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CHAPTER 1- Introduction

1.1 Introduction to Pesticides

Pesticides are a wide range of chemical compounds or biological agents that are carefully applied to battle pest, insect, and rodent populations. They are characterized by their diverse chemical compositions and physical qualities [1]. These compounds are becoming more common across the world, expanding beyond agricultural applications to include household usage such as pest control powders or sprays [2]. With over 1.8 billion people engaged in agriculture reliant on pesticides to protect crops and maintain food security, their proper use offers increased productivity and economic advantages by lowering labour inputs [3]. In contrast, indiscriminate pesticide use endangers both the environment and human health. Pesticide residues on food items are a major public health concern because they can cause a variety of ailments[4]. Human exposure occurs by oral intake, cutaneous contact, ocular exposure, and inhalation, and non-target species are also negatively impacted [5]. To properly limit these dangers, strict safety precautions and correct pesticide disposal methods must be implemented.

1.2 Toxicological classification of pesticides

Pesticides are classified into several types, including rodenticides, fungicides, herbicides, insecticides, and others. All of them are intended to eliminate the population of undesired and hazardous insects, rodents, weeds, and pests. All of them have unique physical and chemical features, emphasizing the significance of categorizing them for study. They should be researched according to their different categories. While some are present in nature, the majority of pesticides are synthetic and created through a variety of chemical techniques [6]. Drum recommended three of the most often used pesticide classification techniques based on their qualities and requirements [7]. There are three major groupings depending on mode of entry.

1) Mode of Entry
2) Pesticides and the pests they kill

3) Pesticide chemistry

1.2.1 Classification of Pesticides based on Mode of Entry

The mechanism of entrance relates to how pesticides make contact with or enter the target.

A) Systematic pesticides - Plants and animals absorb them and transport them to untreated tissues. Example: Glyphosate

B) Repellents- Their unpleasant taste deters bugs from visiting the targeted location and affects their capacity to identify targeted pests [7]. Example: Methiocarb

C) Fumigants- When they enter a pest's trachea, they emit vapor that kills the target pest [7]. Example: Azoxystrobin

D) Touch insecticides - When it comes into touch with pests, it defends itself. Example: Paraquat

E) Stomach poisons - These enter the pest's mouth and digestive tract and subsequently work against it. Example: Malathion

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

Pesticide classification by function and pest organisms killed are Pesticides are classified based on the creatures they target, with particular names indicating their effects. These pesticides' group names are derived from the Latin word *cide*, which means "kill" or "killer," and have been incorporated in the names of the pests they destroy; however, not every pesticide finishes with *-cides*. Pesticide categories based on target cells include the following:

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, also known as chlorinated hydrocarbon. These are the first kind of pesticides to be developed and used on agricultural areas. An organic compound consists of five or more chlorine atoms. They affect insects' neurological systems, resulting in muscular spasm, paralysis, and, finally, death. It is biodegradable and causes less pollution to the environment, such as lindane [4].

B) Organophosphates are phosphoric acid derivatives. It is a broad-spectrum insecticide that eliminates a wide variety of pests. They are biodegradable and hence less hazardous to the environment [8]. Their effects cause a long-term overlay of acetylcholine over the synapse, impairing impulse movement and killing insects by paralyzing them [9]. Examples: Malathion.

C) Carbamates are structurally similar to organophosphate insecticides but differ in origin. Carbamates are carbamic acid derivatives; organophosphates are phosphoric acid derivatives. They operate on the same basis as organophosphate (affecting nerve transmission). They are also biodegradable and so less hazardous to the environment[10]. Example: Carbaryl.

D) Pyrethroids are among the safest pesticides. They are more stable than natural pyrethrins because they imitate their structure. When pyrethrins are pulverized, active chemicals are produced. They poison fish, mammals, insects, and other animals. When exposed to light, they degrade and become non-persistent [11].

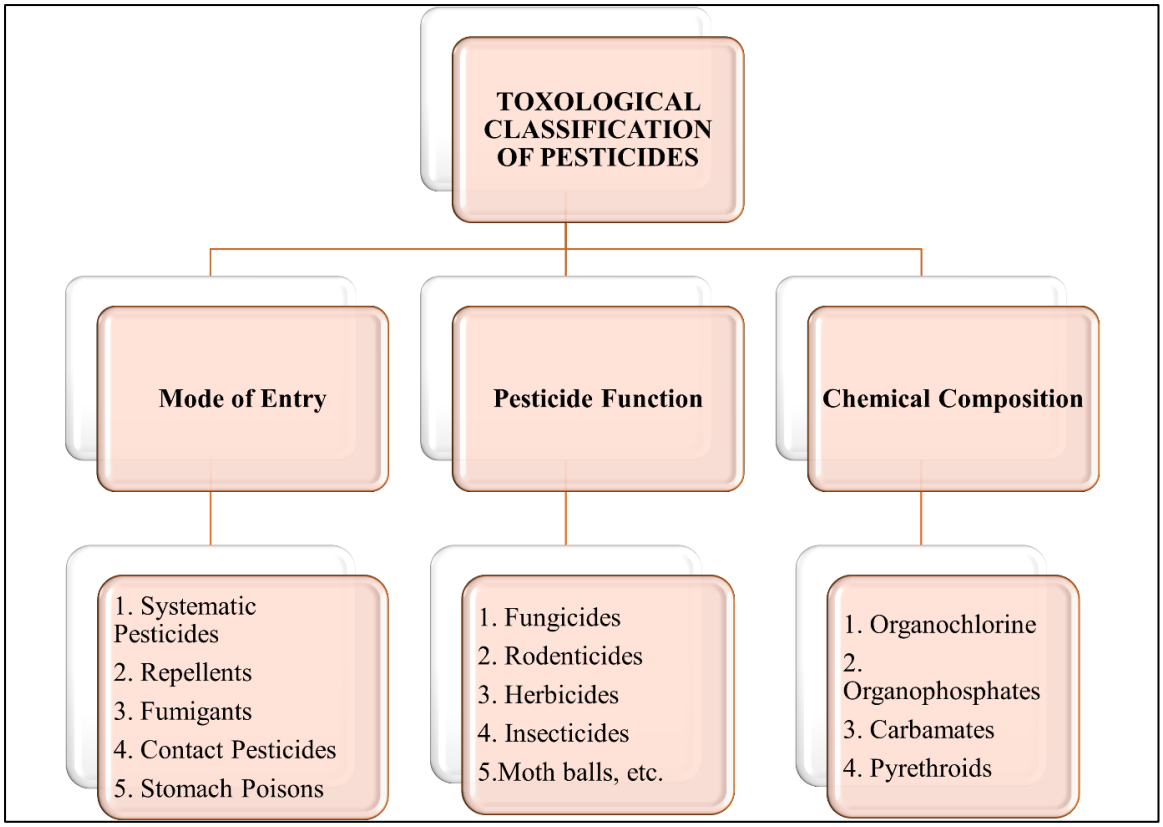


Figure 1.1 shows the toxicological categorization of pesticides

1.3 Other Minor Classes of Pesticides

1.3.1 Classification according to sources of origin

Pesticides are classified into two types based on their origin: biopesticides and chemical pesticides.

A. Biopesticides are derived from natural sources such as animals and plants. They are less harmful, effective in smaller quantities, biodegradable, and target specific pests, hence they are utilized more than other synthetic pesticides [11]. It falls into three categories:

1) **Plant-based:** Plants create insecticides naturally.
2) **Biochemical insecticides:** are natural compounds that employ a non-toxic approach to eliminate pests. For example, insect sex pheromones.
3) **Microbial insecticides:** are composed mostly of microorganisms such as bacteria[12]. For example, Bacillus thuringiensis kills mosquitos.

B. Chemical Pesticides are not present in nature. These are non-biodegradable and hence detrimental to the environment. These are created by diverse chemical processes and are broad spectrum pesticides, which means they can combat a variety of insecticides. Their endurance power is quite great, resulting in environmental contamination and ecological

degradation. They are further classified as organochlorine, organophosphate, carbamates, and pyrethroids[13]. For example, consider Lindane. Because chemical pesticides are more dangerous than biopesticides, categorizing pesticides based on their origin helps to replace chemical pesticide use with biopesticides.

1.3.2. Classification based on toxicity

WHO conducted the experiment in the lab on rats, administering dosages orally and dermally. They discovered that the LD50 (median lethal dosage) caused death in 50% of the rats subjected to such experimental settings[14].

1.4 Advantages of pesticide

1) The advantage of pesticide use is financial gain for farmers. Pesticides are used to safeguard crop quality and productivity, resulting in reduced labor and other inputs [15].
2) Control superfluous vegetation- Herbicides eliminate the overabundance of invasive weeds on highways, gardens, and other locations.
3) Maintaining aesthetic quality- Keeping an attractive- perspective entails removing unneeded plants and safeguarding endangered species from dangerous pests [16].
4) Human health protection: Disease-causing bugs and other hazardous germs are eradicated [17].
5) Contamination prevention- Using pesticides and insecticides throughout the packaging and manufacturing process reduces the possibility of packaged goods and raw materials being contaminated.

TABLE 1.1: Classification of pesticides recommended by WHO

WHO Classification	**LD50 for rats (mg/Kg body weight)**	**Examples**
Class	*Oral*	*Dermal*
Class I a- Extremely hazardous	Below 5	Below 50
Class I b- Highly hazardous	5-50	50-200
Class II - Moderately hazardous	50-2000	200-2000
Class III - Slightly hazardous	Above 2000	Above 2000
Class IV – Unlikely to present acute hazard	5000 or higher	5000 or higher

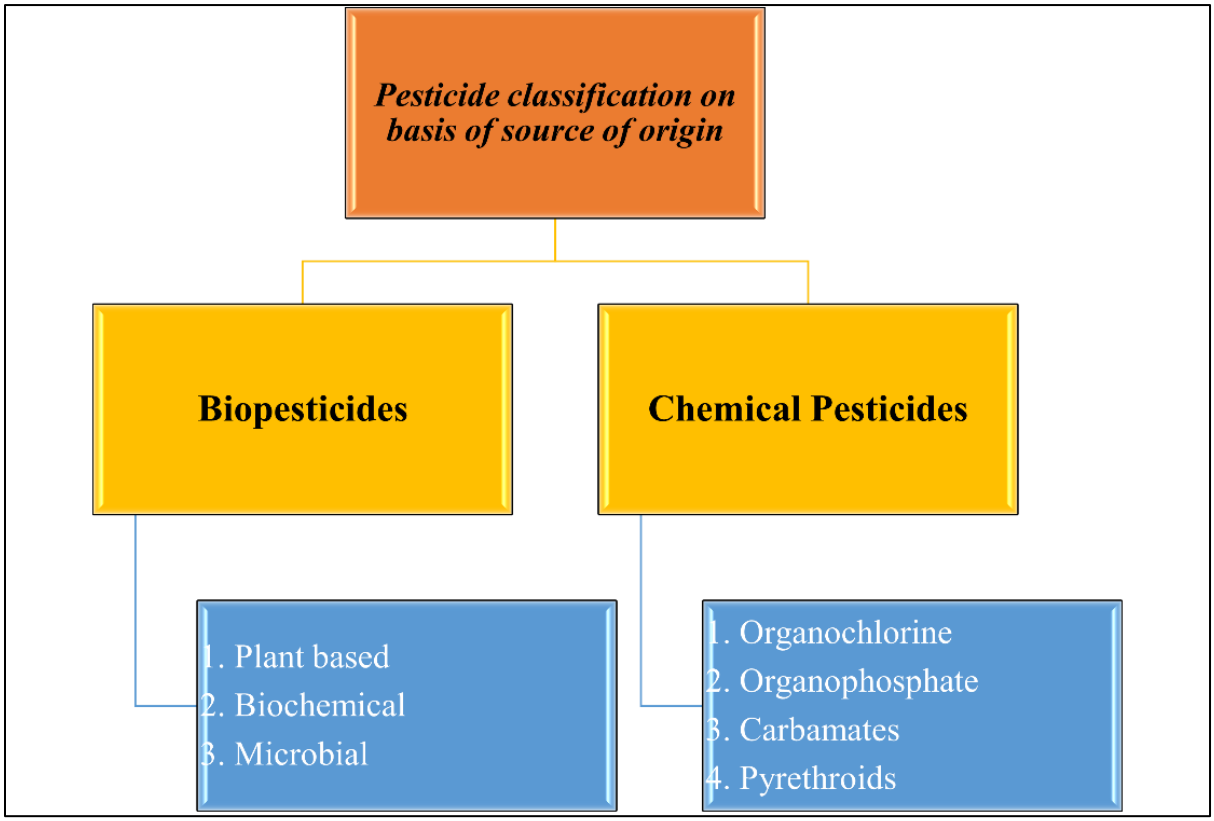


Figure 1.2 depicts a diagrammatic depiction of pesticide categorization according to source of origin

1.5 Effect of pesticides

1) Impact on human health: There are several ways for pesticides to enter the human body, such as through the consumption of contaminated food, inhaling polluted air, or coming into direct touch with them through the skin. Pesticides are sprayed on food, especially fruits and vegetables, and can pollute drinking water, soil, and groundwater [18]. In addition to causing air contamination and floating, the pesticide application can have both short-term and long-term negative impacts on human health.

A single touch through any of the entrance points—the mouth, eyes, lungs, and skin—causes acute consequences. A single touch through any of the entrance points—the mouth, eyes, lungs, and skin—causes acute consequences. Animal tests for dermal, inhalation, and oral toxicity as well as eye and skin irritation are used to determine acute toxicity. Its symptoms include headaches, body pains, skin and eye irritation, and light-headedness [17]. Each year, there are over 3 million cases of acute poisoning. The immediate impacts of pesticides on human health are depicted in figure below.

