Graphitic carbon nitride decorated molybdenum sulfide-based Electrochemical biosensor for Trichlorfon detection

A Dissertation Submitted

In Partial Fulfillment of the Requirements for the Degree of

MASTERS OF SCIENCE

in

CHEMISTRY

by

SRIJITA CHATTERJEE

(2K22/MSCCHE/63)

&

HARSHITA SINGH

(2K22/MSCCHE/13)

Under the Supervision of

Prof. D. Kumar

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

JUNE, 2024

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

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We, Srijita Chatterjee (2K22/MSCCHE/63) and Harshita Singh (2K22/MSCCHE/13), hereby certify that the work which is being presented in the dissertation entitled "**Graphitic carbon nitride decorated molybdenum sulfide-based Electrochemical biosensor 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 2023 to 2024 under the supervision of Prof. D. Kumar.

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

Place: Delhi **Srijita Chatterjee** **Harshita Singh**

(2K22/MSCCHE/63) **(2K22/MSCCHE/13)**

Date: 06/06/2024

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

CERTIFICATE

Certified that Srijita Chatterjee (2K22/MSCCHE/63) and Harshita Singh (2K22/MSCCHE/13) have carried out their research work presented in this thesis entitled **"Graphitic carbon nitride decorated molybdenum sulfide-based Electrochemical biosensor 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

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ABSTRACT

Organophosphorus pesticides as insecticides are extensively used in agricultural products to enhance agricultural production. Organophosphate pesticides instigate toxicity by inhibiting the function of the AChE enzyme and finally affecting the central nervous system. In recent years, acetylcholinesterase (AChE) biosensors have emerged as highly sensitive tools for the detection of toxicity in pesticides. These biosensors can replace classical methods owing to their high selectivity and quick response. Here, graphitic carbon nitride (g-C_3N_4) has been strengthened with molybdenum disulfide (MoS_2) to create an electrochemical biosensor for the detection of trichlorfon. AChE enzymes were covalently attached to the g-C_3N_4@MoS_2 composite integrated electrodes, and potentiometric response was measured using differential pulse voltammetry. The developed biosensors exhibit enhanced electrochemical sensing performance and increased electrocatalytic properties due to the combined action of g-C_3N_4@MoS_2. By utilizing glutaraldehyde as a cross-linking agent to immobilize acetylcholinesterase (AChE) on the g-C_3N_4@MoS_2 film, a responsive biosensor is developed via electrophoretic deposition of g-C_3N_4@MoS_2 on the surface of ITO substrate. The produced biosensor performed admirably because g-C_3N_4 improves electron mobility by forming a 2D–2D hybrid interface with MoS_2 nanosheets. The fabricated biosensor exhibits a low detection limit of 2.1 nM with a broad linear range of 5-100 nM.

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

ACKNOWLEDGEMENT

We would like to express our deepest gratitude to our supervisor, Prof. D. Kumar, Department of Applied Chemistry, Delhi Technological University, Delhi. His consistent guidance, invaluable suggestions, and ongoing encouragement have helped us to complete this work. It has been a privilege to work under such a kind, motivated, and supportive mentor.

We owe a great debt of gratitude to Prof. Ram Singh and Dr. Anil Kumar for their unwavering guidance and for providing the facilities necessary for our project work.

We were fortunate to experience a positive work environment in the laboratory and are grateful to all the laboratory staff and research scholars for their cooperation. We are particularly thankful to Ms. Divya Hudda for her extensive help in teaching us the fundamentals of techniques and for her patience in addressing our numerous questions throughout our research. We are also thankful to Mrs. Sweety Jain and Ms. Tanushee for their support of our project. We are deeply appreciative of all the faculty members of the Department of Applied Chemistry at DTU for their ongoing support and cooperation. Additionally, we extend our thanks to both the technical and non-technical staff for their assistance whenever it was needed.

Finally, we want to express our heartfelt gratitude to our family and friends for their encouragement, support, and motivation throughout this journey.

Srijita Chatterjee **Harshita Singh**

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ABBREVIATIONS

g-C_3N_4	Graphitic Carbon Nitride
MoS_2	Molybdenum Sulfide
AChE	Acetylcholinesterase
ATCl	Acetyl thiocholine chloride
OPP	Organophosphorus Pesticide
TF	Trichlorfon
CV	Cyclic Voltammetry
DPV	Differential Pulse Voltammetry
XRD	X-Ray Diffraction
FT-IR	Fourier Transform Infrared
PBS	Phosphate Buffer Saline
ITO	Indium tin oxide
EPD	Electrophoretic Deposition
RSD	Relative Standard Deviation
EDAX	Energy Dispersive X-Ray Spectroscopy
FESEM	Field Emission Scanning Electron Microscope
LOD	Limit of detection

CHAPTER 1

Introduction

1.1 Introduction to pesticides

Pesticides represent a broad spectrum of chemical compounds or biological agents meticulously deployed to combat pest, insect, and rodent populations, which are distinguished by their varied chemical compositions and physical properties [1]. These substances are increasingly prevalent worldwide, transcending agricultural applications to encompass domestic uses such as powders or sprays for pest control [2]. With approximately 1.8 billion individuals employed in agriculture relying on pesticides to safeguard crops and ensure food security, their judicious application promises heightened productivity and economic benefits by reducing labour inputs [3]. However, indiscriminate pesticide usage poses significant risks to environmental integrity and human health. The persistence of pesticide residues on food commodities poses a notable public health concern, given their potential to induce a range of diseases [4]. Human exposure occurs through oral ingestion, dermal contact, ocular exposure, and inhalation, while non-target organisms are also adversely affected [5]. Hence, it is imperative to adopt stringent safety measures and implement proper pesticide disposal practices to mitigate these risks effectively.

1.2 Toxicological classification of pesticides

Pesticides word include various classes of rodenticides, fungicides, herbicides, insecticides [3], etc. All of these are made to eradicate the population of unwanted and harmful insects, rodents, weeds, pests, etc. All of them differ in their physical and chemical properties, hence highlighting the importance of classifying them for their study. They should be studied under

their respective classifications. While some of them are found in nature, mainly major pesticides are synthetic and obtained by various chemical methods [6].

Drum suggested three of the most utilized pesticide classification methods on the basis of their properties and needs [7] . The three classifications that are popular are based on the

1) Mode of Entry

2) Pesticide function and pest organisms they kill

3) Chemical composition of pesticides

1.2.1 Classification of Pesticides based on Mode of Entry

The mode of entry refers to how pesticides come into contact with or enter the target.

A) Systematic pesticides - Plants and animals absorb these and transfer them to untreated tissues. Example- glyphosate

B) Repellents- Their bad taste prevents pests from approaching the targeted area and impairs their ability to recognize targeted pests [8]. Example- Methiocarb

C) Fumigants- They produce vapor when they enter a pest's trachea, killing the intended pest[8]. Example- Azoxystrobin

D) Contact pesticides -When in contact with pests, it takes action against them. Example- Paraquat

E) Stomach poisons -They enter the pest's mouth and digestive tract and then take action against them. Example- Malathion

1.2.2 Classification of pesticides based on function and pest organism they kill

Pesticides are categorized according to the organisms they target, and specific names indicate their actions. These pesticides' group names derive from the Latin word cide, which means "kill" or "killer," and have been included in the names of the pests they eradicate, but not always every pesticide ends with *–cides.* Some of the pesticide classifications by target cells are:

A) Insecticides - They kill and eradicate insects and arthropods.

B) Fungicides – They eradicate the population of fungi.

C) Rodenticides – They kill mice and rodents.

D) Herbicides – They inhibit the growth of plants.

E) Mothballs - Prevent damage to cloth by larvae of the moth.

1.2.3 Classification of pesticides based on chemical composition

It is the most widely used and practical method for pesticide classification, and it is based on pesticides' chemical makeup.

A) Organochlorine – It is also known as chlorinated hydrocarbon. These are the initial classes of pesticides to be synthesized and applied to agricultural fields. An organic compound contains five or more chlorine atoms. They harm insects' neurological systems, causing muscle contraction, paralysis, and eventually insect deaths. It is biodegradable, and less pollution is caused to the environment [9] for example – lindane.

B) Organophosphate – They are derivatives of phosphoric acid**.** It is a broad- spectrum pesticides that eradicate a large range of pests. They are biodegradable and, hence, are less harmful to the environment [10]. Their actions result in the long-term overlay of acetylcholine across the synapse, which impairs impulse movement across the synapse and kills insects by paralyzing them [11]. Examples – Malathion

C) Carbamates – They are similar to organophosphate pesticides in their structure but are different from them in their origin. Carbamates are carbamic acid derivatives, whereas organophosphates are phosphoric acid derivatives. They work on the same principle of organophosphate (affecting nerve transmission). They are also biodegradable and, hence, are less harmful to the environment [9]. Example – Carbaryl

D) Pyrethroids – They rank among the safest insecticides. They have a higher degree of stability than natural pyrethrins because they are made by mimicking their structure. When pyrethrins are ground, active compounds are created. They poison fish, mammals, insects, and other creatures. When exposed to light, they break down and become non-persistent [12].

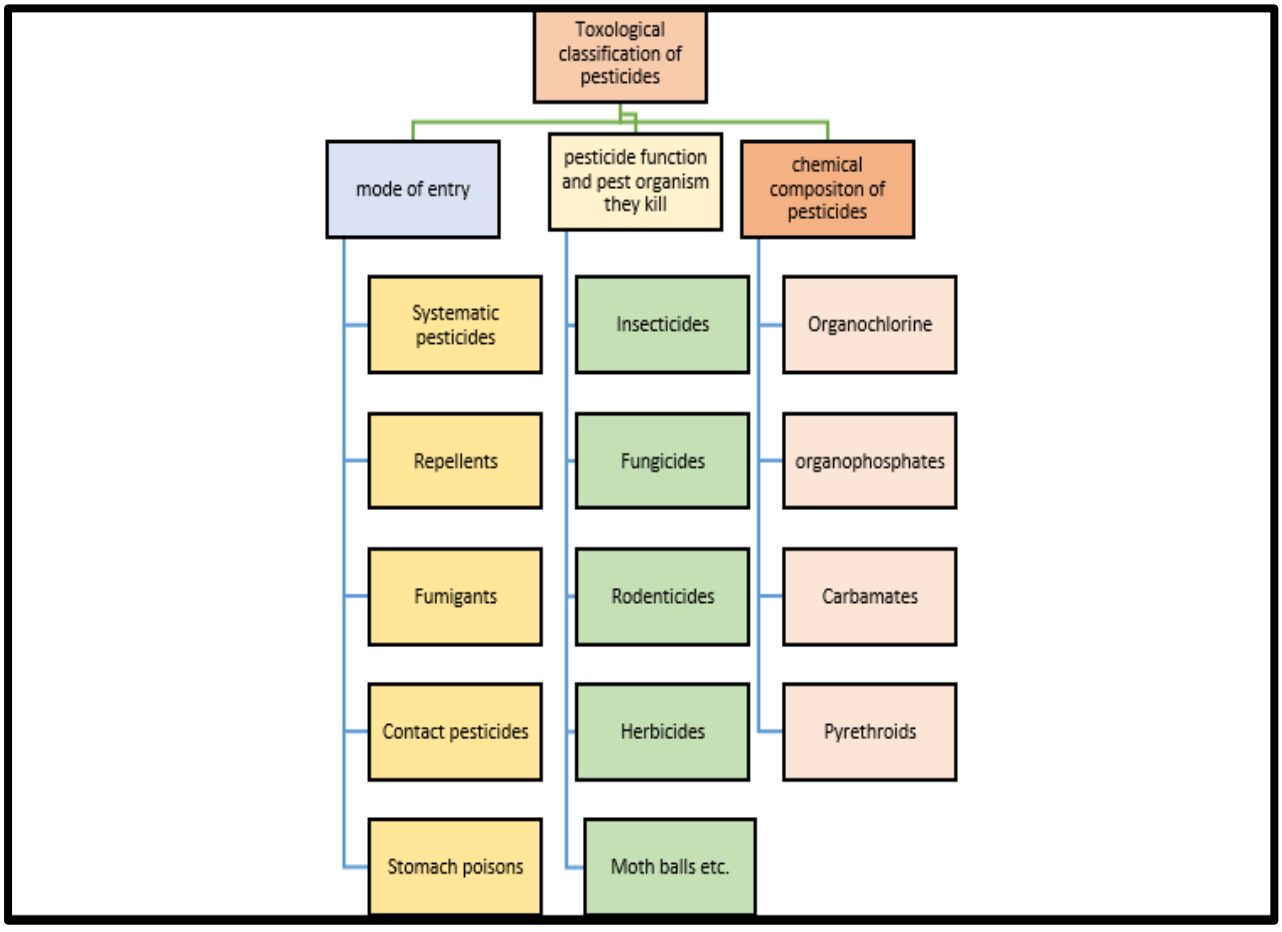


Fig 1.1: Toxicological classification of pesticides

1.3 Other Minor Classes of Pesticides

1.3.1 Classification according to sources of origin

Depending on where they come from, pesticides are divided into two categories: Bio-pesticides and Chemical pesticides.

(A) Bio-pesticides -They arise from natural sources like animals, plants, etc. They are less toxic, effective in less quantity, biodegradable, and attack specific pests; hence, they are used more than other synthetic pesticides [13]. It is categorized into three categories:

1) **Plant-based:** They are pesticides produced naturally by plants.
2) **Biochemical pesticides:** These include natural materials that use a non-harmful mechanism to eradicate pests. An example is insect sex pheromones.
3) **Microbial pesticides –** they have microorganisms like bacteria as their main active ingredient [14] . Example - *Bacillus thuringiensis* acts on mosquitoes.

(B) Chemical Pesticides – They are not found in nature. These are non-biodegradable and, hence, are harmful to the environment. These are obtained by various chemical processes and are broad spectrum pesticides, hence can attack on various types of pesticides. Their persistence power is very high, hence causing environmental pollution and destroying the ecosystem. They are further subcategorized into organochlorine, organophosphate, carbamates, and pyrethroids [15]. Example: Lindane.

Since chemical pesticides are more hazardous than biopesticides, classifying pesticides according to their origin aids in replacing chemical pesticide use with biopesticide use. **Fig. 1.2** shows a diagrammatic representation of a classification of pesticides on the basis of source of origin.

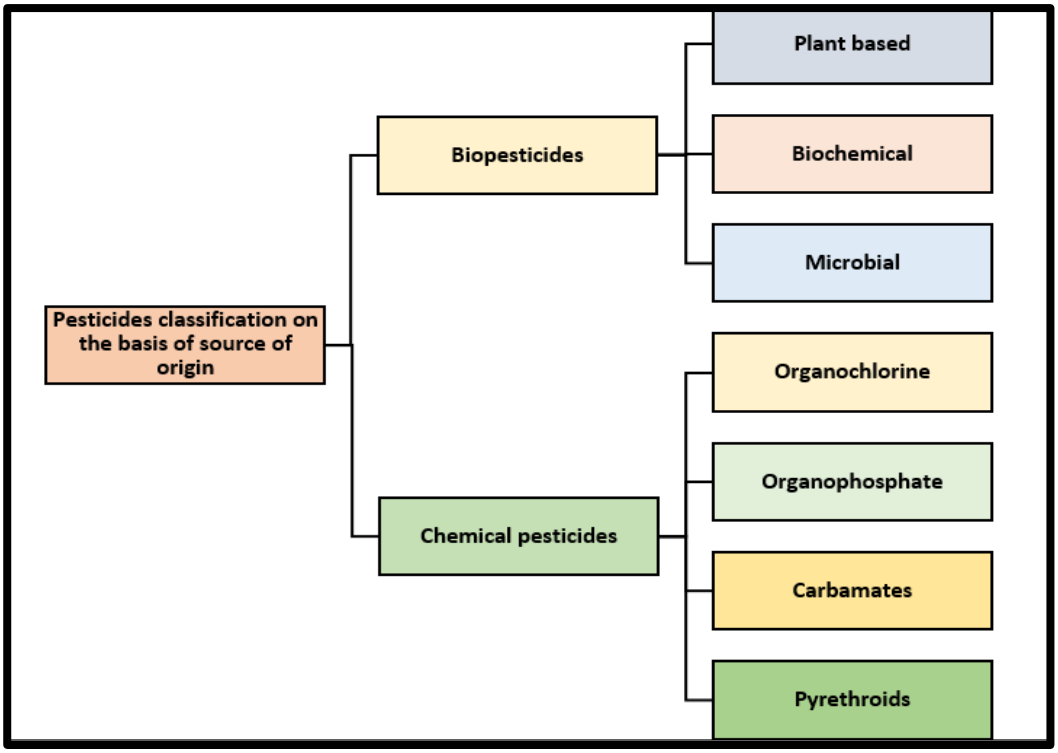


Fig 1.2: Classification according to source of origin

1.3.2. Classification based on toxicity

WHO performed the experiment in the lab on rats by applying doses to them orally and dermally. They found the LD_{50} (median lethal doses) causes death in 50 % of the rat population that is exposed to such laboratory conditions [16].Classification of pesticides recommended by WHO is shown in **Table 1.1.**

TABLE 1.1: Classification of pesticides recommended by WHO

WHO classification	LD_{50} for rats (mg/kg) body weight)		Example
	Oral	Dermal	
Extremely hazardous (class Ia)	Less than 5	Less than 50	Parathion
Highly hazardous (class Ib)	from 5 to50	from 50 to200	Dichlorvos
Moderately hazardous (class ii)	from 50 to 2000	from 200 to 2000	DDT
Slightly hazardous (class iii)	more than 2000	More than 2000	Malathion
Unlikely to present acute hazard (class iv)	5000 or more	5000 or more	Cycloprothrin

1.4 Advantages of pesticide

1) Agricultural benefit to farmers- The primary advantage of using pesticides is the financial gain experienced by farmers. By using pesticides to protect crop quality and yield, labor and other inputs are subsequently reduced [17].

