ELECTROCHEMICAL DETECTION OF CAFFEIC ACID IN GREEN TEA

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE

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Submitted by

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(2K21/MSCCHE/16)

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CANDIDATE'S DECLARATION

I, **Ekta**, Roll No. 2K21/MSCCHE/16 student of M.Sc (Chemistry), hereby declare that the Dissertation titled "**Electrochemical Detection of Caffeic Acid in Green Tea**" which is submitted by me to the Department of Applied Chemistry, Delhi Technological University, in partial fulfillment of the requirement for the award of the degree of Master of Science, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

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CERTIFICATE

I hereby certify that the Dissertation titled "**Electrochemical Detection of Caffeic Acid in Green Tea**" which is submitted by **Ekta**, Roll No. 2K21/MSCCHE/16, Department of Applied Chemistry, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full time for any Degree or Diploma to this University or elsewhere.

Place: Delhi **(Prof. D. Kumar)**

Date: 29/05/2023

ABSTRACT

One of the representatives of phenolic acids with potential carcinogenic properties is caffeic acid (CA), which is frequently found in red wines, coffee, olive oil, and some fresh fruits and vegetables. Understanding that the qualitative and quantitative study of CA in our everyday diet is of great significance. For the purpose of detecting caffeic acid, an enzymatic electrochemical biosensor was designed. In this study, we synthesized a nanocomposite comprising graphitic carbon nitride and copper sulphide for the electrochemical detection of caffeic acid. Thermogravimetric analysis, energy dispersive X-ray spectroscopy, X-ray diffraction, FTIR spectroscopy, and field emission scanning electron microscopy were used to systematically characterize and confirm the morphology, composition, and structure of the resulting nanocomposites. As expected, in comparison with the bare electrode the nanocomposite-modified electrode showed significantly higher charge transfer efficiency and electro-catalytic activity to the oxidation of caffeic acid (CA). The electrochemical and analytical techniques used for the oxidation of caffeic acid were cyclic voltammetry and chronoamperometry.

The study revealed that under optimized conditions, the nanocomposite-modified ITO electrode showed a broad linear range of 1–100 μM with 0.37 μM as the lowest limit of detection for CA detection. Moreover, results indicated that the composite-modified electrode offered good sensitivity, superior reproducibility, selectivity, long-term stability, and excellent anti-interference capabilities, and it is ready for use in the real sample analysis. The fabricated enzymatic biosensor seems like a good real sample candidate for quality control analysis. Since, it effectively demonstrated its capacity to directly identify CA in commercially accessible coffee products, wine, fruit, etc. without any pretreatment. These outcomes give insightful information about the development of novel modified electrodes based on two-dimensional metal sulfide nanocomposites for high-performance electrochemical biosensor.

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LIST OF SYMBOLS, ABBREVIATIONS

CuS	Copper Sulphide
g-C_3N_4	Graphitic Carbon Nitride
CV	Cyclic Voltammetry
TGA	Thermogravimetric Analysis
XRD	X-ray Diffraction
FT-IR	Fourier Transform Infrared
EDC	N-ethyl-N'-(3-dimethyl aminopropyl) carbodiimide
NHS	N-hydroxysuccinimide
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 Microscopy
LAC	Laccase

CHAPTER 1
Introduction

1.1 Introduction of biosensor

A biosensor is a device that connects a biological component with a physicochemical detector to detect and analyze a particular biological or chemical substance. It is formed to convert a biological response into a quantifiable signal, allowing for the detection, quantification, and monitoring of various biological and biochemical processes. The biological component of a biosensor can be an enzyme, antibody, microorganism, cell, or tissue, depending on the target analyte. This biological component interacts selectively with the analyte or target molecule, initiating a specific biological reaction. The physicochemical detector then translates this biological response into a measurable signal, such as an electrical, optical, or electrochemical signal. The operation of a biosensor typically involves three main steps: recognition, transduction, and signal processing. During the recognition step, the biological component interacts with the target molecule, leading to a specific binding or reaction. In the transduction step, the response of a biological reaction is converted into a detectable signal by the physicochemical detector. Finally, in the signal processing step, the obtained signal is measured, processed, and analyzed to provide quantitative or qualitative information about the target analyte [1-6].

Biosensor have a broad range of applications in various fields which includes healthcare, environmental monitoring, food safety, and bioprocessing. They offer numerous advantages such as high sensitivity, specificity, portability, fast response, and real-time monitoring capabilities. Biosensor have been used for detecting biomarkers in medical diagnostics and monitoring glucose levels in diabetes management, detecting pathogens in food and water, and analyzing environmental pollutants, among many other applications. Advancements in nanotechnology, microfabrication techniques, and biotechnology have led to the development of miniaturized, highly sensitive, and versatile biosensor. These advancements have expanded the possibilities for point-of-care testing, personalized medicine, and remote monitoring of health conditions. It can be concluded that biosensor are powerful analytical devices that harness the specificity and sensitivity of biological components to detect and analyze a wide range of biological and chemical

substances. Their applications continue to grow, offering promising solutions in various fields for improving healthcare, ensuring food safety, and monitoring the environment [7-10].

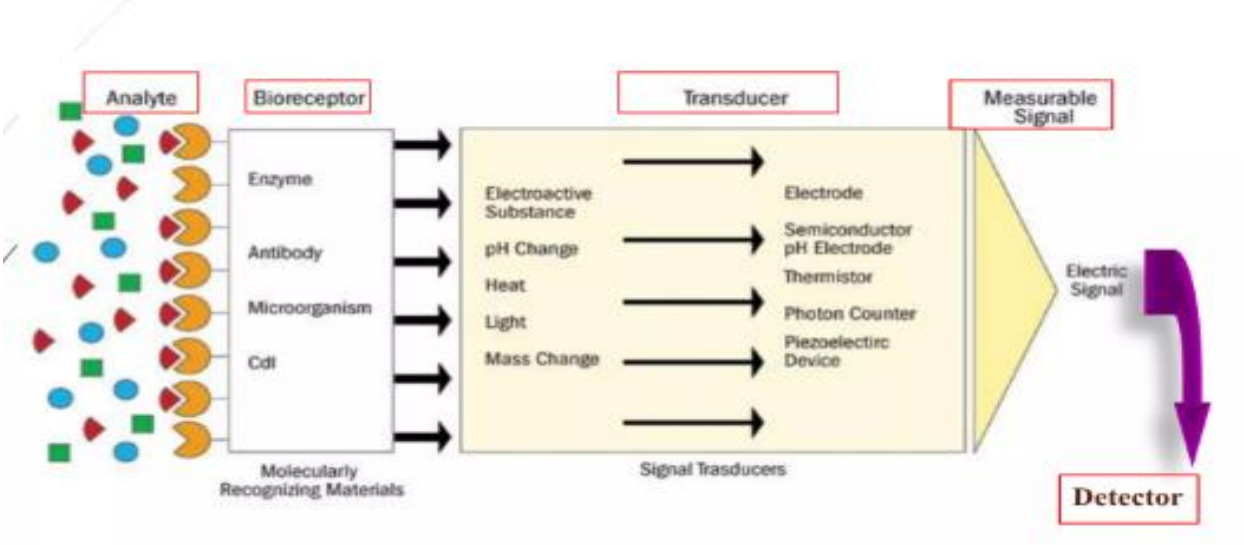


Figure 1.1 Components of biosensor

1.2 Components of biosensor

Mainly there are three components that have been recognized to constitute a biosensor. The three components are biomolecular recognition elements, immobilization of a biomolecule, and a transducer.