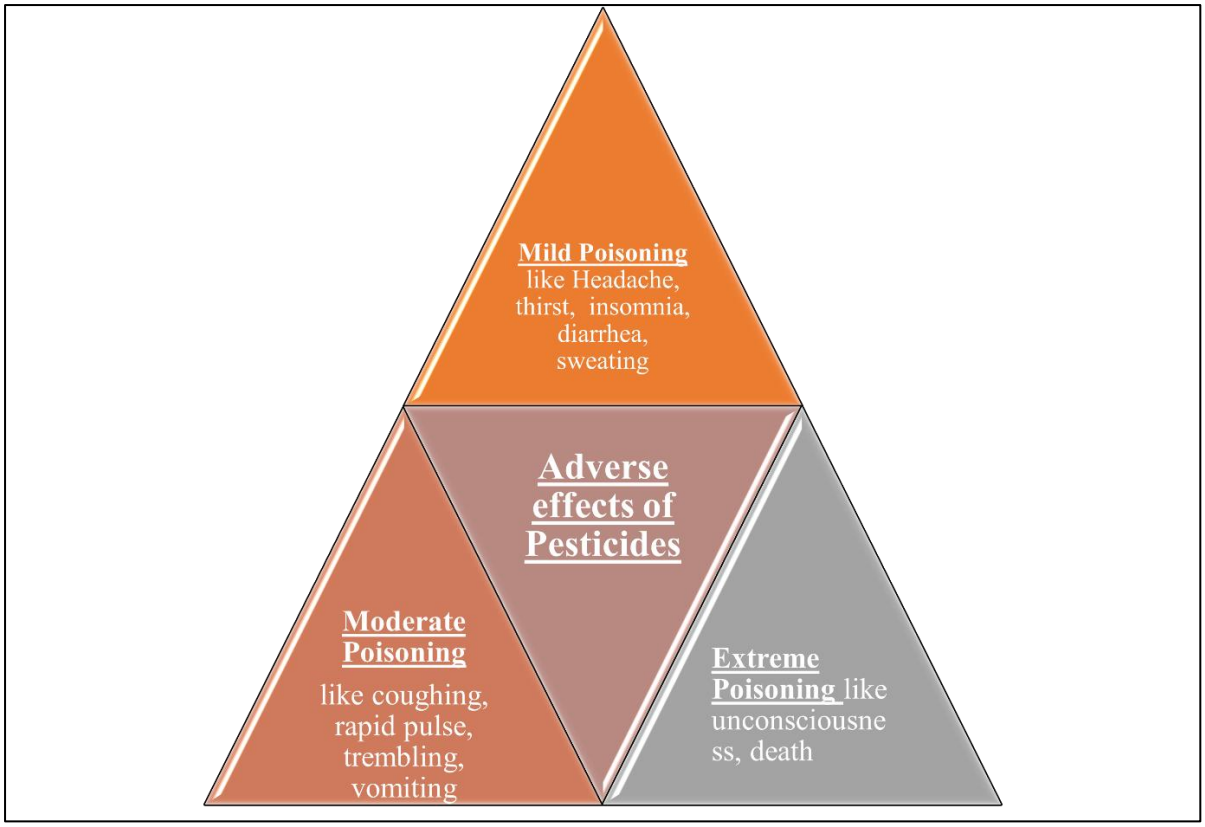
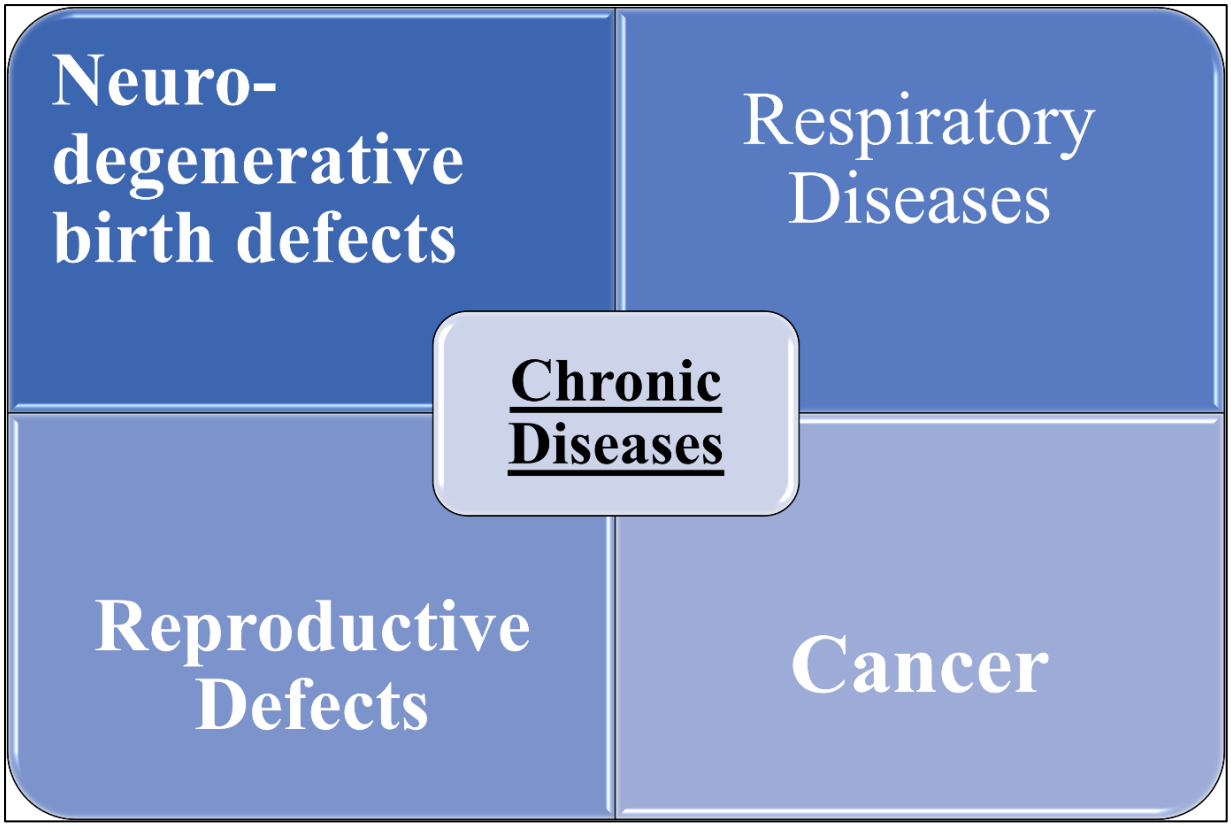


Fig. 1.3. represents the acute effects of pesticides on human health

Chronic impacts are any adverse consequences that occur when tiny dosages are consumed over an extended period of time [19]. In the lab, chronic toxicity is more challenging to assess than acute toxicity. When people are exposed to sublethal pesticide quantities on a regular basis over long periods of time, they acquire chronic diseases. Its main symptoms, which manifested later, include hazardous cancers, birth abnormalities, and genetic alterations [20].



Chronic disorders brought on by pesticides are depicted in Fig. 1.4

2) Effect on biodiversity: There are numerous plants, microorganisms, and animals in the environment. There are many creatures in a healthy environment. In addition to destroying undesired plants, weeds, and other vegetation, pests can also occasionally exterminate other beneficial species, which results in an imbalance in food webs and a loss of biodiversity [21].

3) Effects on water and air ecosystem: Through accidental spills, surface runoff, floating into streams and rivers, etc., pesticides can find their way into waterways. Applications of pesticides from the air produce spray drift into the sky, which throws the water and air ecology out of balance.

1.6 Techniques for detection of pesticides

It is essential to identify and quantify pesticides since they negatively impact human health. For this reason, a variety of techniques have been created.

1.6.1 Traditional methods of analysis

Numerous widely used conventional methods, such as gas chromatography, spectrophotometry, fluorometric analysis, immunoassay[22], and others, can be used to

identify pesticides[23]. These methods are precise and targeted, but they take a lot of time, expensive equipment, and expert operators. Very sensitive methods for detecting pesticides should take their place [24].

1.6.2 Other advanced techniques

Biosensors are utilized for pesticide detection in order to overcome the shortcomings of traditional analytical procedures because they provide so many benefits over them. Their environmental friendliness, rapid reaction time, mobility, selectivity, and specificity all contribute to their widespread use [25].

1.7 Introduction of Biosensors

By generating signals that correspond to analyte concentrations, a biosensor is a device intended to measure reactions of chemical and biological processes [26]. Known as the "Father of Biosensors," Prof. Leland C. Clark coined the word "biosensor" in 1962 [27]. A biological process can involve any biological component, such as tissues, microorganisms, cells, acids, enzymes, and so on [28]. The type of enzyme and substance employed determine the outcome of a biological element. Either voltage or current might be the transducer's electrical form.

At their core, biosensors must be reusable, very accurate, and insensitive to external variables like pH and temperature. These devices have a wide range of uses, including as pharmaceutical research, disease surveillance, and the detection of contaminants, pathogens, and biomarkers in blood, saliva, etc. In 1975, the first biosensor to be used commercially for glucose detection was introduced[29].

- **Analyte -** A target material that has to be found. Glucose, for instance, is regarded as an analyte for glucose detection [30].
- **Bioreceptors -** A molecule that identifies a specific analyte is called a bioreceptor. A signal in the form of light, heat, etc., is created when the bioreceptor and analyte contact. Bioreceptors include things like enzymes and antibodies.

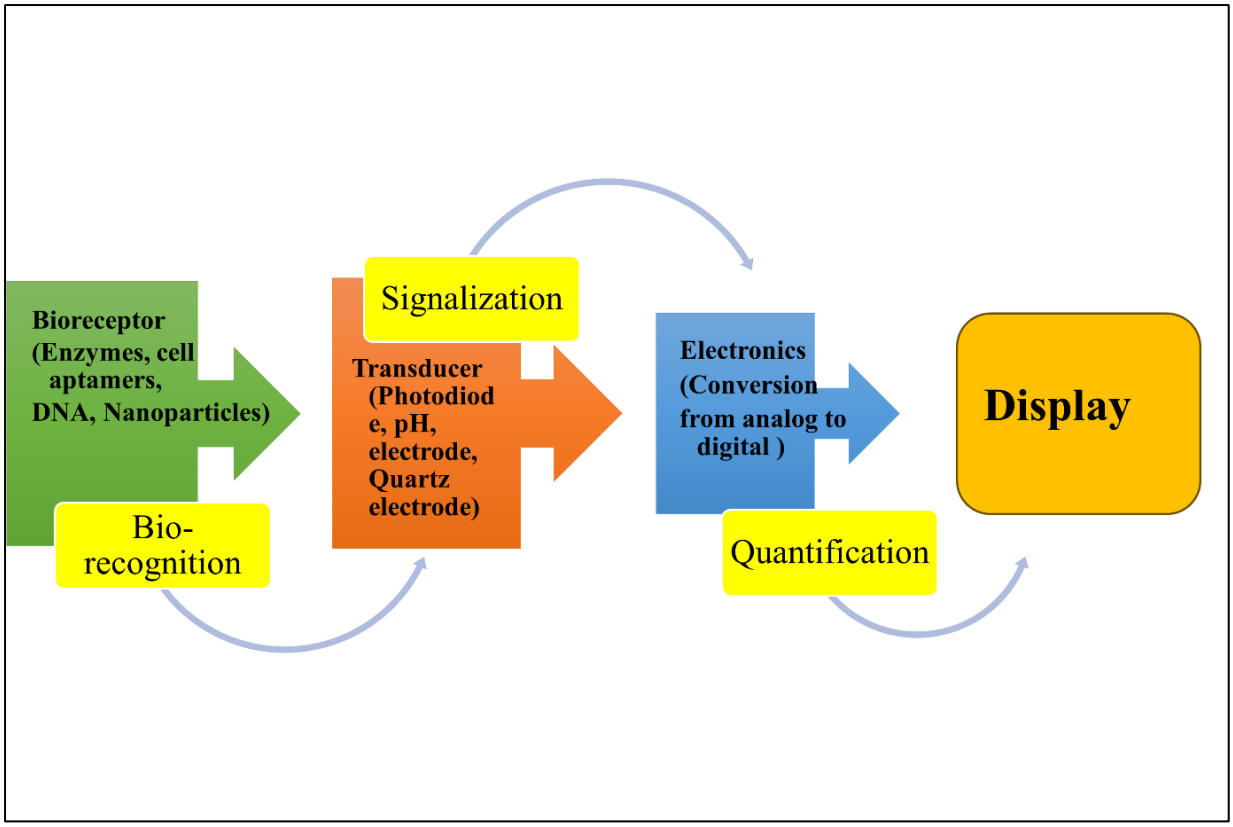


Fig. 1.5 represents a typical biosensor consisting of the following parts

- **Transducer**

  Energy (signalization) is changed from one form to another by the transducer. It aims to transform bio-recognition occurrences into quantifiable signals. The analyte's concentration and the interactions between it and bioreceptors are revealed by the electrical signals that are produced [30].

- **Electronic devices**

  The component of the biosensor prepares the transduced signal for display by processing it. Comprising complex electrical circuitry, it performs various signal-conditioning functions and transforms analogue signals into digital form.

- **Display**

  It comprises of a user-interpretation system that generates comprehensible curves or numbers. To produce the desired outcome in the form of a picture, tabular data, number, etc., it integrates hardware and software.

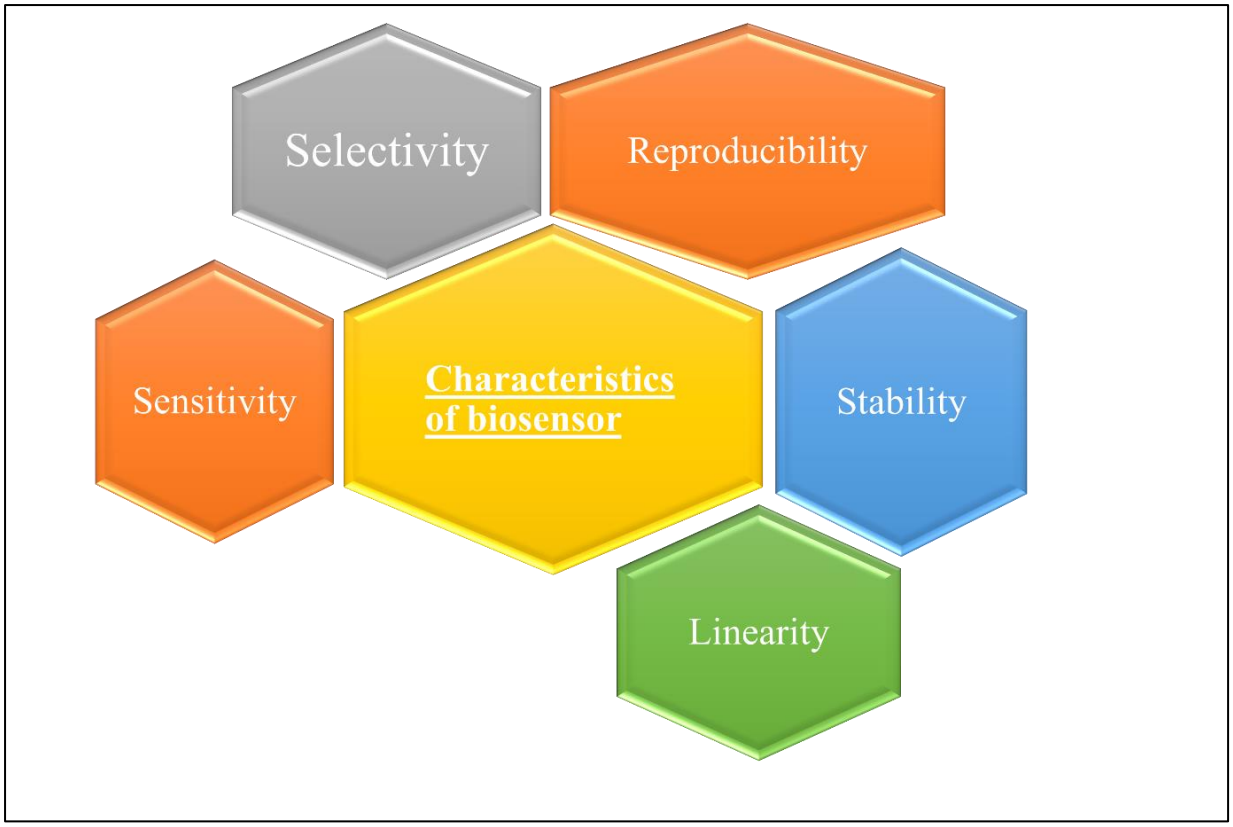


Fig 1.6 represents various parameters of observation for a biosensor

1.8 Characteristics of a Biosensor

Selectivity

Selectivity is the ability of a biosensor to recognize and quantify a specific analyte in a sample that may also contain other chemicals, such as pollutants. The interaction between an antigen and an antibody serves as the greatest example of selectivity in the setting of biosensors. Antibodies often function as bioreceptors and are affixed to the transducer's surface. The transducer is then exposed to a solution, often a buffer that contains both the antigen and salt, where antibodies preferentially attach to antigens. When developing biosensors and selecting bioreceptors, selectivity must be given top priority [31].

Reproducibility

The capacity of biosensors to generate consistent results after several studies is known as reproducibility. The accuracy and precision of biosensors characterize it. Conclusions derived from the biosensor's responses are dependable and robust when the signals are consistent.

Stability

It explains how sensitive the biosensing system is to external environmental disturbances as these disruptions change the output signal measurement and the accuracy and precision of the proposed biosensor. The affinity of the bioreceptors is an important additional element affecting stability. Affinity is the degree to which bioreceptors and the analyte interact. Strong covalent or electrostatic connections are formed between bioreceptors and analytes when affinity is high.

Sensitivity

In applications involving medical and environmental monitoring, biosensors may identify analyte concentrations as low as ng/mL or fg/mL, often known as the limit of detection or sensitivity. It confirms that an analyte is present in a sample.

Linearity

Linearity is the capacity of a measured response to a straight line at a variable analyte concent ration to be linear. The mathematical equation representation of straight line is y = mx+c where y is the output signal, c is the analyte concentration, and m is the sensitivity. Another word associated with linearity is linear range, which refers to the range of analyte concentrations for which the biosensor accordance varies linearly with the concentration. As seen in fig 1.6.

