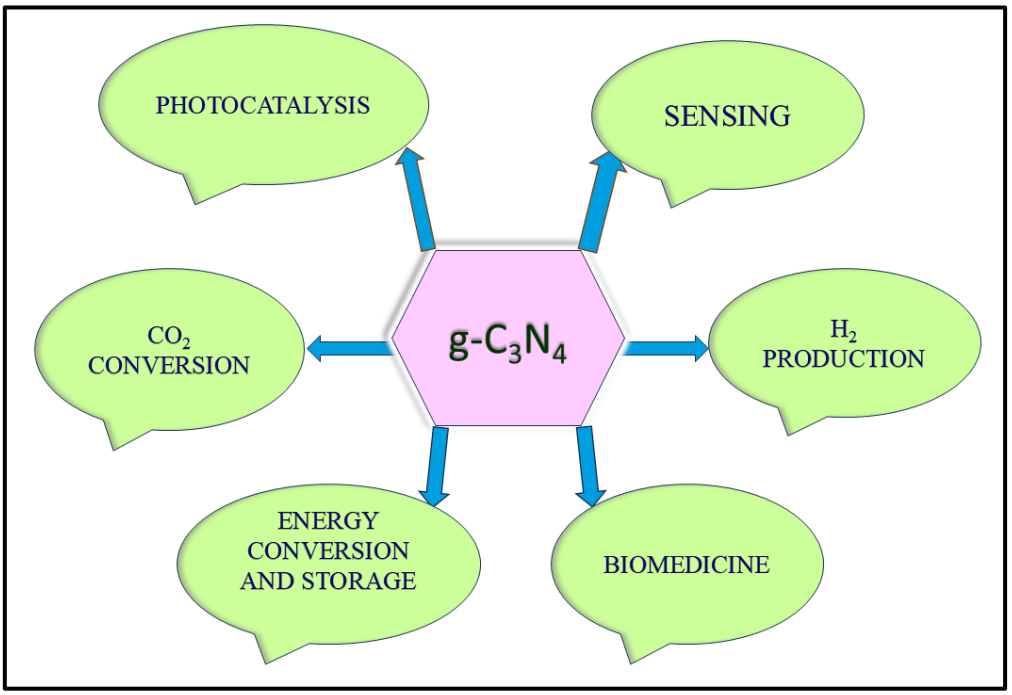


Fig. 2.1 Schematic illustration of applications of g-C_3N_4

2.4.2 Applications of g-C_3N_4

1. Biosensor

g-C_3N_4 nanosheets exhibit excellent thermal stability and high chemical stability because of the graphitic graphene-like structure, which has sp^2 bonds between carbon and nitrogen. Excellent electronic band structure, amazing physicochemical stability, huge specific surface area, ease of hydrogen bonding, extraordinary thermal, electrical, mechanical, and optical capabilities, and simplicity of surface functionalization have made it a valuable and superior substance for sensing material that has garnered a lot of interest in the design of electrochemical sensors and biosensors [111].

2. Energy conversion and storage

In the field of energy conversion and storage, graphitic carbon nitride (g-C_3N_4) has garnered significant interest on account of its distinct layered structure, metal-free nature, great physicochemical stability, adjustable bandgap, and accessibility. The short charge/mass transfer path, large number of reactive sites, and ease of functionalization of 2D g-C_3N_4 nanosheets are advantageous for maximizing their performance in several domains [112].

3. As a photocatalyst

Nowadays, graphitic carbon nitride (g-C_3N_4) is a very good polymer photocatalyst. On the one hand, it can absorb some visible light, decompose water into H_2 and O_2, and reduce CO_2 into substances that are high in energy to address energy concerns; It can break down a wide range of organic and inorganic contaminants into neutral compounds due to its modest 2.7 eV band gap and the appropriate placement of the valance band (VB) and conduction band (CB) at 1.3 and −1.4 eV, respectively. Under extreme heat and chemical conditions, its thermal and chemical stabilities make it very appealing [113].

4. In Biomedicine

Furthermore, g-C_3N_4 has exceptional biocompatibility and distinctive fluorescent properties, which render its employment in diagnostic imaging and biosensors. Light can activate g-C_3N_4 to generate reactive oxygen species (ROS). Additionally, it can operate as a drug carrier by π–π conjugation, which makes it useful in the fields of antibacterial, drug delivery, and photodynamic treatment [114]. Other advantages of g-C_3N_4 over other biological materials include its adaptable solubility and photoelectric characteristics, as well as its compatibility with other materials by surface functional group modification [115].

2.4.3 MXene

MXene, also known as two-dimensional (2D) transition metal carbides, nitrides, or carbonitrides, is a family of innovative nanomaterials that have shown promise in energy storage, sensing, environmental remediation, and catalysis. Their outstanding structural qualities, such as their excellent adsorption-reduction capacity, biocompatibility, large specific surface area, hydrophilicity, wide interlayer spacing, amazing chemical stability, simple process of functionalization, and active metallic hydroxide sites, are what make them the best candidates for a variety of applications [116].

MXene is typically made by a top-down process based on chemical exfoliation and vacuum filtering from its parent phase MAX material (M for early transition metal, A for element from group 12 or 16, and X for carbon and/or nitrogen). Presently, more than 30 MXenes have been effectively synthesized based on the chemical formula $M_{n+1}X_nT_x$ (n = 1–4), where T represents the chemical functional groups such as fluorine (-F), hydroxyl (-OH), chlorine (-Cl), and oxygen = O that are added to the MXene surface during chemical exfoliation [117]. The M and A atoms in the MAX phase have a poor metallic link, while the M and X atoms have a strong covalent, ionic, and metallic mixed bond. In order to produce lamellar-

structured MXene materials that are abundant in functional groups, the weak M−A link is broken during the etching process, the A atom is dissolved, and many functional groups are generated on the surface of the outer M atom [118].

The oldest and most well-researched MXene is $Ti_3C_2T_x$. Ti and C layers are arranged alternately in the structure of $Ti_3C_2T_x$, following exfoliation from the MAX phase. $Ti_3C_2T_x$ exhibits almost 100% photothermal conversion efficiency and the maximum electrical conductivity of nearly 24,000 S cm^{-1} [119]. Furthermore, it is highly processable due to its ability to dissolve in water and a variety of polar organic solvents. Numerous applications in advanced fields are made possible by its special qualities [120]. Due to their outstanding intrinsic conductivity, exceptional mechanical qualities, and chemically active surfaces, materials from the MXene class have found widespread application in electromagnetic interference (EMI) shielding, microwave absorption (MA), gas separation, energy storage, and sensors [121].

MXene, however, have the shortcomings of ultrathin 2D materials, specifically their lack of limited porosity structure and strong susceptibility to restack, that can reduce the number of accessible surface active sites, hinder the movement of ions, guests, and electromagnetic waves, as well as restrict the efficient loading with additional functional materials, resulting in undesired characteristics [122]. Its abundance of surface groups renders it extremely vulnerable to oxidative deterioration. It has a rapid reaction with dissolved oxygen, which restricts its use as a functional material and complicates hydrothermal processing [123].

It has gathered a lot of interest compared to their other 2D counterparts due to their greater specific surface distribution, high electrical conductivity, improved surface mechanism that is attributed to their substantial functionalization potentials, readily dispersed in solvents (like water), and appealing electrochemical features that make them appropriate for energy storage

devices and relatively conductive. A wide range of applications, including bioimaging/sensing, regenerative medicine, tissue engineering, supercapacitors, battery technology, conductive coatings, desalination/water treatment triboelectric nano-generators, gene drug delivery, cancer theragnostic and more, have been investigated for these materials because of their exceptional mechanical qualities, unique architectures, and capacity for reduction/oxidation reactions [124].

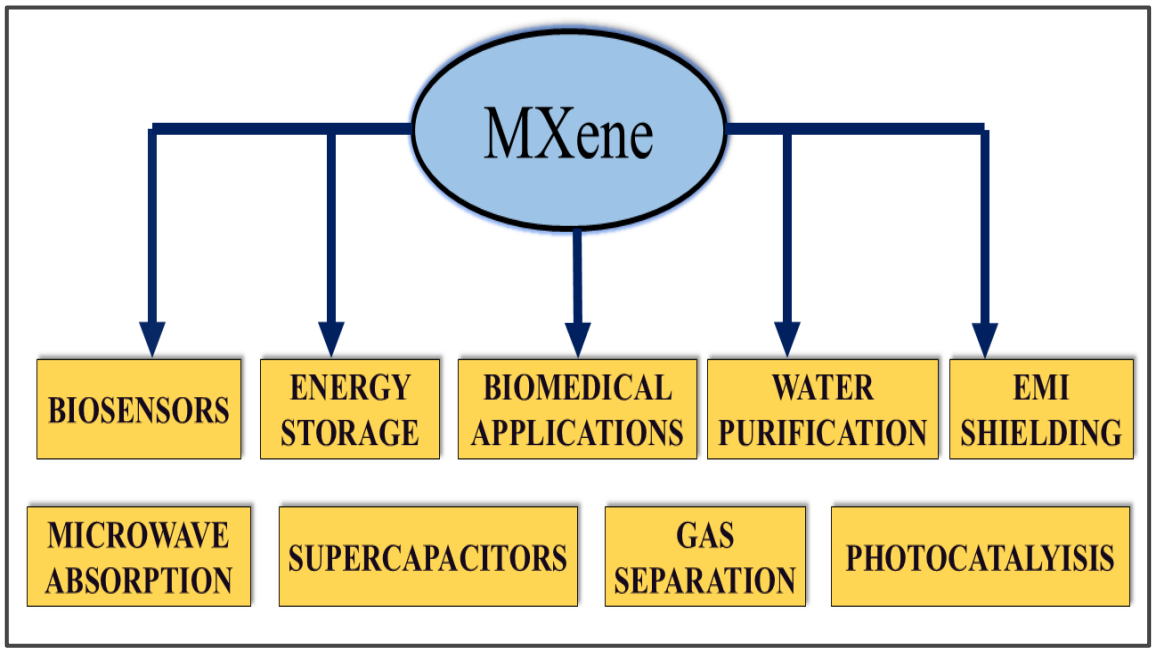


Fig. 2.2 An illustration of the different uses for MXene sheets

2.4.4 Applications of MXene

1. Biosensors

MXene is a great material that works well for biosensor applications. The key factor in choosing it as a transducer for a biosensor application is its high surface area, abundant functional groups, and evidently superior electrical conductivity. Because MXene naturally has a more rapid electron transport rate and negative surface groups, it can generate a signal output with a wide variety of analytes and a lower limit of detection. Direct electron transfer

between the enzyme and electrode is the primary benefit of the MXene-based biosensor and it can be accomplished with ease and without altering the enzyme's natural structure [125].