- **Biomolecular recognition element**

The biomolecule or molecular assemblies that are capable of recognizing the material are used in a biosensor to recognize a target chemical. This component could be a biomolecule or a molecular assembly, like an enzyme, an antibody, a slice of the membrane, or a whole cell. An appropriate environment is necessary for these biomolecules to keep their structure and recognition abilities. They produce a biochemical signal that is recognized by a transducer when they contact with a target material. within the biosensor [11-13]. Enzymes have traditionally been the most commonly used biomolecules in biosensors, but antibodies and protein receptor molecules are now increasingly being used. The specificity of a biosensor relies on the specific binding properties of the biomolecule employed. For instance, although antibodies contain Y-shaped molecules with

two identical antigen binding sites, enzymes have a specific three-dimensional structure that only fits a specific substrate. Antibodies bind to antigens with specificity, but unlike Enzymes, they don't function as catalysts. Their function is to bind and eliminate foreign materials from the body. Similar to this, receptor proteins have a distinct affinity for many biologically active substances, including hormones, antibodies, enzymes, and receptor proteins. These proteins are often present in membranes and are in charge of controlling how membrane channels open and close in order to enable the passage of particular metabolites. [14-15].

- **Immobilization of biomolecules**

Immobilization is the process of fixing biomolecules onto a solid support to enhance their stability and prolong their activity. This process is known as immobilization, and it involves attaching the biomolecules to a matrix, which can be made of various materials such as inorganic substances (e.g., glass), organic materials (e.g., films of polymer), self-assembled monolayers, Langmuir Blodgett films, nanomaterials, or screen-printed electrodes. This can be done for a variety of reasons, such as to improve stability, reusability, or selectivity. There are many different methods of immobilization, each with its own advantages and disadvantages [16,18,19]. The choice of the matrix depends on the mechanical and chemical properties required for the specific application and the biosensor's stability of operation. The matrix's composition and qualities are crucial to the immobilization process. The ideal pH for biomolecule activity, for instance, can be influenced by the charged microenvironment on the matrix surface, potentially expanding the pH range in which the enzyme can function. Immobilization restricts the movement of biomolecules, keeping them in a defined region of space, which enhances their stability and reusability. Physical entrapment, physical adsorption, covalent binding, and cross-linking with multifunctional reagents are only a few of the strategies employed for immobilization. The approach chosen will depend on the intended result as well as how well the active site and reactive groups of the enzyme will be preserved. Enzymes and other biological materials being combined with microelectronics have become a promising field in biotechnology. Immobilized enzymes are used as biocatalysts in extensive biotransformation and as biosensor, which accelerates industrial processes and makes it possible to use sensitive detection systems. It is essential to choose an attachment strategy that maintains the enzyme's activity by preventing alterations to its chemical makeup or the presence

of reactive groups in order to ensure efficient immobilization. To accomplish this goal, an adequate understanding of the enzyme's active site is necessary [17,20].

- **Transducer**

A transducer is a device that changes a biological signal into an electrical signal. A biochemical reaction between a biological component and an analyte produces the biological signal. The transducer then passes the electronic signal to a detector system, which can be used to measure the concentration of the analyte [21]. In essence, the transducer performs the function of a translator, converting the biological signal into another signal that the processor can understand. The biochemical transducer, or bio-component, imparts selectivity or specificity to the biosensor [22]. It is responsible for recognizing and interacting with the target analyte. The transducer, on the other hand, responds to this interaction in a manner that allows the signal to be electronically amplified and displayed. The type of transducer employed depends on the nature of the signal received and it includes four types of transducers: electrochemical, optical, thermal, and piezoelectric [23,24]. Alterations in current or voltage are measured by electrochemical transducers. Optical transducers track variations in reflectance, absorbance, or fluorescence. Thermal transducers track temperature variations. Piezoelectric transducers track frequency variations. and acoustic transducers track frequency variations brought on by tiny changes in mass attached to their surface. [25].

Table 1.1: Principles of different types of biosensor

Type of transducer	**Principle**
Amperometric	A change in current is measured at a constant voltage when electrons are transferred between a biological system and an electrode.
Conductive	Measures change in conductivity between two electrodes
Optical	Change in concentration or mass to direct changes in the characteristics of light via fluorescent or luminescence.
Piezoelectric	Changes in mass during reaction
Potentiometric	Changes in potential at constant current
Thermal	Changes in temperature during the reaction

1.3 Methods of immobilization

(i) Physical adsorption

Immobilizing biomolecules on a matrix's surface through physical adsorption. One of the earliest and simplest techniques, it hardly alters the structure of the enzyme or destroys its active center. However, the matrix could allow the adsorbed enzyme to escape, due to the weak force of interaction between the matrix and the biomolecule during use. A major advantage of physical adsorption is that it is a simple and inexpensive method that requires no reagents or activation steps. The weak binding forces are involved in physical adsorption are also advantageous, as they allow the biomolecule to be easily released from the matrix when desired. However, the weak binding forces also make physical adsorption a less stable method of immobilization than other methods. As a result of variations in temperature, pH, ionic strength, or even the presence of substrate, the adsorbed enzyme may desorb from the matrix. In general, physical adsorption is a simple and affordable technique for immobilizing biomolecules that is appropriate for a wide range of applications. However, its stability is limited by the weak binding forces involved [26-28].

Here are some additional details about physical adsorption:

- The weak binding forces involved in physical adsorption are typically van der Waals forces, hydrogen bonds, and electrostatic interactions.
- The rate of physical adsorption is typically controlled by the diffusion of the biomolecule to the surface of the matrix.
- The extent of physical adsorption is typically limited by the availability of surface sites on the matrix.
- Physical adsorption is reversible, and the biomolecule can be easily released from the matrix by changing the conditions, such as by increasing the temperature or pH

(ii) Covalent binding

Covalent binding is a method of immobilizing biomolecules to a support matrix by forming covalent bonds between the two. This is the most widely used immobilization technique, as it is applicable to a wide range of biomolecules and support matrices. The most common functional groups used for covalent binding are amino, carboxylic, sulfhydryl, imidazole, thiol, hydroxyl,

phenolic, threonine, and indole. Covalent binding can be more complicated and harsh than physical adsorption, as it requires specific conditions to form the covalent bonds. This can lead to changes in the conformation and activity of the biomolecule. However, the binding force between the biomolecule and the support matrix is very strong, which prevents leakage of the biomolecule, even in the presence of any substrate or solution of high ionic strength [29-33].

Here are some of the advantages of covalent binding:

- It is a versatile method that can be used to immobilize a wide range of biomolecules.
- The bonds are very strong, which prevents leakage of the biomolecule.
- The biomolecule can be reused multiple times.

Here are some of the disadvantages of covalent binding:

- It can be more complicated and severe than physical adsorption.
- The bonds can alter the conformation and activity of the biomolecule.

Overall, covalent binding is a powerful immobilization technique that offers a number of advantages. However, it is necessary to be aware of the potential disadvantages before using this technique.