2) Control unnecessary vegetation- Herbicides eradicate the unnecessary population of invasive weeds on the roads, gardens, and other areas.

3) Maintaining aesthetic quality- Maintaining an aesthetic view involves eliminating unnecessary vegetation and protecting endangered species from harmful pests [18].

4) Human health protection- Disease-causing pests and other harmful microorganisms are eliminated [19].

5) Preventing contamination- Using pesticides and insecticides during the packing and manufacturing process helps prevent the contamination of packaged goods and raw materials.

1.5 Effect of pesticides

1) Effect on human health- Pesticides can enter the human body through various routes, including eating contaminated food, breathing in a contaminated atmosphere, or coming into direct contact with them through the skin.

Food, particularly fruits and vegetables, is sprayed with pesticides, which also leak into the groundwater and soil and contaminate drinking water [18] . The pesticide spray can also float and contaminate the air and show both acute and chronic effects on human health [20].

Acute effects result from a single contact through any of the entry points, including the mouth, eyes, lungs, and skin. Acute toxicity is found by testing dermal, inhalation, and oral toxicity and eye and skin irritation in animals. Headaches, aches in the body, irritation of the eyes and skin, and dizziness are among its symptoms [21]. About 3 million cases of acute toxicity occur every year. **Fig 1.3** represents the acute effects of pesticides on human health.

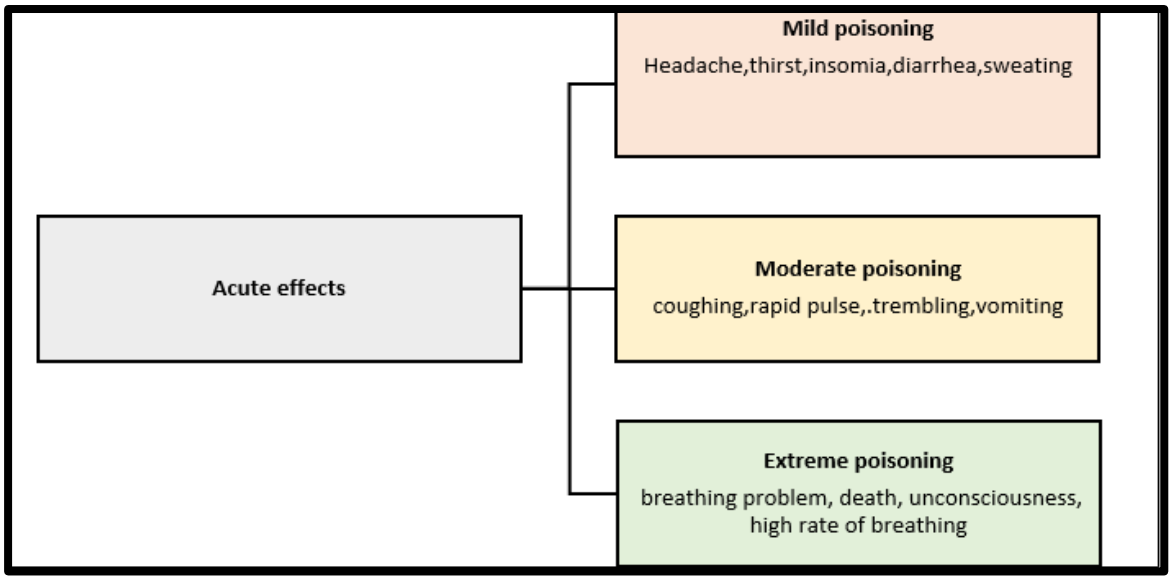


Fig 1.3 Acute effects of pesticides on human health

Any harmful effects that happen when small doses are taken repeatedly over time are referred to as **chronic effects** [22]. Chronic toxicity is more difficult to determine than acute toxicity in the laboratory. Human develops chronic illnesses when they are exposed repeatedly to sublethal pesticide concentrations over extended periods of time [23]. It has genetic changes,

birth defects, and dangerous tumors as its major symptoms, which appeared at a later stage [24]. **Fig 1.4** represents chronic diseases caused by pesticides.

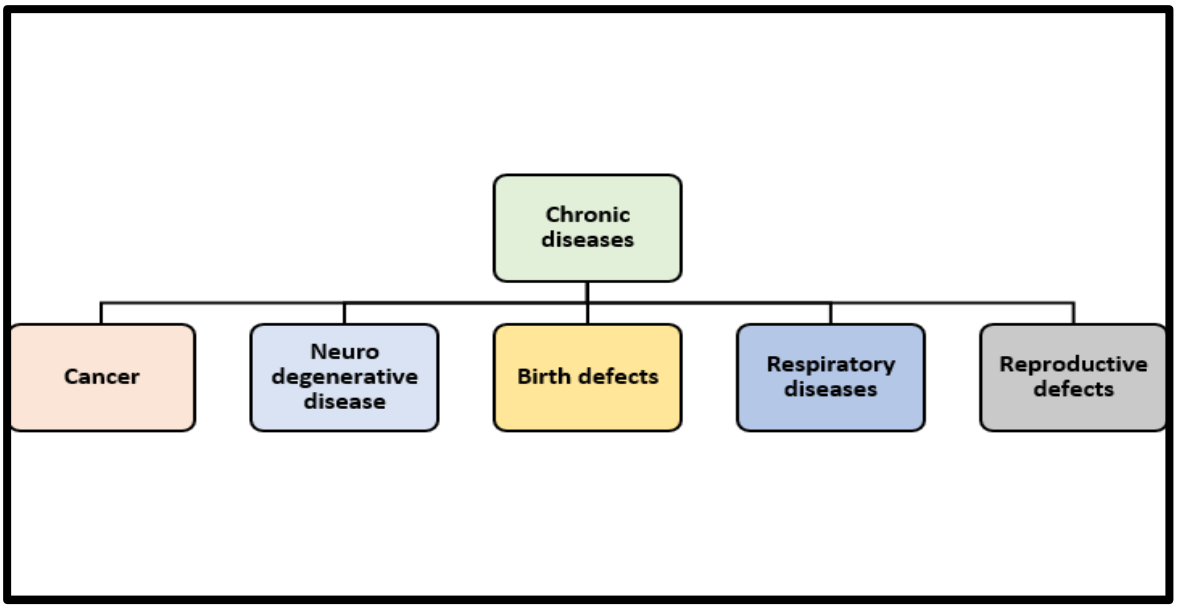


Fig 1.4: Chronic diseases caused by pesticides

2) Effect on biodiversity- the environment has many plants, microbes, and animals. A healthy environment has a large number of organisms. While pests can destroy unwanted plants, weeds, and other vegetation, they can also occasionally wipe out other beneficial species, leading to biodiversity loss and an imbalance in food webs [25].

3) Effects on water and air ecosystem- Pesticides can end up in waterways through unintentional spills, surface runoff, drifting into streams and rivers, etc. Pesticides that are applied aerially cause spray drift to enter the atmosphere, leading to an imbalance in the ecosystem of water and air, causing disbalance in the water and air ecosystem [2] .

1.6 Techniques for detection of pesticides

Given that pesticides have an adverse effect on human health, it is crucial to detect and measure them. Numerous methods have been developed for this purpose.

1.6.1 Conventional analytical techniques

Pesticides can be detected using several commonly employed conventional techniques, including gas chromatography [26], spectrophotometry [27], fluorometric analysis [28], immunoassay [29], and others. These techniques are accurate and specific but require skilled operators, are time-consuming, and require expensive equipment. They should be replaced by extremely sensitive approaches for pesticide detection [30].

1.6.2 Other advanced techniques

Because biosensors have so many advantages over conventional analytical techniques, they are used for pesticide detection to eliminate the drawbacks of these methods. Their extensive usage results from their selectivity, specificity, quick response time, portability, and environmental friendliness [31] .

1.7 Introduction of Biosensors

A biosensor is an instrument designed to quantify reactions of chemical and biological processes by producing signals that correlate to analyte concentrations [32]. Prof. Leland C. Clark demonstrated the term biosensor in 1962, hence called the Father of biosensors [33].

Any biological element, including tissues, microbes, cells, acids, enzymes, and so on, can be a part of a biological process [34]. The result of a biological element depends on the kind of enzyme and material used. The electrical form of the transducer could be either voltage or current.

Fundamentally, biosensors need to be extremely precise, unaffected by environmental factors like temperature and pH, and able to be reused. Such devices are used for wide applications, including disease surveillance, pharmaceutical development, and for detecting pollutants, disease-causing microorganisms, and biomarkers within blood, saliva, etc. The first

commercially used biosensor in the market was used in 1975 for glucose detection [35]. **Fig. 1.5** represents a typical biosensor consisting of the following parts**.**

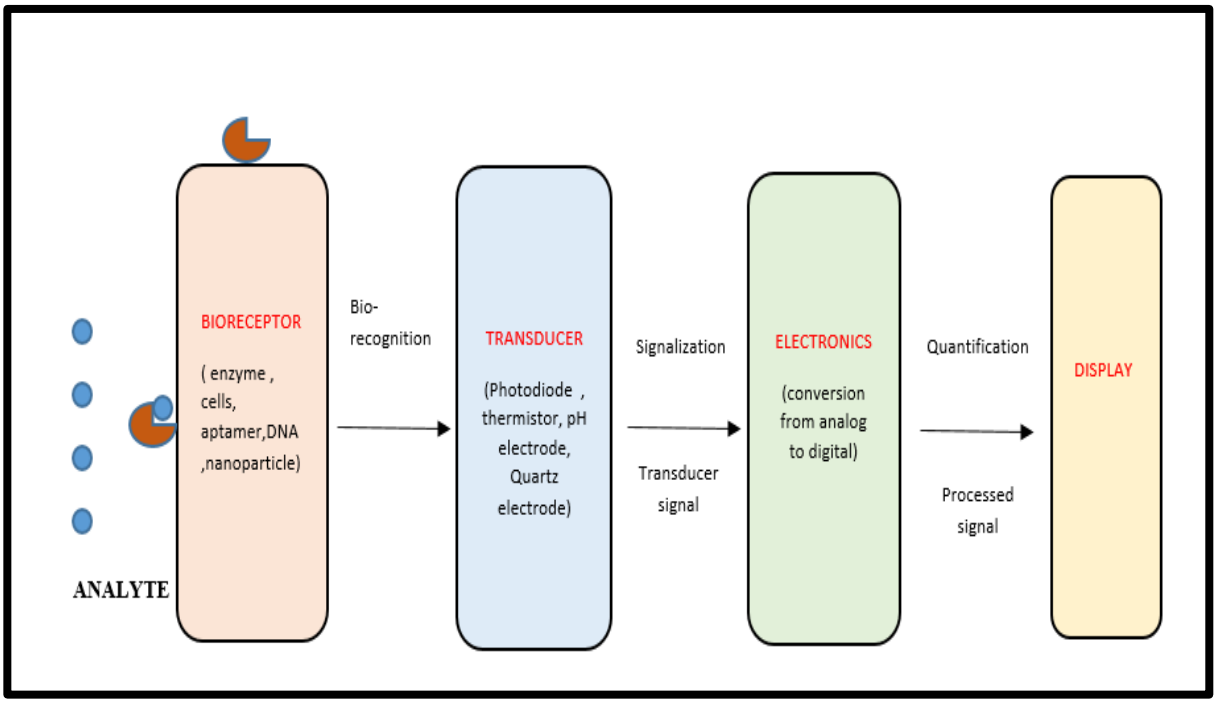


Fig.1.5 Schematic representation of Components of Biosensor

Analyte

A target substance that requires detection. For example, glucose is considered an analyte for the detection of glucose [36].

Bioreceptors

A bioreceptor is a molecule that recognizes a particular analyte. Upon interaction between the bioreceptor and analyte, a signal is produced in the form of light, heat, etc. Examples of bioreceptors are antibodies, enzymes, etc.

Transducer

The transducer converts energy (signalization) from one form to another. It attempts to convert bio-recognition events to measurable signals. Electrical signals are obtained, which tell the concentration of the analyte and the interactions of the analyte and bioreceptors [37].

Electronics

The biosensor's component processes the signal that is transduced to make it ready for display. It is made up of intricate electronic circuitry that converts analog signals into digital form and performs other signal-conditioning tasks.

Display

It consists of a system for user interpretation that produces numbers or curves that the user can understand. It combines hardware and software to give the required result in the form of an image, tabular data, numeral, etc.

1.8 Characteristics of a Biosensor

Selectivity- The capacity of a biosensor to identify and measure a particular analyte in a sample that may also contain other substances, such as contaminants and impurities, is known as selectivity. Selectivity in the context of biosensors is best illustrated by the relationship between an antigen and an antibody. Traditionally, antibodies are attached to the surface of the transducer and act as bioreceptors. After that, the transducer is exposed to a solution, usually a buffer containing salt and the antigen, where antibodies bind to antigens selectively. Selectivity must be prioritized when creating biosensors and choosing bioreceptors [38].

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

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

Sensitivity- In medical and environment monitoring uses, biosensor detects a minimum amount of analyte concentration, i.e., the limit of detection or sensitivity, as low as ng/mL or fg/mL. It validates the presence of an analyte in a sample**.**

Linearity –The ability of a measured response to a straight line at a different analyte concentration to be linear is known as linearity. The straight line is represented mathematically by y = mc, where m is sensitivity, c is the concentration of the analyte, and y is the output signal. Linear range, which is the extent of analyte concentration for which the biosensor accordance varies linearly with the concentration, is another term related to linearity [32].

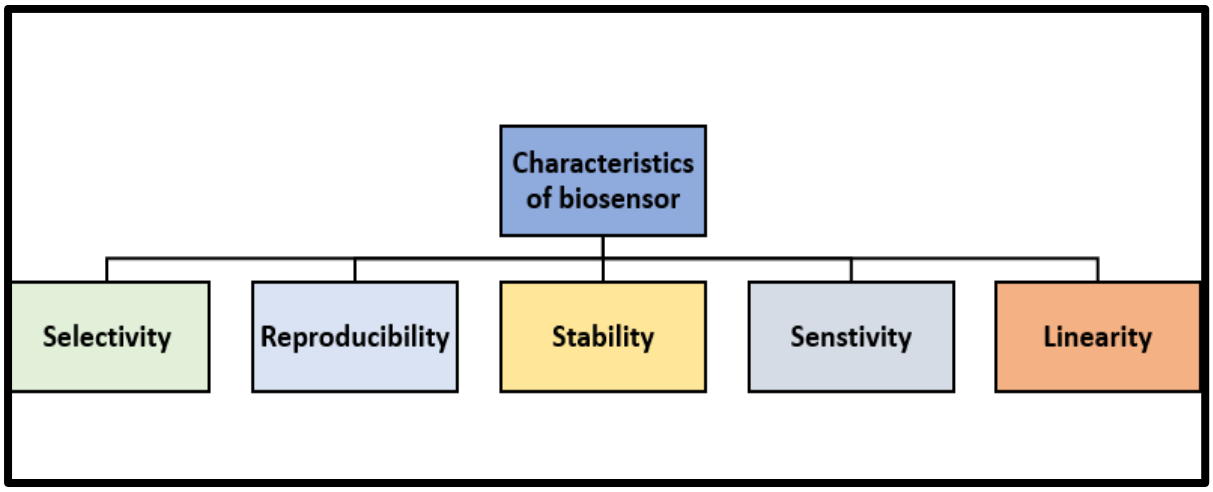


Fig. 1.6 Characteristics of Biosensors

1.9 Types of Biosensors

Sensors are classified into two ways according to Bio-component and transducer.