2. Supercapacitors

The two-dimensional, structured MXene is appropriate for adaptable supercapacitor technology due to its superior mechanical qualities. Strength, elasticity, tractability, and machinability are among the material's mechanical properties; additionally, its flexibility permits it to adopt any shape. Because of their flexible and 2D-lamellar structure, MXene becomes an intriguing candidate for such devices. In recent years, there has been a significant increase in the need for compact, portable "micro-electronic" system devices, such as micro-SCs and flexible SCs. Two-dimensional MXene nanosheets are easy to fabricate as film electrodes, and most of the research to date has focused on improving their structural properties [126].

3. Gas Separation

The materials of the MXene family offer special benefits in a variety of membrane-based separation procedures, including solvent/water separation, gas separation, pervaporation, and desalination [127]. For the first time, exfoliated MXene nanosheets were utilized as building blocks to create 2D laminated membranes for selective gas separation utilizing a model system of CO_2 and H_2. Beyond the capabilities of the most advanced membranes, the MXene membranes provide exceptional performance in terms of hydrogen permeability and H_2/CO_2 selectivity. Numerous applications, including carbon dioxide capture and hydrogen synthesis, require such high-permeability hydrogen-selective membranes [128].

4. Biomedical Applications

Bioimaging, antimicrobial, biosensing, drug delivery, tissue engineering, and other therapeutic approaches are among the primary uses of MXene in the biomedical area. It can be used for tumor ablation, also known as photothermal therapy, which can penetrate deep into bodily tissues, target cancer cells specifically, and cause minimal harm to humans due to its great light absorption and near-infrared conversion capabilities. Additionally, as contrast agents, bioimaging can be used to track the location of tumors in real time throughout cancer treatment. Furthermore, anticancer medications and targeted drug release are also possible with the modified MXenes. Thus far, the combination of photothermal therapy and chemotherapy, along with real-time bioimaging monitoring, has significantly increased the efficacy of cancer treatment [129].

5. EMI Shielding

Special qualities of MXene make them highly promising for EMI shielding. They are electrically conductive, which is a crucial prerequisite for efficient EMI shielding. It can be utilized in difficult conditions without losing their usefulness as a shielding material since they are resistant to corrosion; Because MXene are lightweight and flexible, they are ideal for use in applications where these qualities are crucial, such as wearable technology or other applications where weight is a crucial consideration or when the EMI shield needs to be moulded or curved to meet a certain geometry [130].

CHAPTER – 3

MATERIALS AND METHODS

3.1 Introduction

An overview of the material, including the formation of graphitic carbon nitride, MXene, and their nanocomposites, and different characterisation techniques, including morphological, structural, and electrochemical techniques used for the fabrication of biosensors for the detection of OP pesticide, is discussed in this chapter. This chapter also covers the parameters, processes, and protocols relevant to the performance-developed biosensors.

3.2 Materials

Below is a thorough description of every material:

3.2.1 Chemicals

Lithium fluoride (LiF), Ti_3AlC_2, acetylcholinesterase (AChE), acetylthiocholine chloride (ATCl), trichlorfon (TF), glutaraldehyde solution, glucose (Glu; ≥ 99.5%), ascorbic acid (AA; 99%), uric acid (UA; ≥ 99%) and sodium chloride (NaCl) were bought from Sigma-Aldrich. The actual samples (fenugreek, guava, carrot) are gathered from the neighbourhood market, while all of the solvents, such as acetone, ethanol, and hydrochloric acid, are purchased from Thermo-Fischer Scientific, India. Every chemical is of analytical quality and is utilized without purification.

3.2.2 Solutions and buffer

- 0.1 M Phosphate buffer saline (PBS), pH 7

- 5mM $[Fe(CN)_6]^{3-/4-}$ used in PBS solution as redox initiator.

3.3 Characterisation techniques

All of the modified electrodes and the synthesized materials have been characterized for structural investigation using Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction. In the current study, morphological and elemental characterisation methods such as energy dispersive X-ray spectroscopy (EDX) and scanning electron microscopy (SEM) were employed. Additionally, cyclic voltammetry (CV) and differential pulse voltammetry (DPV), two electrochemical characterisation techniques, were also employed.

3.3.1 Fourier transform infrared spectroscopy (FTIR)

One of the most important analytical methods for characterizing different materials is Fourier transform infrared (FTIR) spectroscopy. The Fourier transform infrared spectrometer (FTIR) uses infrared spectroscopy. The study of the interactions between the IR electromagnetic radiation and matter in IR spectroscopy allows for the qualitative and quantitative evaluation of materials [131]. FTIR works on the concept that molecules absorb specific energy and frequencies of infrared radiation through their atomic vibration and transmit it through the sample. Absorbing electromagnetic radiation at different wavelengths causes a material or chemical compound to create different spectral lines, reflecting its chemical composition [132]. The resulting spectrum, which displays the molecule's absorption and transmission (**Fig. 3.1**), produces the sample's molecular fingerprint [133]. Thus, organic, inorganic, and polymeric materials are analysed using infrared spectroscopy [134].

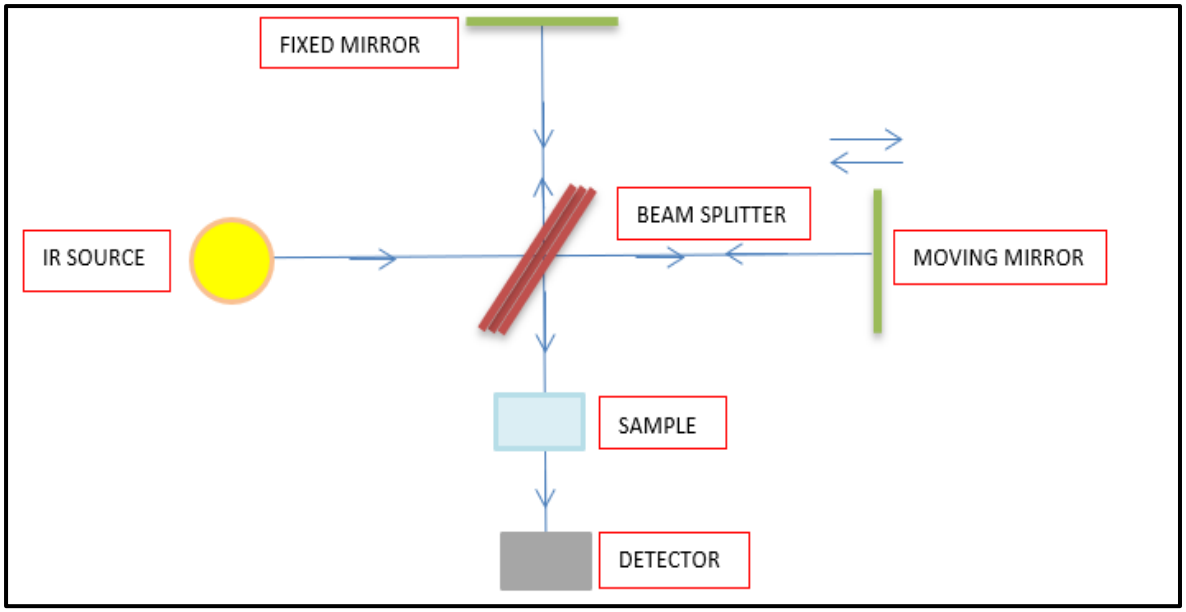


Fig. 3.1 Diagrammatic illustration of Michelson interferometer

The Michelson interferometer (**Fig. 3.1**) has been utilized in FTIR spectroscopy to investigate the chemical composition of any substance. Its fundamental configuration consists of four parts: (a) a filament light source; (b) a beam splitter; (c) two mirrors arranged as shown in **Fig. 3.1**; and (d) a detector.

An infrared radiation beam produced by a black body source is released by the FTIR instrument. The beam eventually crosses to the interferometer, where spectral encoding occurs. When beams with varying travel lengths recombine, constructive and destructive interference are produced, leading to an interferogram. As soon as this beam enters the sample's compartment, the sample begins to accrue specific energy frequencies that are only visible in the interferogram. Additionally, for each frequency, the detector analyses the unique interferogram signal in energy vs time. In that time, the beam is imposed to provide a backdrop or point of reference for the instrument's operation [135]. Finally, the computer program known as the Fourier transformation (FT) produced the desired spectrum by subtracting the interferogram's background from the sample's spectrum [136] [137].

By using proper beam detectors and splitters, FTIR spectroscopy can be applied to a wide variety of frequencies, including UV (ultraviolet), near-infrared, visible, far-infrared, and mid-infrared. None of the other dispersive approaches can cover such a vast range of frequencies [138]. FTIR has several advantages over dispersive measurements, including a larger spectrum range, higher sensitivity, faster data acquisition, lower sample requirements, higher spectral resolution, and superior signal-to-noise ratios [139].