(iii) Cross-linking

The method of cross-linking involves attaching biomolecules to a stable support matrix. Compounds with several functional groups that can bind various materials under various circumstances can be used to accomplish this by utilizing multifunctional or bifunctional reagents, such as glutaraldehyde, 1,5-difluoro-2,4-dinitrobenzene, 4-azido-1-fluoro-2-nitrobenzene, and bis-diazobenzidine-2,2'-disulphonic acid, intermolecular cross-linking of biomolecules has been achieved. There is little to no desorption because of the covalent connection between the biomolecule and the support matrix. The most widely used reagent for cross-linking is glutaraldehyde. Cross-linking can be used to improve the properties of biomolecules, such as their stability, solubility, and reactivity. Researchers are actively seeking new cross-linkers that can bind different biomolecules under mild conditions, combining strong binding to prevent leaching and loss of activity during the binding process [34-36].

(iv) Entrapment

The entrapment method of immobilizing enzymes involves confining them within a matrix or membrane. This process allows the desired biomolecules to be retained within the matrix while still enabling the penetration of the substrate. One way to achieve this is by preparing a polymeric gel in a solution containing biomolecules, which results in their entrapment within the gel matrix (e.g., polyacrylamide, sol-gel films). However, this technique has a drawback in the form of a significant diffusion barrier that hinders the transport of substrate and product, thereby increasing the response time compared to other methods. Unlike covalent or cross-linking techniques, the biomolecules themselves do not bind to the matrix or membrane during entrapment. The ions in the matrix and the immobilized biomolecules occasionally interchange during entrapment or physical binding, resulting in the biomolecules' ionic interaction. Ionic binding often necessitates softer circumstances than covalent binding, which causes the biomolecule's shape and active site to change very little.

As a result, immobilized enzymes produced using this approach frequently have higher activity. The attachment of biomolecules to the matrix is stronger with ionic binding than with physical adsorption, however, it is still weaker than with covalent binding. However, in solutions with high ionic strength or when there are variations in pH, there may be a risk of biomolecule leakage from the matrix [37-42].

There are several advantages to using the entrapment method of enzyme immobilization. First, it is a relatively simple and inexpensive method. It does not require any covalent modification of the enzyme, which can sometimes damage the enzyme, also the entrapment method can be used to immobilize a wide variety of enzymes.

However, there are also some disadvantages to the entrapment method. First, the diffusion of the substrate to the enzyme can be slow, which can reduce the rate of the reaction. Second, the enzyme can sometimes leak out of the matrix or membrane which can reduce the efficiency of the immobilized enzyme. Overall, the entrapment method is a versatile and effective method of enzyme immobilization. It is a good choice for applications where simple, inexpensive, and non-covalent immobilization is required [43-46].

Here are some additional details about the entrapment method:

- The matrix or membrane can be made from a wide range of materials, including polymers, hydrogels, and silica.
- The size of the pores in the matrix or membrane can be controlled to allow the desired substrate to diffuse to the enzyme, while preventing the enzyme from diffusing out.
- The entrapment method can be used to immobilize a wide variety of enzymes, including hydrolases, oxidases, and transferases.
- The entrapment method is a versatile and effective method of enzyme immobilization. It is a good choice for applications where simple, inexpensive, and non-covalent immobilization is required.

Table 1.2: Advantages and disadvantages of various methods of immobilization

Method	Advantages	Disadvantages
Physical adsorption	No modification of biocatalyst. Low cost and re-generable matrix.	Binding forces are susceptible to changes in pH, temperature, and ionic strength.
Entrapment	Only physical confinement of biocatalyst near transducer.	The diffusion barrier is high.
Cross-linking	Minimum loss of biocatalyst	Harsh treatment of biocatalysts by toxic chemicals.
Covalent binding	Stable under adverse conditions	Matrix not re-generable

1.4 Configuration of biosensor

In general, biosensor have been categorized into a number of groups based on the bio-recognition component and the techniques of detection i.e. transducers.

1.4.1 Biosensor based on the biological recognition element

(i) Bio-affinity sensors

A receptor that is weakly attached to a determinant analog that is immobilized on a transducer surface is used in bio-affinity sensors. Bio-affinity sensors are devices that use the specific binding interactions between biomolecules to detect the presence of target analytes. These sensors are frequently employed in applications for environmental monitoring, medical diagnosis, and food safety. The most common type of bio-affinity sensor is the antibody-based sensor [47]. Antibodies

are proteins that can bind to specific antigens with high affinity and selectivity. In a bio-affinity sensor, an antibody is immobilized on a transducer surface. When the target analyte is introduced into the sensor, it binds to the antibody, which produces a measurable signal. These biosensors are commonly used for environmental applications and rely on antibodies due to their availability and ability to target various environmental pollutants [48-49]. An example of such biosensors is the avidin-biotin system combined with an oxygen electrode. Other examples include hormones, drug receptors (drugs and drug metabolites), lectins (saccharides), and antibodies (antigens). Affinity sensors take advantage of physicochemical changes, such as variations in layer thickness, refractive index, light absorption, or electrical charge, that take place during the formation of complexes. After a measurement is taken, the initial state is restored by separating the complex. However, these sensors are not suitable for continuous monitoring or tracking decreasing concentrations of analytes. Ramanathan et al. demonstrated a simple and biomolecular-friendly method to create a polypyrrole nanowire biosensor with controlled dimensions, composition, aspect ratio, and precise positioning. This biosensor enables label-free bio-affinity sensing. Pei et al. introduced a quartz crystal microbalance bio-affinity sensor for the rapid identification of glycosyl-disulfide lectin inhibitors from a dynamic combinatorial library. They investigated carbohydrate-lectin interactions using a pool of thiol components and a quartz crystal microbalance setup. Their findings highlighted the most active inhibitors as dimers based on 1-thio- and 6-thio-mannose analogues, with the 6-thio-mannose exhibiting unique characteristics compared to its oxygen-containing counterpart [50-53]. Other types of bio-affinity sensors include sensors that use enzymes, nucleic acids, and other biomolecules. These sensors offer a variety of advantages over antibody-based sensors, including increased sensitivity, selectivity, and stability. Recent studies have presented various approaches for the development of bio-affinity sensors.

(ii) Immunosensors

An immunosensor is a device that uses the specific binding of antibodies to antigens to detect and quantify the presence of an analyte. There are many different types of immunosensors, but they all work on the same basic principle. The analyte of interest is first brought into contact with a surface that has been immobilized with antibodies specific to that analyte. The antibodies bind to the analyte, forming an immune complex. This immune complex is then detected by a transducer, which converts the binding event into a measurable signal. Immunosensors are created

by combining biosensors with enzyme-linked immunosorbent assays (ELISA). ELISA is a method that detects and amplifies the reaction between antigens and antibodies. The amount of free and conjugated antigens, as well as the rate of an enzymatic reaction, define the amount of enzyme-linked antigen attached to an immobilized antibody. To enhance the sensitivity of these assays, enzyme-catalyzed reactions that produce highly colored, fluorescent, or bioluminescent products can be utilized. Researchers have developed a chemiluminescent immunosensor for chloramphenicol, which involves the competition between chloramphenicol as an analyte and chloramphenicol-horseradish peroxidase conjugate as a tracer for binding to an anti-chloramphenicol antibody on a solid support. This sensor provides a response that correlates with the concentration of the analyte and follows a linear relationship when plotted on a semi-logarithmic scale. The limit of detection for chloramphenicol is 10^{-8}M. Immunosensors offer a number of advantages over traditional immunoassays as they are typically faster, more sensitive, and more specific than immunoassays. Immunosensors are also more portable and easier to use, making them ideal for point-of-care testing. However, immunosensors also have some disadvantages. Immunosensors can be expensive to develop and manufacture. Immunosensors can also be susceptible to interference from other substances in the sample [54]. Despite their disadvantages, immunosensors are a promising technology with a wide range of potential applications. Immunosensors are already being used in a variety of clinical, environmental, and food safety applications. As the technology continues to develop, immunosensors are likely to become even more widely used in the future.