1.9.1 Biosensor classification based on Bio-component

Biocomponents, including enzymes, antibodies, tissue, organisms, and nucleic acids, are used in many designs of biosensors [39].

1. **Enzyme biosensor**

For enzyme-based biosensors, enzymes are employed as biocomponents. Made up of protein biomolecules, enzymes catalyze reactions to produce signals that are measured indirectly by biosensors through the formation of enzyme-substrate complexes [40]. Enzyme biosensors are widely used because of their various prospective applications. An enzyme is kept near the sensor surface in enzyme biosensors, and the enzymatic reaction detects the substrate concentration. It happens via two different reaction processes- diffusion in the product enzyme layer and enzymatic conversion in the substrate. Moreover, there are four types of enzyme-based biosensors used commercially: urea, lactate, glucose, and glutamate biosensors. Enzymes are immobilized in the matrix on the transducer surface to sustain enzyme activity. The analyte concentration is detected by measuring changes such as proton concentration, release and uptake of gases, etc [41]. These changes are transformed into measurable signals by the transducer. Generally, acetylcholinesterase (AChE) and butyrylcholinesterase (AChE) are used as enzymes for the detection of pesticides [42]. Enzyme-based biosensors are very popular due to the following reasons-

- Sensitivity-They can detect analyte even at very low concentrations. Hence, shows high sensitivity [43].
- Selectivity- They are highly selective, recognizing particular analytes among complex samples.

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

2. Immunosensors

The immunosensor uses antibodies, which are glycoproteins generated by the immune system, as a biocomponent. It functions by interacting with 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 are widely used in many potential fields, including the detection of cancer biomarkers, environment monitoring, and food safety [44].

3. Nucleic acid biosensors

Nucleic acid is utilized as a bio component in nucleic acid biosensors. Nucleic acid biosensors are used to determine the sequences of nucleic acids (DNA, RNA). It operates on the basis of complementary base pairing between the probe molecule (DNA or RNA) immobilized on a surface and the target nucleic acid sequence [45].

4. Microbial biosensors

Microorganisms are utilized in microbial biosensors as biocomponents [46]. It is based on the ability of microorganisms to identify different substances in their surroundings. When certain target molecules interact with these microorganisms, measurable reactions, such as light or electrical conductivity, are produced. Due to their affordability, sensitivity, and ability to detect a wide variety of target molecules in the form of light, color, electrical signals, etc., these biosensors are more favored than chemical biosensors. These biosensors are employed in several industries, including detecting contaminants in water and food safety. It has several disadvantages also as appropriate biosensors for molecules and macromolecules that are unable to cross the membrane cannot be created because the structure of the cell membrane acts as a diffusion barrier. The response time of microbial biosensors and the time required to return to the basic signal points have a lengthy after-use period. Among the most significant issues

during immobilization are contamination and decreased activity.

5. Lactate biosensors

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

1.9.2 Biosensor classification based on transducer

A transducer is a device for analysis that yields an output quantity that is correlated with the input quantity. Based on their transduction mechanism, biosensors are categorized [26]. The following are the classifications:

Amperometric biosensors - Amperometric biosensors work by transferring electrons to an electrode surface through the action of an oxidoreductase enzyme. The majority of these biosensors work with an enzyme system that catalytically converts electrochemically inactive analytes into products that can be reduced or oxidized at a working electrode. The potential of this electrode is maintained in relation to a reference electrode. Using an amperometric transducer, an electrochemical reaction, either reduction or oxidation, can take place at the electrode surface, producing a current proportional to the bulk substrate concentration.

Potentiometric biosensors- Potentiometric biosensors measure the potential in equilibrium at the electrode [47]. They work on the principle of charge formation in the sample via the Nernst equation in relation to the analyte activity a_1.

$$\mathbf{E = E_0 \pm (RT/nF) \ln a_1} \qquad \textbf{(eq.1)}$$

When the standard electrode potential (E_0) is 1 mol/L, then the activity(a_1) is 1. Here, T is the temperature in Kelvin, R is the gas constant, F is the Faraday constant, and n is the total number of charges on the ion. Anions and cations are denoted by the signs ±, -, and +, respectively.
In potentiometric biosensors, an inert electrode (reference electrode) and a working electrode are used to calculate potential. Various analyte concentrations are detected as the potential calculated is proportional to the logarithm concentration of electro-active species

Optical biosensors– The oldest techniques are optical techniques, which are also thought to be the most sensitive and effective. creation of superior optical fibers intended for biosensors have been expanded into the field of communication systems. Due to a number of factors, including **(a)** the optical signal is not affected by any electrical or magnetic field, **(b)** the optical sensors do not require a reference signal, and **(c)** they are simple to fabricate with high efficiency, hence, optical fiber biosensors are advantageous in biochemical and clinical analysis [48].

Piezoelectric biosensors- These transducers are thought to be exceptionally sensitive for use in biosensor applications. These biosensors' fundamental idea is based on the binding of a molecular species to the surface of the crystal, causing a mass change that ultimately causes the crystal's oscillation frequency to change [49]. It can also measure changes in viscosity and density on the sensor surface. The piezoelectric crystal is coated with high-selectivity compounds containing biological materials such as enzymes or antibodies to harness the piezoelectric effect. Practical immune applications and immune identification fields use piezoelectric transducers. Real-time tracking, tag-free identification, and ease of use are a few benefits of utilizing this kind of converter.

Thermal biosensor- It functions by measuring the amount of heat that is absorbed during a biological reaction [50]. A physical transducer and biomaterial (a calorimetric biosensor) are

combined to form it. It is utilized for clinical detection, environmental control, and the determination of antigen-antibody interaction (thermometric Elisa test). However, one drawback of the method is the high cost of the instruments.

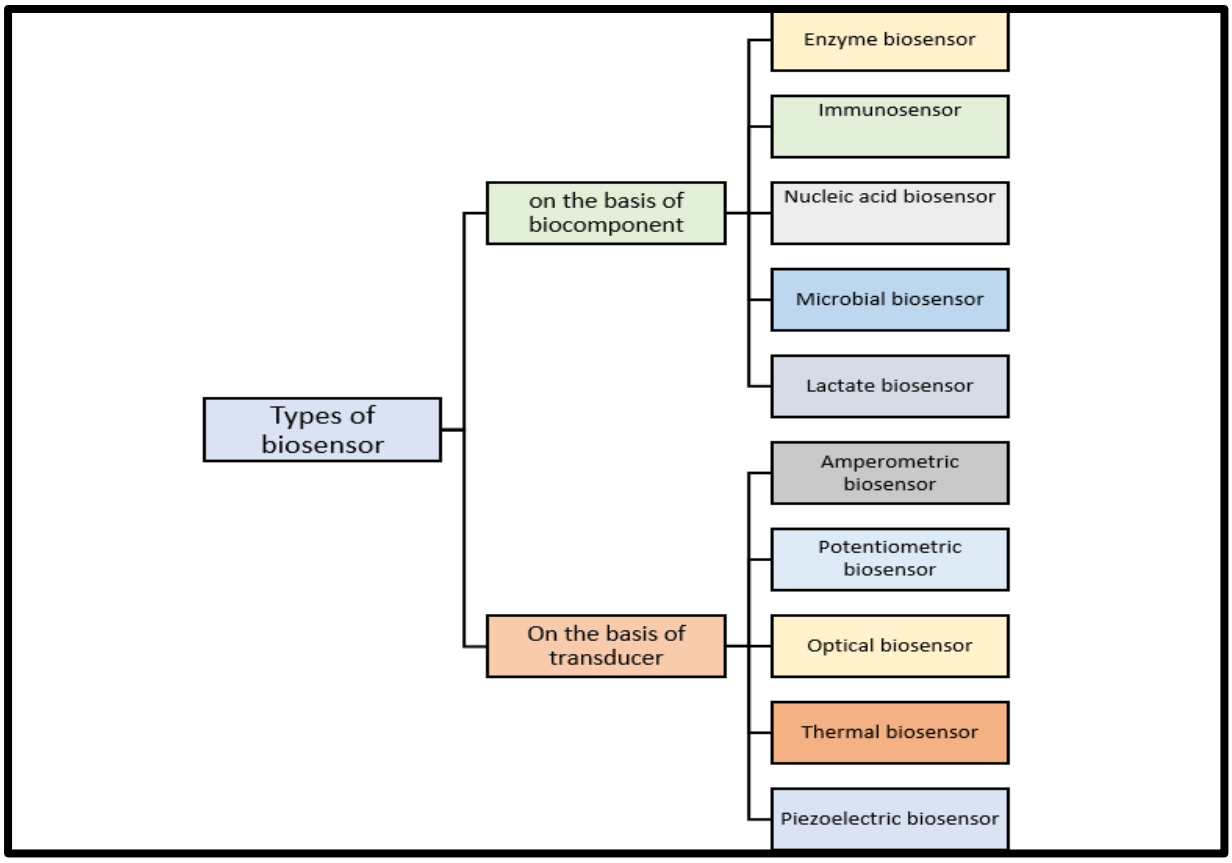


Fig. 1.7 Schematic representation of types of biosensors

1.10 Immobilization of biomolecules

The method of attaching biomolecules to solid supports so they can stay active for extended periods of time is known as immobilization [51]. Matrix refers to the stable solid surface an enzyme can bond to. The immobilization process is determined by the matrix's mechanical and chemical properties. As a matrix, materials such as glass, screen-printed electrodes, conducting and non-conductive polymer films, etc. Immobilization improves stability by limiting the mobility of biomolecules and fixing them in a specific location. When selecting an immobilization technique, care should be taken to ensure that the reactive group of the

enzyme's binding site remains unchanged and that no enzyme activity is lost. Physical entrapment, physical immobilization, covalent binding, and cross-linking are frequently employed immobilization techniques [52].

1.11 Methods of immobilization

Cross-linking- In the cross-linking technique, compounds having two or more functional groups that can bind two different materials under distinct conditions are used [51]. Compounds with two or more functional groups, like 4-azido-1-fluro-2-nitrobenzene that can bind two different materials under distinct circumstances are used in the cross-linking technique. Bifunctional and multifunctional reagents are generally used. Glutaraldehyde is most commonly used.

Advantages

1. Cost-effective
2. Less loss of biocatalyst.

Disadvantages

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

Physical adsorption

Using this technique, a biomolecule is physisorbed onto a matrix surface. As a result, the technique does not modify or cause little modification in the conformation of the enzyme. Weak forces like hydrogen bonding and van der Waal force are required for binding the biomolecule, which results in changes in various factors such as temperature, ionic strength, etc.

Advantages

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

Disadvantages

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

Entrapment

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

Advantages

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

Disadvantages

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

Covalent binding

Through this technique, the biomolecule and support matrix establish a covalent bond. Most situations use matrix functional groups that can either be activated or covalently coupled to produce such groups. Typically, nucleophilic functional groups - such as amino, carboxylic, indole, etc. found in the side chains of amino acids of proteins are employed for covalent coupling. The conditions engaged are harsher and more complex than with other techniques because they change the active center, which causes a significant decrease in substrate activity.

Advantages

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

Disadvantages

1. Regeneration of the matrix is not possible.

2. Change in conformation of substrate.

1.12 Applications of Biosensors

In the medical field - Biosensors are extensively employed in the medical field. Glucose biosensors are widely used for diabetes diagnosis. Infectious disease diagnosis is another application for them [53]. They accurately detect biomarkers at low concentrations. It is frequently used to identify biomarkers and detect cancer. They are able to recognize dangerous pathogens as well as other biological elements like enzymes and antibodies. These days, biosensors are built into mobile units and are able to identify cardiac troponin, a protein complex that senses cardiac injury and can identify acute coronary syndrome. These biosensors

make use of the concepts of machine analysis, disk with the reader, and fluorescent microfluidics. COVID-19 was identified in present circumstances using biosensors that were made of nanomaterials.

In food authenticity and processing - Foodborne pathogen detection is accomplished by means of biosensors. Biosensors are used to measure the levels of glucose and lactate in foods like milk and yogurt [54]. Biosensors detect lipids, alcohol, cholesterol, glutamate, glucose, and lactate. They also measure and identify obesity, a disease-causing artificial sweetener. Biosensors are used to assess chemical constituents, residual evaluation of agricultural drugs, food quality, and other factors like freshness and aroma.

For military defense purposes- Biosensors identify threat-causing organisms (biowarfare agents), primarily bacteria and viruses, aiding in the detection of biological attacks. Since diseases are spreading and bioterrorism is on the rise, it is critical to identify and prevent both because they have the potential to wreak massive havoc. Biosensors look for these biowarfare agents, such as viruses, fungi, and bacteria.

In environment protection - It finds the causes of soil disease as well as the concentrations of pesticides, fertilizers, and heavy metals in the soil and water [55]. Biosensors detect harmful algal blooms, algal RNA, and halogenated compounds in water bodies. They help in pollution monitoring by detecting the concentration of harmful pollutants.

Nanotechnology - By leveraging the unique characteristics of nanomaterials, biosensors that employ nanotechnology can greatly improve their sensitivity, specificity, and overall performance when it comes to identifying biological materials. High surface area to volume ratio nanomaterials, like nanoparticles, nanotubes, etc, improve the interaction between sensors and the target molecules. It reduces the time needed to analyze small molecules with intricate structures. Its selectivity rises if the value found is high when the surface area is proportionate

to the volume. There is no diffusion issue, and very little energy is needed for the research. Thus, a lengthy life is guaranteed for the biosensors. It guarantees the study's completion without endangering the cells.

1.13 Future scope of biosensors

The biomarker approaches of the future will investigate the development of several options for producing precision diagnostics, medications, and equipment [56]. Drawing samples has to be disruptively improved by the human person. Implantable biosensors have the potential to greatly speed up the manufacturing of tailored drugs. It will enable researchers to precisely monitor how possible drugs interact with the body and assess whether a drug has a chance to advance medicine. Moreover, biosensor chip technology might be added to the body to identify intricate blood DNA alterations before the onset of illness in the initial growth phases. Biosensor technology can be applied to low-cost, reversible care point equipment. It's also applicable for ongoing implanted equipment tracking.

Numerous sensors that use nucleic acid hybridization detection as their basis are making exciting progress in this field. One such sensor is the "Environmental Sample Processor," which is being established at the Monterey Bay Aquarium Research Institute. Its objective is the automated detection of toxic algae in situ from moorings using ribosomal RNA probes. One of the main aims is also the monitoring of pollutants, toxic metals, and pesticides.

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

1.14. 2-D based nanocomposite

Progress has been made in the field of material science due to the formation of nanocomposites. Nanocomposites are special materials made up of combining two or more particles of nano-size [57]. They have wide, unique, and unique physical and chemical properties due to the combination of properties of their processor materials [58]. They are used in wide applications. In the perspective of having unique designs and combining properties not found in traditional composites, they are said to be the materials of the twenty-first century [59]. 2-D materials are made up of thin layers joined by van der Waals force [60].The layers are a few nm thick. Electrons easily move in the layers of 2-D materials. Materials like g-C_3N_4, graphene hexagonal boron nitride, and transition metal dichalcogenides are some examples of 2-D materials [61].They show excellent mechanical strength, electrical conductivity, rigidity, and flexibility.