3.3.2 X-ray diffraction (XRD)

X-ray diffraction is used to characterize unknown materials and determine their crystalline structure. This approach determines the atomic structure of macromolecules, while for tiny materials, it determines crystallinity, strain, grain size, crystal structure, and composition [140]. X-ray diffraction is based on the constructive interference of monochromatic X-rays and crystalline substances. CRTs (cathode ray tubes) produce these X-rays and are then further filtered to produce a monochromatic beam that is targeted and concentrated on the sample [141]. The X-ray diffractometer is depicted graphically in **Fig. 3.2**. This approach uses Bragg's law to determine the structure and composition of a material by reflecting an X-ray beam at specific angles on its crystal lattice. This law defined the correlations between the wavelength of the incident beam, the angle of the diffracted beam, and the atomic spacing that could be measured in the sample's crystalline structure.

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

where, integer **n -:** order of diffraction, **λ -:** the wavelength of produced X-rays, **d -:** interplanar distance of the planes of the crystal that diffract through the beam, and **Θ -:** angle between the surface of the reflecting crystalline lattice and the incident ray [142].

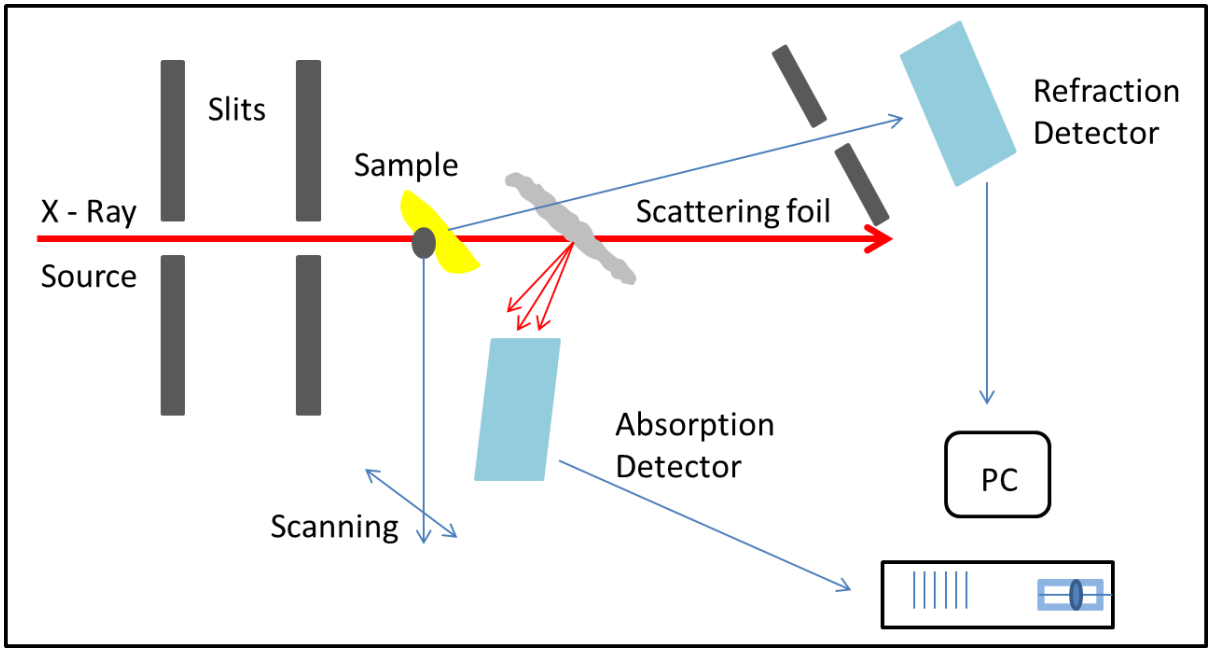


Fig. 3.2 Graphical representation of X-Ray diffractometer

3.3.3 Scanning electron microscopy (SEM)

An effective tool for analysing and examining surface topography, chemical composition, microstructure morphology, and crystal structure [143]. The SEM uses electrons instead of light to capture images of samples. To understand the fundamentals of electron microscopy, one must have a solid understanding of light optics. The capacity to capture signals from the interactions between the specimen and the electron beam is essential for the creation of SEM images. Elastic and inelastic interactions are the two basic categories into which these interactions can be generally separated.

When the sample atomic nucleus deflects the incident electron or when outer shell electrons with comparable energy deflect it, elastic scattering occurs; however, multiple interactions between the arriving electrons and the sample's atoms and electrons cause inelastic scattering. Compared to other microscopes, SEM has an advantage because it can reach 45 specimens at once due to its relatively greater depth of field. A better resolution of SEM is suggested by the specimens' focus, which provides a greater degree of magnification.

The SEM creates signals on a solid's surface using a concentrated, high-energy electron beam, as illustrated in **Fig. 3.3**.

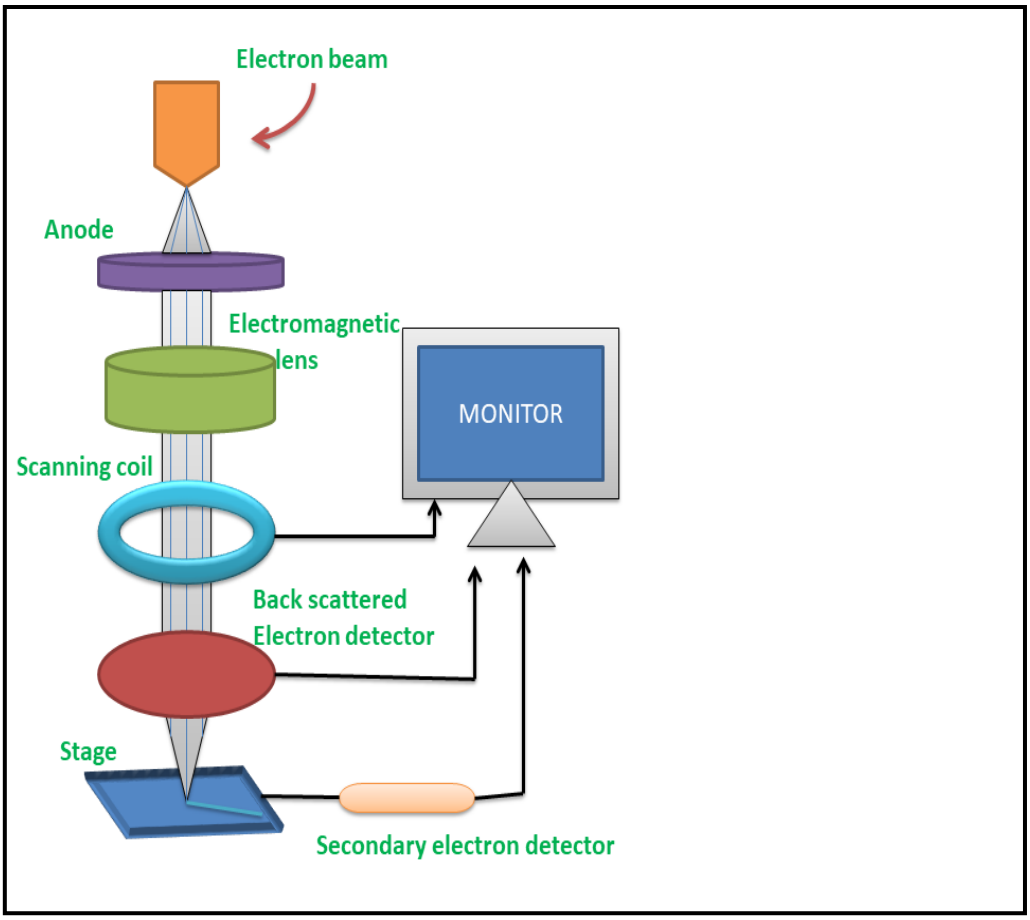


Fig. 3.3 Visual representation of scanning electron microscopy (SEM)

3.3.4 Energy Dispersive X-ray Spectroscopy (EDX)

EDX spectroscopy detects the elemental composition of a material using scanning electron microscopy. EDX can identify elements with an atomic number greater than boron and thus detect at least 0.1% concentration [144] [145]. When the sample collides with an electron beam, it reacts with it and produces X-rays. Because neither of the elements has a similar X-ray spectrum, they may be measured and targeted based on the sample concentration [146] [147]. When the primary beam contacts with an atom's nucleus, the electron is ejected, releasing X-rays. The emitted X-rays include both characteristic and continuum X-rays.

3.4 Electrochemical techniques

Electrochemical approaches use electrochemical principles to examine chemical processes, concentrations, and characteristics of substances. Electrochemical approaches offer additional advantages over surface modification technologies as it utilize the interaction of electricity and chemical species at the electrode-electrolyte interface. These techniques consist of electrodes, an electrolytic solution, and an external circuit. In this study, the three electrodes which are named as, reference electrode (using Ag/AgCl), counter electrode (using platinum) and working electrode are coupled to a potentiostat (using an Autolab (Ecochemie, Netherlands) Galvanostat/Potentiostat), which monitors current by regulating the voltage of the working electrode and hence, graph is plotted as current versus time. The pictorial representation of a potentiostat is shown in **Fig. 3.4.** Some of the Electrochemical techniques include: Differential pulse voltammetry (DPV), Cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and chronoamperometry [148].