(iii) Whole-cell biosensor

Biosensor can be fabricated using whole cells from various biological organisms. Whole-cell biosensor are analytical devices that make use of living cells to detect and respond to specific molecules or environmental conditions. The cells are typically genetically engineered to express a reporter gene that is activated in the presence of the analyte. The reporter gene can encode for a protein that produces a fluorescent signal, or an enzyme that catalyzes a reaction that produces a measurable change in pH or electrical potential. These biosensors take advantage of the natural biological capabilities of cells, such as their ability to recognize and interact with specific compounds or produce a measurable response [55-57]. These cells contain a wide range of chemicals such as enzymes, nucleic acids, proteins, and small organic molecules. However, one

of the main challenges in using whole cells for biosensor is achieving selective detection of specific biochemical events. It is crucial to maintain the stability of these biological cells for successful biosensor applications. Isolating and preserving cells from higher organisms is difficult and requires sustained viability, which is often challenging in sensor development [58-59]. On the other hand, cells from less complicated organisms like yeast, bacteria, and algae are more robust and can be easily stabilized in controlled environments. Incorporating these organisms into biosensor allows for the inclusion of truly "living" biological components.

Here's a general overview of how whole cell biosensors work:

Cells: The cells used in whole cell biosensor are typically bacteria, yeast, or mammalian cells. They are selected based on their specific characteristics and ability to interact with the target molecule or environmental parameter of interest.

Receptors: The cells in a whole cell biosensor are typically engineered to express receptors or proteins that specifically bind to the target molecule. These receptors can be natural receptors present in the cells or synthetic receptors designed to have high affinity and selectivity.

Signal transduction: When the target molecule binds to the receptor on the surface of cell, it triggers a cascade of signaling events within the cell. This can involve changes in gene expression, activation of specific enzymes, or production of reporter molecules.

Reporter system: The signal generated by the signaling events in the cell is measured using a reporter system. The reporter system can be a fluorescent protein, an enzyme, or a genetically encoded biosensor. The signal generated by the reporter system is then correlated with the concentration or presence of the target molecule.

Whole-cell biosensor have a variety of applications in various fields, including environmental monitoring, biomedical research, food safety, and drug discovery. They can be used to detect pollutants, pathogens, toxins, and other analytes in different samples, providing real-time and sensitive measurements. It's important to note that the design and construction of whole-cell biosensors require careful consideration of factors such as cell viability, signal specificity, and sensitivity. Researchers continue to develop and optimize these biosensors to improve their performance and broaden their applications.

(iv) DNA biosensor

DNA biosensor are devices that use DNA to detect the presence of specific molecules. DNA biosensor work by binding the target molecule to a specific DNA sequence. The completion of genome projects, such as the human genome project, has led to the development of new screening technologies that go beyond examining genes one at a time. DNA hybridization arrays, which allow for the simultaneous examination of hundreds to thousands of genes, are one frequently used screening technique. However, traditional microarray analysis requires complex optical equipment and number of molecules. To address this, researchers have proposed new electrochemical methods to create sequence-specific readouts of microarrays. Diagnostic microarrays rely on polymerase chain reactions to amplify viral target sequences from patient samples, and electrochemical monitoring has been suggested as a means of detecting DNA hybridization. Different approaches have been explored, including using oligonucleotides labeled with enzymes or electroactive tags, as well as incorporating external labels like anticancer drugs, metal complexes, or organic dyes into hybridization biosensor [60-67].

1.4.2 Classification of biosensor based on transducer

(i) Amperometric biosensor

An amperometric biosensor is a type of biosensor that measures the current produced by the oxidation or reduction of an analyte at an electrode. Amperometry is an electrochemical technique that measures the current generated during an electrochemical reaction. In the case of amperometric biosensor, the biological component acts as a recognition element that selectively interacts with the target analyte, leading to a measurable current response.

Amperometric biosensor are analytical devices that utilize the transfer of electrons generated by an enzyme to an electrode surface. These biosensors are commonly used for the detection and quantification of various analytes in biological samples. In amperometric biosensor, the analyte of interest, which is typically electrochemically inactive, undergoes catalytic conversion by an enzyme system. This conversion generates products that can be oxidized or reduced at a working electrode. The working electrode is maintained at a particular potential with respect to a reference electrode. The amperometric transducer plays a crucial role in these biosensors by facilitating the electrochemical reaction at the electrode surface. The current

generated by the electrochemical reaction is directly proportional to the analyte concentration in the sample as the reaction progresses. There are different generations of amperometric biosensor based on the type of electron transfer mechanism employed. Oxygen and peroxide-based systems are classified as "first-generation" biosensor. These systems typically utilize oxygen or hydrogen peroxide as electron acceptors or donors, respectively. Mediated systems are categorized as "second-generation" biosensor. In these systems, a mediator compound is used to shuttle electrons between the enzyme and the electrode, enabling the electrochemical reaction. More recently, "third generation" biosensor have gained interest, which involves the use of chemically modified electrodes. Conducting polymers are commonly employed in these biosensors, serving both as immobilization matrices for the enzyme and as electron transfer agents. The conducting polymers facilitate efficient electron transfer between the electrode material and biological molecules (enzyme), results in enhancing the sensitivity and stability of the biosensor [68-76].

Here are some of the advantages of amperometric biosensors:

- They are relatively simple to design and manufacture.
- They can be used to measure a wide range of analytes.
- They are highly sensitive and can detect analytes at very low concentrations.
- They are relatively fast and can provide results in a matter of seconds or minutes.

Here are some of the disadvantages of amperometric biosensors:

- They can be susceptible to interference from other substances in the sample.
- They can be affected by changes in temperature and pH
- They can be prone to fouling and calibration drift.

(ii) Potentiometric biosensor

Potentiometric biosensor is a type of biosensor that measures the potential difference or voltage generated in an electrochemical cell as a result of a biochemical reaction. These biosensors are commonly used for detecting and quantifying the concentration of various analytes, such as ions, gases, small molecules, proteins, and enzymes, in biological samples. Potentiometric biosensor is a type of biosensor that rely on measuring the potential at an electrode immersed in a solution. Unlike amperometric biosensor, which measure current, potentiometric biosensor operate

under equilibrium conditions where no current flows. The fundamental principle behind potentiometric measurements is based on the Nernst equation, which relates the potential of an electrochemical cell to the activity of the analyte:

$$E = E_O \pm \left(\frac{RT}{nF}\right) ln\ a_1 \quad (1.1)$$

In this equation, E is the measured potential, E_0 is the standard potential of the analyte at a concentration of 1 mol/L, R is the gas constant, T is the temperature in Kelvin, F is the Faraday constant, n is the number of charges on the ion, and a_1 represents the activity of the analyte.