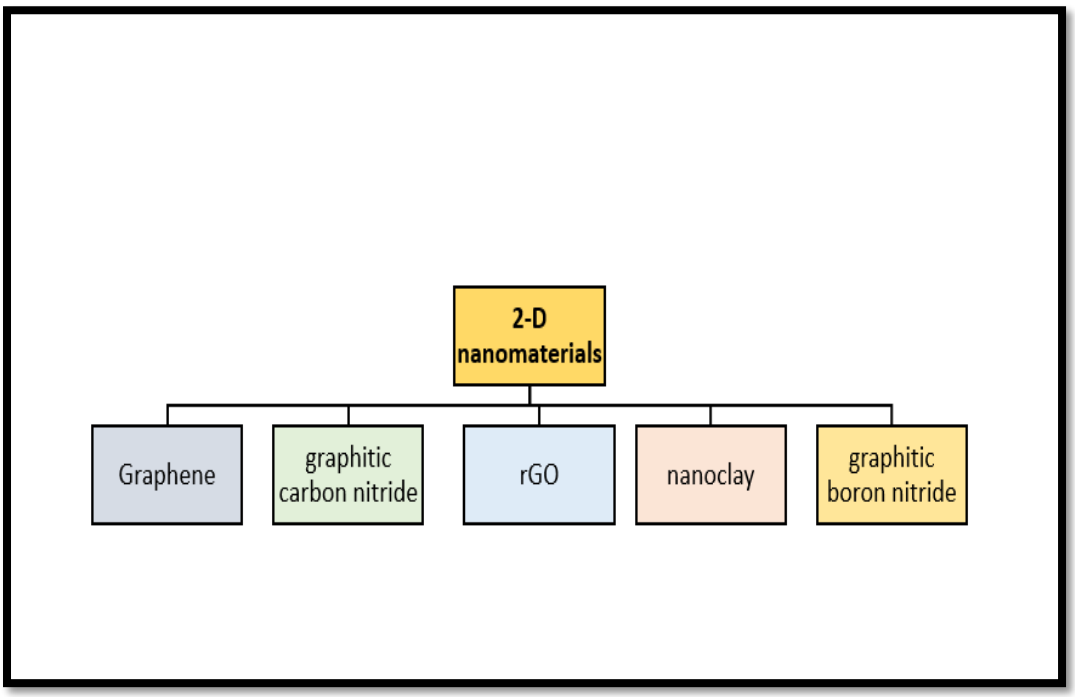


Fig.1.8 Example of 2-D nanomaterial

1.14.1 Properties of 2-D nanocomposite

They possess unique properties, such as

1. **Improved mechanical property**- They show improved strength and toughness compared to other traditional materials due to the large surface area of the nanocomposite, which reinforces the matrix [62].
2. **Enhanced thermal stability**- By adding nanoparticles, composite materials can become more thermally stable and resistant to heat-related damage. This feature is especially handy for uses that involve high temperatures [63].
3. **Improved Electrical Conductivity**- Nanocomposites can have either improved electrical conductivity or insulation properties, depending on the kind of nanoparticles used and how dispersed they are within the matrix. Therefore, they can be used in a variety of electronic applications [64].
4. **Enhanced Optical Properties**- Nanocomposites can exhibit special optical properties, such as increased transparency, fluorescence, or light scattering, depending on the nanoparticles mixed into the matrix [65].
5. **Barrier Properties**- Because of the high surface area and fragility of the areas within the matrix that are filled with nanoparticles, nanocomposites can offer better protection against gases, moisture, and other environmental variables.

1.14.2 Applications of 2-D nanocomposite

1. **Structural Materials:** Metals, ceramics, and polymers are materials whose mechanical qualities can be improved using nanocomposites. Integrating nanoparticles increases the durability, rigidity, and strength of these materials, making them appropriate for use as building materials, automobile parts, and aerospace components [66].

2. **Packaging:** By strengthening the barrier qualities of food packaging and preventing oxygen, moisture, and other impurities from passing through the material, nanocomposites help prolong the shelf life of perishable items [67].
3. **Electronics:** To create conductive substances with better electrical and thermal conductivity, nanocomposites are used.
4. **Energy Conversion and Storage**: Batteries and supercapacitors are two examples of devices that extensively use nanocomposites for energy storage. They are also utilized in fuel.

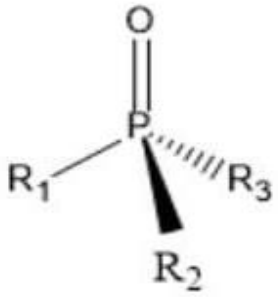
CHAPTER 2

Literature Review

2.1 Introduction to Organophosphate Pesticides

Organophosphorus pesticides are a diverse class of pesticides that are commonly used to safeguard crops from insects and are highly toxic in nature [68][69]. These compounds are usually esters, amides, or thiol derivatives of phosphonic acid. Despite the fact that fewer pesticides were developed at that time, insects acquired resistance to the pesticides due to their frequent exposure, ultimately resulting in less effectiveness of the pesticide [70]. Hence, new pesticides are needed. Pesticides range in toxicity and can be categorized as poisonous, common substances, or deleterious.

The structure of Organophosphate pesticides is -:



The widespread consumption of these pesticides in fruits, vegetables, and contaminated water sources have adversely affected human health and other organisms such as fishes, amphibians, birds, etc [71]. Residues of OPPs show acute toxicity by the irreversible inhibition of the acetylcholinesterase (AChE) enzyme via phosphorylation, which subsequently arrests the hydrolysis of the substrate acetylcholine (AChE) and blocks choline and acetic acid (Ch)

production [72][73]. Thus, AChE is incorporated in the synaptic ends, which leads to the overstimulation of nicotinic and muscarinic receptors in the human nervous system [74]. Overstimulation of OPPs causes diseases such as:

Four neurotoxic disorders are caused by OPPs in humans. These syndromes are-

1. Intermediate Syndrome - This syndrome arises within 24 to 96 hours after exposure to organophosphates [75]. This syndrome largely affects patients without cholinergic symptoms. Even though intermediate syndrome is identified as a neuromuscular junction disorder, its exact risk factors, etiology, and incidence are not understood clearly. It causes weakness of respiratory muscles and motor cranial nerves, followed by numbness of neck flexors [76]. IMS typically occurred in patients with severe and acute inhibition of AChE, leading to continuous excess of AChE in the neuromuscular junctions, though it did not affect all patients. Other risk factors include slowed metabolism of OPPs, muscle respiratory weakness, increased muscle enzymes, and insufficient or delayed therapy with pyridinium or atropine oximes.

2. The cholinergic Syndrome - Signs and symptoms associated with cholinergic syndrome in cases of severe OPP poisoning are predictable because of their biomedical mode of action and are directly correlated with acetylcholinesterase activity [77]. In cases of poisoning in humans, general intense symptoms of peripheral and nicotinic poisoning are clearly evident. These symptoms are sweating, tremors, lacrimation, cramps in the abdomen, and other gastrointestinal symptoms. These symptoms are followed by central effects such as dizziness, headache, fatigue, and paraesthesia. Lastly, seizures, coma, and convulsions may occur.

3. Organophosphate Induced Delayed Polyneuropathy (OPIDP) – It is a distinct neurological phenomenon that is caused by a single exposure to OPPs, with symptoms showing after 10-20 days or longer. OPIDP is a rare neurogenerative disease in humans distinguished by the loss of function and ataxia of the distal portions of sensory and motor axons within

peripheral nerves and in the ascending and descending spinal cord tracts [78][79]. The early neurological signs are usually cramp-like sensations in the calves and tickling in the hands and feet, accompanied by distal numbness and parenthesis. In severe cases of OPIDP quadriplegia along with foot and wrist, foot drop and mild pyramidal signs were noticed.

4. Chronic organophosphate-induced neuropsychiatrist disorder (COPIND) - Prolonged OPP exposure has been related to impaired neurobehavioral performance; however, not all epidemiological investigations [80]. COPIND occurs without cholinergic symptoms and is not dependent on inhibition of AChE [81]. COPIND usually appears with a delay and lasts for a long period, possibly indicating permanent damage to the central nervous system [82]. The most common signs of COPIND are changes in mood (depression, emotional lability, anxiety), cognitive deficit (impairment in learning, memory, and concentration, problems with processing, attention, and information), chronic fatigue, peripheral neuropathy, autonomous dysfunction, and extrapyramidal functions resting tremor, postural instability [83].

2.2 Importance and need for detection of Organophosphate pesticides

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

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

2. Adverse effects on humans, plants, and ecosystems - It can negatively impact humans, plants, and animals even at minimal concentrations [84]. OPPs typically enter the body by ingestion, inhalation, cutaneous, and injection. Contamination to OPPs might occur in workers manufacturing pesticides, floriculturists, pesticide applicators, and farmworkers. Prolonged exposure to OPPs is related to a number of issues: memory and verbal difficulties, visual-spatial performance, coordination, etc [85][86]. Moreover, OPPs are observed to affect the health of

humans during both embryonic and adult stages and to raise morbidity and mortality in patients with chronic poisoning [87]. The presence of residues in agricultural products can cause chronic toxicity to the health of humans [88].

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

2.3 Literature Review

In a recent study, nanomaterials were utilized for the fabrication of OPP sensing devices because of their excellent biocompatibility, electrical conductivity, and magnetic and optical properties [90]. Nanomaterials offer numerous advantages, such as the large specific surface area to obtain more sensitive detection, and thus, it is an intense research hotspot in biosensor construction [91]. For the detection of OPPs, a range of nanomaterials are used, such as nanoparticles of metals and their oxides, graphene, carbon nanotubes, polymer with nanomaterial composites, etc. Thus, the analytical performance of the developed electrode reported in previous studies has been compared with the present work.

Table 2.1: Recent advances in the analytical capability of electrochemical biosensors for the detection of trichlorfon

S.No.	Fabricated electrode	Detection methods	Linear range	LODs	Ref.
1.	MWCNT@Au-g-PMAEFc/GCE	DPV	50 nM-10 µM	27 nM	[92]
2.	AChE/Pbo_2/TiO_2/Ti	Amperometry	0.01-20 µM	0.1 nM	[93]
3.	CHIT/ Au electrode	SGGT	300-3000 nM	10 nM	[94]
4.	MHPBC/GCE	DPV	0.1 nM-10µM	35 nM	[95]
5.	AChE/Ag@CuO/ITO	CV	5-35 nM	1.59 nM	[96]
6.	(PDDA/Chox)(PDDA/AChE)$_2$	LBL	0.005 mM-0.4 mM	0.001µg/ml	[97]
7.	Poly (FBThf)fMNPs/AChE	CV	0.125 mM-2.6 mM	6.66×10^{-3} mM	[98]
8.	T-MIP/GCE	CV	10^{-8}-10^{-6} g/ml	2.8×10^{-9} g/ml	[99]

2.4 Characteristics of the material

In performing electrochemical procedures selection of the suitable electrode material is necessarily important. For the detection of OPPs numerous electrode materials have been developed and reported. Recently, 2D nanomaterials have been widely used for the detection of OPPs due to their unique chemical and physical properties, which further enhance the

performance of electrochemical sensors [100]. Their catalytic effect and large surface area affect the sensitivity of the biosensor [101].

2.4.1 Graphitic carbon nitride (g-C_3N_4)

Graphitic carbon nitride or g-C_3N_4 is a polymeric consisting of nitrogen, carbon, and a few hydrogen impurities linked via tris-triazine patterns [102]. In comparison with the majority of materials of carbon. It exhibits electron-rich features, basic surface functionalities, and hydrogen bonding motifs as it contains N and H atoms [103]. Ideal g-C_3N_4 comprises an entire assemblage of C-N bonds without localization of electrons in the Π state. g-C_3N_4 is a Π conjugated polymer, in which its defect-abundant nitrogen-bridged poly-heptazine structure provides it with enhanced thermal stability in organic solvents, N-type distinctive electronic structure, acidic and alkaline conditions, a metal-free and polymeric semiconductor with band gap (2.7eV). g-C_3N_4 are ideal for environmental photocatalytic and a range of energy applications, particularly degradation of harmful pollutants, reduction of carbon dioxide, etc [104][105]. Properties such as good biocompatibility and bio-metabolizability make them suitable for relevant biomedical applications [106][107][108] **(Fig. 2.1)**. g-C_3N_4 is a promising nano platform over a variety of applications such as photoluminescence, photo-electrochemistry, electrochemiluminescence, bioimaging, hydrogen evolution, metal-free

photocatalysis and its electron-donating property [109][110]. g-C_3N_4 nanostructures play a crucial role in the construction of g-C_3N_4-derived materials utilized for electrochemical sensing. Modification of g-C_3N_4 has proven to be effective for the determination of analytes by using electrostatic contact of the electroactive species by supporting the large molecules between the C1s and N1s atoms [111].

To summarize, g-C_3N_4 has enormous applications in various fields due to its intriguing properties, such as enhanced thermal stability in organic solvents, biocompatibility, bio-

metabolizability, electrochemiluminescence, fast electron transfer conjugation structure, catalytic applications, etc [112] . Due to its high sensitivity, selectivity, and easy preparation, it is used as a sensor material.

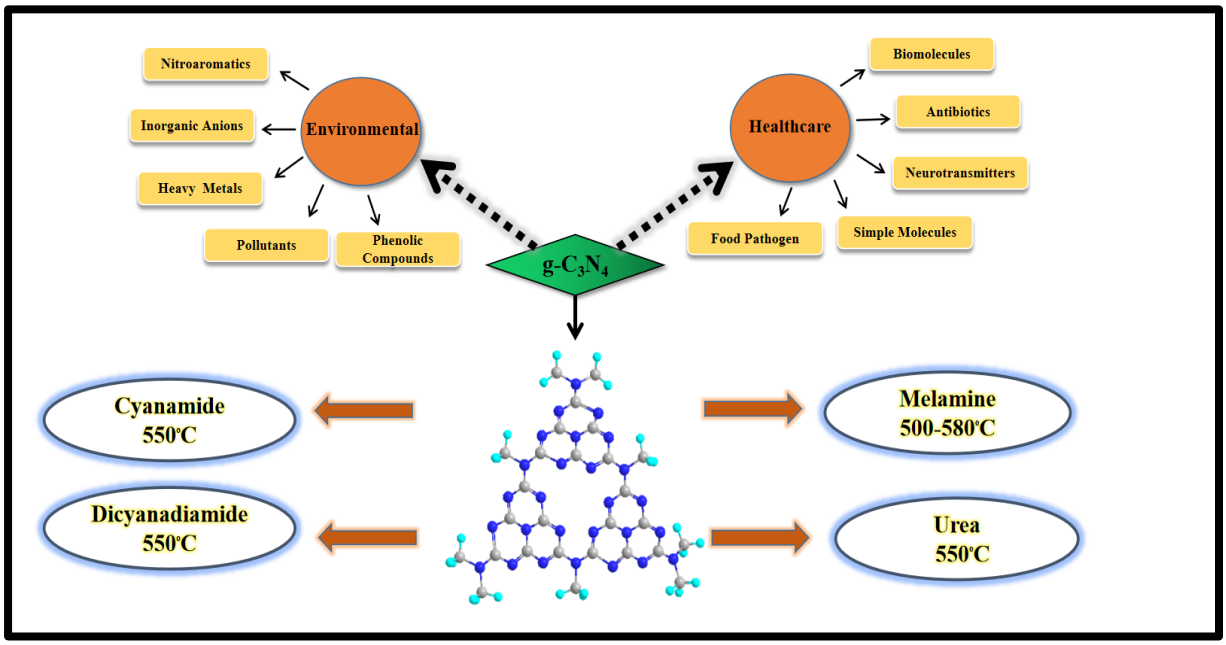


Fig. 2.1 Diagrammatic representation of g-C_3N_4 and its uses

2.4.2 Applications of g-C_3N_4

1. As a photocatalyst

g-C_3N_4 exhibits a suitable mid-wide band gap for effective visible light absorption. Moreover, its reduction and oxidation potential is appropriate for splitting water. Additionally, its flexibility in modifying with metals further entrapping to create active sites containing multiple melon moieties is another point to increase photocatalytic performance. All these characteristics make g-C_3N_4 a promising material as a photocatalyst [113][114][115].

2. In CO_2 conversion

In green technology, photocatalytic reduction of carbon dioxide (CO_2) into fuels based on carbon is regarded as green technology for directing shortage of fuel energy and greenhouse

effect globally [116]. However, the elevated recombination of the hole and photo-induced electrons in pure g-C_3N_4 greatly suppresses the efficiency of CO_2 photoreduction conversion.

3. Degradation of pollutants in wastewater

Recently elimination of different emerging contaminants from water and wastewater has gained increasing attention [117][118]. Heavy metals and organic pollutants could be eliminated by photo-generated holes and photo-induced electrons [119]. However, pure g-C_3N_4 has fast charge recombination due to its low pollutant removal capacity. Consequently, a lot of effort has been spent enhancing its rising property via increasing photolytic capacity.