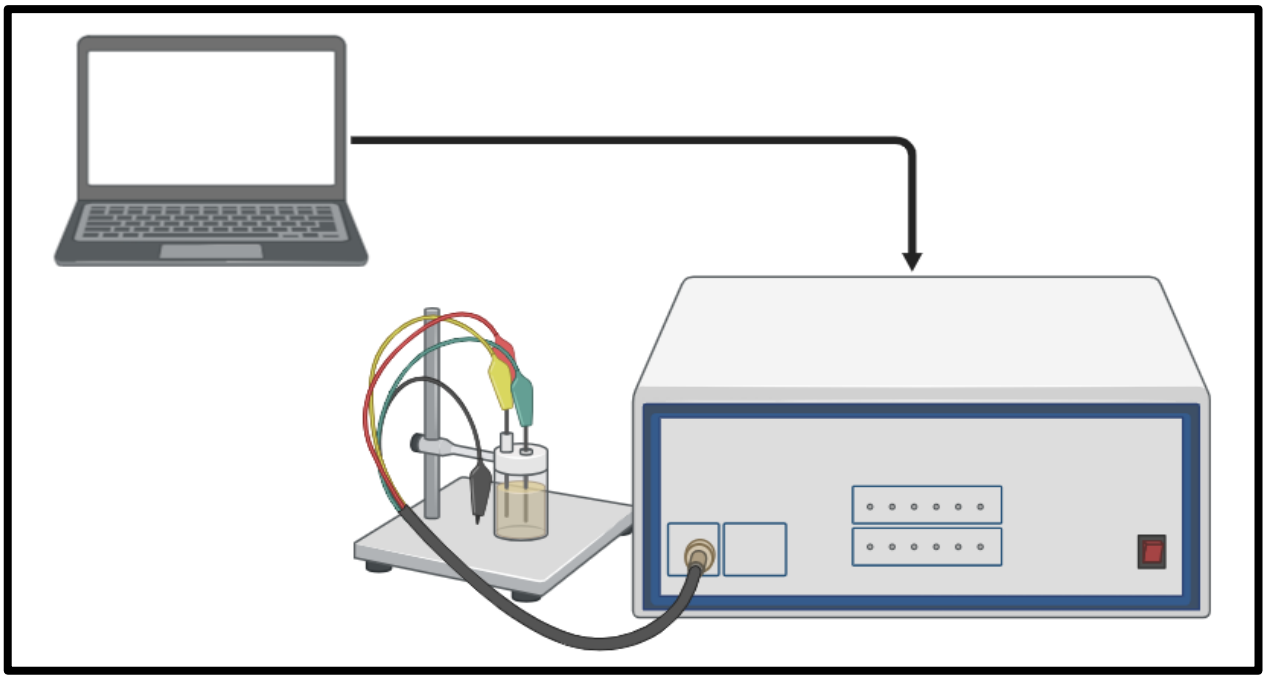


Fig. 3.4 Pictorial representation of potentiostat

3.4.1 Differential pulse voltammetry (DPV)

The most precise and widely used voltammetric technique is differential pulse voltammetry. It offers both qualitative and quantitative details regarding the types of oxidation or reduction reactions. Voltammetry entails applying a time-varying potential excitation signal to the working electrode, altering its potential in relation to the reference electrode's fixed potential, and measuring the current flowing between the working and counter electrode [149]. Furthermore, sequences of pulses with moderate amplitude are superimposed on the voltage, and the current is displayed within the pulse voltage and ramping baseline voltage. Every pulse's current has been measured before and after the pulse (noted as the first and second points, respectively). The differences in the measurement of current after each pulse at those places are recorded [150] [151].

3.4.2 Cyclic voltammetry (CV)

Cyclic Voltammetry is the most commonly used electro-analytical technique for characterizing the electrochemical behaviour of electrochemically active compounds [152]. In cyclic voltammetry, a scan can be completed in either direction. Cyclic voltammetry measures electrochemical reactions of electroactive substances with known redox potential. In the first step, the potential scan is moved to a more positive value, as a result following oxidation reaction for the species R occurs -:

$$\mathbf{R \rightleftharpoons O + ne^-}$$

Then, in the second step, the scan's orientation changes to greater negative potentials when the potential reaches the pre-established switching potential. As a result, the product of the forward scan is reduced back to R.

$$\mathbf{O + ne^- \rightleftharpoons R}$$

During the reduction-oxidation process, the ions are supplied to the electrodes using an electrolytic solution. It has been demonstrated that alkali metals and tetraalkylammonium salts are particularly useful in this method [153].

3.4.3 Electrochemical impedance spectroscopy (EIS)

One effective method for analysing the mechanism of electrochemical reactions is electrochemical impedance spectroscopy (EIS), which can also be used to calculate the transport and dielectric properties of materials, examine the properties of porous electrodes, and analyse passive surfaces [154]. It has been widely used for characterization of charge transport, elucidation of corrosion mechanisms, battery optimization, and solution/membrane interfaces. It is suitable for the detection of binding events on the transducer surface [155]. It is common practice to compute electrochemical impedance and generate a pseudo-linear response within the electrochemical cell using a brief excitation pulse. The Nyquist plot depicts EIS data for the electrochemical cell, with imaginary impedance representing both the cell's inductive and capacitive properties.

In this thesis, the EIS approach is used to compute the R_{ct} value from the obtained EIS spectra. Equations (1) and (2) were used to calculate the exchange current per geometric unit area and apparent electron transfer rate constant (K_{app}) of various electrodes.

$$\mathbf{i_0 = nRT/ R_{ct}F} \qquad \textbf{(i)}$$

$$\mathbf{K_{app} = RT/n^2F^2AR_{ct}C} \qquad \textbf{(ii)}$$

Where C is concentration and F is Faraday's constant. The geometrical area of the electrode is denoted by A, while n denotes the number of electrons. T is temperature, and R is the gas constant.

CHAPTER 4

MXene anchored graphitic carbon nitride-based electrochemical biosensor for trichlorfon detection

4.1 Introduction

This chapter presents a new and effective acetylcholinesterase-based electrochemical biosensor for the detection of trichlorfon that uses MXene-anchored graphitic carbon nitride (g-C_3N_4). The synthesized nanocomposite (g-C_3N_4/MXene) has an electrostatic interaction that greatly boosts the electroactive surface area, enhances catalytic activity, speeds up electron transport, and has exceptional biocompatibility. The fabricated biosensor showed a wide linear range (0.1 pM-1 μM), as well as good reproducibility, low detection limit (0.16 pM), increased sensitivity, good selectivity, and acceptable stability. Moreover, the developed biosensor AChE/g-C_3N_4/MXene/ITO was effectively used for trichlorfon detection in three distinct real samples to verify its accuracy and practicality. **Fig. 4.1** displays the schematic representation of biosensor fabrication.

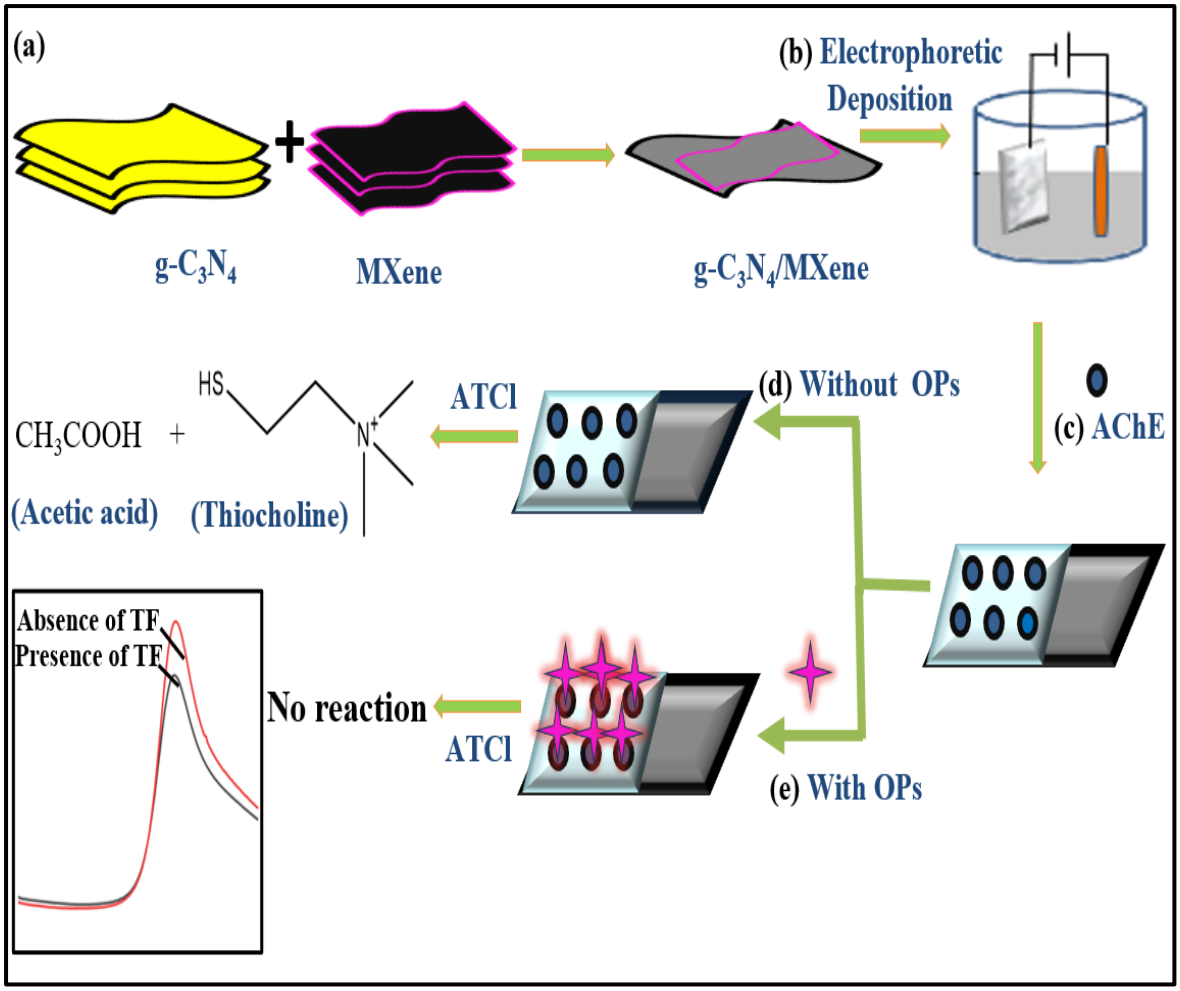


Fig. 4.1 Schematic illustration of TF detection using fabricated AChE/g-C_3N_4/MXene/ITO bio electrode

4.2 Experimental section

4.2.1 Synthesis of g-C_3N_4 nanosheets

A high-temperature condensation method was used to yield g-C_3N_4 powder at standard atmospheric pressure. Usually, 10 g of melamine was heated directly at 550°C for four hours in a muffle furnace. The resulting pale-yellow substance was cleaned with distilled water and ethanol (1:1) and dried in a vacuum oven.