1.9 Biosensor Types

Sensors are divided into two categories based on the transducer and bio-component.

1.9.1 Classification of biosensors by bio-component

Numerous biosensor designs make use of biocomponents, such as enzymes, antibodies, tissue, organisms, and nucleic acids [32].

1. Enzyme Biosensor

Enzymes are used as biocomponents in enzyme-based biosensors. Enzymes, are composed of protein biomolecules, catalyse processes to generate signals that biosensor monitors indirectly by forming complexes between enzyme and substrate [33]. The vast range of potential uses of enzyme biosensors has led to their widespread adoption. In enzyme biosensors, an enzyme is maintained close to sensor surface, and the concentration of the substrate is determined by an enzymatic reaction. It occurs through two distinct reaction processes: enzymatic conversion in the substrate and diffusion in the product enzyme layer. Additionally, urea, lactate, glucose, and glutamate biosensors are the four categories of enzyme-based biosensors that are commercially available. To maintain enzyme activity, the transducer surface matrix immobilizes the enzymes. Changes like proton concentration, gas absorption and release, etc., are measured to determine

the analyte concentration[34]. The transducer converts these changes into quantifiable signals. Acetylcholinesterase (AChE) and butyrylcholinesterase (AChE) are typically employed as enzymes for pesticide detection [35]. The following factors contribute to the widespread use of enzyme-based biosensors:

- Sensitivity: They are able to identify analytes at extremely low concentrations. exhibits significant sensitivity as a result[36]
- Selectivity: They are able to identify specific analytes in complicated samples because to their high level of selectivity.
- Quick reaction: They identify the analyte efficiently.

2. Immunosensors

Antibodies are glycoproteins produced by the immune system and are used as a biocomponent in immunosensors. It works by interactions with antibodies and antigens. A measurable signal, such as a change in mass or conductivity, is created when an antibody interacts with antigens. Food safety, environmental monitoring, and the identification of cancer biomarkers are just a few of the numerous possible applications for immunosensors[37].

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3. Nucleic acid biosensors

In nucleic acid biosensors, nucleic acid is used as a bio component. The sequences of nucleic acids (DNA, RNA) can be ascertained using nucleic acid biosensors. It works by using complementary base pairing between the target nucleic acid sequence and the probe molecule (DNA or RNA) that is immobilized on a surface [38].

4. Microbial biosensors

Microbial biosensors use microorganisms as biocomponents [39]. It is predicated on microbes capacity to distinguish between various chemicals in their environment. Measurable reactions, like light or electrical conductivity, are created when certain target molecules contact with these microbes. These biosensors are preferred over chemical biosensors because of their low cost,

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high sensitivity, and capacity to identify a broad range of target molecules in the form of light, colour, electrical signals, etc. These biosensors are used in a number of sectors, such as food safety and water contamination detection. It has a number of drawbacks as well, such as the inability to develop suitable biosensors for chemicals and macromolecules that cannot pass through the membrane due to the cell membrane's structure acting as a diffusion barrier. Microbial biosensors have a long after-use period due to their reaction time and the time needed

to return to the fundamental signal points. Contamination and reduced activity are two of the most important problems during immobilization.

5. Lactate biosensors

Lactate biosensors assess the quantity of lactate in biological materials like blood, sweat, or saliva, which is indicative of anaerobic metabolic activity. These biosensors typically have a biorecognition component, such lactate oxidase or lactate dehydrogenase, mounted on a transducer substrate. A metabolic process initiated by the combination of the recognition element with lactate produces detectable alterations in electrical conductivity, pH modulation, or fluorescence emission.

1.9.2 Biosensor classification based on transducer

An analytical tool that produces an output quantity that is correlated with the input amount is called a transducer. Biosensors are classified according to their transduction mechanism[40]. The classes are as follows:

Amperometric biosensors: Amperometric biosensors function by using an oxidoreductase enzyme to transport electrons to an electrode surface. These biosensors utilize enzymatic systems that facilitate catalytic conversion of target compounds through electrochemical processes to generate measurable analytes that can be detected or quantified during working of electrode powers the majority of these biosensors. This electrode's potential is preserved in respect to a reference electrode. An electrochemical- processes, involving electron transfer or charge reduction, can occur on electrode interface with the use of an amperometric transducer, leading to a measurable current signal that corresponds directly to the target analyte concentration in the bulk substrate.

Potentiometric biosensors: These devices assess the electrode's equilibrium potential [39]. Using the Nernst equation in connection with the analyte activity a1, they operate on the basis of charge generation in the sample.

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

The activity (a1) is 1 when the standard electrode potential (E0) is 1 mol/L. Here, n is the total number of charges on the species, R represents the universal constant, F denotes the faraday constant, and T indicates the absolute temperature in kelvin. The symbols ±, -, and + stand for anions and cations, respectively.

Potential is determined in potentiometric biosensors using a working electrode and an inert

electrode (reference electrode). Since the computed potential correlates directly with the logarithmic concentration of the electroactive analyte, different analyte concentrations can be determined**.**

Optical biosensors: The most sensitive and efficient methods are believed to be optical methods, which are also the oldest. The development of high-quality optical fibres for biosensors has been extended to communication systems. Optical fibre biosensors are useful in biochemical and clinical analysis because of several reasons, such as (a) the optical signal is unaffected by any electrical or magnetic field, (b) the optical sensors don't need a reference signal, and (c) they are easy to fabricate with high efficiency [41].

Piezoelectric biosensors: It is believed that these transducers are quite sensitive enough to be used in biosensor applications. The basic principle of these biosensors is based on the binding of a molecular species to the crystal surface, which results in a change in mass, which in turn alters the oscillation frequency of the crystal [42]. Changes in density and viscosity on the sensor surface can also be measured by it. To capture the piezoelectric action, high-selectivity compounds containing biological components, such enzymes or antibodies, are applied to the piezoelectric crystal. Piezoelectric transducers are used in the domains of immune identification and practical immune applications. Benefits of using this type of converter include convenience of use, tag-free identification, and real-time tracking.

Thermal biosensors: The way a thermal biosensor works is by calculating how much heat is absorbed during a biological process [43]. It is made up of a biomaterial (a calorimetric biosensor) and a physical transducer. It is used for environmental control, clinical detection, and the thermometric Elisa test, which determines the antigen-antibody interaction. The method's high instrument costs are a disadvantage, though.

1.10 Immobilization of biomolecules

Immobilization is the process of binding biomolecules to solid substrates so they can remain active for long periods of time[44]. The stable solid surface that an enzyme may attach to is called a matrix. The mechanical and chemical characteristics of the matrix dictate the immobilization process. materials including glass, conducting and non-conductive polymer sheets, screen-printed electrodes, etc., as a matrix. By restricting biomolecules' movement and putting them in one place, immobilization increases stability. Care should be given while choosing an immobilization method to guarantee that no enzyme activity is lost and that the reactive group of the enzyme's binding site stays unaltered. Commonly used immobilization

methods include covalent binding, cross-linking, physical trapping, and physical immobilization [45].

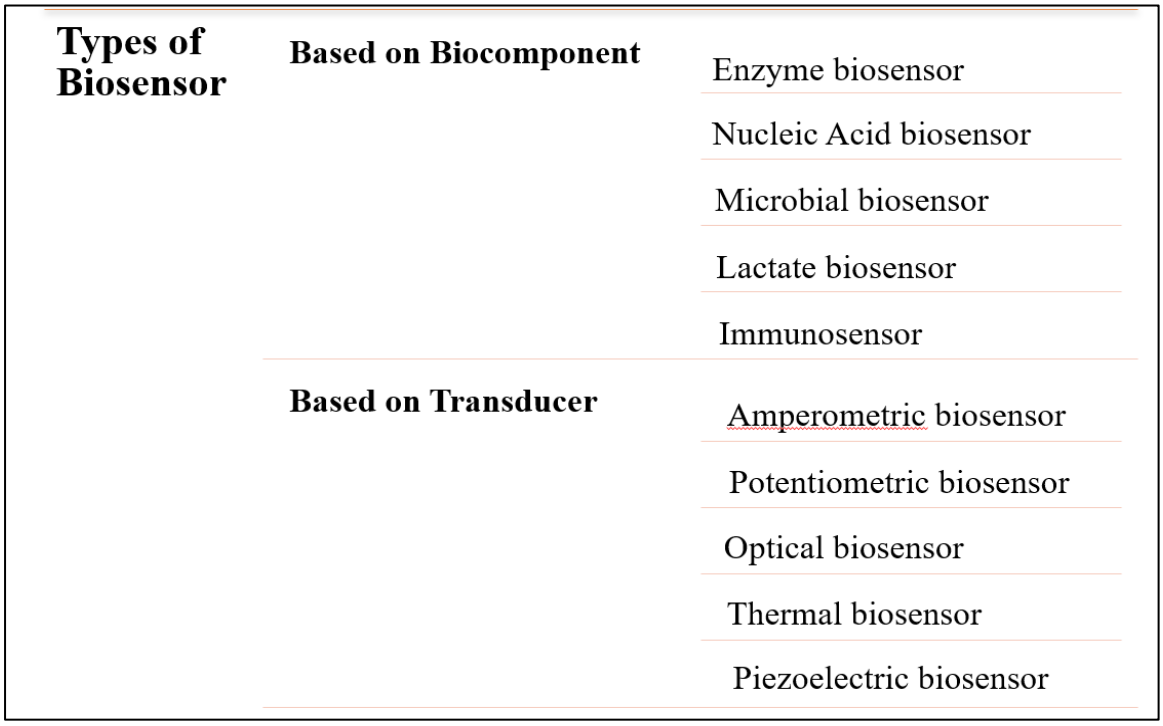


Fig. 1.7 Schematic representation of types of biosensors

1.11 Methods of immobilization

Cross Linking: Compounds with two or more functional groups that can bind two different materials under various circumstances are employed in the cross-linking process [46]. The cross-linking approach uses compounds with two or more functional groups, such as 4-azido-1-fluro-2-nitrobenzene, which may bind two different materials under various conditions. Reagents with two or more functions are typically used. Most often, glutaraldehyde is used.

Benefits:

1.Cost-effective

2. Reduced biocatalyst loss.

Drawbacks

1. The active sites of enzymes and biomolecules can be changed by chemical reagents.
2. It isn't appropriate for all biomolecules and enzymes since it needs certain conditions.

Physical adsorption: A biomolecule is physisorbed onto a matrix surface using this method. As a result, the method either leaves the enzyme's structure unchanged or just slightly alters it.

The biomolecule must be bound by weak forces like hydrogen bonding and van der Waal force, which alters a number of variables including temperature and ionic strength.

Benefits:

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

Drawbacks

1. The adsorbed molecules have a considerable possibility of separating off the support because of the weak binding forces.
2. Compared to alternative immobilization methods, adsorbed molecules have a reduced loading capacity.

Entrapment: The targeted biomolecules are able to stay in the matrix because the enzyme layer restricts the other biomolecules. After mixing the polymer solution or gel precursor (polyacrylamide) with the biorecognition element, the combination is left to gel or polymerize on the sensor surface. Care must be used while choosing the entrapment conditions because they are severe and result in inactivity**.**

Benefits:

1. Enzyme activity is preserved using this technique.
2. Adaptable to various matrix types.

Drawbacks:

1. A high diffusion barrier.
2. A remarkably high reaction time.

Covalent binding: This method creates a covalent link between the biomolecule and the support matrix. In the majority of cases, matrix functional groups are used, which can be created via covalent coupling or activation. Covalent coupling usually uses nucleophilic functional groups, such amino, carboxylic, indole, etc., that are present in the side chains of proteins' amino acids. Since they alter the active center, which results in a marked reduction in substrate activity, the circumstances involved are more severe and intricate than with other methods.

Benefits:

1. Because of the high binding force between the enzyme and the carrier, there is no loss of enzyme.
2. Consistent even under difficult circumstances.

Drawbacks:

1.There is no way to regenerate the matrix.
2. The substrate's change in conformation.

1.12 Applications of Biosensors

38 **In the medical industry:** Biosensors are widely used in the medical industry. A lot of people use glucose biosensors to diagnose diabetes. They can also be used to diagnose infectious diseases [53]. Even at low concentrations, they can identify biomarkers with accuracy. It is widely utilized for cancer detection and biomarker identification. Along with other biological components like enzymes and antibodies, they can identify harmful microorganisms. These days, biosensors that can detect cardiac troponin—a protein complex that detects heart damage and can detect acute coronary syndrome—are integrated into mobile units. These biosensors utilize the ideas of fluorescent microfluidics, disk with the reader, and machine analysis. In the current situation, COVID-19 was detected by means of nanomaterial-based biosensors.

For Food Industry: Biosensors are used in food processing and authentication to detect foodborne pathogens. Lactate and glucose levels in meals like milk and yogurt are measured using biosensors [47]. Lipids, alcohol, cholesterol, glutamate, glucose, and lactate are all detected using biosensors. Additionally, they monitor and detect obesity, an artificial sweetener that causes illness. Food quality, chemical components, residual assessment of agricultural medications, and other aspects like freshness and scent are all evaluated using biosensors.

For Military: Biosensors help detect biological attacks by identifying threat-causing organisms (biowarfare agents), mainly bacteria and viruses. Given the potential for catastrophic destruction, it is imperative to detect and prevent both illnesses and bioterrorism, which are on the rise. These biowarfare agents, which include bacteria, fungus, and viruses, are detected using biosensors.

In environmental protection: it determines the levels of pesticides, fertilizers, and heavy metals in the soil and water, as well as the causes of soil disease [48]. In aquatic bodies, biosensors identify halogenated chemicals, algal RNA, and toxic algal blooms. By determining the concentration of dangerous contaminants, they aid in pollution monitoring.

Nanotechnology: Biosensors that use nanotechnology can significantly increase their sensitivity, specificity, and overall performance when it comes to recognizing biological components by taking use of the special properties of nanomaterials. Nanomaterials with a high surface area to volume ratio, such as nanoparticles, nanotubes, etc., enhance the communication between the target molecules and the sensors. It cuts down on the amount of time required to examine tiny molecules with complex structures. If a high value is obtained when the surface area is comparable to the volume, its selectivity increases. Very little energy is required for the investigation, and there is no diffusion problem. As a result, the biosensors are assured of a long lifespan. It ensures the completion of the investigation without putting the cells in jeopardy.

1.13 Future scope of biosensors

Future biomarker techniques will explore the creation of many choices for manufacturing precise diagnostics, drugs, and equipment [49]. The human being must disruptively enhance sample drawing. Importable biosensors might significantly accelerate the production of customized drugs. Furthermore, the body may be equipped with biosensor chip technology to detect complex changes in blood DNA throughout the early stages of intricate blood DNA alterations before onset of illness in its initial stage. Low-cost, reversible care point devices can incorporate biosensor technology. It can also be used for continuous tracking of implanted devices.

In this area, a number of sensors that rely on nucleic acid hybridization detection are making fascinating advancements. The "Environmental Sample Processor," which is being developed by the Monterey Bay Aquarium Research Institute, is one such sensor. Its goal is to use ribosomal RNA probes to automatically detect hazardous algae in situ from moorings. Monitoring of contaminants, hazardous metals, and pesticides is another primary goal. Because biosensors are used in so many medical and healthcare operations, there is a growing need for them. Additionally, biosensors have made strides in a number of areas, such as human health management, illness detection, patient wellness monitoring, and diagnosis.

1.14. 2-D based nanocomposite

The creation of nanocomposites has led to advancements in the field of material science. The creation of nanocomposites has led to advancements in the field of material science. Special materials known as nanocomposites are created by mixing two or more nanoparticles [50]. Because of the combination of their processing materials' features, they have a wide range of

distinct physical and chemical characteristics. There are many uses for them. They are regarded as the materials of the twenty-first century because of their distinctive designs and ability to combine qualities not present in conventional composites [51]. Van der Waals force holds thin layers together to form 2-D materials [52]. The thickness of the layers is a few nm. The layers of two-dimensional materials allow electrons to travel freely. Materials such as transition metal oxides and MXene.