2.4.3 Molybdenum Sulphide

Molybdenum Sulphide is a crystal structure comprising loosely coupled layers of S-Mo-S bond where the Mo layer is sandwiched within two layers of sulfur atoms and composed of a large direct bandgap at a range of 1.8eV [120]. Molybdenum disulfide naturally occurs in a layered solid and has applications in both bulk and dispersed forms. Crystals of MoS_2 comprise weakly interacting, vertically stacked layers connected through weak van der Walls forces [121][122]. The length of the Mo-S bond is 2.42Å, and the optimized lattice constant of a monolayer of MoS_2 has a thickness of about 3.18Å, which is then consistent with the earlier predictions [123]. In electrochemical biosensors, MoS_2 offers two key advantages. Firstly, it offers more electrochemically active sites. Secondly, incorporating additional components like metal nanoparticles or biorecognition (e.g., antibodies, aptamers, enzymes) can enhance the properties of the transducer [124]. Structural defects in MoS_2 include grain boundaries, point defects, and edges, which play a crucial role in sensing applications. For instance, defects in MoS_2 facilitate the possibility for functionalization and surface modification, which can provide significant applications in optical, chemical, and physical properties.

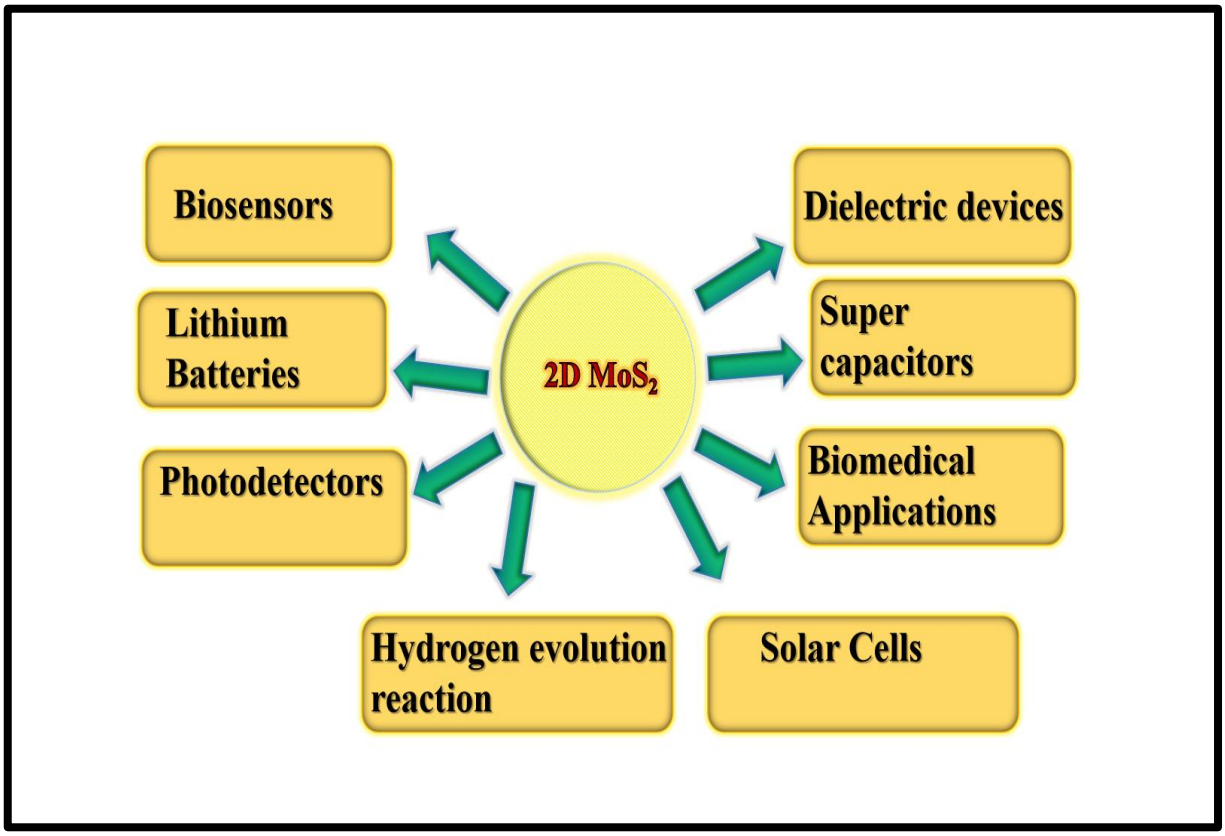


Fig. 2.2 Figure depicting the various applications of MoS_2 nanoparticles.

The most important feature of MoS_2 is its plane ultrathin structure, where the holes/electrons are confined within a plane of atomic thickness. Thus, two-dimensional MoS_2 is sensitive to the environment. In general, MoS_2 would be an excellent material for biosensors [125] **(Fig. 2.2)**. There are various types of 2D MoS_2 biosensors, such as reverse electroluminescent systems, electrode-based devices, and electrodeless optical systems.

In summary, the presence of electrochemically active sites and the incorporation of various biorecognition elements can increase the characteristics of transducers. Additionally, MoS_2 finds applications as solar cells, photodetectors, supercapacitors, dielectric devices, etc.

2.4.4 2D MoS_2 in the field of biosensor

1. Electrochemical biosensor based on 2D MoS_2

Developments in characterizations and nanofabrication electrochemical sensors, mainly nano-electrochemical sensors, exhibit strong potential in areas of food packaging, healthcare, monitoring of the environment, etc [126]. Ultrathin 2D nanomaterials and some wide-range bandgap 2D nanomaterials have gained a lot of attention because of their unmatched benefits in the construction of electrochemical biosensors. It is commonly known that 2D MoS_2 is greater than bulk MoS_2. In comparison with excellent conductors like graphene and metal. 2D MoS_2 shows no advantages in electrochemical biosensors. Moreover, MoS_2 prepared by sonication-assisted extrapolation in specific solvents has enhanced catalytic activity.

2. Fluorescent biosensor based on 2D MoS_2

Fluorescent biosensors based on 2D MoS_2 are divided into 2 types. First is 2D MoS_2 as an efficient quencher which uses (FRET) fluorescence biosensor energy transfer [127].Second is as a type of intrinsic emission of 2D MoS_2 instead of labeled fluorophores. Various fluorescent biosensors based on 2D MoS_2 have been created due to their strong biocompatibility, large surface area, efficient fluorescence quenching feature for amplification of signal [128].

3. Electrochemiluminescence biosensor based on 2D MoS_2

A combination of chemiluminescence and electrochemical methods is called electrochemiluminescence (ECL) is called electrochemiluminescence. It consists of a light emission process in a redox reaction of an electrogenerated reactant at the surface of electrons [129]. 2D MoS_2 cannot generate electrochemiluminescence itself, but can be utilized in other roles like amplification of signal or acting as a strong substrate. Thus, the ECL signal is produced through another nanomaterial, like quantum dots, which are polymer-coated or quantum dots.

4. Calorimetric biosensor based on 2D MoS_2

MoS_2 has a robust characteristic optical absorbance across a wide spectrum of wavelengths. Confinement of electronic motions, as well as the absence of interlayer interference, leads to monolayer band gap [130].

5. Field effect transistor (FET) based on 2D MoS_2

Field effect transistors provide an efficient, simple, real-time, and inexpensive sensing platform for a range of target analytes due to their fast electronic detection without biomolecule labeling, portability, low consumption of power, as well as on-chip integration possibility of both measurement and sensor systems [130]. 2D MoS_2 exhibits rapid saturable absorption and tends to interact efficiently with donor-like targets, which have distinctive potential for detecting gases like ammonia, NO, and triethylamine. In general, a Field effect transistor (FET) based on 2D MoS_2 exhibits N-type behavior; the presence of a band gap would enhance its sensitivity [131][132].

CHAPTER 3

Material and Methods

3.1 Introduction

This chapter gives an overview of the materials utilized for fabricating graphitic carbon nitride, molybdenum sulfide, and their nanocomposites for the detection of organophosphate pesticides. It covers all the morphological, structural, and electrochemical techniques for the characterization of the synthesized materials and the fabricated electrode. It also discusses methods employed for immobilizing AChE on the surface of the electrode.

3.2 Materials

The following are the details of the materials utilized in the following experiment.

3.2.1 Chemicals

Sodium Molybdate dihydrate (Na_2MoO_4; ≥ 99.5%), acetylthiocholine chloride (ATCl), Thiocholine (CH_4N_2S), trichlorfon (TF), glutaraldehyde solution (Glu; ≥99.5%), uric acid (UA; 99%), ascorbic acid (AA; 99%), were bought from Sigma-Aldrich, India. Ethanol, acetone, melamine, and sodium chloride were purchased from Thermo–Fischer Scientific, India. All the chemicals are used without purification and are of analytical grade.

3.2.2 Solutions and Buffers

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

3.2.3 Characterization Methods

Several techniques, such as Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD), are used for the structure analysis, and thus, all developed electrodes and synthesized materials are characterized. The morphological and elemental techniques of characterization, like scanning electron microscopy and energy-dispersive X-ray spectroscopy, were utilized in the current study. Electrochemical characterization techniques, such as cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS), were also used for characterization.

3.2.4 Fourier transform infrared spectroscopy (FTIR) spectroscopy

In IR spectroscopy, IR spectra are transmitted through a sample, where one part is absorbed, and the other part is transmitted through the sample. The sample's molecular fingerprint is produced by the resulting spectrum, which shows the absorption of the molecule and transmission **(Fig. 3.2)** depicting IR spectroscopy [133].Thus, IR spectroscopy is used for the analysis of organic, inorganic, and polymeric materials. The fingerprint of the sample is shown by IR spectroscopy, with the absorption peak that matches the vibrational frequency within the bonds of the atoms [134][135].

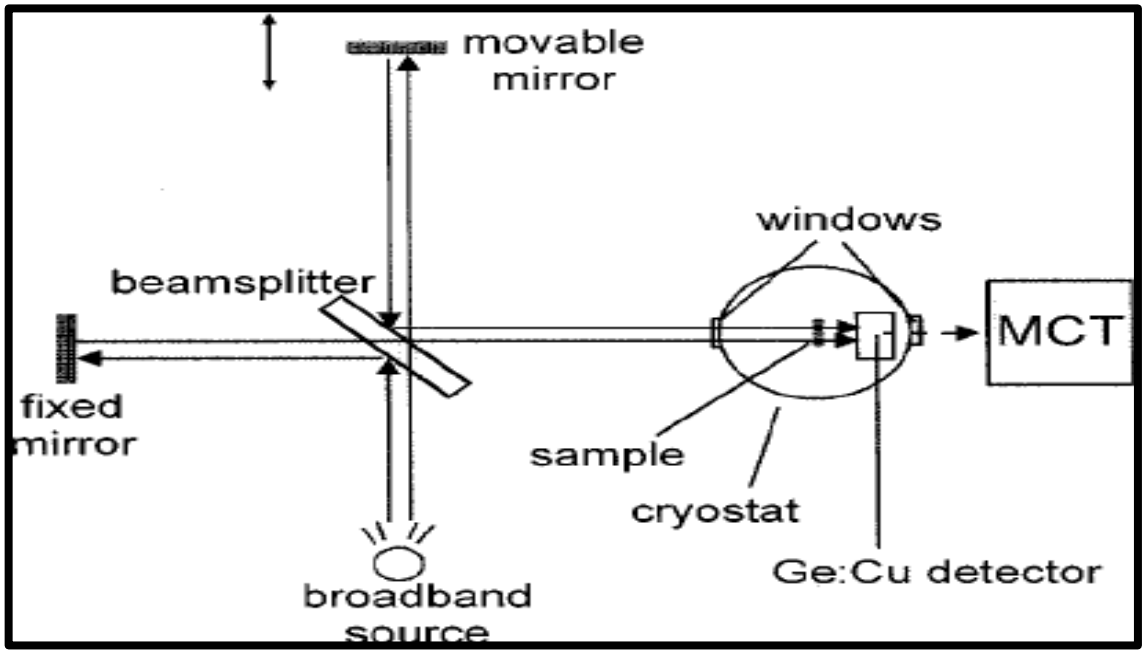


Fig. 3.1 Diagrammatic representation of Michelson interferometer

FTIR spectroscopy can be used for a broad range of frequencies shown as UV (ultraviolet), near-infrared, visible, far infrared, and mid-infrared by distinguishing appropriate beam detectors and splitters. None of the other dispersive techniques can encompass such a broad range of frequencies [136]. One of the biggest advantages of FTIR is that it works as a quantitative tool in order to carry out multicomponent studies [137]. Beer's law additive nature and available software are the basis of the quantitation of several components, which

can thus obtain the absorbance values, compute the calibration factor matrix, and estimate the unknown concentrations. The majority of FTIR multicomponent analysis techniques depend on the K/P values of the matrix, which uses a mixture of certain concentrations as a calibration standard and directly the mixture of unknown concentrations [138]. FTIR differs fundamentally from traditional dispersive IR(infrared) spectroscopy as it relies on interferometry [139]. Spectrophotometry is the device that identifies a compound's absorption spectrum. In contrast to traditional spectrophotometers, a Fourier transform (FT) spectrophotometer gives a spectrum of IR more rapidly.

The FTIR instrument emits an infrared radiation beam which is generated from a black body source. Eventually, the beam transverses to the interferometer, where spectral encoding takes place. An interferogram is a result that is caused by the recombination of the beam with different path lengths, thus creating constructive and destructive interference. This beam now reaches the compartment of the sample, and the sample accumulates certain frequencies of energy that are only present in the interferogram. Moreover, the detector monitors the distinct interferogram signal in energy against time for every frequency. Meanwhile, the beam is imposed to serve as a background or reference for the operation of the instrument [140].

Lastly, the desired spectrum was generated as the interferogram subtracted the spectrum's background from the spectrum of the sample by Fourier transformation (FT) computer software [141][142].

3.2.4 X-ray diffraction (XRD)

The X-ray diffraction method **(Fig. 3.2)** is a commonly used method for analyzing atomic spacing and crystal structure [143]. The principle of X-ray diffraction relies on the constructive interference of crystalline samples and monochromatic X-rays. These X-rays are generated by CRT(cathode ray tube) and then filtered to generate monochromatic radiation aligned to focus and directed toward the sample [144]. X-ray has a 10nm wavelength covalent bond length and radius of atoms. Employing longer wavelength radiations (ultraviolet) would produce a low resolution to determine atomic position. Conversely, shorter wavelength radiations (Gamma radiations) would result in the phenomenon of inelastic scattering [145]. The position of the polycrystalline and the crystalline structure are determined by this method. Bragg devised a law called Bragg's law from which relationships can be established between the incident beam's wavelength, the diffracted beam's angle, and the atomic spacing that could be determined in the sample's crystalline structure.

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

where integer n signifies the order of diffraction, λ signifies the wavelength of X-rays that are emitted, d signifies the interplanar distance of the planes of crystal that diffract through the beam, Θ signifies the angle among the surface of the reflecting crystalline lattice and the incident ray [146]. In studies of XRD, the material is scanned across a range of predefined 2Θ angles, and all the X-rays that are diffracted are obtained from the sample. After monitoring the diffraction pattern, the obtained 2Θ angles for every single peak would be changed to d-spacing in order to carry out a qualitative analysis of the sample i.e., identifying the chemical component of the substance [147].

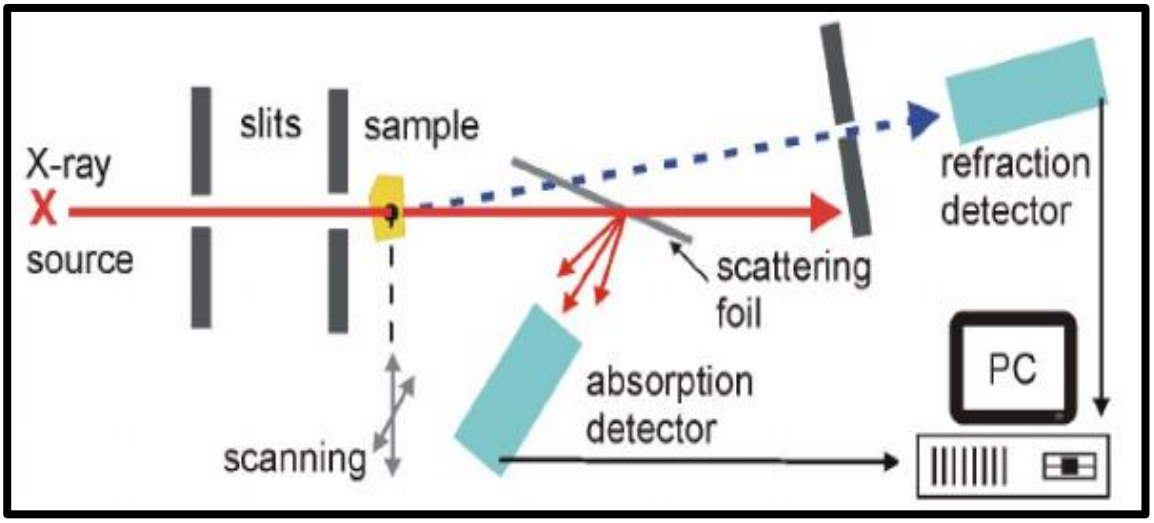


Fig. 3.2 Diagrammatic representation of XRD.