4.2.2 Synthesis of MXene

The hydrothermal process was used to prepare MXene. Initially, a LiF/HCl mixture was created by dispersing 1 g of lithium fluoride (LiF) powder in 20 mL of 6M hydrochloric acid and stirring the mixture for 30 minutes at room temperature. After that, Ti_3AlC_2 (1 g) powder was gradually added to the above suspension and constantly agitated for half an hour at room temperature. The final suspension was transferred to a 100 mL Teflon-lined autoclave and hydrothermally treated for 16 hours at 180 °C. After this, the precipitate was obtained, and it was centrifuged at 3500 rpm through DI water until the pH of the supernatant reached 6. After that, the resultant MXene powder was dried in a vacuum oven for 12 hours at 60 °C.

4.2.3 Synthesis of g-C_3N_4/MXene nanocomposite

To synthesize the nanocomposite g-C_3N_4/MXene, 100 mg of g-C_3N_4 was dissolved in 40 mL of distilled water, and the mixture was ultrasonicated for an hour before being stirred for another hour. A 30% MXene solution was prepared in 20 ml of deionized water, which was then ultrasonicated for an hour and agitated for an additional hour. Next, the MXene solution is introduced into the g-C_3N_4 solution gradually and dropwise. The resultant grey solution was stirred overnight. Ultimately, grey precipitate was centrifuged at 4000 rpm and washed with distilled water-ethanol (1:1). The composite was then dried in a vacuum oven set to 80°C.

4.2.4 Electrophoretic deposition of g-C_3N_4/MXene nanocomposite

The resultant g-C_3N_4/MXene hybrid was electrophoretically deposited onto the pre-hydrolysed ITO-coated glass substrate (1.4 x 0.7 cm). A two-electrode system was used for EPD, with the reference electrode (platinum wire) positioned 1 cm from the working electrode (ITO). Prior to deposition, a colloidal suspension of g-C_3N_4/MXene (1 mg) is

created by ultrasonically dispersing it in 20 mL of deionized water. The film was deposited smoothly by applying a DC potential of 10 V for 8 seconds.

4.2.5 Fabrication of g-C_3N_4/MXene nanocomposite-based biosensor

This electrode (g-C_3N_4/MXene/ITO) is incubated with 0.2% glutaraldehyde for 1 hour at room temperature in order to fabricate the biosensor. After letting the electrodes dry at room temperature, the solution of AChE and 1% chitosan solution (made of 1% acetic acid) in the ratio of 1:1 was immobilized on the prepared electrode and incubated for 12 hours at 4°C. The constructed biosensor AChE/g-C_3N_4/MXene/ITO is cleaned with PBS before the electrochemical analysis in order to eliminate the unbound enzyme.

4.3 Results and Discussion

4.3.1 Structural Characterisation

Fig. 4.2 (A) illustrates the XRD pattern of g-C_3N_4 and g-C_3N_4/MXene. The plot of g-C_3N_4 demonstrated two distinct peaks at $2\theta = 13.0°$ and $2\theta = 27.5°$. The peak at 13.0° indicates tri-s-triazine rings (100), while the peak at approximately 27.5° indicates conjugated aromatic ring stacking (002) and a feature of the hexagonal phase diffraction planes of the graphitic form of carbon nitride [156]. The prepared nanohybrid g-C_3N_4/MXene displays the $2\theta = 6.4°$ peak from MXene and the $2\theta = 27.5°$ peak characteristic of g-C_3N_4. The absence of additional XRD peaks in nanocomposites suggests that electrostatic interactions are the means by which g-C_3N_4 and g-C_3N_4/MXene bond together [157].

Fig. 4.2 (B) represents the FTIR spectra of g-C_3N_4 and g-C_3N_4/MXene. In case of g-C_3N_4, the absorption peaks at 1575 and 1658 cm^{-1} are due to the stretching vibration modes of C=N, whereas the prominent peak at 808 cm^{-1} represents tri-s-triazine units in the bending mode. The peaks at 1239, 1321, and 1401 cm^{-1} indicate aromatic C-N stretching, and a broad peak at 3244 cm^{-1} corresponds to terminal NH_2 or NH groups [158]. For g-C_3N_4/MXene, no new

peaks were observed; instead, peaks corresponding to both g-C_3N_4 and MXene were identified [159].

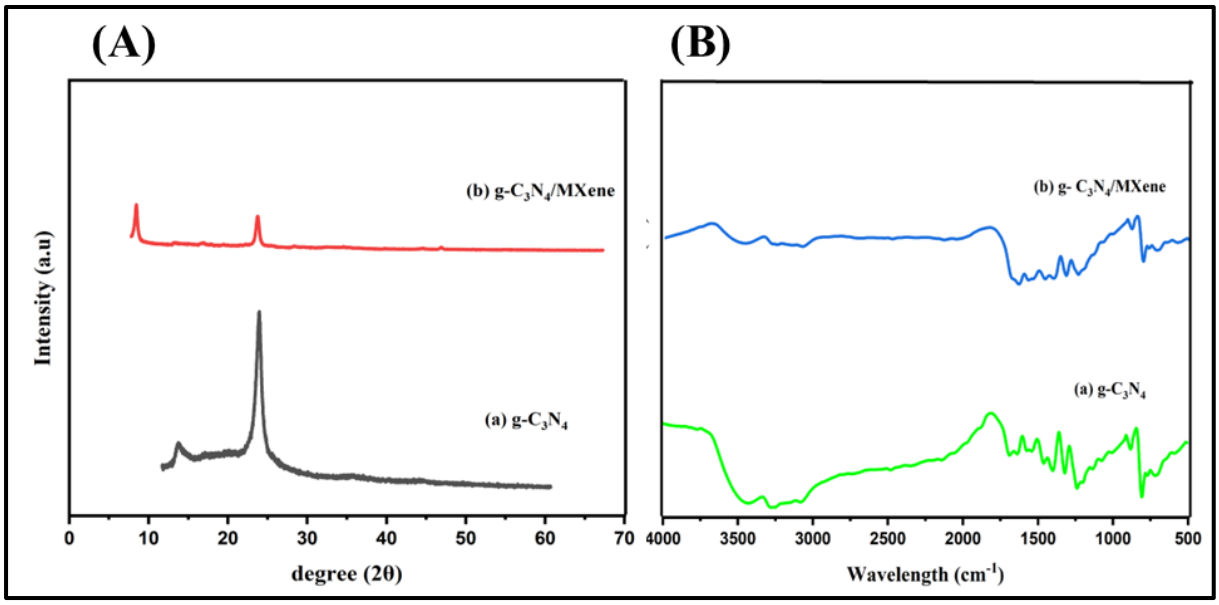


Fig. 4.2 (A) XRD and **(B)** FTIR spectra of (a) g-C_3N_4, and (b) g-C_3N_4/MXene

4.3.2 Morphological Studies

The surface morphology of g-C_3N_4 and g-C_3N_4/MXene nanocomposite is examined using a Scanning Electron Microscope (SEM). The **Fig.4.3 (A)** illustrates, g-C_3N_4 has been characterized by a lamellar structure made up of irregular, thin sheets that are backfolded at the edges [160]. The morphology of g-C_3N_4/MXene revealed that g-C_3N_4 is anchored on MXene as represented by **Fig. 4.3 (B).**

EDX analysis is conducted to ascertain the components present in g-C_3N_4 and g-C_3N_4/MXene. The existence of C, N, O, Ti, and F indicates that all the materials are synthesised with high purity as displayed in **Fig. 4.3 (C, D)**.