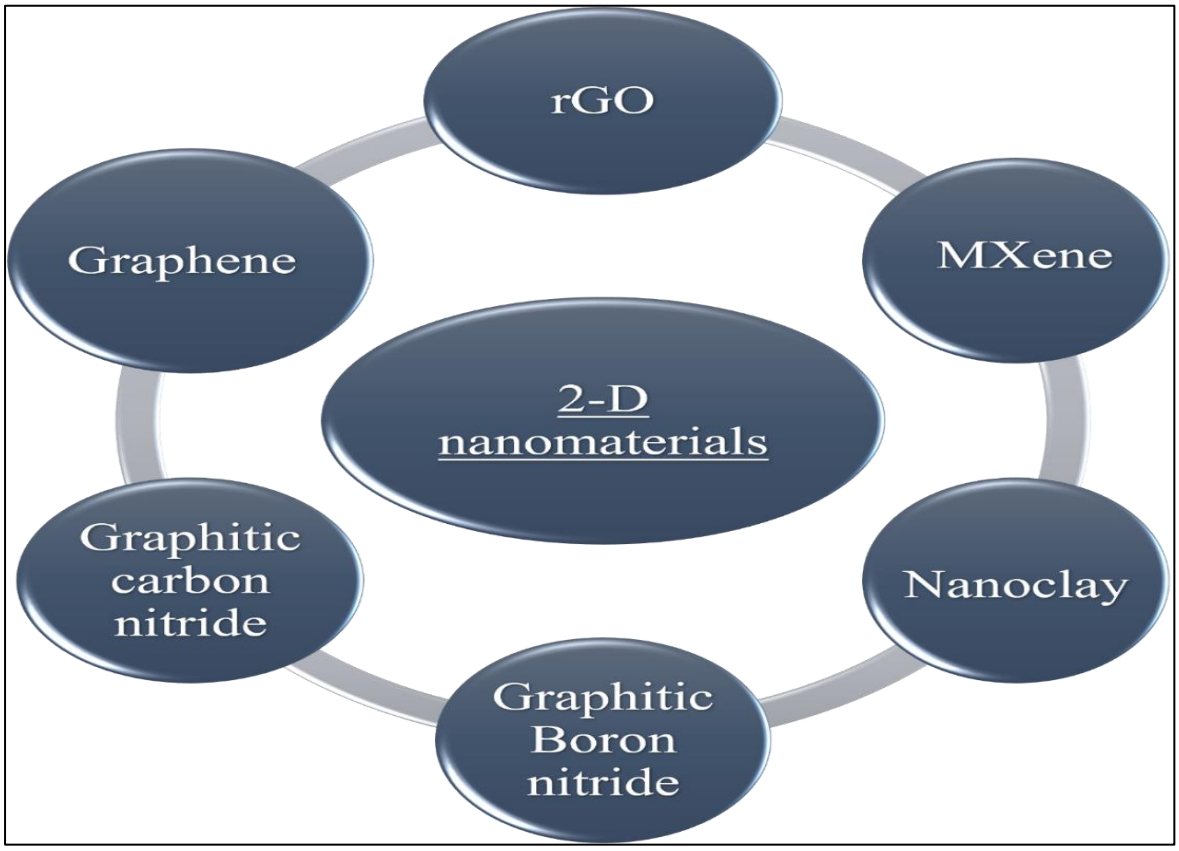


Fig.1.8 Example of 2-D nanomaterial

1.14.1 Properties of 2-D nanocomposite

They possess unique properties, such as

1. **Mechanical property:** Nanocomposite materials have higher strength and toughness than standard materials due to their enormous surface area that strengthens the matrix [53].
2. **Enhanced thermal stability:** Nanoparticles can make composite materials more thermally stable and resistant to heat damage. This capability is particularly useful for applications that need high temperatures [54].
3. **Better Electrical Conductivity:** Depending on the type of nanoparticles utilized and their dispersion within the matrix, nanocomposites can have better electrical conductivity or

21 insulating capabilities. As a result, they are suitable for a wide range of electronic applications [55].

4. **Enhanced Optical features:** Depending on the nanoparticles added to the matrix, nanocomposites can have unique optical features such as improved transparency, fluorescence, or light scattering [56].
5. **Barrier Properties:** Because of the large surface area and fragility of the nanoparticle-filled portions inside the matrix, nanocomposites can provide improved protection against gases, moisture, and other environmental factors.

1.14.2 Applications of 2-D nanocomposite

1. **Nanocomposites can increase mechanical properties:** of structural materials such as metals, ceramics, and polymers. Integrating nanoparticles improves the endurance, stiffness, and strength of these materials, making them suitable for usage as construction materials, automotive parts, and aerospace components [57].

2. **Nanocomposites improve food packaging:** by preventing oxygen, moisture, and other contaminants from flowing through, extending shelf life for perishable foods [58].

3. **Electronics:** Nanocomposites improve electrical and thermal conductivity.

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

CHAPTER 2- Literature Review

2.1 Introduction to Organophosphate Pesticides

Organophosphorus pesticides are a varied family of pesticides that are widely employed to protect crops from insects and are extremely hazardous in nature. These chemicals are often esters, amides, or thiol derivatives of phosphonic acid[59]. Despite the fact that fewer pesticides were created at the time, insects developed resistance to pesticides owing to repeated exposure, resulting in reduced pesticide efficiency[60]. Hence, new insecticides are required. Pesticides vary in toxicity and can be classified as poisonous, common, or detrimental. Organophosphate insecticides are structured as follows:

Overstimulation of OPPs leads to illnesses such OPPs induce four neurotoxic diseases in humans. These syndromes are–

1. **Intermediate Syndrome** - This syndrome appears between 24 - 96 hours following exposure to organophosphates[61]. This condition primarily affects people without cholinergic symptoms. Even though intermediate syndrome has been recognized as a neuromuscular junction condition, the specific risk factors, etiology, and incidence remain unknown. It weakens the breathing muscles and motor cranial nerves, followed by numbness in the neck flexors[62]. IMS often occurred in individuals with severe and acute AChE inhibition[63], resulting in a constant excess of AChE at the neuromuscular junctions, although it did not impact all patients. Other risk factors include decreased OPP metabolism, muscle respiratory weakness, elevated muscle enzymes, and inadequate or delayed treatment with pyridinium or atropine oximes[64].
2. **The Cholinergic Syndrome** – The signs and symptoms of cholinergic syndrome in situations of severe OPP poisoning are predictable due to their biological method of action and are closely connected to acetylcholinesterase activity [65]. In situations of human poisoning, the typical severe symptoms of peripheral and nicotinic toxicity are readily apparent. Sweating, tremors, lacrimation, abdominal pains, and other gastrointestinal problems are some of the symptoms[66]. These symptoms are followed by central effects such dizziness, headache, weariness, and paraesthesia. Finally, seizures, unconsciousness, and convulsions can ensue[67].

3. **Organophosphate Induced Delayed Polyneuropathy (OPIDP)** - It is a unique neurological condition generated by a single exposure to OPPs, with symptoms appearing 10-20 days or more later. OPIDP is a rare neurodegenerative illness in humans characterized by the loss of function and ataxia of the distal parts of sensory and motor axons in peripheral nerves as well as ascending and descending spinal cord tracts [62], [68]. Early neurological indications often include cramp-like feelings in the calves and tickling in the hands and feet, as well as distal numbness and parenthesis. In severe instances of OPIDP, quadriplegia, foot and wrist drop, and minor pyramidal symptoms were observed.

4. **Chronic organophosphate-induced neuropsychiatric disease (COPIND)** - Prolonged OPP exposure has been linked to poorer neurobehavioral function, but not in all epidemiological studies [69]. COPIND occurs without cholinergic symptoms and does not need AChE inhibition [59], [62], [68]. COPIND generally emerges gradually and lasts a long time, perhaps indicating chronic injury to the central nervous system [8]. The most common symptoms of COPIND are mood changes (depression, emotional lability, anxiety), cognitive deficits (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)[28].

2.2 Importance and need for detection of Organophosphate pesticides

The detection of organophosphorus pesticides is required for the following reasons.

1. **Threat to the environment**- Organophosphate insecticides are purposely hazardous to non-target creatures such as mammals, birds, and aquatic animals.
2. **Adverse impacts on humans, plants, and ecosystems-** Even at low concentrations, it can harm humans, plants, and animals. OPPs generally enter the body by ingestion, inhalation, cutaneous application, or injection. Workers who manufacture pesticides, floriculturists, pesticide applicators, and farmworkers may be exposed to OPP contamination. Prolonged exposure to OPPs is associated with a variety of impairments, including memory and linguistic difficulty, visual-spatial performance, coordination, and so on [70], [71]. Furthermore, OPPs have been shown to influence human health at both the embryonic and adult stages, as well as to increase morbidity and death in individuals with chronic

poisoning [72]. Residues in agricultural goods can have a long-term negative impact on human health [73].

3. **Causes dangerous illnesses in the human body** - Some research shows a link between OPPs and diseases like leukemia and lymphoma. Many studies have found that OPPs like chlorpyrifos and diazinon enhanced the risk of Parkinson's disease. Furthermore, OPPs have an adverse effect on the human reproductive system, reducing male fertility. They also produce non-neurological consequences such as cardiac arrest, infertility, chronic disruption, and so on [74]. The presence of OPP metabolites in the body reduces the levels of sex hormones like testosterone. Thus, it is obvious that quantifying OPPs has a significant influence on the environment. As a result, it is critical to create simple, quick, and low-cost methods for determining organophosphate pesticides, taking into account health, toxicity, and environmental safety.

2.3 Literature Review

Nanomaterials were used in a recent work to fabricate OPP sensing devices because to their superior biocompatibility, electrical conductivity, magnetic and optical characteristics [[75]. Nanomaterials have various benefits, such as a large specific surface area for more sensitive detection, and are thus a popular study topic in biosensor building [76]. OPPs are detected using a variety of nanomaterials, including metal and oxide nanoparticles, graphene, carbon nanotubes, polymer-nanomaterial composites, and so on. Thus, the analytical performance of the produced electrode reported in earlier research was compared to the current work.

Table 2.1: Correlation of sensing of the $CeO_2/Ti_3C_2T_x$@ITO electrode with various sensors reported.

S No	Working Electrode	Method	Linear Range	Limit of Detection	Reference
1.	CeO_2/rGO/GCE	LSV	0.025–2 uM	3.0 nM	[77]
2.	ZnTFMPCAPc@MWCNTs/ GCE	DPV	10–310 uM	1.358 nM	[78]
3.	PANI/GCE	ADSV	0.01–100 uM	0.007 nM	[79]
4.	MWCNTs/GCE	SWV	0.2–60 uM	80 nM	[80]

5.	CNTs	CV	0.54–6.84 uM	90 nM	[81]
6.	SiO_2/MWCNTs/RuPc	DPV	3.0–66 uM	1620 nM	[82]
7.	PGO/SPCE	SWV	0.02–250 uM	61 nM	[83]
8.	NbC/Mo	DPV	0.01–1889 uM	0.15 nM	[84]
9.	Nano-silver/dodecane/GCE	DPV	1.00×10^{-4} – 0.700 µM	0.600 nM	[85]
10.	**$CeO_2/Ti_3C_2T_x$@ITO**	**DPV**	**1-100 pM**	**1 pM**	**This work**

PANI= Polyaniline; AuNPs = gold nanoparticles; CeO_2/rGO = cerium oxide/reduced graphene oxide; MWCNTs = multiwall carbon nanotubes; RuPc = ruthenium phthalocyanine; NbC/Mo = niobium Carbide/Molybdenum; ZnTFMPCAPc = Zinc (II) tetra trifluoromethyl carboxamide phthalocyanine.

2.4 Characteristics of the material

When performing electrochemical processes, it is necessary to pick the appropriate electrode material. Several electrode materials for detecting OPPs have been developed and published. Because of their unique chemical and physical characteristics, 2D nanomaterials have recently gained popularity for the detection of OPPs, significantly improving the efficacy of electrochemical sensors [86]. Their catalytic action and vast surface area influence the sensitivity of the biosensor [87].

2.4.1 Titanium aluminium carbide ($Ti_3AlC_2T_x$) [MAX Phase]

The term MXene (pronounced M-X-ene, max-ene, or M-zene depending on phonetics) comes from its chemical formula, $M_{n+1}X_nT_y$, where M is an early transition metal (most often Ti, Zr, Hf, V, or Nb) and X is carbon or nitrogen. MXenes were called after graphene, which has been extensively investigated; the termination group T has since been recognized as an important part of the chemical formula of most MXene compositions, and it typically relates to

chalcogens (O, S, Se, Te) and halides. The construction of an MXene sheet is straightforward: n+1 M layers are intercalated with nX layers and covered by T groups[88]. Early transition metal carbides and nitrides were among the hardest and most refractory substances known. They are vital in anti-wear coatings, machine tools, and structural materials, but they have one drawback: they are brittle, as are most superhard substances.

After many decades of engineering study on carbides, Barsoum and colleagues published work on ternary carbides including the M and X elements as well as a third element, this time from the early p block of the periodic table. The component became known as A, and the ternary carbides as MAX phases[89]. The layered structure of MAX phases, for example, Ti_3AlC_2, is made up of Ti_3C_2 (Ti-C-Ti-C-Ti) layers that alternate with single Al layers[90]. Enzyme/protein-based MXene biosensors have been extensively studied for electrochemical detection of various biomolecules. The long-term stability of MXene sensors/biosensors allows for great repeatability of data over time, making them ideal for diagnostic applications. Titanium carbide is the sole known MXene for sensing, with few reports on alternative transition metal-based detection methods.

Extensive research is needed to utilize various transition metal-based MXenes in sensing technologies, given their exceptional sensing capabilities. While there are limited publications on electrochemical enzymatic MXene biosensors for tiny biomolecules, there is a huge need to create more biosensing platforms. MXenes' large surface area and biocompatibility make them ideal for developing nano(bio)hybrid systems with bioreceptors such as aptamers (DNA/miRNA)[91], antigeneantibodies (AgeAb), whole cells, proteins, and enzymes[92]. These systems can easily immobilize biomolecules onto their surface, acting as a sensitive detection interface. Advancements in MXene biosensor design will enable advanced detection methods, including fluorescence, luminescence, colorimetry, and electrochemistry, for early disease detection and point-of-care diagnostics[93]. Fig. 2.1 shows various uses of MXene.

2.4.2 Applications of $Ti_3C_2T_x$

1. As a Photocatalyst

$Ti_3C_2T_x$ derived photocatalysts are typically prepared by calcination or hydrothermal oxidation of precursors in-situ. Over the last few decades, TiO_2. Semiconductor photocatalysts are widely studied due to their enhanced photocatalytic performance, superior light absorption characteristics, excellent chemical stability. These materials demonstrate exceptional

photodegradation capabilities and are challenging materials for advanced energy applications. Individual TiO_2 has two limitations that are challenging to overcome: a large band gap and a high photo-generated carrier recombination rate [94]. Various techniques, including optical measurement methods for testing, modern spectrophotometric analysis, sophisticated diffused to enhance photogenerated co-catalyst loading, and heterojunction building, have been used to enhance photogenerated carriers' light response range and usage rate[95].