3.2.5 Scanning electron microscopy (SEM)

A scanning electron microscope is a useful instrument used for the analysis and examination of the morphology of the microstructure, crystalline structure, surface topography, and characterizations of chemical composition [148] **(Fig. 3.3)**. It is important to understand the fundamental principle of light optics to comprehend the basics of electron microscopy. The human eye can distinguish objects at about 1/60˚ visual angle, resulting in ~ 0.1mm. The limit

of microscopy of an optical microscope is ~2000 Å, which is due to the enlarging of the visual angle [149][150]. In SEM image formation depends on the ability to acquire signals emitted from the interactions of the specimen and the electron beam. These interactions can be broadly divided into two main groups, namely elastic and inelastic interactions.

Elastic scattering takes place when the incident electron is deflected by the sample atomic nucleus or due to similar energy outer shell electrons. This type of interaction is characterized by a wide range of directional changes and collisions. Electrons that are scattered elastically when the incoming electron is deflected by an angle of more than 90˚ are known as back-scattered electrons. Inelastic scattering takes place due to multiple interactions within the incoming electrons and the atoms and electrons of the sample and leads to primary beam electrons transferring a significant amount of energy to the atom.

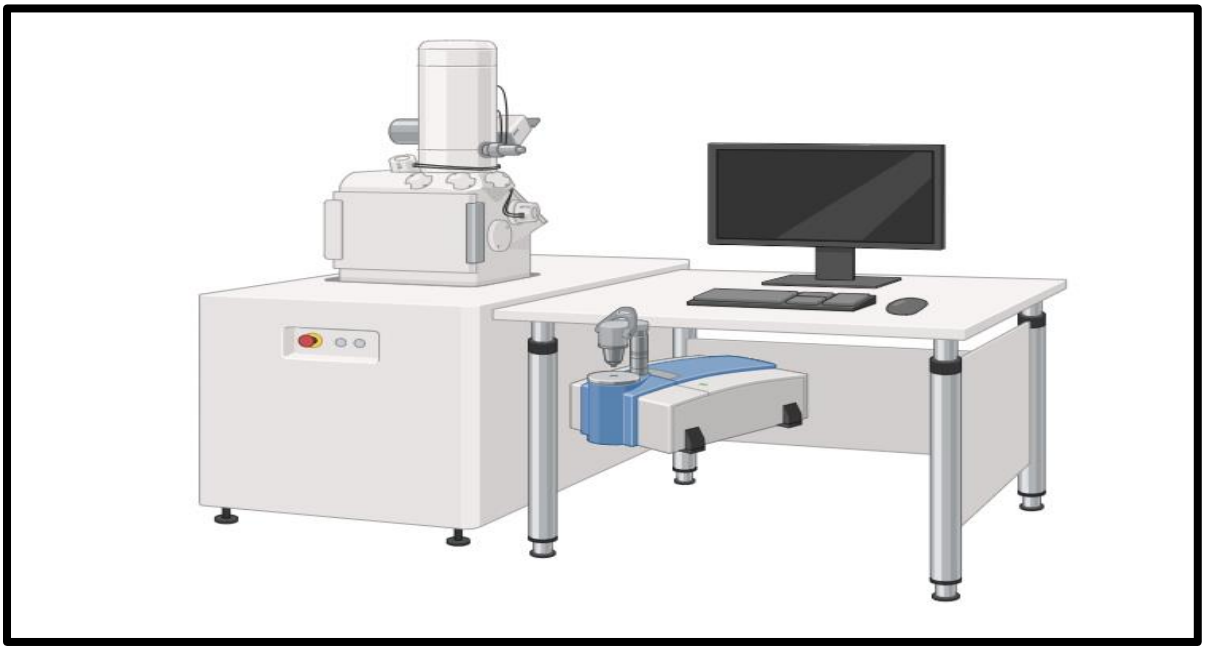


Fig. 3.3 Diagrammatic representation of SEM machine

3.2.6 Energy Dispersive X-ray Spectroscopy (EDX)

The elemental composition of a sample is detected by EDX spectroscopy by utilizing scanning electron microscopy. EDX has the ability to determine the elements that contain an atomic number greater than boron and thus detect at least 0.1% concentration [151][152]. EDX's numerous applications are the evaluation of material, analysis of spot detection of areas up to a diameter of 10cm, identification, contamination, etc. On collision with the beam of electrons, the sample interacts with the beam, and X-rays are produced. Because of the principle that neither of the elements has a similar X-ray spectrum, they can be measured and differentiated for the sample concentration [153][154]. X-ray emission occurs when the primary beam interacts with the nucleus of an atom, leading to the ejection of the electron and the release of X-rays. The X-rays that are emitted comprise characteristic and continuum X-rays.

3.2.7 Electrochemical Techniques

Electrochemical techniques are a category of analytical methods that utilize electrochemical principles to analyze and examine chemical reactions, investigate substance’s properties, and determine concentrations. Electrochemical techniques present more benefits than technologies of surface modification. Moreover, electrochemical dissolution gradually maintains the surface characteristics, such as the composition and orientation of crystals, unchanged. Now, the electrochemical methods consist of measurements, potentiometry, cyclic voltammetry, electrochemical impendence spectroscopic (EIS), and chronoamperometry [155]. In electrochemical techniques, three electrodes, namely working electrodes. reference electrode and counter electrode are present in an electrochemical cell. Now, these electrodes are linked via potentiostat, which monitors the associated current response. In this type of electrochemical experiment, the potential is shown by a working electrode, and the value of the current is plotted against time. In this thesis, the electrochemical response of all the developed electrodes is

examined by using an Autolab (Ecochemie, Netherlands) Galvanostat/Potentiostat, and reference electrodes such as Ag/AgCl and Platinum (Pt) are used as counter electrodes.

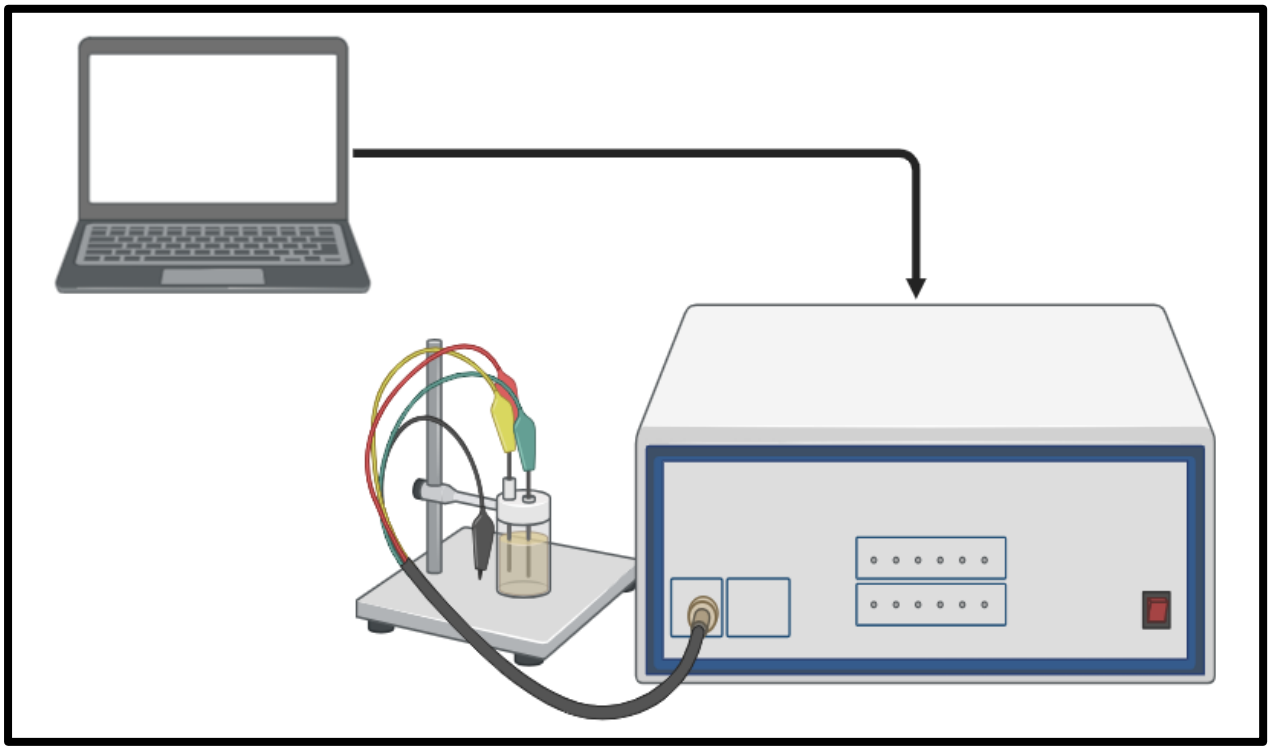


Fig. 3.4 Diagrammatic representation of potentiostat.

3.2.8 Cyclic Voltammetry (CV)

Cyclic Voltammetry is the most widely used electro-analytical technique for the characterization of the electrochemical behavior of electrochemically active substances [156]. Reducible mass transport or oxidizable species that are electroactive in CV are restricted to diffusion. Thus, to avoid currents' migration and ensure conductivity, 0.1M supporting or ground electrolyte is introduced to the solution. Tetraalkylammonium salts and alkali metals are proven to be majorly effective in this technique. In the CV technique, the x-axis represents the parameter directed onto the system where (E) is the applied potential while the axis represents the resultant current (i) is passed. Two conventions are widely used to report data of CV that shows the sign convention for plotting and acquiring data [157]. To better understand the electrochemical reaction within the electroactive species between a given reduction-

oxidation potential. Throughout the reduction-oxidation process, ions are introduced to electrodes in an electrolytic solution [158].

3.2.9 Differential pulse voltammetry (DPV)

Differential pulse voltammetry is the most accurate and commonly used voltammetric method besides anodic stripping voltammetry. This method was developed to achieve a large differential between the charging current and the analytical current in the mercury drop's half-life at the end. As the need is increasing for trace analysis for essential oxidizable materials (e.g., vitamins, drugs, and carcinogens), the method is utilized for different solid electrodes like platinum or carbon that have a greater anodic range (potential) as compared to mercury electrodes [159]. Further, a series of pulses with moderate amplitude are overlaid on voltage; then, the current is shown within pulse voltage and ramped baseline voltage. Current is recorded before the application of the pulse (1^{st} point) and the end of the pulse (2^{nd} point). The variations in the measurement of current following each pulse at those locations are recorded [160][161].

3.2.10 Electrochemical impendence spectroscopic (EIS)

Electrochemical impendence spectroscopic (EIS) is a powerful technique for examining the mechanism of electrochemical reactions for calculating the transport and dielectric characteristics of materials, investigating the characteristics of porous electrodes, as well as for examining the passive surfaces [162]. It has been intensively utilized for charge transport characterization, explanation of the mechanisms of corrosion, battery optimization, and interfaces of solution/membranes. In the application of biosensors, it is appropriate for the detection of events of binding on the surface of the transducer [163].

An electrochemical cell is supplied with alternating current (AC) voltage to measure its flow of current. Moreover, a sinusoidal potential stimulation was utilized, leading to AC current

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

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

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

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

where C is concentration, and F is Faraday's constant. n is the number of electrons, and A is the electrode's geometrical area. T is temperature, and R is gas constant.

CHAPTER 4

Graphitic carbon nitride decorated molybdenum sulfide-based Electrochemical biosensor for Trichlorfon Detection

4.1 Introduction

This chapter discusses a novel and efficient acetylcholinesterase-based electrochemical biosensor using graphitic carbon (g-C_3N_4) decorated molybdenum sulfide (MoS_2) for trichlorfon detection. The synergistic effect of synthesized nanohybrid (g-C_3N_4@MoS_2) significantly increases the electroactive surface area, improves catalytic activity, rapid electron transfer, and remarkable biocompatibility. The fabricated biosensor exhibits good selectivity, high sensitivity (0.51µA $(nM)^{-1}$), and low detection limit (2.1 nM). Additionally, the biosensor was successfully applied for trichlorfon detection in three different real samples.

4.2 Experimental section

4.2.1 Synthesis of g-C_3N_4 nanosheets

The thermal condensation method was used to prepare g-C_3N_4 nanosheets. In this method, 10 g of melamine was put in a crucible covered with a lid and then heated in a muffle furnace at a temperature of 550°C for a time period of four hours. The resultant material underwent washing with distilled water and ethanol and was further dried in a vacuum oven.

4.2.2 Synthesis of g-C_3N_4@MoS_2 nanocomposite

To prepare g-C_3N_4@MoS_2 nanocomposite, 500 mg of sodium molybdate and 950 mg of thiourea were dissolved in 50 ml of distilled water and stirred to create a homogenous solution. After that, 100 mg of g-C_3N_4 was added to the above mixture and agitated for two hours. The mixture was then ultrasonicated for 30 minutes, poured into a 100 ml Teflon vessel, and then

kept at 180°C for 20 hours. Finally, the black precipitate was centrifuged and washed with distilled water and ethanol. Then, the composite was dried at 80°C in a vacuum oven.

4.2.3 Electrophoretic deposition of g-C_3N_4@MoS_2 nanocomposite

Using a two-electrode system, the g-C_3N_4@MoS_2 nanocomposite was deposited on a pre-hydrolyzed ITO. Prior to deposition, 0.5 mg of nanocomposite was dispersed in 15 ml of distilled water and sonicated until a clear solution was obtained. The film was deposited on the surface of ITO at 10 V for a duration of 10 sec.

4.2.4 Fabrication g-C_3N_4@MoS_2 nanocomposite-based biosensor

To fabricate the AChE/g-C_3N_4@MoS_2/ITO biosensor, 0.06 mg/mL of AChE enzyme (20µL) was drop-cast onto the g-C_3N_4@MoS_2/ITO electrode. Prior to the electrochemical investigation, the biosensor was incubated in a refrigerator at 4°C for 12 hours. The schematic representation is shown in **Fig 4.1**.