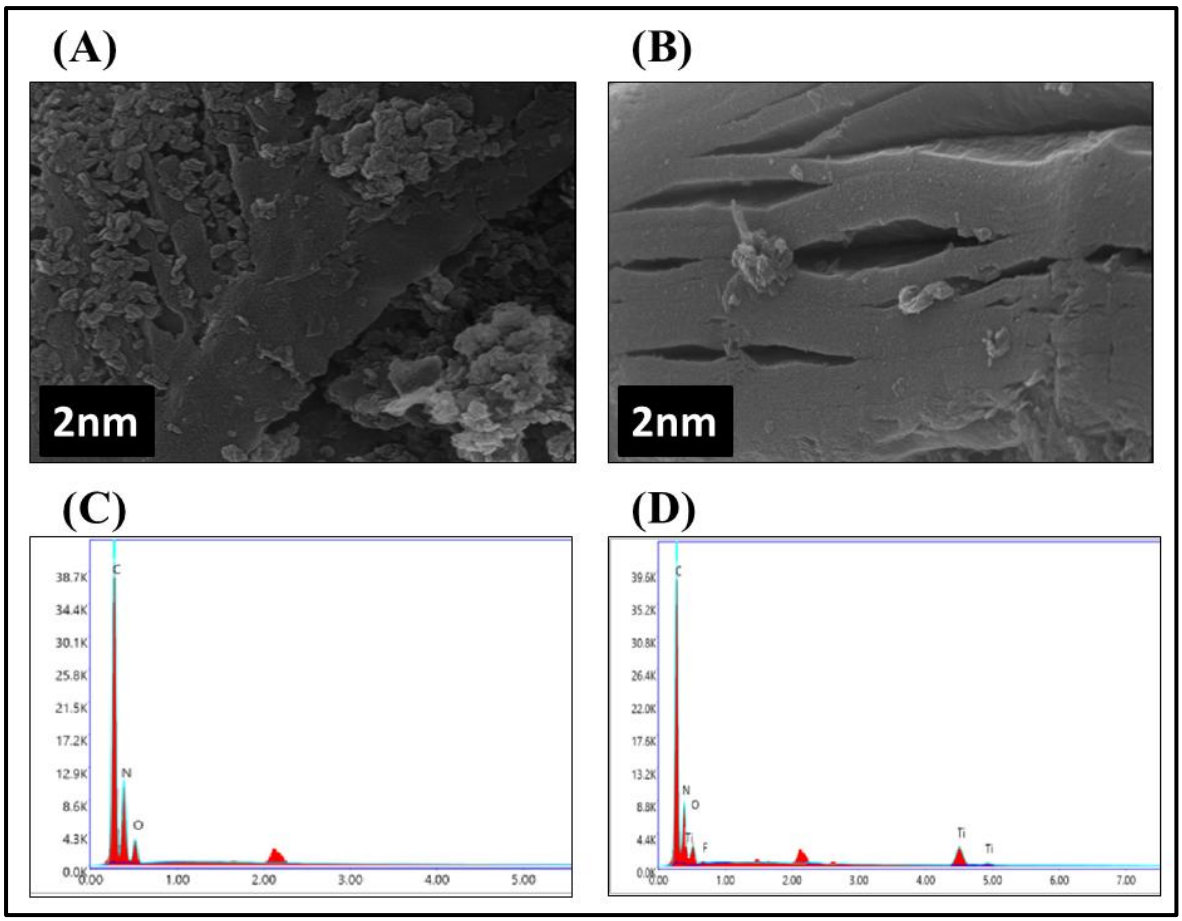


Fig. 4.3 Morphology structures of **(A)** g-C_3N_4, **(B)** g-C_3N_4/MXene, and EDX of **(C)** g-C_3N_4, (D) g-C_3N_4/MXene

4.3.3 Electrochemical Characterisation

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) methods were used to electrochemically characterize each of the fabricated electrodes. The **Fig.4.4 (A)** depicts the CV curves at a scan rate of 50 mV/sec for g-C_3N_4/ITO, g-C_3N_4/MXene/ITO, and AChE/g-C_3N_4/MXene/ITO in 0.1 M of PBS (consisting of 5mM $[Fe(CN)_6]^{3-/4-}$). As observed in **Fig. 4.4 (A),** g-C_3N_4/MXene/ITO displayed the maximum redox peak current (0.49 mA, curve b) when compared to g-C_3N_4/ITO (0.33 mA, curve a). This is due to the high surface area and great electrical conductivity of the g-C_3N_4/MXene nanocomposite. The least current

(0.24 mA, curve d) and low electron mobility were noticed for AChE/g-C_3N_4/MXene /ITO as a result of the non-conductivity of enzyme AChE.

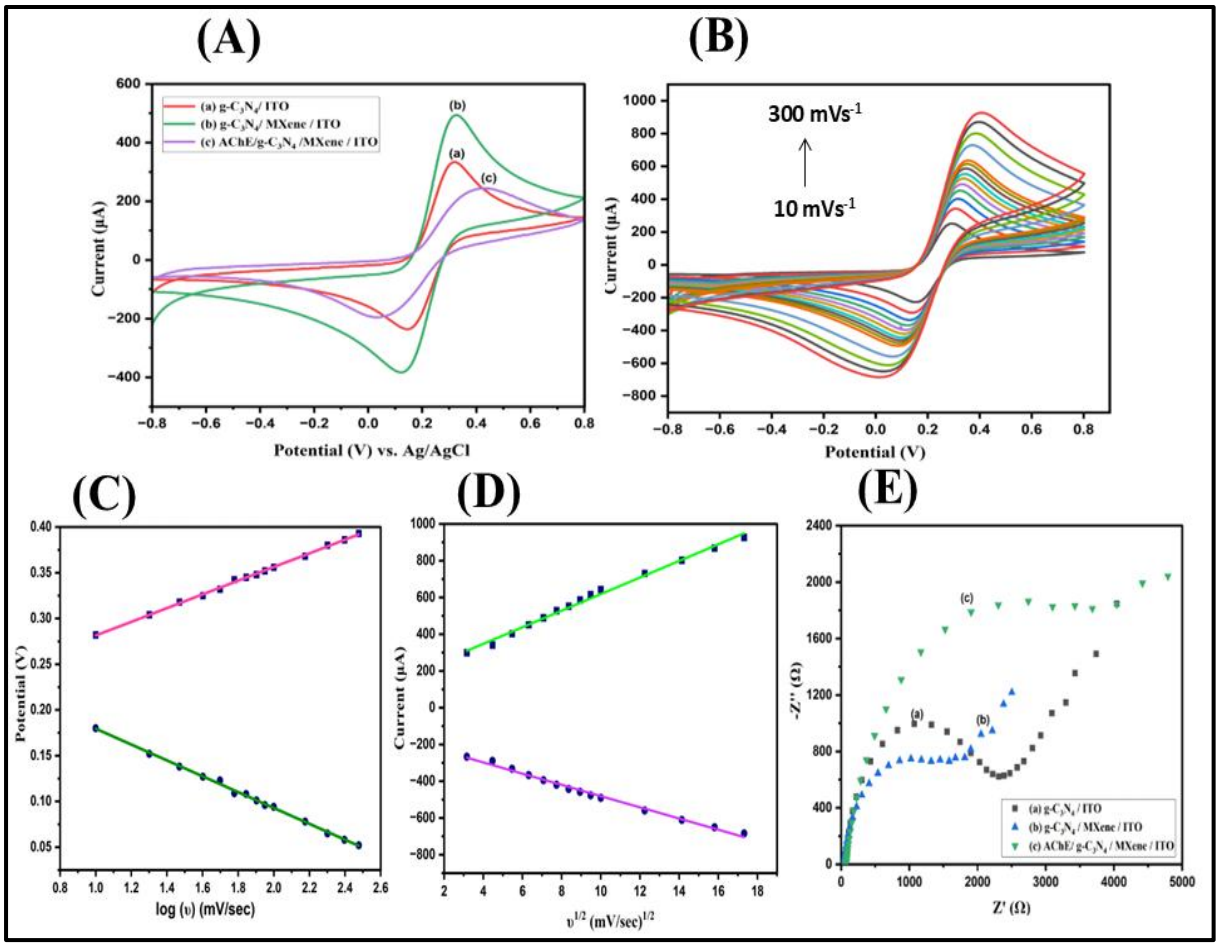


Fig 4.4 (A) Cyclic voltammogram for different fabricated electrodes (a) g-C_3N_4/ITO, (b) g-C_3N_4/MXene/ITO, and (c) AChE/g-C_3N_4/MXene/ITO and **(B)** CV response of electrode g-C_3N_4/MXene/ITO in a solution of 0.1 M PBS containing 5 mM $[Fe(CN)_6]^{3-/4-}$ at various scan rates (10-300 mVs^{-1}), **(C)** Plot between peak potential versus log scan rate and **(D)** Plot of peak current versus square root of scan rate for g-C_3N_4/MXene/ITO and **(E)** EIS plots of different fabricated electrodes (a) g-C_3N_4/ITO, (b) g-C_3N_4/MXene/ITO, and (c) AChE/g-C_3N_4/MXene/ITO

The CV studies g-C_3N_4/MXene/ITO electrodes at scan rates ranging from 10 to 300 mV/sec. This graph demonstrates that the anodic peak current (I_{pa}) shifts to more positive values and

the cathodic peak current (I_{pc}) shifts to more negative values when the scan rate increases from 10 to 300 mV/sec. This implies that the anodic and cathodic peak potentials (E_{pa} and E_{pc}) have good linearity against the logarithm value of the scan rate (log v) **(Fig. 4.4 C)**, as suggested by Equations **(4.1)** and **(4.2)**.

E_{pa} [g-C_3N_4/MXene/ITO] = 0.0748 (V) log (v) +0.2075 (V); R^2 = 0.9904 **(4.1)**

E_{pc} [g-C_3N_4/MXene/ITO] = -0.0887 (V) log (v) +0.2718 (V); R^2 = 0.9976 **(4.2)**

Considering the slopes of these equations, the electron transfer coefficient (α) is calculated. The determined values of α and the charge transfer rate constant (K_s) for the g-C_3N_4/MXene are 0.923 and 0.0269, respectively. Furthermore, the anodic and cathodic peak currents (I_{pa} and I_{pc}) exhibit a linear increase with the square root of the scan rate ($v^{1/2}$), suggesting a surface-regulated process for g-C_3N_4/MXene **(Fig. 4.4 D)**, as indicated by Equations **(4.3)** and **(4.4)**.

I_{pa} [g-C_3N_4/MXene/ITO] = 46.46 μA $(mV/s)^{1/2} \times v^{1/2}$ mV $s^{1/2}$ + 151.45 μA; R^2 = 0.986 **(4.3)**

I_{pc} [g-C_3N_4/MXene/ITO] = -31.57 μA $(mV/s)^{1/2} \times v^{1/2}$ mV $s^{1/2}$ - 163.38 μA; R^2 = 0.984 **(4.4)**

Effective surface area (A) and diffusion coefficient (D) have also been calculated using the slopes of the aforementioned equations and the Randles–Sevcik equation **(4.5),** demonstrated below.

$$I_p = (2.69\times10^5)\, n^{3/2}\, AC\, D^{1/2}\, v^{1/2} \quad \textbf{(4.5)}$$

Here, I_p represents the peak oxidation or reduction current, n is the number of electrons that migrated during a redox reaction, C is the concentration of $[Fe(CN)_6]^{3-/4-}$ (mole cm^{-3}) and υ denotes the scan rate (V s^{-1}) of the modified electrode. For the g-C_3N_4/MXene/ITO electrode, A was found to be 0.41 cm^2, and D was computed as 6.89 × 10^{-12} cm^2 s^{-1}, respectively.