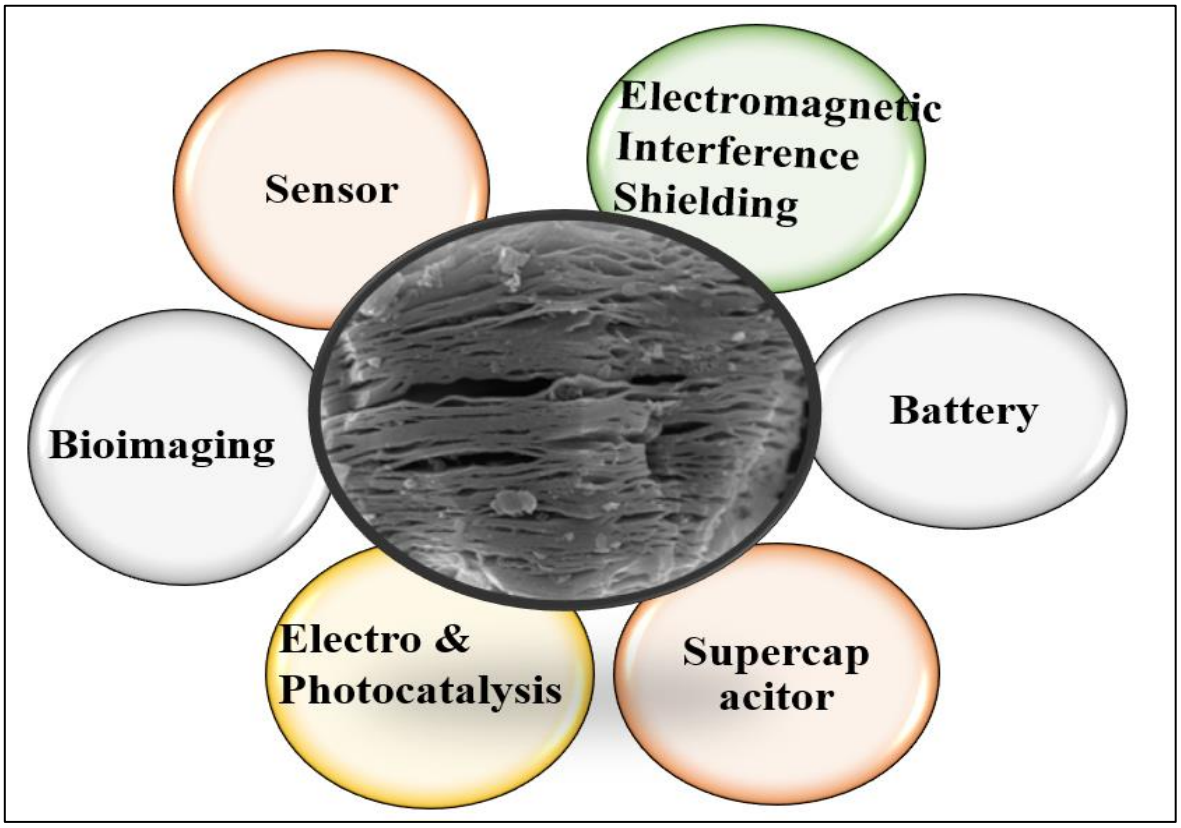


Fig. 2.1 Diagrammatic representation of $Ti_3C_2T_x$ and its uses

2. As a Supercapacitor

$Ti_3C_2T_x$ are widely employed in a variety of sectors due to their enhanced electrical characteristics and exceptional electrochemical properties. These have superior energy storage capabilities resulting from their optimized structural composition up to 600-900° F. They demonstrate advanced charge discharge characteristics due to unusual mix of metallic (transition metal atoms) and ceramic (carbon/nitrogen atoms) characteristics. $Ti_3C_2T_x$ have excellent surface chemistry, resulting in high metallic conductivity up to 6000-8000 S cm^{-1}[96], good thermal conductivity[97], excellent charge transfer [98], exceptional optical

composition [99], sublime improved optimized charge transfer mechanisms[99], [100], malleable surface functional groups, and intercalation ability.[101]

3. As a sensor

Sensors are becoming increasingly important in our daily lives as electronic and information. The sensing materials, without a doubt, have the greatest impact on their performance [102]**.** Layered nanomaterials having a two-dimensional (2D) planar structure outperform their bulk counterparts in a number of ways, making them ideal for the development of a variety of high-performance sensors [103]. As an emerging 2D material, $Ti_3C_2T_x$ have numerous advantages, including customizable surface characteristics, tunable bandgaps, and great mechanical strength, making them appealing in a variety of applications[104]. Informative materials with improved detecting capabilities are included into advanced sensing technologies. Without a doubt, their performance is most affected by detecting materials that are both active and informative [105]. Finally, we examine the primary problems and future prospects of $Ti_3C_2T_x$ -based materials in sensor applications.

2.4.3 Cerium Oxide (CeO_2)

Cerium is highly reactive in the presence of oxygen, resulting in stable oxides with varying compositions, sometimes referred to as ceria. Pure cerium oxide exhibits dual stable stoichiometric: cerium compounds (CeO_2) along with cerium sesquioxide (Ce_2O_3), also referred commonly as ceria[106]. Pure CeO_2 demonstrates a crystal lattice arrangement featuring a distinctive *Fm3m* group, particularly within the operational temperature range of ambient conditions up to the fusion temperature. It is a reduction transition caused by the production and migration of oxygen vacancies. Skorodumova et al. hypothesized a quantum method for ionization and delocalization of cerium's 4f electrons [107]. The thermodynamic stability of CeO_2 and Ce_2O_3 allows for a reversible transition between the two compounds, resulting in partly modified CeO_{2-x} compositions which function as oxygen storage materials through producing or eliminating oxygen defects[108]. This investigation of translational compositions has been going throughout the past two decades. Advancements in technology have led to the use of ceria for oxygen storage. It's important to note that unwanted qualities, including thermal volume changes due to increased heat levels, might occur while modifying environmental conditions. The formations of oxygen vacancies lead to the creation of an individual oxygen-vacancies represents a fundamental step during the conversion of CeO_2 to Ce_2O_3.

The formation of oxygen vacancies causes a rise within the Ce^{3+} concentration in the crystal lattice, maintaining electroneutrality. CeO_2's deficient ionic and structural characteristics exhibit dynamic behaviour, changing naturally or through reaction under varying environmental conditions including elevated temperatures, reduced oxygen levels, interaction with additional ions, electric field, and mechanical stress. The energy cost for generating a single oxygen deficiency in pure ceria (387 ± 13 KJ/mol [108]) is significantly lower than the activation energy required to reduce CeO_2 to Ce_2O_3 (about 382 KJ/mol). When oxygen is eliminated, resulting in a vacancy formation through the subsequent chemical reaction:

$O_2^x + 2Ce_4^x \rightarrow V_o + 2\ Ce_4^x + ½O_2(g)$[106]

In the aforementioned process, the development of the oxygen defect leads to the concentration and positioning of charge carriers in cerium tetravalent oxidation states, resulting in the formation of dual Ce^{3+} sites [106]. Despite the fact that it was lengthy, current theoratical studies show that the Ce^{3+} generated arising through oxygen vacancy formation is situated in locations further distant away from the defect place, as the cations in the nearest neighbour position are decreased [108].

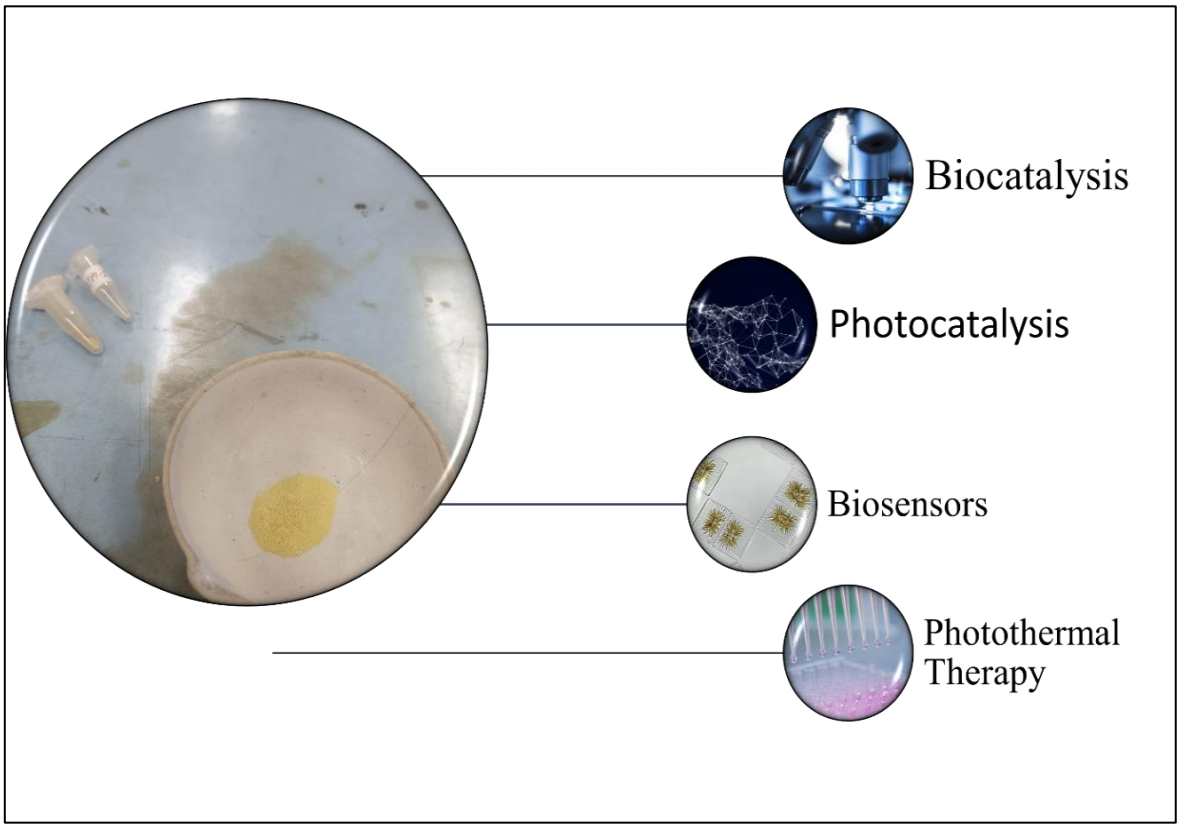


Fig. 2.2 Figure depicting the various applications of CeO_2 nanoparticles.

2.4.4 CeO_2 in the field of biosensor

1. Gas sensing: CeO_2-based nanoparticles are highly effective in gas sensing due to their oxygen vacancy-rich architectures that improve gas adsorption and electron transfer[106]. Choi et al. (2009) used CeO_2 thin films to create a highly sensitive gas sensor for detecting hydrogen sulfide (H_2S), demonstrating how doping and nano-structuring may improve sensitivity and response time[109]. Combining CeO_2 with other metal oxides, such ZnO, improves selectivity and lowers detection limits for ammonia and VOCs through synergistic effects[109].

2. Electrochemical biosensor based on CeO_2: CeO_2 has remarkable electrocatalytic characteristics due to its ability to redox cycle between Ce^{3+} and Ce^{4+}. This is especially beneficial in biosensors, where fast electron transport is required. CeO_2 nanoparticles were utilized for non-enzymatic glucose detection due to their catalytic nature, resulting in increased sensitivity and stability[110]. CeO_2-modified electrodes improve the electrochemical response to H_2O_2, a frequent indicator in oxidative stress-related illnesses[111].

3. Hybrid nanocomposites sensor: Combining CeO_2 with carbon materials (such as graphene) or metal oxides improves sensor performance by increasing electron transport and surface area[112]. CeO_2/Graphene-based composites have been studied for their ability to enhance conductivity and sensitivity in various sensing platforms. CeO_2/ZnO Nanocomposites: These hybrids have better detection and recovery capabilities for environmental contaminants[113].

4. Photoluminescence and Optical sensors: CeO_2's photoluminescence capabilities have been utilized in optical sensing techniques, particularly for detecting heavy metal ions and other dangerous chemicals through fluorescence quenching or amplification processes[114]. CeO_2-Based Optical Sensors CeO_2's strong luminous response allows for low-concentration detection of diverse analytes, providing rapid and sensitive optical sensing platforms[115].

CHAPTER 3- Material and Methods

3.1 Introduction

This chapter gives an overview of the materials utilized for fabricating $Ti_3C_2T_x$, cerium oxide, 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.

3.2 Materials

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

3.2.1 Chemicals

All chemicals including Cerium nitrate hexahydrate ($CeNO_3 \cdot 6H_2O$) (99%), Whatman filter paper, Hydrochloric acid (HCl), Sodium Hydroxide (NaOH), Urea ($CO(NH_2)_2$), Ethanol, Sodium Tetrafluoroborate ($NaBF_4$) and Titanium aluminium carbide (MAX phase) are purchased from Sigma-Aldrich, India. Monobasic and dibasic potassium phosphate [$K_2HPO_4(H_2O)_x$], and potassium chloride (KCl) are purchased from Fisher-Scientific, India. Fenugreek leaves, Tomato, Mandarin Orange and banana fruit were procured from the local vendor, Rohini, New Delhi. All solutions were prepared in Milli Q water to avoid contamination.

3.2.2 Solution and Buffers

- 0.1mM PBS (pH 7)
- 5mM $[Fe(CN)_6]^{3-/4-}$ is utilized as a redox initiator in PBS solutions

3.2.3 Characterization Techniques

Structure analysis is carried out using a variety of methods, including Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD), and all created electrodes and synthetic materials are described accordingly. The current work included both morphological and elemental characterisation methods, such as scanning electron microscopy and energy-dispersive X-ray spectroscopy. Characterization was also accomplished using electrochemical methods such as cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS).

3.2.4 Infrared spectroscopy using Fourier transform (FTIR)

IR spectra are transmitted through a sample in IR spectroscopy, with one portion of the spectrum being absorbed and the other part passing through the material. The resultant

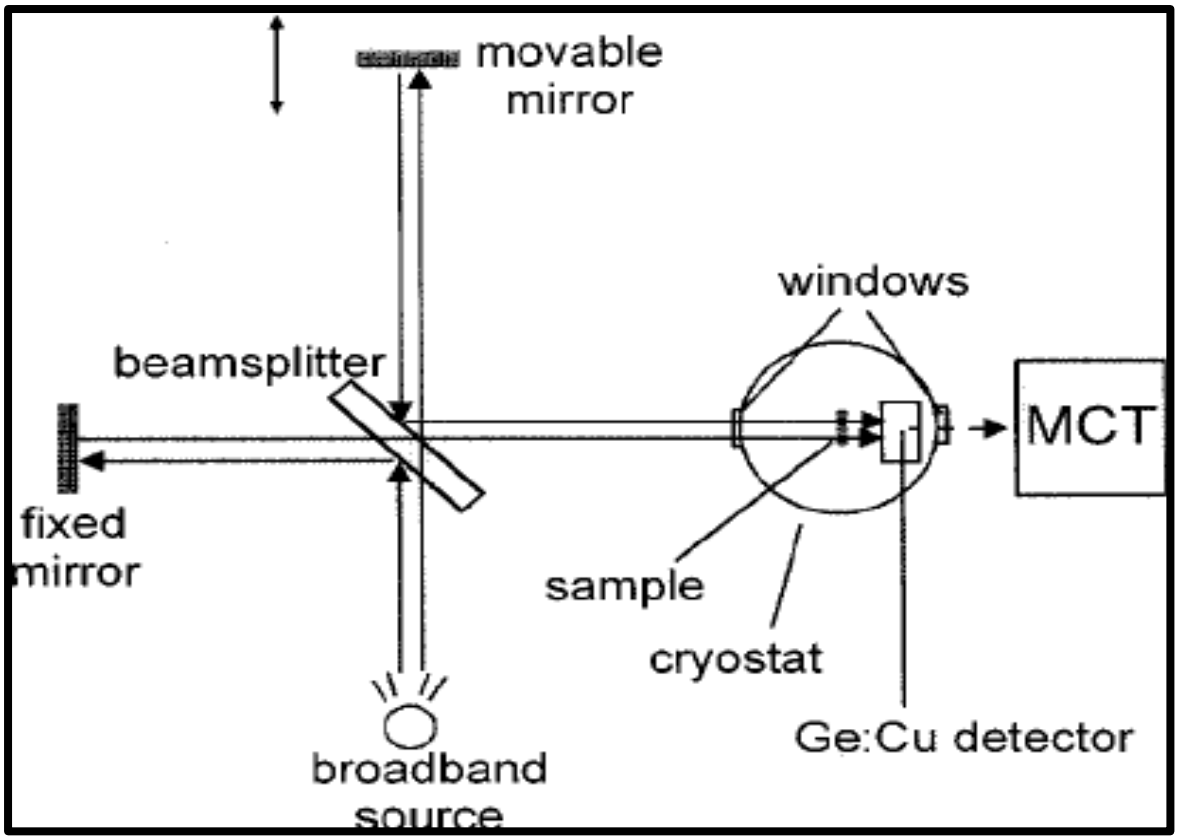


Fig. 3.1 Represents Michelson interferometer

spectrum, which displays the molecule's absorption and transmission (Fig. 3.2), which illustrates IR spectroscopy, creates the sample's molecular fingerprint [133]. Thus, organic, inorganic, and polymeric materials are analysed using infrared spectroscopy. With an absorption peak that corresponds to the vibrational frequency within the atoms' bonds, infrared spectroscopy reveals the sample's fingerprint [134][135].