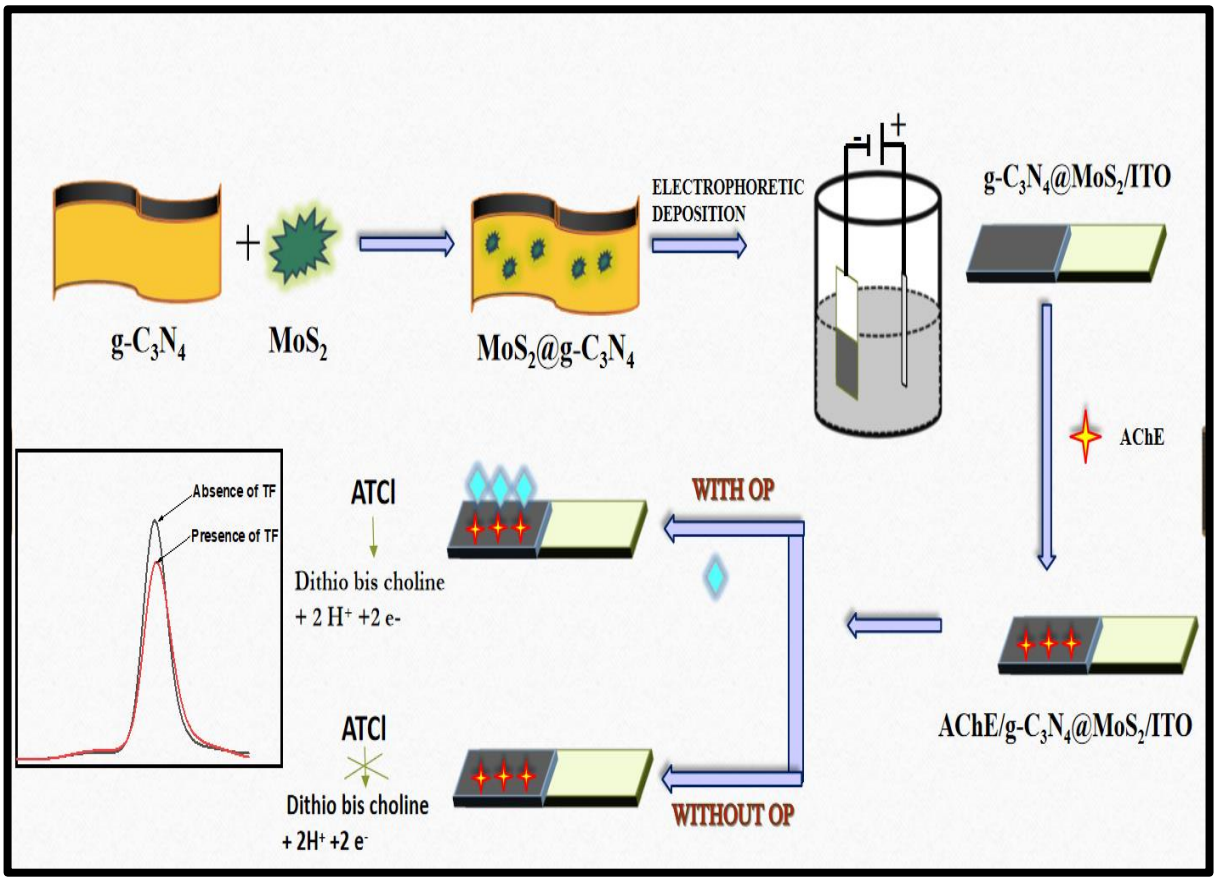


Fig. 4.1 Schematic representation of fabrication of bioelectrode.

4.3 Results and Discussion

4.3.1 Structural Characterisation

Fig. 4.2 (A) displays the XRD patterns of g-C_3N_4 and g-C_3N_4@MoS_2 nanohybrid. The characteristic peaks of g-C3N4 at 2θ =13.1° and 27.5° correspond to the tri-s-triazine ring (100) and the aromatic interlayer stacking ring (002), respectively (**curve a**). The XRD pattern for g-C_3N_4@MoS_2 is represented by **curve b**, which displays peaks at 14.65°, 35.15°, 39.68°, 49.85° and 58.9° which further correlates to (002), (101), (103), (105) and (110) MoS_2 planes but no peaks for g-C_3N_4 was seen as it was added in less amount [164].

Fig. 4.2 (B) displays the FT-IR pattern for g-C_3N_4 and g-C_3N_4@MoS_2. The peaks at 1578 cm^{-1} and 1647 cm^{-1}in the FT-IR spectra of pure g-C_3N_4 (**curve (a)**) correspond to the stretching vibration modes of C=N bonds. The peaks at 1228 cm^{-1}, 1318 cm^{-1}, and 1403 cm^{-1} are responsible for aromatic C-N stretching vibrations [165][166].A strong peak at 807 cm^{-1} is linked to the bending mode of tri-s-triazine units, whereas a broad peak at 3168 cm^{-1} is suggestive of terminal NH_2 or NH groups. These distinctive peaks of pure g-C_3N_4 remain in the FT-IR spectra of the g-C_3N_4@MoS_2 nanocomposite, as seen in **curve (b)**. Furthermore, a prominent peak at 497 cm^{-1} is noted, which is associated with the Mo-S vibration [167].

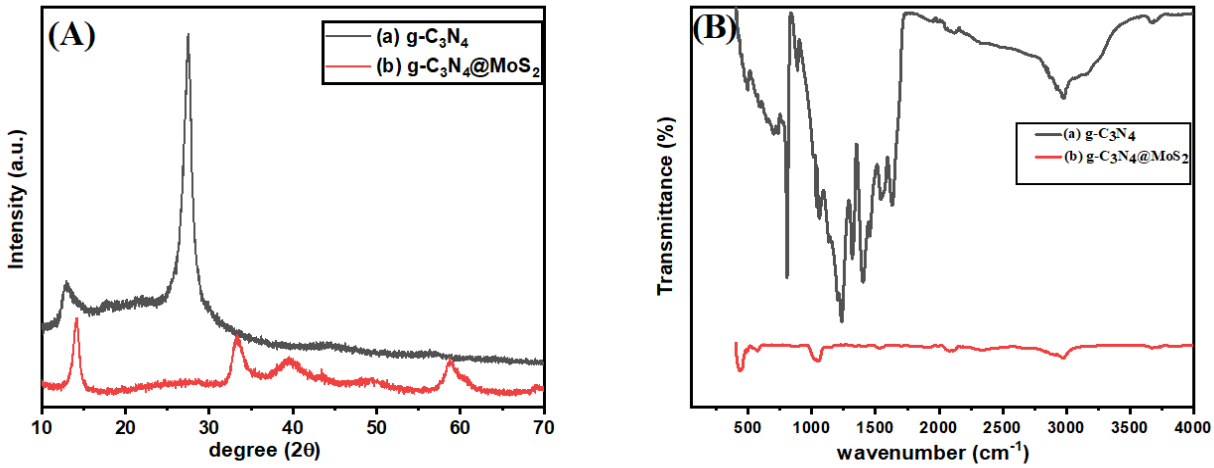


Fig. 4.2 (A) XRD and **(B)** FTIR of **(a)** g-C_3N_4 and **(b)** g-C_3N_4@MoS_2

4.3.2 Morphological Studies

The surface morphology of the compound was examined with SEM. SEM images of g-C_3N_4 are shown in **Fig.4.3 (A)**, and SEM images of g-C_3N_4@MoS_2 are shown in **Fig.4.3 (C)**. SEM pictures of g-C_3N_4 show a structure that resembles a sheet folding inward at the edges. Sheet-like structure of g-C_3N_4 is present on the flower like structure of MoS_2 in SEM images of g-C_3N_4@MoS_2.

EDX analyses are performed to determine the elements contained in the sample as well as its purity. The EDX analysis of g-C_3N_4@MoS_2, which represents a peak for C, Mo, S, and N, is shown in **Fig.4.3(D)**.

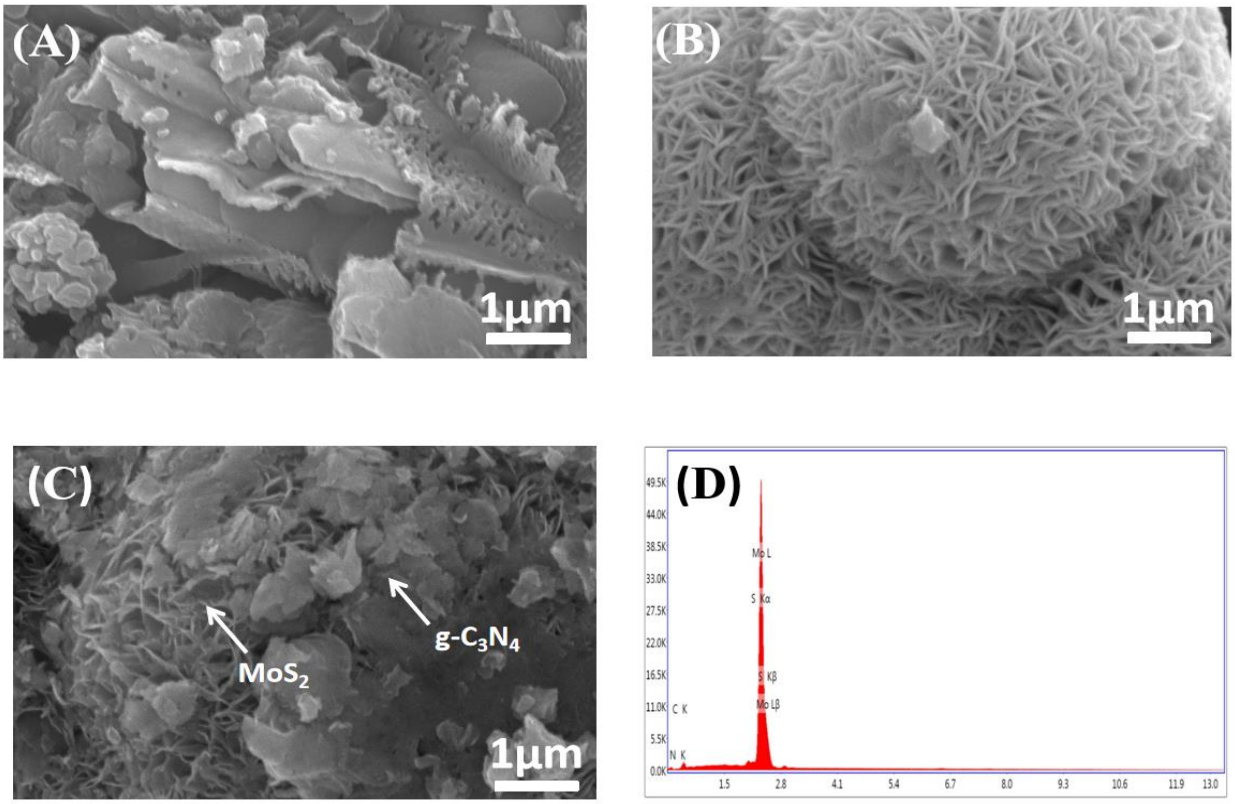


Fig. 4.3 SEM images of **(A)** g-C_3N_4; **(B)** MoS_2; **(C)** g-C_3N_4@MoS_2; and **(D)** EDAX of g-C_3N_4@MoS_2

4.3.3 Electrochemical Characterisation

Electrochemical characterization of all the fabricated electrodes was done by EIS and CV techniques. Electron transfer resistance (R_{ct}) of all developed electrodes was found by EIS using 0.2 M PBS (containing 5mM $[Fe(CN)_6]^{3-/4-}$) at the potential of 0.01 V and frequency range of 0.01-10^5 Hz. With the help of the Nyquist plot, a linear pattern was observed for the transfer process of electrons at low frequencies, and a semicircular pattern was observed for the electron transfer process at higher frequencies [168]. R_{ct} values for different modified

electrodes are 2.19kΩ (ITO-curve**(a)**), 1.14kΩ (g-C_3N_4/ITO-curve**(b)**), 845Ω (g-C_3N_4@MoS_2/ITO-curve**(c)**), 1.05kΩ (AChE/g-C_3N_4@MoS_2/ITO-curve**(d)**). The values of i_0 were found to be 2.252×10^{-5} Acm^{-2} and $3.039\times10^{-5}Acm^{-2}$, and K_{app} to be 2.032×10^{-4} cms^{-1}, and 2.522×10^{-4} cms^{-1} for g-C_3N_4/ITO and g-C_3N_4@MoS_2/ITO respectively by using equations **4.1** and **4.2.**

$$i_0 = nRT/ R_{ct}F \quad \textbf{4.1}$$

$$K_{app} = RT/n^2F^2AR_{ct}C \quad \textbf{4.2}$$

C is concentration, n is the number of electrons, T is temperature, and R is the gas constant.CV was performed at a 50 mV/sec scan rate, with the -0.8 V to 0.8 V range of potential. In **Fig. 4.4 (B)**, the highest peak of current was observed at 0.43 mA for g-C_3N_4@MoS_2/ITO **(curve c)** because of its high area of surface and excellent electrical conductivity, in contrast to g-C_3N_4/ITO **(curve b)**, which was observed at 0.32 mA. The lowest current and less electron mobility were observed for AChE/g-C_3N_4@MoS_2/ITO because of the non-conductivity of AChE. The scan rate was done in the range of 10- 300mV/sec (**Fig. 4.4 (C)**). It was seen that with the increase in scan rate, the peak current of oxidation goes to a more positive side value and the peak current of reduction to a more negative side value. It was observed that E_{pa} and E_{pc} are in a linear relationship with the logarithmic value of scan rate (log ν). This relationship is illustrated by the following equations.

$$E_{pa}\ [g\text{-}C_3N_4@MoS_2/ITO] = 0.1033\ (V) \log (v) + 0.1709\ (V);\ R^2 = 0.976 \quad \textbf{4.3}$$

$$E_{pc}\ [g\text{-}C_3N_4@MoS_2/ITO] = -0.1266\ (V) \log (v) + 0.2689\ (V);\ R^2 = 0.970 \quad \textbf{4.4}$$

The slopes found by the above equations are used to calculate the electron transfer coefficient (α) and electron transfer rate constant (Ks). The value of α was found to be 0.9245. With the help of α, the value of Ks was found to be 0.0844 s^{-1}. It was observed that I_{pa} and I_{pc} are in a linear relationship with $v^{1/2}$. This relationship is illustrated by the following equations.

I_{pa} [g-C_3N_4@MoS_2/ITO] = 38.89 µA $(mV/s)^{1/2}$ × $v^{1/2}$ mV $s^{1/2}$ + 136.97 µA; R^2 = 0.987 **4.5**

I_{pc} [g-C_3N_4@MoS_2/ITO] = -26.80 µA $(mV/s)^{1/2}$ × $v^{1/2}$ $mV^{1/2}$ - 137.60 µA; R^2 = 0.996 **4.6**

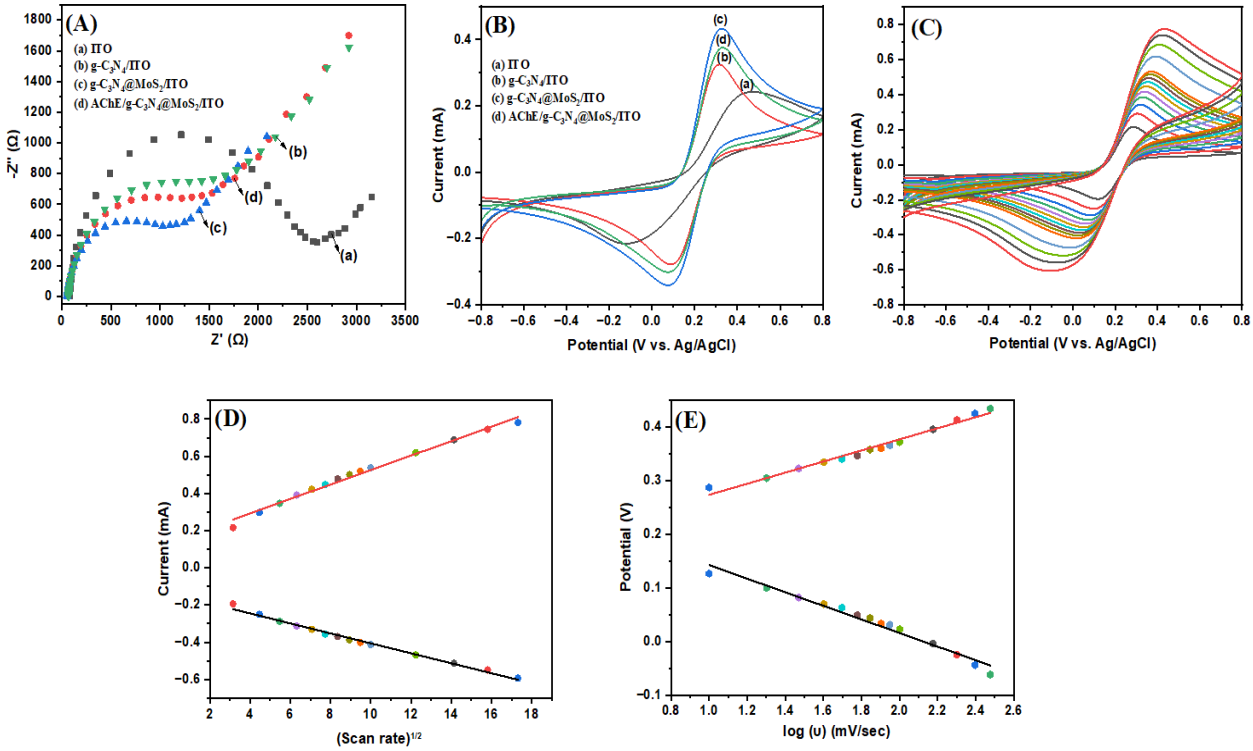


Fig. 4.4 (A) EIS study plot; **(B)** Cyclic voltammogram for different developed electrodes **(a)** ITO; **(b)** g-C_3N_4; **(c)** g-C_3N_4/ITO; **(d)** AChE/g-C_3N_4@MoS_2/ITO; **(C)** Different scan rate of g-C_3N_4@MoS_2/ITO electrode (10-300 mV/sec); **(D)** Graph of current v/s square root of scan rate of g-C_3N_4@MoS_2/ITO; **(E)** Graph for potential v/s log (scan rate) of g-C_3N_4@MoS_2/ITO

By using Randles – Sevcik equation ($I_p = 2.69\times10^5\ n^{3/2}ACD^{1/2}\ \nu^{1/2}$), we found the values of effective surface area to be 0.25 cm^2 and diffusion coefficient to be 7.146 $\times10^{-6}\ cm^2\ s^{-1}$, where n is number of electrons, C is concentration of $[Fe(CN)_6]^{3-/4-}$ in mole cm^{-3},ν is scan rate in V/s.