Furthermore, the surface coverage of the modified electrode (g-C_3N_4/MXene/ITO) was determined by employing the formula [$I_p = n^2F^2A\tau v/4RT$], where F, T, and R denote Faraday's constant, temperature, and gas constant, respectively.

The electrochemical impedance spectroscopy (EIS) for g-C_3N_4/ITO, g-C_3N_4/MXene/ITO, and AChE/g-C_3N_4/MXene/ITO electrodes in 0.1 M of PBS (containing 5mM $[Fe(CN)_6]^{3-/4-}$) in the 0.01 to 10^5-Hz frequency range (set potential 0.01 V) is displayed in **Fig. 4.4 (E)** This technique is also used to analyze the interfacial charge transfer properties at each step of electrode modification. The Nyquist plot was used to show that the semicircular pattern was seen for the electron transfer process at high frequencies, whereas a linear pattern was seen for the electron transfer process at lower frequencies [161]. The charge transfer resistance (R_{ct}) values for g-C_3N_4/ITO (curve a), g-C_3N_4/MXene/ITO (curve b), and AChE/g-C_3N_4/MXene /ITO (curve c) are 3.55, 1.17, and 4.06 kΩ, respectively. Since the g-C_3N_4/MXene nanocomposite fabricated ITO electrode has a lower R_{ct} value (1.17 kΩ) than g-C_3N_4, it is more conducting and has a greater capacity for charge transfer. But the AChE immobilized AChE/g-C_3N_4/MXene/ITO electrode exhibits the highest R_{ct} value (4.06 kΩ) because charge transfer across the electrode interface is hindered by the non-conductive nature of the enzyme AChE. Additionally, R_{ct} is used to calculate the exchange of current per geometric unit surface area (i_o) and an apparent electron transfer rate constant (K_{app}) for g-C_3N_4/MXene/ITO electrodes by using the following formulas **(4.6)** and **(4.7)**.

$$i_o = nRT / R_{ct} F \quad \textbf{(4.6)}$$

$$K_{app} = RT / n^2 F^2 AR_{ct} C \quad \textbf{(4.7)}$$

Here, R, T, F, C, and A denote the gas constant, temperature, the Faraday constant, concentration of the $[Fe(CN)_6]^{3-/4-}$, and electrode area, respectively. The values of i_o and K_{app} for g-C_3N_4/MXene/ITO are 2.19×10^{-5} A cm^{-2} and 9.35×10^3 cm s^{-1}, respectively.

4.3.4 Optimization studies

Optimizing the created biosensor's hyperparameters, including the concentration of ATCl, the amount of chitosan, the pH of the electrolyte, the amount of AChE, and the pesticide inhibition period, is necessary to get a more reliable performance. Firstly, in order to optimize the concentration of AChE enzyme (0.04–0.12 mg/ml) deposited onto the constructed electrode surface, the DPV response of the electrode in the presence of 2 mM ATCl is examined. **Fig. 4.5 (A)** illustrates that the maximum current can be observed at 0.08 mg/ml. Thus, AChE at a concentration of 0.08 mg/mL is optimal for catalyzing the hydrolysis of ATCl.

The pH of the electrolytic solution (6–8 pH) is optimized by the DPV response of the prepared electrode in the presence of 2 mM ATCl. The optimal pH for the subsequent biosensing studies, where the maximum current is observed, is 7, as shown in **Fig. 4.5 (B)**.

Furthermore, the effect of the ratio of AChE to chitosan on the efficacy of the biosensor was also investigated. The results showed that as the chitosan concentration rose from 1:1 to 1:3, the current increased and subsequently decreased as the chitosan concentration increased further, as illustrated in **Fig. 4.5 (C)**. Thus, to create a responsive and reliable biosensor, a ratio (1:3) of AChE to chitosan is employed in the further studies.

Moreover, the catalytic activity of AChE can be maximized by varying the concentration of ATCl. **Fig. 4.5 (D)** illustrates that the rate at which OPs inhibit AChE rises progressively when the concentration of ATCl is increased from 1 to 2 mM and declines when it is further increased from 2 to 4 mM. This suggests that the activity of AChE is at its peak when the concentration of ATCl is 2 mM.

The incubation time is yet another significant aspect that influences the efficiency of the designed biosensor AChE/g-C_3N_4/MXene/ITO. When the pesticide incubation period extends up to 10 minutes, it is obvious from **Fig. 4.5 (E)** that the peak current value increases. After 10 minutes, the current stabilizes, signifying that the enzyme and TF reaction is over.

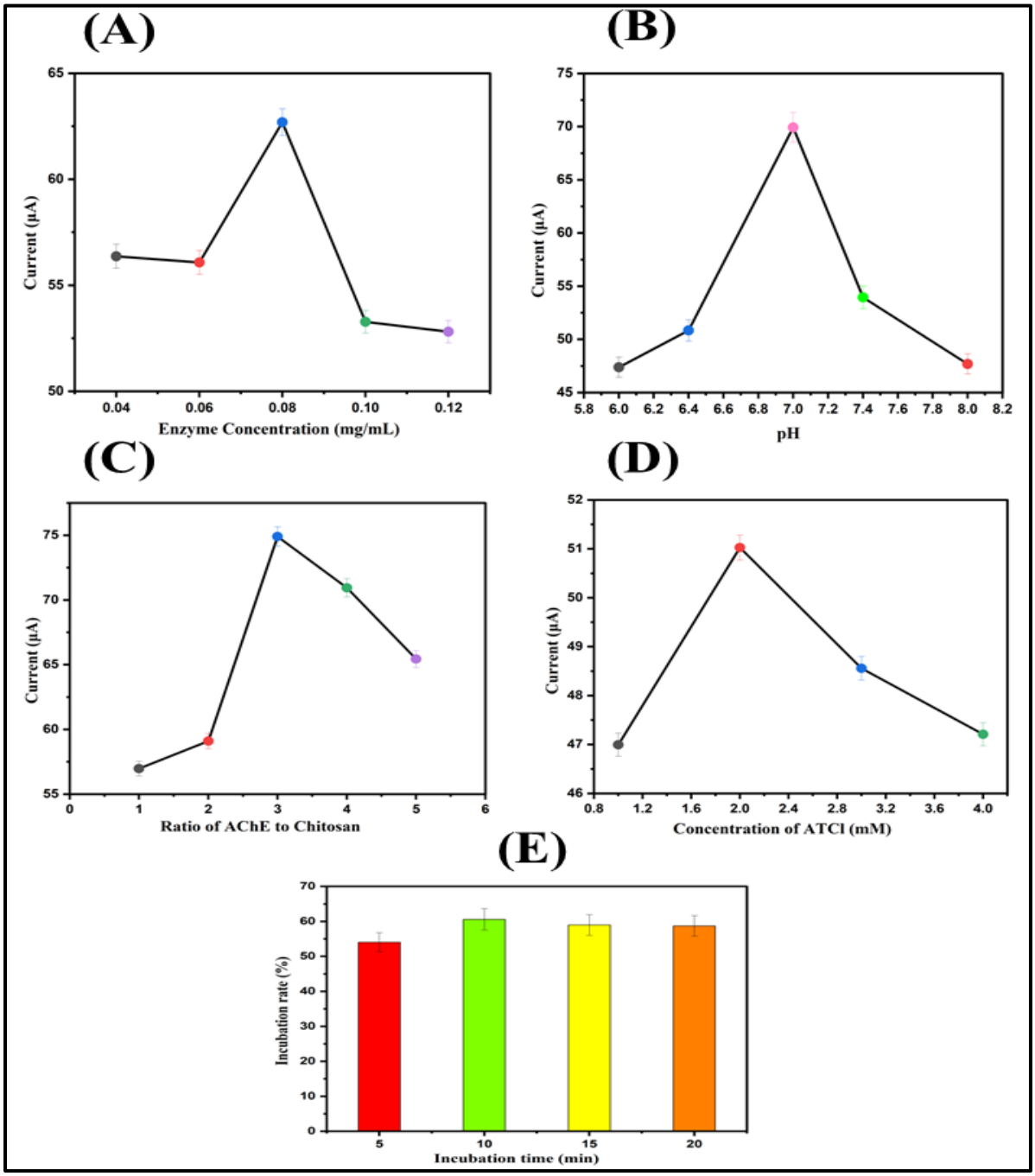


Fig. 4.5 Optimization studies of **(A)** AChE concentration, **(B)** Impact of pH of electrolyte, **(C)** AChE: Chitosan ratio, **(D)** ATCl concentration, and **(E)** Incubation time

4.3.5 Electrochemical Biosensing Study of AChE/g-C_3N_4/MXene/ITO

Differential pulse voltammetry (DPV) was employed in order to examine the electrochemical biosensing response of the fabricated AChE/g-C_3N_4/MXene/ITO electrode towards TF. The constructed bioelectrode's current was evaluated while the TF concentration was increased from 1 pM to 1 µM in 0.1M PBS with a pH of 7 and ATCl (2 mM). As TF concentration increases, the current has been observed to steadily drop as illustrated in **Fig. 4.6 (A),** indicating that TF inhibits the activity of the enzyme AChE. The calibration curve **Fig. 4.6 (B)** illustrates the linear relationship between peak current (I) and TF concentration, which was obtained from the equation:

$I = 3.194\ \mu A/pM + 8.593\ \mu A \times C_{TF}$; $R^2 = 0.998$

Using the slope of the linear curve of the proposed bioelectrode, the sensitivity (slope/ effective surface area) of the biosensor was determined to be 7.79 µA $(pM)^{-1}$ cm^{-2}. The limit of detection (LOD) is computed to be 0.16 pM, applying the formula: 3σ/S, where σ and S correspond to the standard deviation and sensitivity of the bioelectrode.