The basic structure of a potentiometric biosensor typically consists of a working/sensing electrode and a reference electrode immersed in an electrolyte solution. The reference electrode is usually an inert electrode with a stable and known potential, serving as a reference point. The working electrode, on the other hand, is specifically designed to interact with the analyte and undergo a potential change based on its concentration. The working electrode is modified with a bio receptor, such as enzymes, antibodies, or DNA, which specifically interacts with the target analyte. When the analyte binds to the bio receptor, it triggers a biochemical reaction that generates a change in the electrical potential at the electrode-electrolyte interface. The potential difference between the sensing electrode and the reference electrode is measured using a voltmeter or a potentiostat. This potential difference also known as the electromotive force (EMF), is proportional to the concentration of the target analyte in the sample. By calibrating the biosensor with known concentrations of the analyte, a calibration curve can be established, enabling quantitative analysis of unknown samples. The potential measured by the working electrode in a potentiometric biosensor is directly proportional to the logarithmic concentration of the electro-active species being detected. This logarithmic relationship allows for a wide dynamic range of detection and facilitates the measurement of both high and low concentrations of analytes [77-78].

(iii) Thermistor-based biosensor

Many biochemical reactions involve the absorption or release of heat, which can be further used to detect the occurrence and extent of the reaction. Thermistor-based biosensor is a type of biosensor that uses a thermistor to measure the heat generated by an enzymatic reaction. Thermistors are resistors that have a negative temperature coefficient, implying that their resistance decreases as temperature increases. When an enzyme catalyzes a reaction, it releases

heat. This heat is absorbed by the thermistor, causing its resistance to decrease. The change in resistance is proportional to the amount of heat generated, which can be used to determine the concentration of the analyte being measured. A thermistor is a temperature-sensitive resistor whose electrical resistance changes with temperature variations [79-81]. By incorporating a thermistor into a biosensor design, it is possible to measure temperature changes associated with biochemical reactions or interactions.

Thermistor-based biosensors offer several advantages, including simplicity, rapid response, and high sensitivity. They can be used in various fields, such as medical diagnostics, environmental monitoring, and food safety. However, it's important to note that different biochemical reactions may generate different temperature changes, and the design and calibration of the biosensor need to be tailored to the specific reaction of interest.

(iv)Piezoelectric biosensor

Piezoelectric biosensor are a type of biosensor that uses the piezoelectric effect to detect the presence of specific molecules. The piezoelectric effect is the ability of certain materials to produce an electric charge when they are subjected to mechanical stress. In a piezoelectric biosensor, a piezoelectric crystal is used to measure the changes in frequency or amplitude of oscillation that occur when a specific molecule binds to the surface of the crystal. The basic working principle of piezoelectric biosensor involves the binding of a target molecule, such as a biomolecule or analyte, to the surface of a piezoelectric crystal. This binding event causes a change in the mass of the crystal due to the added molecular species. As a result, the mechanical stress on the crystal changes, leading to a corresponding change in the frequency of oscillation of the crystal. The piezoelectric crystal is typically made of materials such as quartz, which exhibit the piezoelectric effect. These crystals are often cut in a specific orientation, such as the AT-cut, to optimize their sensitivity and stability [82,83].

(v) Optoelectronic biosensor (OEBs)

Optoelectronic biosensors (OEBs) are a class of biosensor that utilize optical detection principles to detect and quantify target analytes. They combine the fields of optics and electronics to provide sensitive and selective detection capabilities. OEBs typically consist of a transducer that converts the analyte signal into an optical signal, and an optical detector that measures the

optical signal. The transducer is responsible for converting the signal from the analyte into an optical signal that can be measured. This conversion is often achieved through various mechanisms, such as changes in light absorption, emission, or scattering properties induced by the presence of the analyte. The transducer material used in OEBs can vary depending on the specific application and desired properties such as the analyte being detected, the required sensitivity, stability, and compatibility with the detection system [84-85]. Examples of transducer materials include semiconductors, polymers, or metal oxides. The optical detector can be a variety of devices, such as photodiodes, photomultiplier tubes, or spectrometers. The optical detector in an OEB is responsible for measuring the optical signal generated by the transducer. There are various types of optical detectors that can be employed, including photodiodes, photomultiplier tubes (PMTs), spectrometers, or other light-sensitive devices. These detectors convert the optical signal into an electrical signal that can be further processed and analyzed.

(vi) Optical biosensor

Optical biosensor are analytical devices that use the interaction of light with biological molecules to detect the presence or concentration of an analyte. They have been widely used in various fields, including biomedical research, clinical diagnostics, environmental monitoring, and food safety. Optical biosensor offer several advantages over other sensing techniques, making them highly desirable for many applications.

- Sensitivity and efficiency: Optical biosensor are known for their high sensitivity, allowing for the detection of even small quantities of target analytes. They can achieve extremely low detection limits, making them suitable for applications requiring high sensitivity. Additionally, optical methods are highly efficient, enabling rapid and accurate measurements.
- No Reference Signal Required: Unlike some other sensors, optical biosensor do not require a reference signal for their operation. This simplifies the sensing system and eliminates the need for additional calibration steps.
- Long-Distance Detection: Optical fiber biosensor, in particular, offer the advantage of long-distance detection. Optical signals can travel through fiber optic cables for several hundred meters without significant loss or degradation. This allows for flexible placement of the sensing element, making them suitable for remote or distributed sensing applications.

However, there are also some disadvantages associated with optical biosensors:

- Ambient light interference: Optical biosensor can be affected by ambient light, which may interfere with the accurate detection of the target analyte. This limitation necessitates the use of shielding or advanced filtering techniques to minimize the impact of ambient light.
- Long-term stability: Maintaining long-term stability can be a challenge for optical biosensor. Factors such as drift in light sources, changes in sensor components, and surface fouling can impact the stability of the sensor over time. Regular calibration and maintenance are necessary to ensure reliable and accurate measurements.
- Response time: Optical biosensor typically have longer response times compared to some other biosensor technologies. The time required for the formation of an antibody-antigen complex and subsequent measurement of the attenuated light signal can introduce a delay in obtaining results. However, advancements in sensor design and signal processing techniques have helped to improve the response times of optical biosensors.

In total internal reflectance spectroscopy (TIRS)-based optical biosensor, the evanescent wave phenomenon is used. This technique involves passing a beam of light through a waveguide, such as a fiber optic or a pair of parallel plates. At each reflection of the beam within the waveguide, an evanescent wave a component of light interacts with an antibody immobilized on the waveguide's outer surface. The interaction between the evanescent wave and the antibody is measured as an attenuation of the light signal. When a complex of an antibody-antigen is formed, the alteration in the evanescent wave's properties gives insights into the presence or concentration of the target analyte. This principle is the basis for the detection and quantification of various biological substances using TIRS-based optical biosensor [86-88].