4.3.4 Optimization studies

The DPV method optimizes the electrolytic solution's pH, amount of AChE, and incubation period. **Fig. 4.5 (A)** shows the impact of pH values ranging from 6 to 8 on the sensor's efficiency. The highest current was recorded at pH 6.5, as the following graph illustrates. Therefore, at this pH, all electrochemical studies have been performed.

To optimize the enzyme concentration, the DPV response of the AChE/g-C3N4@MoS2/ITO electrode was performed in the presence of 2 mM ATCl and 5 nM TF. As shown in **Fig. 4.5 (B)**, when the concentration of AChE increases from 0.04 mg/mL to 0.12 mg/mL, the current rises, and at 0.06 mg/mL, it reaches its maximum point; after that, it decreases due to large mass transfer resistance. Hence, 0.06 mg/mL is taken as the optimum amount for the study. Furthermore, as illustrated in **Fig. 4.5 (C)**, the incubation period is determined by varying the duration from 2 to 14 min. The result shows that the peak current decreases with an increase in time and saturates within 8 min, which signifies that the optimum amount of TF binding to the AChE enzyme has been achieved during this time.

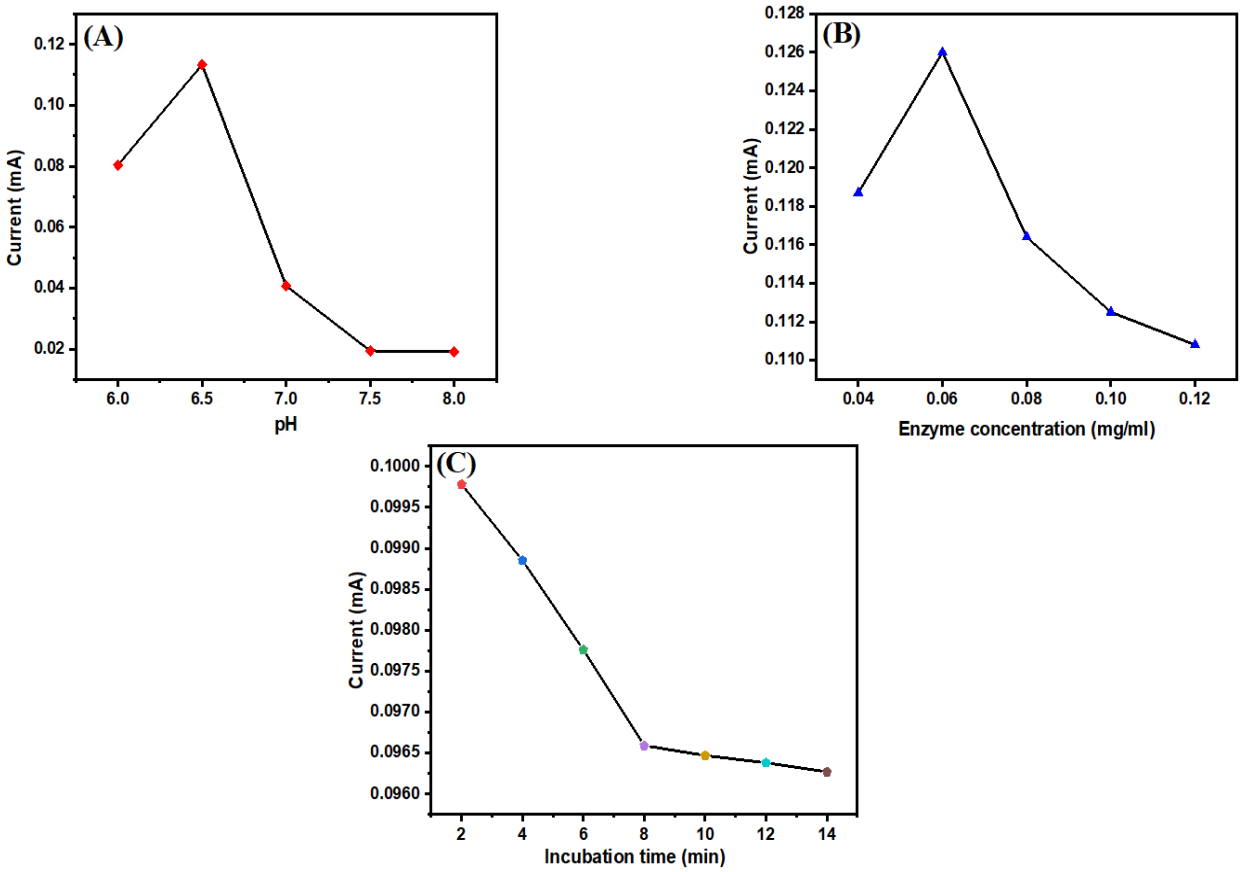


Fig 4.5 Optimization studies of **(A)** Effect of pH; **(B)** Concentration of an enzyme; **(C)** Incubation time

4.3.5 Electrochemical Biosensing Study

Electrochemical biosensing response of the developed AChE/g-C_3N_4@MoS_2/ITO electrode as a function of the concentration of TF (5 to 100 nM) in 0.2 M PBS of pH 6.5 comprising of 5mM $[Fe(CN)_6]^{3-/4-}$was carried out by utilizing differential pulse voltammetry (DPV) technique. A decrease in the anodic peak current was seen as the fabricated biosensor was subjected to increased TF concentration, as shown in **Fig. 4.6 (A)**. Peak current appeared to be decreasing because of inhibition by TF, which impacted the activity of the AChE enzyme. **Fig. 4.6 (B)** represents the calibration curve, which shows linearity within peak current (I) and TF concentration and thus follows the equation given below:

$I = -0.127\ \mu A/nM + 9.185\ \mu A \times C_{TF}$; $R^2 = 0.993$

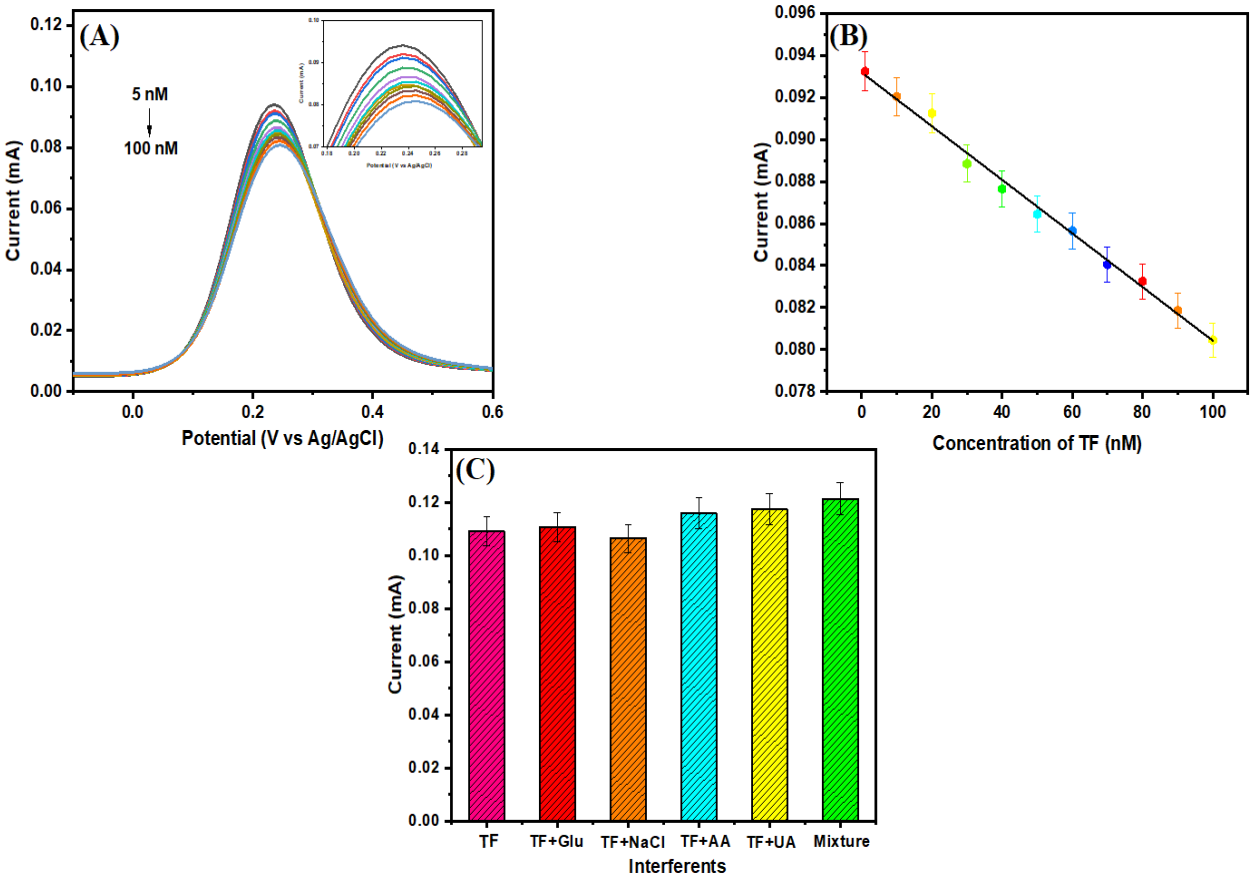


Fig. 4.6 (A) DPV response study of AChE/g-C_3N_4@MoS_2/ITO developed electrode at different concentrations of TF (5-100 nM); **(B)** Linearity plot for current v/s TF concentration; **(C)** Study of selectivity of AChE/ g-C_3N_4@MoS_2/ITO electrode

The biosensor's sensitivity (AChE/g-C_3N_4@MoS_2/ITO) was $0.51\mu A(nM)^{-1}cm^{-2}$, with a regression value of 0.993. The limit of detection (LOD) is detected as 2.1 nM using the relation $3\sigma/S$, where σ and S denote the bioelectrode's standard deviation and sensitivity.

4.3.6 Interference Study and Real Sample Analysis

The selectivity of the fabricated biosensor (AChE/g-C_3N_4@MoS_2/ITO) was studied by incubating the developed biosensor with 100 nM Glucose, uric acid, ascorbic acid, and NaCl in addition to 5 nM TF. **Fig. 4.6 (C)** demonstrates that there was no consequent change in

current when the developed biosensor was subjected to interferent analytes, which shows the high selectivity for TF detection.

The efficiency and applicability of the AChE/g-C_3N_4@MoS_2/ITO biosensor were examined in spiked samples (Pulse, Carrot, and Banana) at known concentrations of 5, 10, 20, and 30 nM. The results exhibited satisfactory recovery (97-108%), further depicting the biosensor's reliability. The TF detection results in spiked samples are presented in **Table 4.1**.

Table 4.1 Detection of TF in three real samples using AChE/g-C_3N_4@MoS_2/ITO electrode.

Samples	Added amount (nM)	Found amount (nM)	Recovery (%)	RSDs (%)
Pulse	5	4.86	97.3	1.93
	10	10.5	105	4.02
	20	21	105	3.98
	30	31.2	104.2	2.92
Carrot	5	5.4	108	5.66
	10	10.4	104	3.13
	20	21.4	107	4.89
	30	31.2	104	3.17
Banana	5	5.05	101	0.87
	10	9.88	98.8	0.84
	20	20.1	100.5	0.38
	30	29.8	99.4	0.39

4.3.7 Reproducibility and Stability Studies

The reproducibility of the AChE/g-C_3N_4@MoS_2/ITO electrode was detected using the DPV technique, utilizing five fabricated similar electrodes in the presence of 5nM TF **(Fig. 4.7 (A))**. The result demonstrates that the RSD value is 2.45%, which suggests good reproducibility.

The AChE/g-C_3N_4@MoS_2/ITO electrode was stored in a refrigerator maintained at 4°C, and the stability of the biosensor was monitored by recording its response every 5 days (**Fig. 4.7 (B)**). Furthermore, after 20 days, the electrochemical response remained at 82.4% of its original value, demonstrating the developed biosensor's good stability.

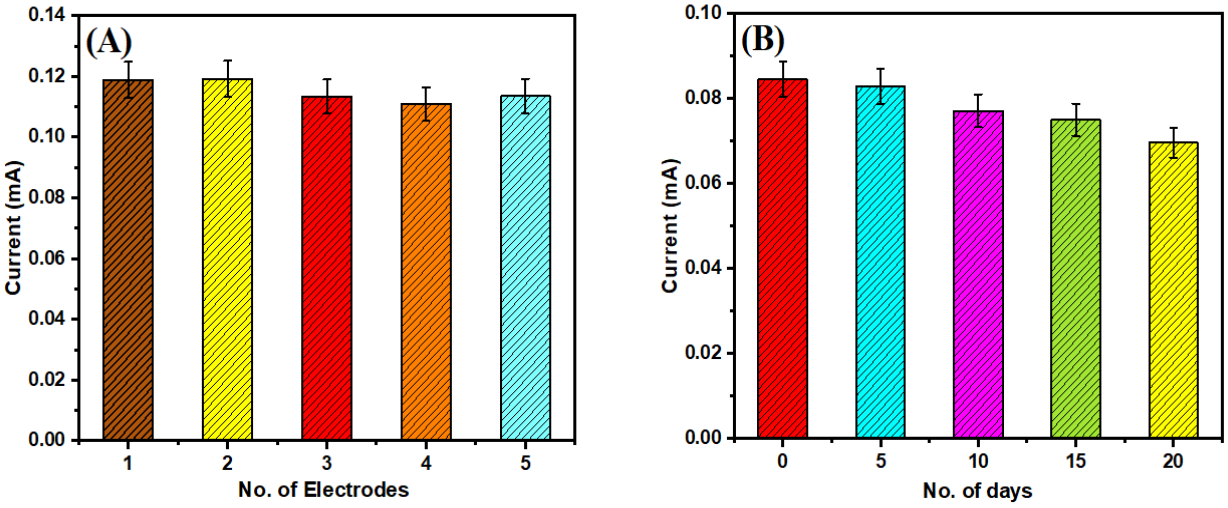


Fig. 4.7 (A) Reproducibility study of TF using different electrodes; **(B)** Stability study of the fabricated electrode (AChE/ g-C_3N_4@MoS_2/ITO) over a period of 20 days

CHAPTER 5

CONCLUSION

In this chapter, g-C_3N_4@MoS_2 nanohybrid has been used to create an efficient electrochemical biosensor for the detection of trichlorfon. The synthesized g-C_3N_4@MoS_2 nanohybrid enhanced the electrochemical characteristics due to its large surface area and good biocompatibility. The biosensor's validation using actual samples demonstrates its potential for field applications for the detection of trichlorfon. The developed biosensor was highly stable, sensitive, and specific. Three real samples were used to successfully validate the developed g-C_3N_4@MoS_2 nanocomposite-based platform, demonstrating its applicability and dependability for organophosphorus pesticide detection.

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Conference Attended

Presented our work at the International Conference on “Current Trends in Chemical Sciences for Sustainable Living” held at Shyam Lal College, University of Delhi, on 5th April 2024.

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Prof. Kusha Tiwari
IQAC, Director

Dr. Reeta Sharma
Convenor

(Dr. Rabi Narayan Kar)
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PAPER NAME

MSC THESIS Final (1).pdf

AUTHOR

Ram Singh

WORD COUNT

16544 Words

CHARACTER COUNT

95369 Characters

PAGE COUNT

71 Pages

FILE SIZE

1.7MB

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Jun 7, 2024 11:25 AM GMT+5:30

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