By identifying the proper beam detectors and splitters, FTIR spectroscopy may be used to a wide variety of frequencies represented as UV (ultraviolet), near-infrared, visible, far-infrared, and mid-infrared. Such a wide range of frequencies cannot be covered by any of the other dispersive methods [136]. The fact that FTIR may be used as a quantitative technique to conduct multicomponent research is one of its greatest benefits [137]. The quantification of many components is based on Beer's law additive nature and the software that is now

accessible. This allows for the calculation of the calibration factor matrix, the estimation of unknown concentrations, and the acquisition of absorbance values. The K/P values of the matrix, which employ a mixture of certain concentrations as a calibration standard and directly the mixture of unknown concentrations, are the foundation of most FTIR multicomponent analysis methods [138]. Because it uses interferometry, FTIR is fundamentally different from conventional dispersive IR (infrared) spectroscopy [139]. The tool used to determine a compound's absorption spectra is called spectrophotometry. A Fourier transform (FT) spectrophotometer provides an infrared spectrum faster than a standard spectrophotometer.

An infrared radiation beam produced by a black body source is released by the FTIR instrument. Spectral encoding occurs when the beam finally transverses to the interferometer. When beams with varying travel lengths recombine, constructive and destructive interference are produced, leading to an interferogram. As soon as this beam enters the sample's compartment, the sample begins to accrue certain energy frequencies that are only visible in the interferogram. Additionally, for each frequency, the detector tracks the unique interferogram signal in energy vs time. In the meanwhile, the beam is imposed to provide a backdrop or point of reference for the instrument's functioning [140]. Finally, using Fourier transformation (FT) software, the interferogram removed the background spectrum from the sample's spectrum to get the required spectrum [141][142].

3.2.5 X-Ray Diffraction (XRD)

A widely used method for examining crystalline structure and atomic spacing is X-ray diffraction (Fig. 3.2) [143]. The constructive interference amongst monochromatic X-radiation and crystalline materials forms the basis of the X-rays, which undergo further processing to produce monochromatic radiation that is concentrated and aimed at the specimen [144]. The wavelength of an X-ray is 10 nm, as is the radius of an atom and its covalent bond length. To identify atomic location, using longer wavelength radiation (ultraviolet) would result in a low resolution. On the other hand, inelastic scattering would occur from radiations with shorter wavelengths (Gamma radiations) [145]. This technique establishes the location of the crystalline and polycrystalline structures. Bragg developed a formula known as Bragg's law that establishes correlations between the frequency of the incident radiation, the orientation of the diffracted radiation, and the atomic sepration that may be found in the crystalline structure of the sample.

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

where n represents an integer that indicates the order of diffraction, λ denotes the wavelength of the X-rays that are released, d refers to the interplanar distance of the crystal planes that diffract through the radiation, and Θ represents the angle between the incident ray and the surface of the reflecting crystalline lattice [146]. The material is examined across a range of predetermined 2Θ angles in XRD experiments, and all diffracted X-rays are extracted from the sample. To do a qualitative examination of the sample, that is, to determine the chemical component of the material, the acquired 2Θ angles for each individual peak would be converted to d-spacing after the diffraction pattern was monitored [147].

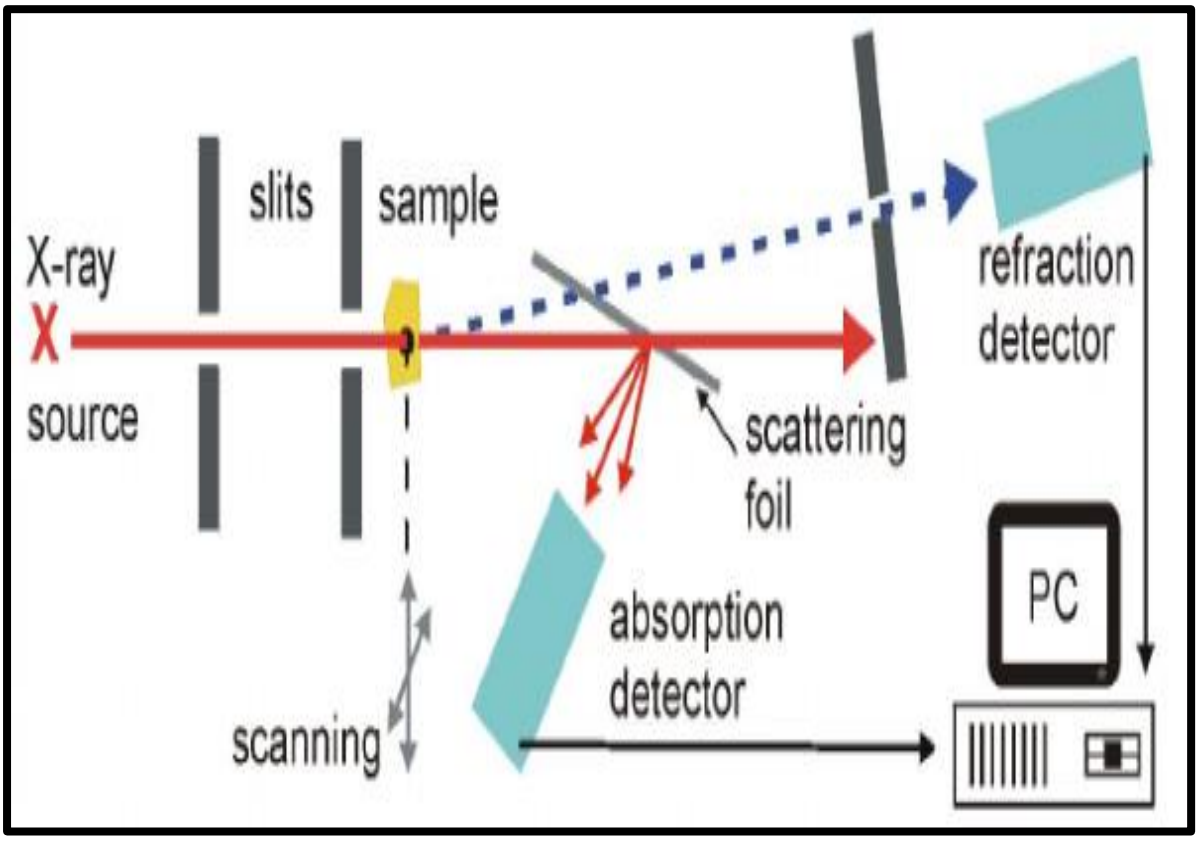


Fig. 3.2 Diagrammatic representation of XRD process.

3.2.6 Field Emission- Scanning electron microscopy (FE-SEM)

An effective tool for analyzing and examining surface topography, crystalline structure, microstructure morphology, and chemical composition characterizations is a scanning electron microscope [148] (Fig. 3.3). To grasp the fundamentals of electron microscopy, one must have a solid understanding of light optics. The visual angle at which the human eye can discern objects is approximately 1/60˚, or ~0.1mm. The enlargement of the viewing angle causes the

optical microscope's limit of microscopy to be around 2000 Å [149][150]. The capacity to capture signals from the interactions between the specimen and the electron beam is essential for the creation of SEM images. Elastic and inelastic interactions are the two basic categories into which these interactions may be generally separated. When the incident electron is deflected by the atomic nucleus of the sample or by outer shell electrons of comparable energy, elastic scattering occurs. Numerous directional shifts and collisions are characteristics of this kind of engagement. Back-dispersed electrons are those that are elastically scattered when the incoming electron is deflected by an angle greater than 90˚. Multiple interactions between the arriving electrons and the sample's atoms and electrons cause inelastic scattering, which results in primary beam electrons giving the atom a significant energy transfer.

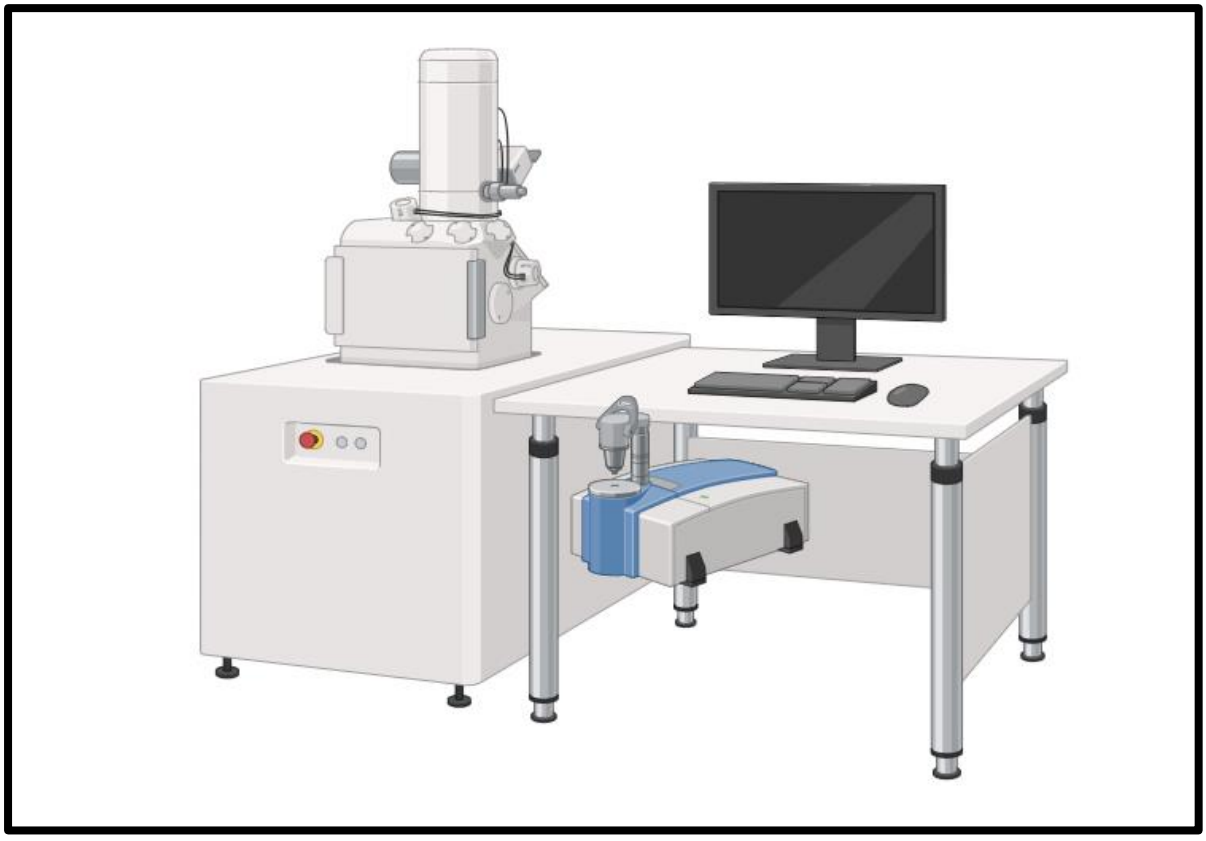


Fig. 3.3 Diagrammatic representation of FE-SEM machine

3.2.7 Energy Dispersive X-ray Spectroscopy (EDX)

EDX spectroscopy uses scanning electron microscopy to determine a sample's elemental makeup. Elements with an atomic number higher than boron can be identified by EDX, which can then detect concentrations of at least 0.1% [151][152]. Evaluation of material, analysis of

spot detection of regions up to 10 cm in diameter, identification, contamination, etc. are just a few of the many uses for EDX. X-rays are created when the sample interacts with the electron beam after colliding with it. Both elements may be detected and distinguished for the sample concentration according to the notion that their X-ray spectra are not identical [153][154]. When an atom's nucleus interacts with the main beam, the electron is ejected and X-rays are released. This process is known as X-ray emission. Characteristic and continuum X-rays are among the X-rays released.

3.2.8 Electrochemical Techniques

A subset of analytical techniques known as electrochemical techniques makes use of electrochemical principles to study and evaluate chemical processes, look into the characteristics of substances, and calculate concentrations. The advantages of electrochemical

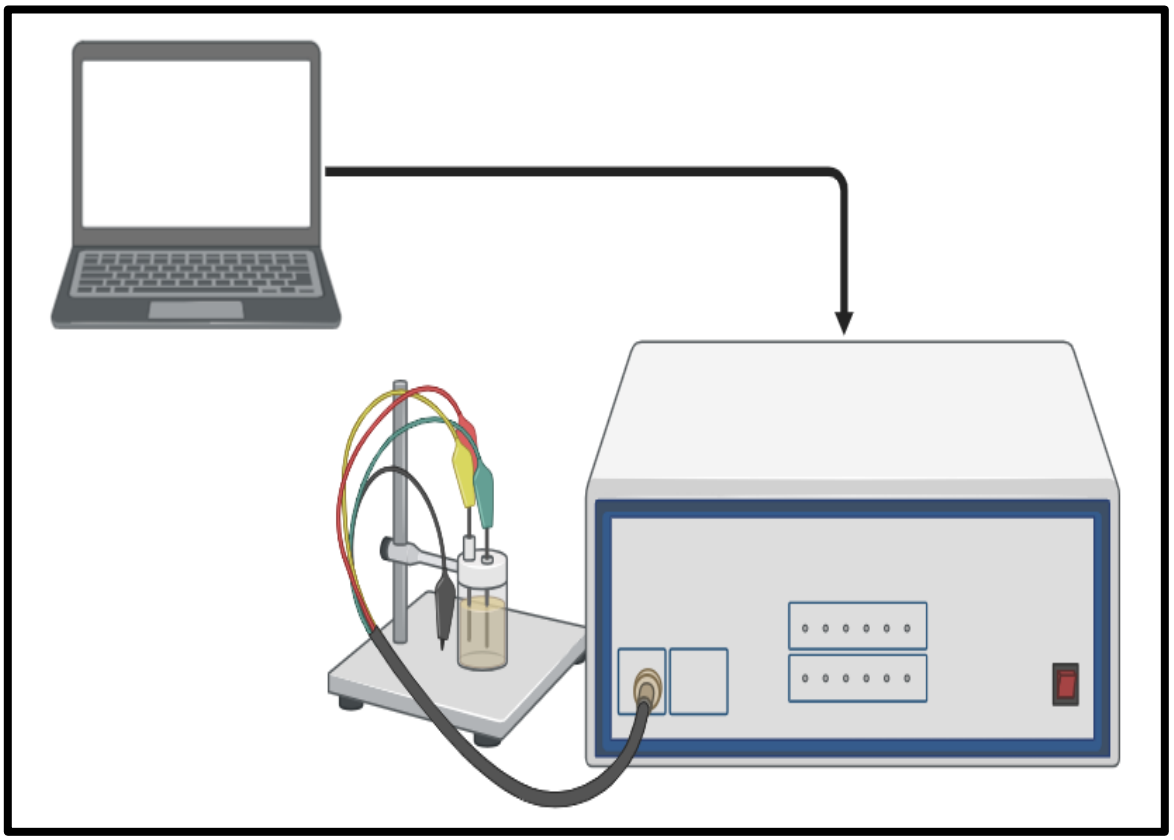


Fig. 3.4 Representation of potentiostat

methods outweigh those of surface modification technologies. These days, electrochemical techniques include chronoamperometry, electrochemical impendence spectroscopy (EIS), cyclic voltammetry, potentiometry, and measurements [155]. Three electrodes—the working electrode, reference electrode, and counter electrode—are found in an electrochemical cell when using electrochemical methods Furthermore, surface properties including crystal

composition and orientation are gradually preserved after electrochemical breakdown. These electrodes are now connected via a potentiostat, which keeps track of the corresponding current response. A working electrode displays the potential in this kind of electrochemical experiment, and the current value is shown against time. In this thesis, reference electrodes like Ag/AgCl and Platinum (Pt) are employed as counter electrodes, and an Autolab Galvanostat/Potentiostat (Ecochemie, Netherlands) is used to analyze the electrochemical response of all the created electrodes.