1.5 Future of biosensor

The future of biosensor is very promising. Biosensor are devices that can detect and measure biological substances, such as DNA, proteins, and cells. There are several reasons why the future of biosensor is so bright. Firstly, biosensor technology is constantly improving. New materials and methods are being developed that make biosensor more sensitive, accurate, and portable. Second, the demand for biosensors is growing as they have a large number of applications in fields such as healthcare, environmental monitoring, food safety, and biosecurity. In the future, biosensor are

expected to undergo significant advancements and developments, leading to several exciting aspects. Here are some future aspects of biosensor:

- Miniaturization and wearable devices: Biosensor will continue to become smaller in size, leading to the development of wearable devices. These wearable biosensor can be integrated into clothing, accessories, or even implanted inside the body, enabling continuous monitoring of various health parameters. They will revolutionize personalized healthcare by providing real-time data on vital signs, biochemical markers, and disease conditions.
- Multiplexing and integration: Future biosensor will allow the simultaneous detection of multiple analytes. Multiplexing will enable the detection of several targets in a single analysis, enhancing efficiency, reducing costs, and providing comprehensive diagnostic information. Integration with other technologies, such as microfluidics, nanotechnology, and artificial intelligence, will further enhance the performance and functionality of biosensors.
- Point-of-care testing (POCT): Biosensor will continue to advance toward point-of-care testing, bringing diagnostic capabilities closer to the patient. POCT biosensor will enable rapid and accurate diagnosis at the bedside, in remote areas, or in resource-limited settings. They will contribute to early disease detection, monitoring of chronic conditions, and timely intervention, improving healthcare outcomes and reducing healthcare costs.
- Internet of things (IoT) and connectivity: Biosensor will be integrated into the internet of things ecosystem, allowing seamless connectivity and data sharing. They will communicate with other devices, such as smartphones, smartwatches, or cloud platforms, enabling remote monitoring and data analysis. This connectivity will facilitate personalized health management, remote patient monitoring, and real-time tracking of environmental parameters.
- Enhanced sensitivity and selectivity: Future biosensor will exhibit improved sensitivity and selectivity, enabling the detection of analytes at extremely low concentrations and in complex biological samples. Advancements in nanomaterials, signal amplification techniques, and bioengineering will contribute to enhanced sensor performance. This will

be particularly beneficial in areas such as early disease detection, environmental monitoring, and food safety.

- Non-invasive and continuous monitoring: Non-invasive biosensor will gain prominence, enabling monitoring without the need for invasive procedures or blood sampling. They will utilize non-invasive techniques like sweat, tears, saliva, or exhaled breath analysis to obtain valuable diagnostic information. Continuous monitoring will become a reality, allowing real-time tracking of physiological parameters over extended periods, leading to better disease management and prevention.
- Bioelectronics medicine: Biosensor will integrate with therapeutic interventions to create bioelectronics medicine. By interfacing with the nervous system or specific organs, biosensor can modulate physiological processes, treat chronic conditions, and restore health. This emerging field holds promise for personalized medicine and targeted therapies.

These future aspects of biosensor demonstrate the immense potential and impact they will have on various sectors, transforming healthcare, diagnostics, environmental monitoring, and beyond. Continued research and technological advancements will drive these developments, making biosensors more accessible, efficient, and versatile in the coming years.

CHAPTER 2

Literature review

2.1 Introduction of caffeic acid

Caffeic acid is a phenolic compound that is naturally found in many foods, including coffee, tea, fruits, vegetables, and olive oil. It is a member of the hydroxycinnamic acid family of compounds, which are also known as flavonoids. Flavonoids are a diverse group of plant secondary metabolites with numerous health benefits.

Chemically, caffeic acid is classified as a hydroxy derivative of cinnamic acid, with the molecular formula $C_9H_8O_4$. It is characterized by the presence of a hydroxyl group (-OH) attached to the aromatic ring of cinnamic acid. This hydroxyl group gives caffeic acid its distinct properties and contributes to its biological activities.

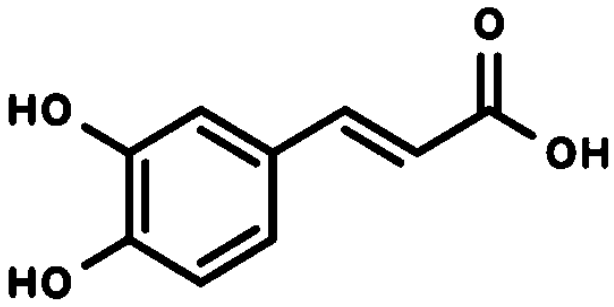


Figure 2.1 Structure of caffeic acid

Caffeic acid is primarily known for its potent antioxidant properties. Since caffeine is an antioxidant, it can aid in preventing cell deterioration brought on by free radicals. Free radicals are unstable chemicals that have the potential to harm cells and have a role in the emergence of diseases including cancer and heart disease. Caffeic acid can help to scavenge free radicals and prevent them from damaging cells. Additionally, caffeic acid exhibits anti-inflammatory properties, which may contribute to its potential health effects. Although inflammation is a natural immunological reaction to injury or infection, it can also be a factor in the development of chronic illnesses including cancer, heart disease, and arthritis. Caffeic acid can help to reduce inflammation by blocking the production of inflammatory compounds. Caffeic acid's anti-inflammatory properties make it a subject of interest in the field of natural medicine and research. Caffeic acid

may also have anti-carcinogenic properties. Cancer is a disease in which cells grow uncontrollably. Caffeic acid may help to prevent cancer by killing cancer cells or by preventing them from growing. It also plays a role in modulating enzymatic activity and gene expression, highlighting its influence on cellular processes and signaling pathways [89-95].

Due to its widespread presence in commonly consumed foods, caffeic acid is often regarded as a beneficial dietary component. However, its concentration can vary depending on the source and processing methods. Overall, caffeic acid's presence in various foods, along with its antioxidant, anti-inflammatory, and other potential biological activities, make it an intriguing subject for further investigation in the field of nutrition and health.

However, caffeic acid can also have some side effects, including:

- Insomnia: Caffeic acid has a mild stimulating effect, which could make it difficult to fall asleep.
- Stomach upset: Caffeic acid can irritate the stomach lining, leading to nausea, vomiting, and diarrhea.
- Allergic reactions: In some people, caffeic acid can cause allergic reactions, such as hives, itching, and swelling.
- Drug interactions: Caffeic acid can interact with certain medications, such as blood thinners.

2.2 Need for detection

The detection of caffeic acid is necessary for several reasons:

- Quality control in food and beverages: Caffeic acid is a natural compound found in various plant-based foods and beverages, including coffee, tea, fruits, and vegetables. Its presence and concentration can serve as an indicator of the quality and authenticity of these products. Detecting caffeic acid ensures that the desired levels are present, contributing to the taste, aroma, and potential health benefits associated with these food and beverage items.
- Health and safety considerations: Caffeic acid possesses antioxidant and anti-inflammatory properties, and it is being studied for its potential health benefits. By accurately detecting and quantifying caffeic acid, researchers and scientists can better understand its role in

human health and develop appropriate dietary recommendations or pharmaceutical applications. Furthermore, the identification of caffeic acid in certain herbs, supplements, or medications is important to ensure product safety and prevent any potential adulteration or contamination issues.

- Environmental monitoring: Caffeic acid is a phenolic compound that can be released into the environment through natural processes or anthropogenic activities. Its detection can serve as an indicator of environmental contamination or the presence of certain plant species. Monitoring caffeic acid levels can provide insights into the impact of human activities on ecosystems and contribute to environmental conservation efforts.

Therefore, there is no question that the quantitative determination of CA has significant implications for people's diets. Therefore, the accurate, fast, and inexpensive determination of CA is of great significance for the development in the detection and analysis of food texture the detection of caffeic acid is necessary for quality control, health and safety considerations, pharmacological research, and environmental monitoring.

2.3 Literature review

In recent years, various nano-catalyst modified electrode has been used for the detection of caffeic acid using various techniques. The analytical performance of the previously reported modified electrode has been compared with the present work.