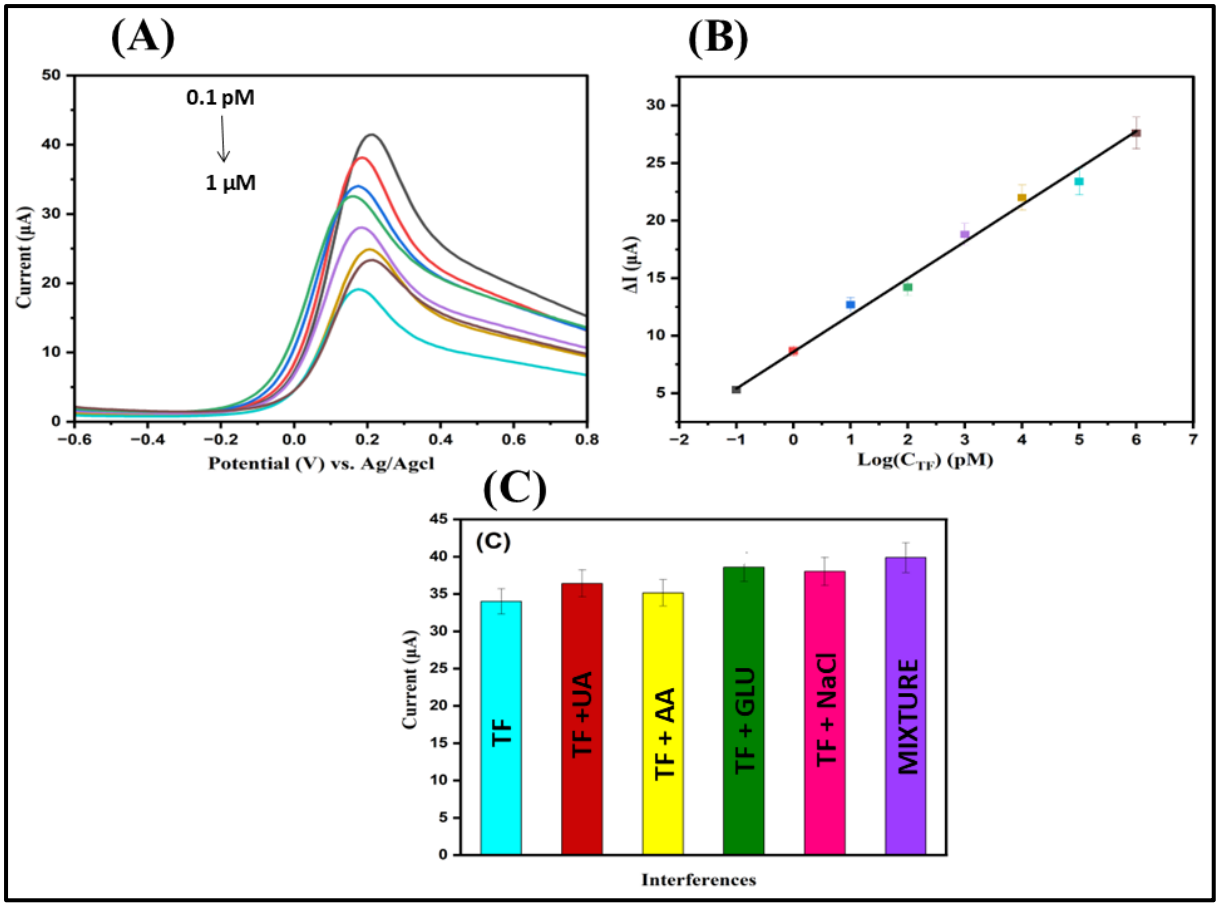


Fig. 4.6 (A) DPV response of AChE/g-C_3N_4/MXene/ITO electrode with different concentrations of TF (0.1 pM to 1µM), **(B)** Calibration curve between log of TF concentration with change in current, and **(C)** Study of selectivity of AChE/g-C_3N_4/MXene/ITO electrode

4.3.6 Interference Study and Real Sample Analysis

The selectivity of the created biosensor (AChE/g-C_3N_4/MXene/ITO) was analysed using a few interferent analytes, including glucose, uric acid, ascorbic acid, and NaCl. In this study, 1 nM of the listed interferent species was mixed with 0.01 nM of TF. The bar diagram **Fig. 4.6 (C)** illustrates excellent selectivity for TF detection, with no apparent changes in peak current when the produced biosensor was exposed to interferent analytes.

The efficiency and practicality of the biosensor AChE/g-C_3N_4/MXene/ITO have been evaluated in spiked samples such as carrot, guava, and fenugreek at known concentrations of 0.01, 0.1, and 1 nM by the DPV method. The results indicate a satisfactory recovery rate (91.9–108.1%), which further demonstrates the accuracy of the biosensor.

Table 4.1 Detection of TF in three real samples using AChE/g-C_3N_4/MXene/ITO electrode

Samples	Added amount (nM)	Found amount (nM)	Recovery (%)	RSDs (%)
Carrot	0.01	0.015	105.3	3.66
	0.1	0.108	108.1	3.13
	1	0.988	98.8	2.76
Guava	0.01	0.010	104.7	3.29
	0.1	0.091	91.9	5.94
	1	0.963	96.3	2.62
Fenugreek	0.01	0.009	98.2	1.27
	0.1	0.107	107.8	5.37
	1	1.036	103.6	2.51

4.3.7 Reproducibility and Stability of AChE/g-C_3N_4/MXene/ITO biosensor

Reproducibility was evaluated by analyzing the DPV response of five different prepared electrodes (AChE/g-C_3N_4/MXene/ITO) in the presence of 0.01 nm TF. The **Fig. 4.7 (A)** clearly shows that the maximum currents for the five electrodes in consideration do not differ substantially from one another. According to the results, the RSD value is 2.62%, indicating good reproducibility of the produced biosensor.

The electrode AChE/g-C_3N_4/MXene/ITO was kept in a refrigerator maintained at 4°C for 28 days in order to assess the biosensor's stability, and the DPV response to TF was evaluated every 7 days as represented by **Fig. 4.7 (B)**. The electrochemical response remained 81.1% of its initial activity after 28 days, indicating that the proposed biosensor is sufficiently stable.

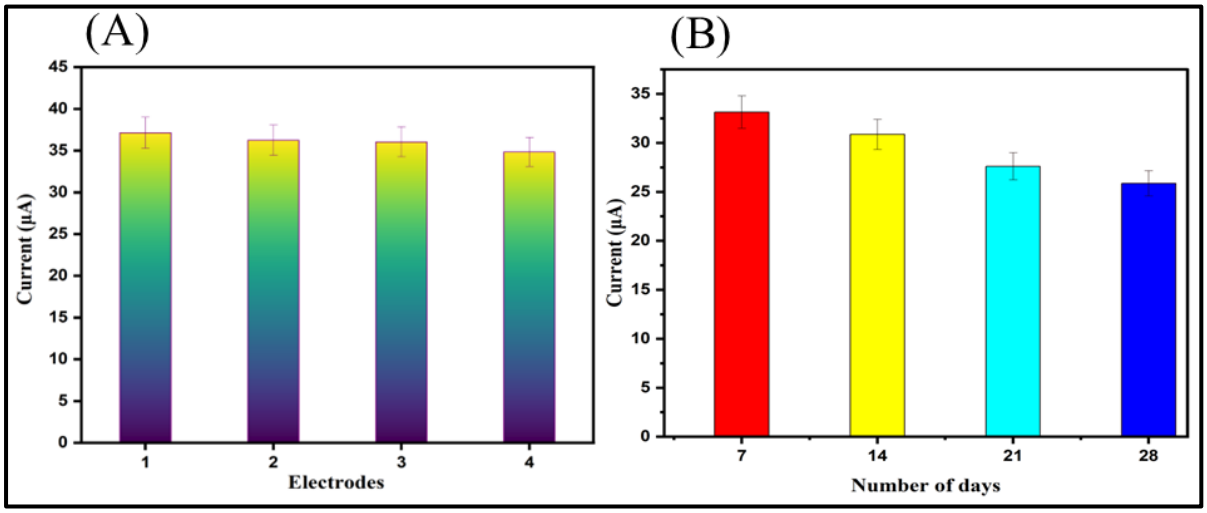


Fig. 4.7 (A) Reproducibility study of (AChE/g-C_3N_4/MXene/ITO) electrodes for TF detection and **(B)** Stability study of the fabricated electrode (AChE/g-C_3N_4/MXene/ITO) over a period of 28 days

CHAPTER 5

CONCLUSION

In summary, using a g-C_3N_4/MXene nanocomposite, a novel and extremely sensitive electrochemical biosensor for TF detection is designed in this work. The constructed biosensor is further adjusted in terms of several factors, such as the pH of PBS, the amount of AChE, chitosan, and ATCl, and the incubation duration, to get the best possible performance. The electron transfer mechanism between the AChE and the electrode surface is accelerated by the g-C_3N_4/MXene-modified transducer, increasing the suggested biosensor's sensitivity in the process. The remarkable performance of the suggested biosensor over TF detection is confirmed by comparison with seven well-known and newly created biosensors in terms of sensitivity (7.79 μA $(pM)^{-1}$ cm^{-2}), LOD (0.16 pM), and linear range (0.1 pM - 1 μM). Comparable recoveries (91.9% to 108.1%) with low RSDs were obtained in the analysis of real food samples, indicating the viability of the constructed biosensor.

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Conference Attended

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