3.2.9 Cyclic Voltammetry (CV)

The most popular electro-analytical method for characterizing the electrochemical behavior of compounds that are electrochemically active is cyclic voltammetry. Diffusion is the only way that oxidizable species or reducible mass transfer may occur in CV. Therefore, 0.1M supporting or ground electrolyte is added to the solution to prevent current migration and guarantee conductivity. Alkali metals and tetraalkylammonium salts have been shown to be particularly useful in this method. In the CV approach, the x-axis shows the parameter applied to the system, while the axis shows the current that is passed as a result (i) and the x-axis shows the applied potential (E). There are two commonly used standards for reporting CV data that illustrate the sign convention for data acquisition and graphing [157]. to have a deeper comprehension of the electrochemical reaction between a certain reduction-oxidation potential inside the electroactive species. Ions are added to electrodes in an electrolytic solution during the reduction-oxidation process [158].

3.2.10 Differential pulse Voltammetry (DPV)

Apart from anodic stripping voltammetry, differential pulse voltammetry is the most accurate and widely used voltammetric technique. This technique was created to provide a significant difference between the charging and analytical currents at the end of the mercury drop's half-life. The technique is used for various solid electrodes, such as carbon or platinum, which have a larger anodic range (potential) than mercury electrodes, due to the growing need for trace analysis for vital oxidizable elements (such as vitamins, medications, and carcinogens) [159]. Additionally, a sequence of moderately amplituded pulses is superimposed on the voltage, and the current is shown within the ramping baseline voltage and pulse voltage. Before the pulse is applied (1st point) and after it ends (2nd point), current is measured. At those sites, the changes in the current measurement after every pulse are noted [160][16].

3.2.11 Electrochemical Impendence Spectroscopic (EIS)

A potent method for analyzing the mechanism of electrochemical reactions, determining the transport and dielectric properties of materials, examining the properties of porous electrodes, and analyzing passive surfaces is electrochemical impendence spectroscopy (EIS) [162]. It has been widely used for characterization of charge transport, elucidation of corrosion processes, battery optimization, and solution/membrane interfaces. It is suitable for the detection of binding events on the transducer surface when biosensors are used [163].

Alternating current (AC) voltage is applied to an electrochemical cell in order to monitor the current flow. AC current was produced with the use of a sinusoidal potential stimulation. To determine electrochemical impedance and get a pseudo-linear response inside the electrochemical cell, a brief excitation pulse is frequently used. The matching current has a frequency that is comparable to the sinusoidal waveform regardless of the phase shift. The current response in the linear system is sinusoidal. R_{ct} (charge transfer resistance), R_s (solution resistance), and C_{dl} (double layer capacitance) are used to characterize the impedance data. Z_w (Warburg element) also serves as an example of mass transport diffusion or the diffusion of the cell. Therefore, the Nyquist plot displays the electrochemical cell's equivalent circuit model EIS data, where imaginary impedance stands for the cell's capacitive and inductive properties.

$$\mathbf{i_0 = nRT/ R_{ct}F} \quad \text{.....(1)}$$

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

This thesis calculates Rct from the collected EIS spectra using the EIS technique. Equations (1) and (2) have been used to calculate the exchange current per geometric unit area (i) and the apparent electron transfer rate constant (Kapp) of the various electrodes based on the acquired values of Rct. where F is Faraday's constant and C is concentration. A is the geometrical area of the electrode, and n is the number of electrons. R is the gas constant, and T is the temperature.

CHAPTER 4- $Ti_3C_2T_x$ decorated Cerium dioxide-based Electrochemical sensor for Fenitrothion Detection

4.1 Introduction

This chapter describes a new and effective electrochemical sensor for fenitrothion detection that uses $Ti_3C_2T_x$ decorated CeO_2. The produced nanohybrid's synergistic impact greatly boosts the electroactive surface area, enhances catalytic activity, speeds up electron transport, and has exceptional biocompatibility. The manufactured sensor has a low detection limit (1pM), excellent sensitivity (0.728 µA pM^{-1}), and good selectivity. Furthermore, the biosensor was effectively used to detect trichlorfon in three distinct actual samples.

4.2 Experimental Section

4.2.1 Synthesis of 2D $Ti_3C_2T_x$ sheets

For synthesis of $Ti_3C_2T_x$, 3g of Sodium Tetrafluoroborate ($NaBF_4$) was dissolved in 35 mL hydrochloric acid (37.5% by wt.) and was stirred for thirty minutes for even dispersion. With continuous stirring, 1g of titanium aluminium carbide was added to the suspension very gradually. For 12 hours, the resulting suspension was autoclaved at 160 °C. After letting the finished combination cool, it was rinsed until the pH of the supernatant was 6-7. The obtained black mass, $Ti_3C_2T_x$ was vacuum dried overnight at 60 °C.

4.2.2 Synthesis of CeO_2 nanoparticles

In order to prepare CeO_2 nanoparticles, green approach was used by washing, drying and crushing the dried banana peels using water as solvent. 10g of dried banana powder was soaked in 75 mL of DI water for 24 hours. The obtained solution was centrifuged for 30 minutes at 5000 rpm. 40 mL of distilled water was added to 10 mL of obtained supernatant. After that, 2.17g of cerium nitrate hexahydrate was added to 50 mL of diluted filtrate under constant stirring for 15 minutes. Then, 4M sodium hydroxide was added gradually till pH 10 was obtained. Obtained solution was stirred at 60°C for 4 hours. Subsequently, the solution was centrifuged with DI water about 10 times for washing of sample. The yellowish mass was dried overnight in oven at 80°C, followed by calcination at 300°C with heating rate of 5°C/min.

4.2.3 Synthesis of $CeO_2/Ti_3C_2T_x$ nanocomposite

In order to prepare the nanocomposite, mixing by stirring method was adopted. 75 mg of $Ti_3C_2T_x$ and 25 mg of CeO_2 were taken respectively in 20 mL DIW each to create a uniform dispersion by sonication for 1 hour separately at room temperature. These solutions were then separately magnetically stirred at 500 rpm. These solutions were then mixed and stirred for 1 hour & washed twice with DI water followed by drying overnight at 60°C in vacuum oven.

4.2.4 Fabrication of cerium oxide-based sensor, $CeO_2/Ti_3C_2T_x$@ITO

Indium tin oxide (ITO) coated glass substrate is used to fabricate working electrode due to its particular characteristics including low capacitance current, substrate adherence, strong electrical conductivity, broad working window, superior optical transparency, and reliable physical and electrochemical characteristics[116]. 0.5 mg of prepared $CeO_2/Ti_3C_2T_x$ nanocomposite and $Ti_3C_2T_x$ were dissolved separately in 20 mL DI water and ultrasonicated for about 2 hours to prepare uniform colloidal solution. Then through electrophoretic deposition, a thin uniform film was deposited on ITO at 10V for 6 seconds. All steps for the fabrication of electrochemical sensor are shown in Fig. 4.1.

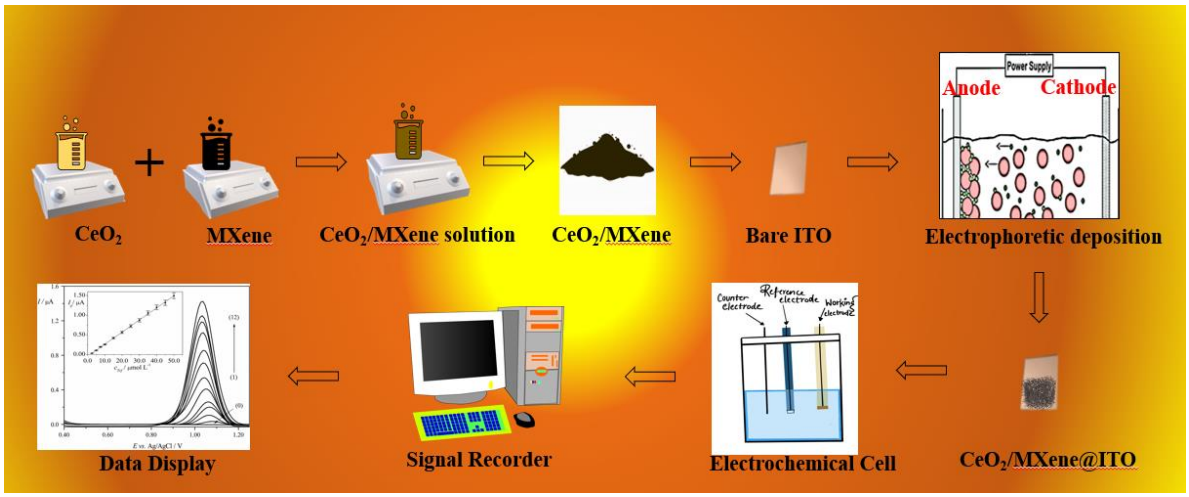


Fig. 4.1 Schematic representation of fabrication of working electrode.

4.3 Result and Discussion

4.3.1 Structural Studies

Fig. 4.2 (A) represents XRD patterns of CeO_2, $Ti_3C_2T_x$ and $CeO_2/Ti_3C_2T_x$ nanocomposite. The XRD pattern of CeO_2 **(curve a)** (JCPDS No # 34-0394) belongs to the face-centered cubic (FCC) system and no impurity peak was observed. Peaks appearing at 28.5°, 33°, 47.5°, 56.4°,

59.2°, 69.4°, 76.8°, 79.2°, 88.3° and 99.3° are corresponds to (111), (200), (220), (311), (222), (400), (331) and (422) crystal planes, respectively. The spectra of $Ti_3C_2T_x$ **(curve b)** shows peaks at 2θ = 5.88°, 17.92°, 24.89° and 60.43°. In the XRD pattern for $CeO_2/Ti_3C_2T_x$ nanocomposite, common peaks observed at 5.88°, 17.92°, 28.5°, 33°, 47.5° and 60.43° in curve c confirms the presence of CeO_2 nanoparticles in 2D-MXene matrix.

Fig. 4.2 (B) displays the FT-IR pattern for $Ti_3C_2T_x$ and $CeO_2/Ti_3C_2T_x$. FTIR data of $Ti_3C_2T_x$ (curve a) and $CeO_2/Ti_3C_2T_x$ (curve b) nanocomposite is displayed in a 400- 4000 cm^{-1} range spectrum, indicating chemical bonding and functional groups in the molecule. The wide band at 3458 cm^{-1} (curve b) and 3475 cm^{-1} (curve a) is attributed to the O–H stretching vibration. The absorption peak at 1607 cm^{-1} and 1396 cm^{-1} pertains to the stretching of C=O and O–H stretching in curve a, while 1408 cm^{-1} corresponds to the bending vibration of C-H bond in curve b. The absorption band at 468 cm^{-1} corresponds to the Ce–O stretching vibration.

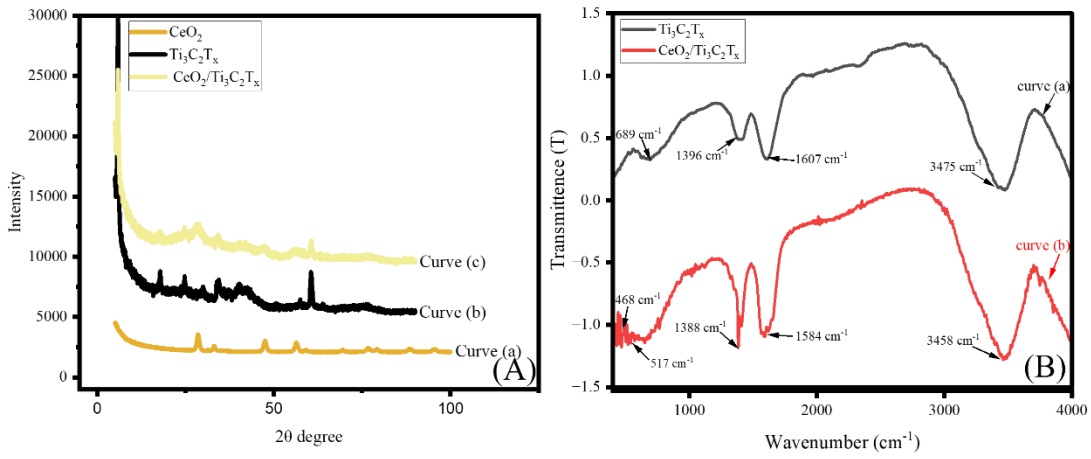
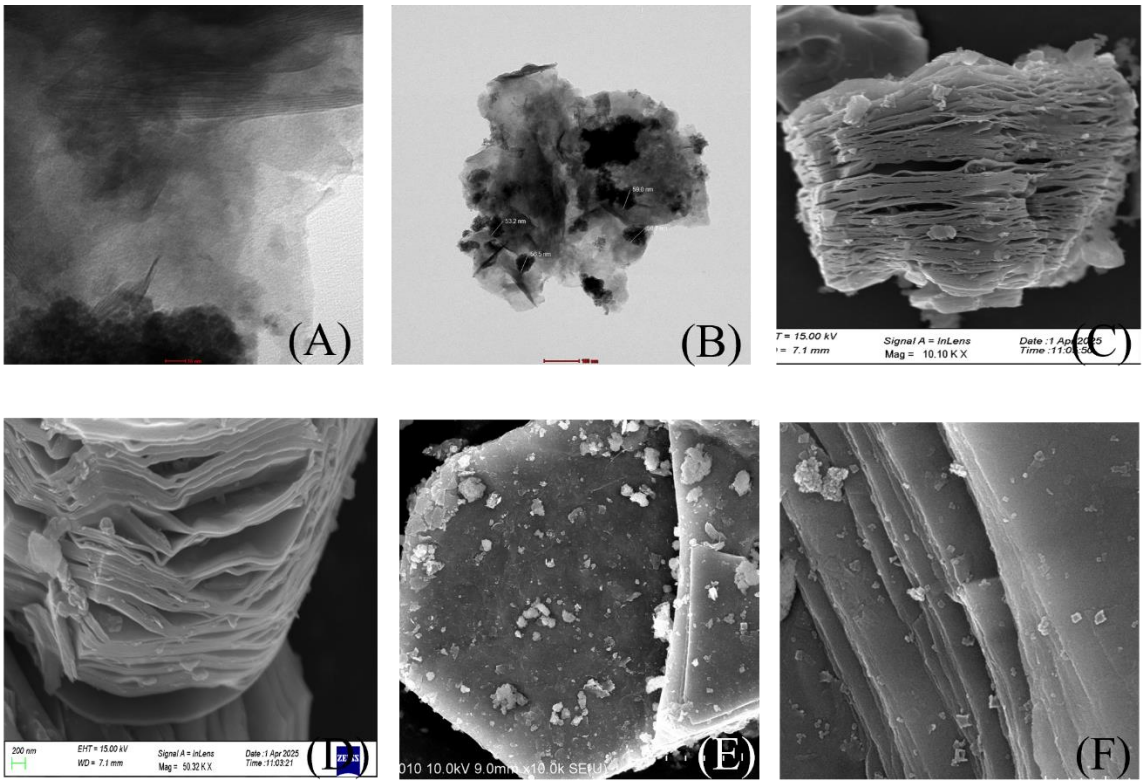


Fig. 4.2 (A) XRD pattern and (B) FT-IR pattern for $Ti_3C_2T_x$ and $CeO_2/Ti_3C_2T_x$

4.3.2 Morphological Studies

FE-SEM and TEM **(Fig. 4.3)** were used to analyse the morphological structure of all samples at different magnifications. Etching weakens the bond between Ti and Al, resulting in Al separation and increased inter-layer gaps in $Ti_3C_2T_x$. Titanium-carbon bonds remain stable due to their intrinsic strength. **Fig. 4.3(A)** illustrates the TEM images of compact layered structure of $Ti_3C_2T_x$ sheet. On the other hand, the incorporated CeO_2 exhibits spherical structure with an average diameter of around 52.5 nm, as shown in **Fig. 4.3(B)**. FESEM study of $Ti_3C_2T_x$ clearly

elucidates the formation of 2D-MXene sheets stacked over each other as shown in **Fig. 4.3(C)** and **4.3(D)**. The mesoporous nature of $CeO_2/Ti_3C_2T_x$ composite with void-structured morphologies is also visible in **Fig. 4.3(E)**. **Fig. 4.3(F)** shows considerable impregnation of CeO_2 nanoparticles into the layers of $Ti_3C_2T_x$. This porous property promotes quick electron transit in addition to offering additional active sites for electrocatalytic activity. The findings of the analysis of EDX spectra on $Ti_3C_2T_x$ and $CeO_2/Ti_3C_2T_x$ are displayed in **Fig. 4.3(G)** and **4.3(H)** without any extra contaminants, the EDX findings unequivocally demonstrate the existence of the predicted elements, such as oxygen (O) and cerium (Ce), Titanium (Ti), Carbon(C), Fluorine(F), Oxygen(O) in the following weight percentage – Titanium (3.97%), Fluorine (12.94%), Carbon (6.40%), Oxygen (8.18%) in $Ti_3C_2T_x$ and Titanium (4.35%), Fluorine (4.12%), Carbon (4.61%), Oxygen (9.57%), Cerium (2.26%) respectively.