Table 2.1 Comparison of the electrochemical sensing performance of different modified electrodes toward CA detection

Electrode	Linear Range(μM)	LOD(μM)	Technique	References
Au@α-Fe_2O_3@rGO	19–1869	0.98	DPV	96
layered double hydroxide film (LDH)	$7–1.8 \times 10^2$	2.6	DPV	97
Poly(Glutamic Acid)	4–30	3.91	CV	98
Carbon fiber-ultra micro electrodes	$1–5 \times 10^2$	0.41	CV	99
PtNi jagged-like nanowires	0.75–591.78	0.5	DPV	100
CuS@g-C_3N_4	1-100	0.37	Amperometry	This work

2.4 Properties of material

The selection of appropriate electrode material is essential for electrochemical procedures. In order to detect CA, a variety of electrode materials have been created and reported. Electrochemical sensors based on carbon are a wonderful substitute for noble metals since they provide a less expensive method of measuring CA. Graphitic carbon nitride (g-C_3N_4) and copper sulphide (CuS) are the two major materials that have received a lot of attention.

2.4.1 Graphitic carbon nitride

Graphitic carbon nitride (g-C_3N_4) is a highly stable form of carbon nitride under normal conditions. It is similar to graphene in structure, consisting of layers held together by weak van der Waals forces. These layers can be exfoliated into nanosheets, resulting in a two-dimensional (2D) material. Additionally, g-C_3N_4 can be further converted into different morphologies such as nanorods/nanotubes (one-dimensional) and quantum dots (zero-dimensional).

The 2D nanosheets of g-C_3N_4 possess molecular thickness and exhibit various desirable physicochemical properties. They belong to a prominent group of metal-free conjugated polymers. Some of the appealing properties of g-C_3N_4 include biodegradability, sustainability, a tunable narrow band gap, and electron-rich characteristics. These properties have led to the application of g-C_3N_4 in several fields, including photo catalysis, sensing, energy storage, and solar cells. As

sensors, g-C_3N_4-based materials offer numerous advantages. They exhibit high sensitivity and selectivity towards analytes, because of their unique surface properties and chemical composition. Moreover, g-C_3N_4-based materials possess a high surface area, enabling efficient adsorption and interaction with target molecules. They also demonstrate excellent chemical stability, ensuring the longevity and reliability of the sensing systems [101-102].

Here are some additional details about the properties and applications of g-C_3N_4

Properties

- g-C_3N_4 is a two-dimensional material with a molecular thickness.
- It is a metal-free, conjugated polymer.
- It has a tunable band gap.
- It is highly stable.
- It is non-toxic and biodegradable.

Applications

- Photo catalysis

g-C_3N_4 is a highly efficient photo catalyst for a variety of reactions, including the degradation of pollutants, the production of hydrogen, and the synthesis of chemicals.

- Sensing

g-C_3N_4-based sensors have been developed for a variety of applications, including the detection of pollutants, toxins, and pathogens.

In summary, g-C_3N_4 is a highly stable and versatile material that finds applications in various fields due to its desirable properties, such as biodegradability, tunable band gap, and electron-rich nature. As a sensor material, g-C_3N_4-based materials offer advantages such as high sensitivity, selectivity, surface area, and chemical stability.

2.4.2 Copper sulphide

Copper sulphide (CuS) is indeed a semiconductor material that has governed a significant amount of interest in recent years due to its unique physical and chemical properties. Its p-type semiconductor nature and distinctive characteristics make it suitable for various technological applications. One of the key areas where CuS has found applications is in solar cell devices. Its tunable band gap, ranging from 1.2 to 2.5 eV depending on the stoichiometric composition (x=1-2), allows for efficient absorption of light across a wide range of the solar spectrum. This property makes CuS a promising material for photovoltaic applications, where it can be used to convert solar energy into electricity.

In addition to solar cells, CuS has also demonstrated potential as a material for gas sensors. Its metal-like electrical conductivity enables the detection of gas molecules through changes in conductivity when exposed to specific gases. This property makes CuS suitable for sensing applications, where it can be used to detect and quantify the presence of gases in various environments. Furthermore, CuS has shown promise in Li-ion batteries, where it can be used as an electrode material. Its unique electrochemical properties and ability to undergo reversible lithium insertion/extraction processes make it a potential candidate for high-performance energy storage devices.

The solution processing ability and relatively low cost of synthesizing CuS are additional advantages that contribute to its attractiveness for various applications. Moreover, the optoelectronic properties of CuS can be tailored by controlling its shape, size, and doping, allowing for further customization and optimization of its performance in specific devices. It's worth noting that CuS exists in different stoichiometric compositions and morphologies, and it also exhibits non-linear optical properties. These characteristics open up opportunities for applications in fields such as non-volatile memory devices, where the properties of CuS can be utilized to store and retrieve information [103-106].

Overall, the distinctive properties of CuS, including its tunable band gap, metal-like electrical conductivity, solution processing ability, and low cost of synthesis, make it a highly promising material for a range of technological applications, including solar cells, gas sensors, Li-ion batteries, non-volatile memory devices, and more.

CHAPTER 3

Materials & methods

3.1 Introduction

This chapter describes the materials used for the fabrication of copper sulfide, graphitic carbon nitride and its nanocomposites-based biosensor for caffeic acid detection employing the electrochemical technique. Further, various analytical and characterization techniques were employed for the characterization of nanoparticles and nanocomposites.

3.2 Materials

Details of the materials utilized while pursuing different experiments are as follows:

3.2.1 Chemicals

Copper chloride dihydrate ($CuCl_2 \cdot 2H_2O$), thiourea (CH_4N_2S) (≥99.0%) N, N-dimethylformamide (DMF), ethanol (C_2H_6O), EDC-NHS solution, laccase (≥50 units' mg^{-1}; Rhusvernicifera) and melamine were bought from Sigma-Aldrich, India. All chemicals and solvents were of analytical reagent grade and used as starting materials without purifying them further. The solutions and glassware were autoclaved before use, and all the solutions were made with deionized water (Mili-Q, 18.2 MΩ.cm).

3.2.2 Buffer and solutions

- 0.1M Phosphate buffer saline (PBS), pH (7.0)
- 5Mm $[Fe(CN)_6]^{3-/4-}$ in PBS solution as redox indicator.

3.3 Characterization techniques

At various stages of preparation, the synthesized nanoparticle, nanocomposite, and bio electrodes have been characterized by utilizing different techniques like fourier transform infrared spectroscopy (FT-IR), field emission scanning electron microscopy (FESEM), X-ray diffraction (XRD), thermogravimetric analysis (TGA) and electrochemical techniques such as cyclic voltammetry (CV) and chronoamperometry.

3.3.1 Fourier transform infrared (FT-IR) spectroscopy

Fourier transform infrared (FTIR) spectroscopy is a technique used to analyze the interaction of matter with infrared light. In FTIR spectroscopy, a sample is placed in a broad range of infrared wavelengths. The sample absorbs specific wavelengths of infrared light that correspond to the vibrational energy levels of its constituent molecules. The remaining light is detected, and the resulting spectrum is obtained. It provides details about the molecular composition and structure of a substance by measuring the absorption, emission, or reflection of infrared radiation and producing various spectral lines. The spectrum is essentially determined by the chemical bonds that exist between the atoms of a molecule. The spectrum can be caused by vibration, rotation, or combination. The frequency of such movements occurs in the electromagnetic spectrum's infrared region. As a result, IR spectroscopy is employed to investigate the information about the analyzed sample's "functional groups, chemical bonds, stoichiometry, and so on, and may be referred to as the material's molecular fingerprint". This is a non-destructive approach that may be used to investigate any sort of sample. The approach is quick, sensitive, and simple to use, and it allows for convenient qualitative and quantitative examination of the spectra. All FT-IR spectrometers are based on the michelson interferometer where a beam-splitter splits the beam into two parts.