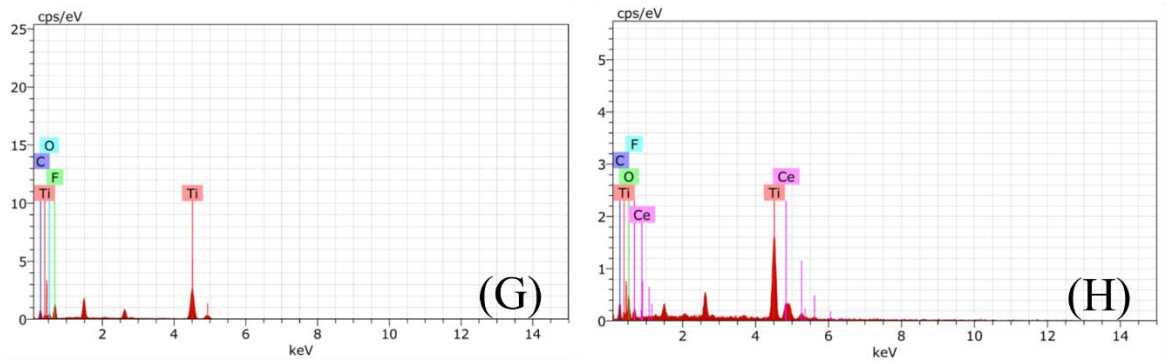


Fig. 4.3 TEM images of $CeO_2/Ti_3C_2T_x$ (A-B); FE-SEM images of $Ti_3C_2T_x$ (C-D) and $CeO_2/Ti_3C_2T_x$ (E-F); EDX Mapping of $Ti_3C_2T_x$ (G) and $CeO_2/Ti_3C_2T_x$ (H).

4.3.3 Electrochemical Studies

The electrochemical behaviour of the prepared electrodes was first examined by cyclic voltammetry (CV) in 0.1 M PBS solution with 10^{-7} hydrogen ion concentration and 5mM $[Fe(CN)_6]^{3-/4-}$ at 50 mV/sec scan rates (v). The $CeO_2/Ti_3C_2T_x$@ITO electrode displayed the maximum current when compared to the $Ti_3C_2T_x$@ITO electrode and bare ITO due to the increase in conductivity after incorporation of CeO_2 NPs in $Ti_3C_2T_x$ **{Fig. 4.4(A)}**.

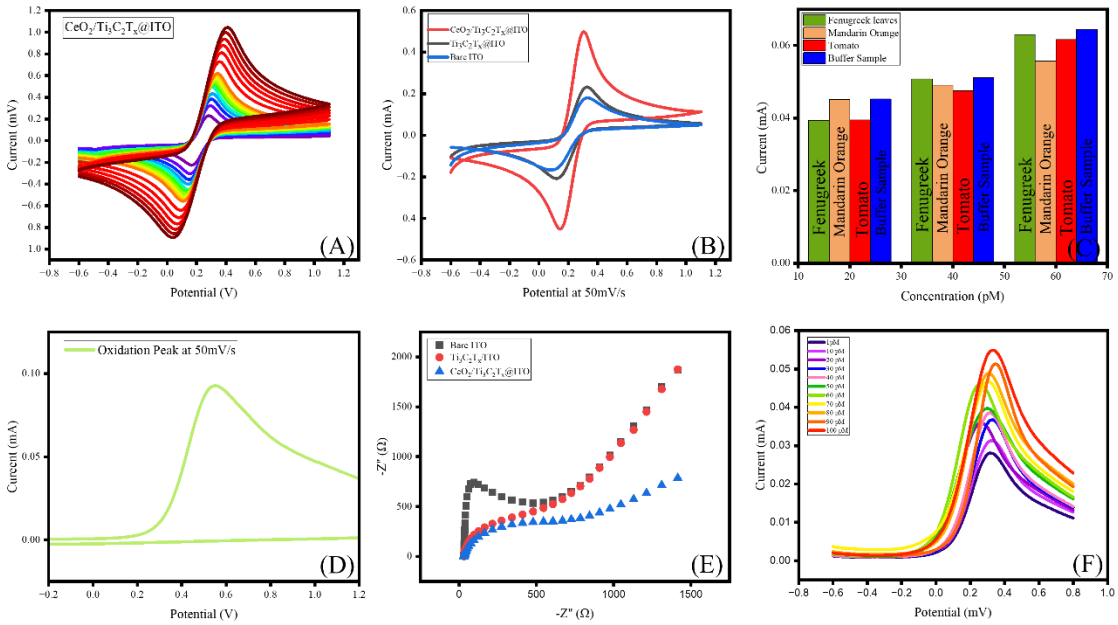


Fig. 4.4 (A) Scan rate of $CeO_2/Ti_3C_2T_x$@ITO from 10 - 400 mV/sec in 0.1 M PBS with 10^{-7} hydrogen ion concentration containing 5 mM $[Fe(CN)_6]^{3/4-}$; (B) Cyclic voltage diagrams of bare ITO, $Ti_3C_2T_x$@ITO, $CeO_2/Ti_3C_2T_x$@ITO; (C) Electrochemical response of spiked real-life samples compared to buffer signals at 1pM; (D) Cathodic peak current of $CeO_2/Ti_3C_2T_x$@ITO observed in 7.0 pH PBS buffer solution; (E) EIS plot for bare ITO, $Ti_3C_2T_x$@ITO and $CeO_2/Ti_3C_2T_x$@ITO; (F) DPV Sensing of $CeO_2/Ti_3C_2T_x$@ITO electrode against FNT (1 – 100 pM).

{**Fig. 4.4(B)**} displays comparative study of CeO_2, $Ti_3C_2T_x$ and $CeO_2/Ti_3C_2T_x$ electrode, respectively. Greater scan rates lead to larger current due to the enhancement of rate of electron transfer at the electrode surface. Higher scan speeds increase the dependence on electron

transfer kinetics by decreasing the amount of time available for the diffusion of the electroactive species to the electrode surface[117], [118]. Randles-Sevick Eq. 1 was used to measure the slopes of these equations and calculate the electrochemical kinetics parameters, such as the diffusion coefficient (D) and effective surface area (A), as follows:

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

where C = $[Fe(CN)_6]^{3-/4-}$ concentration (mole/cm3), ν = scan rate (V/s), Ip = peak current, and n = number of electrons oxidized or reduced. Table 1 displays the D and A values for various electrodes.

$$I_{pa}\,[CeO_2/Ti_3C_2T_x@ITO] = 4.694\mu A(s/mV)^{1/2} v^{1/2} + 0.135\mu A;\ R^2 = 0.9915 \quad \ldots(2)$$

$$I_{pc}\,[CeO_2/Ti_3C_2T_x@ITO] = -3.86\mu A(s/mV)^{1/2} v^{1/2} - 0.152\mu A;\ R^2 = 0.9892 \quad \ldots(3)$$

$$I_{pa}\,[Ti_3C_2T_x@ITO] = 0.237\mu A(s/mV)^{1/2} v^{1/2} + 5.77\mu A;\ R^2 = 0.9931 \quad \ldots(4)$$

$$I_{pc}\,[Ti_3C_2T_x@ITO] = -0.192\mu A(s/mV)^{1/2} v^{1/2} - 6.85\mu A;\ R^2 = 0.99151 \quad \ldots(5)$$

Fig. 4.4(D) displays the cathodic peak current of fabricated working electrode $CeO_2/Ti_3C_2T_x$@ITO no reduction peak in PBS buffer solution. The Electrochemical impedance spectroscopy (EIS) results for Bare ITO, $Ti_3C_2T_x$@ITO and $CeO_2/Ti_3C_2T_x$@ITO with frequencies ranging from 100 Hz to 100 kHz and potential amplitude of 5 mV are presented in **Fig.4.4(E)**. The data indicate that the $CeO_2/Ti_3C_2T_x$ modified electrode exhibits the smallest semicircle in the Nyquist plot, signifying the fastest electron transfer rate and the lowest charge transfer resistance.

4.3.4 Electrochemical Sensing Studies

Differential pulse voltammetry (DPV) is a technique used to record the current just before each potential shift, and the difference in current observed is plotted against the potential. By measuring the current right before the potential changes, the impact of the changing current is minimized. The performance of fabricated electrode was examined via DPV technique with different concentrations of FNT in 0.1 M phosphate buffer solution (PBS) at pH 7.0. Differential pulse voltammetry (DPV) with a potential window from -1.1 to -0.1 V vs Ag/AgCl was employed. The differential pulse voltammograms taken at successive concentrations FNT in a PBS (pH 7.0) electrolyte solution are presented for $CeO_2/Ti_3C_2T_x$@ITO electrode in the

given **Fig. 4.4(F)**, The current increases as the concentration of FNT added is increased from 1pM to 100pM due to enhancement of more reactive sites to be occupied on modified material. The findings show that throughout a wide concentration range of 1 to 100pM, the nanocomposite demonstrated exceptional sensing capability. The peak current increased linearly as the FNT concentration rise, suggesting a close relationship between the analyte concentration and the nanocomposite electrode's electrochemical response. The sensitivity and LOD (3σ/sensitivity) value for the $CeO_2/Ti_3C_2T_x$@ITO electrode were determined to be 0.728μA pM^{-1} cm^2 and 1pM, respectively.

4.4.5 Reproducibility and Stability Studies

Fig. 4.5(A) illustrates the reproducibility study of 40pM and 60pM concentrations respectively. In order to get the reproducibility and repeatability of the working electrode, three times DPV was performed on different electrodes providing the concentration of the pesticide being introduced was kept same each time, this was performed for 40pM and 60pM concentrations respectively. The RSD% value was calculated to be 2.53% and 2.15% for 40pM and 60pM respectively. The results showed high stability for $CeO_2/Ti_3C_2T_x$@ITO electrode as percentage of electrode response after five weeks decreased by 5.62% as provided in **Fig. 4.5(C)**.

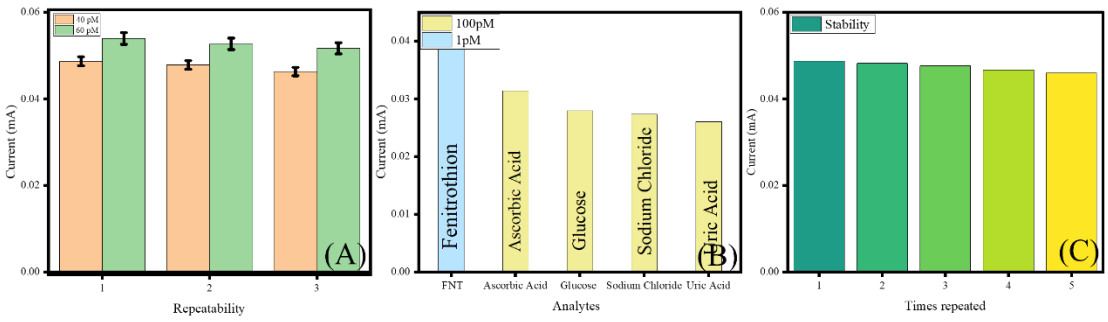


Fig. 4.5 (A) Repeatability investigation of current sensing in triplicate at various electrodes for 40pM and 60pM; (B) Analyte interference research at 100pM; (C) Electrodes' stability for 60pM over a month.

4.3.6 Interference study and Real sample Studies

Ascorbic acid, uric acid, glucose, and sodium chloride were among the several analytes that we tested our functioning electrode against in this investigation. The objective is to examine the operating non-enzymatic electrode's selectivity against analyte concentrations of 100 times

and assess the electrode's reaction to these analytes. As can be seen in **Fig. 4.5(B)**, the investigation demonstrated that even at such high concentrations (100pM) of interfering analyte, the detected current was lower than the current at the lowest concentration (1pM). Thus, the non-enzymatic electrochemical sensor's selectivity was demonstrated. Testing of the $CeO_2/Ti_3C_2T_x$@ITO electrode under actual sample circumstances is shown in **Fig. 4.4(C)**. Fenugreek, tomato, and Mandarin Hybrid Orange were the actual samples that were collected. These were gathered at a Rohini, New Delhi, neighbourhood market store. The samples were cleaned with deionized water to get rid of dust and other debris. Following cleaning, the samples were crushed in a mortar and pestle with 10 milliliters of 0.1 M PBS buffer. They were then centrifuged for five minutes to produce a homogenized solution, and they were then let to filter normally through filter paper. As predicted by data in **Table 4.1**, due to matrix effect of tomato, slight decrease in recovery of sample is seen when compared to fenugreek (methi leaves) and Mandarin orange (kinnu).

Table 4.1: Detection of Fenitrothion pesticide in real life samples using CeO_2-$Ti_3C_2T_x$@ITO.

S. No	Real Sample	Concentration of pesticide added (pg/ml)	Concentration of pesticide found (pg/ml)	Recovery (%)	RSD% Value
1.	Fenugreek	20	19.512	97.56	4.68
2.		40	39.684	99.21	0.56
3.		60	58.62	97.70	1.64
4.	Tomato	20	19.242	96.21	0.20
5.		40	37.86	92.86	2.94
6.		60	57.402	95.67	6.89
7.	Mandarin Orange	20	19.938	99.69	3.95
8.		40	38.364	95.91	5.23
9.		60	58.314	97.19	3.13

CHAPTER 5- CONCLUSION

In conclusion, we effectively synthesized cerium oxide nanoparticles using a straightforward, environmentally friendly, and effective chemical method. A modified working electrode for the electrochemical detection of FNT in various fruit and vegetable samples as well as via electrochemical methods was made using the resultant complex in conjunction with $Ti_3C_2T_x$. Curiously, the manufactured sensor worked better than the unaltered ITO and $Ti_3C_2T_x$@ITO under the identical circumstances, showing distinct redox peaks. Because cerium oxide's extensive fluorite crystal structure and the oxygen vacancies it contains allow it to interact with $Ti_3C_2T_x$'s two-dimensional sheets through π-π stacking, these materials are ideal for improving the adsorption and immobilization of FNT. By using these π-π covalent interactions, van der Waals forces, and H-bonding to adsorb molecules onto the $Ti_3C_2T_x$ surface, this technique significantly improves the stability and functionality of the resulting fenitrothion detecting sensor.