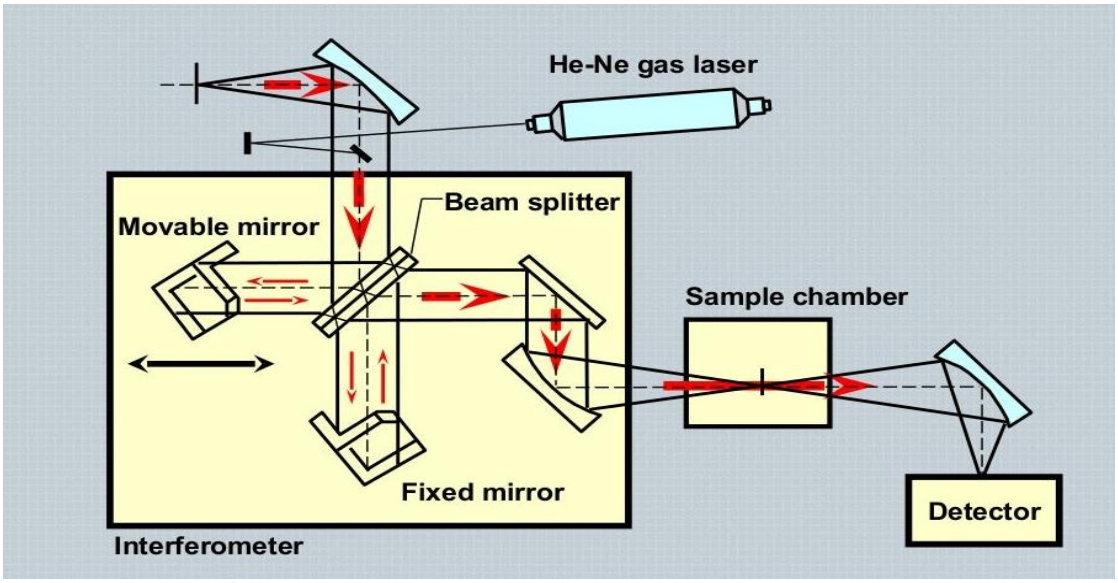


Figure 3.1 Schematic representation of the optical layout of the michelson interferometer

An infrared spectrometer is made up of three key components: (a) the source, which is a filament that is kept at red or white heat by an electric current. (b) Michelson interferometer is the most frequent interferometer used in FTIR spectroscopy. The IR radiation is either transmitted or reflected when it reaches the beam-splitter. A stationary or moving mirror will catch the 50% intensity of the radiation, and reflect it back to the beam splitter where possible beam interactions may occur. When the radiation reaches the detector, it fluctuates due to the movement of the moving mirror. (c) detector: two types of detectors are commonly employed. “one detects the radiation’s heating impact and the other relies on photoconductivity”. In both cases the stronger the impact (temperature or conductivity rise) at a specific frequency, the higher the sample’s transmittance (lower its absorbance) at that frequency in an FTIR spectrometer, an interferogram is recorded in the presence of a sample to trace the spectrum and also in the absence of a sample. The computer performs, fourier transforms on the recorded data, and the ratio of sample and background data is displayed as a transmittance spectrum.

3.3.2 Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) is a method used for analyzing, how a sample's composition and thermal stability change with temperature. It involves weighing a sample as it undergoes a controlled temperature program in a controlled environment. The basic setup of a TGA instrument consists of a sample holder (usually a crucible) and a balance to measure the sample's weight. The sample is typically heated in a furnace, and the weight of the sample is continuously monitored throughout the heating process. During a TGA experiment, the sample is first heated from room temperature to an elevated temperature at a constant rate. As the temperature increases, the sample may undergo various thermal events such as desorption of volatile components, decomposition, phase transitions, or oxidation. These events can be detected as changes in the sample's weight.

The weight change is usually plotted as a function of temperature or time, resulting in a thermogram or thermogravimetric curve. From this curve, various parameters can be determined, including the onset temperature and rate of weight loss associated with different thermal events.

This information can be used to analyze the thermal stability, composition, and decomposition behavior of the sample.

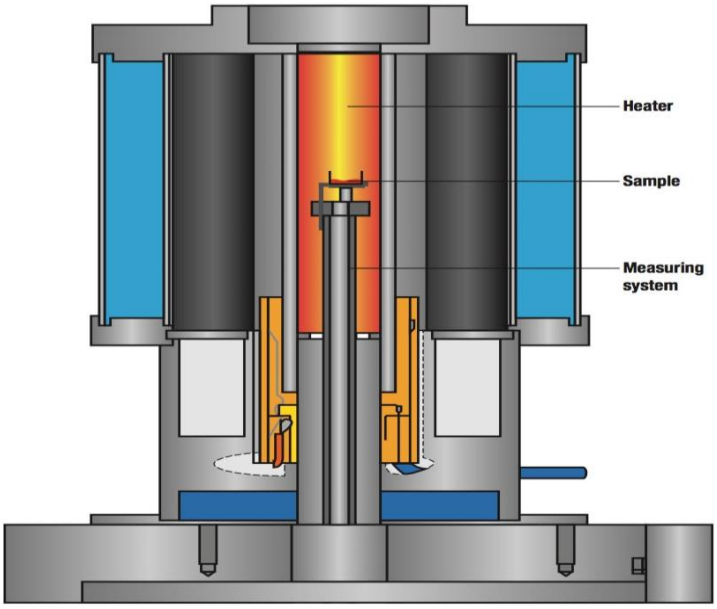


Figure 3.2 Instrument representation of thermogravimetric analysis

3.3.3 X-ray diffraction (XRD)

A method for examining the atomic and molecular structure of materials is known as X-ray diffraction. It is based on the idea that constructive and destructive interference occurs when X-rays interact with a crystal lattice, producing a pattern of scattered X-rays known as a diffraction pattern. When an X-ray beam is focused on a crystalline substance, a diffraction pattern is produced. The X-rays interact with the evenly spaced atoms inside the crystal lattice, causing them to scatter. The resulting pattern is recorded on a detector as a result of the scattered X-rays interfering with one another.

By studying the diffraction pattern, one can find out the arrangement of atoms within the crystal lattice and obtain information about the crystal's structure. The diffraction pattern contains characteristic peaks that correspond to the spacing between planes of atoms in the crystal lattice. The position, intensity, and shape of these peaks provide valuable data about the crystal structure, including the dimensions of a unit cell, crystal symmetry, and atomic positions. X-ray diffraction is widely used in determining the structures of minerals, metals, polymers, proteins, and other

crystalline substances. X-ray diffraction data helps in understanding the properties and behavior of materials, aid in the formation of new materials, and contribute to fields such as drug discovery and protein structure determination.

Instruments called X-ray diffractometers are used to perform X-ray diffraction experiments. These instruments consist of an X-ray source, a sample holder, a detector, and various components to manipulate the X-ray beam. Modern X-ray diffractometers often use rotating anode X-ray sources or sources of synchrotron radiation, which gives intense and tunable X-ray beams for precise measurements.

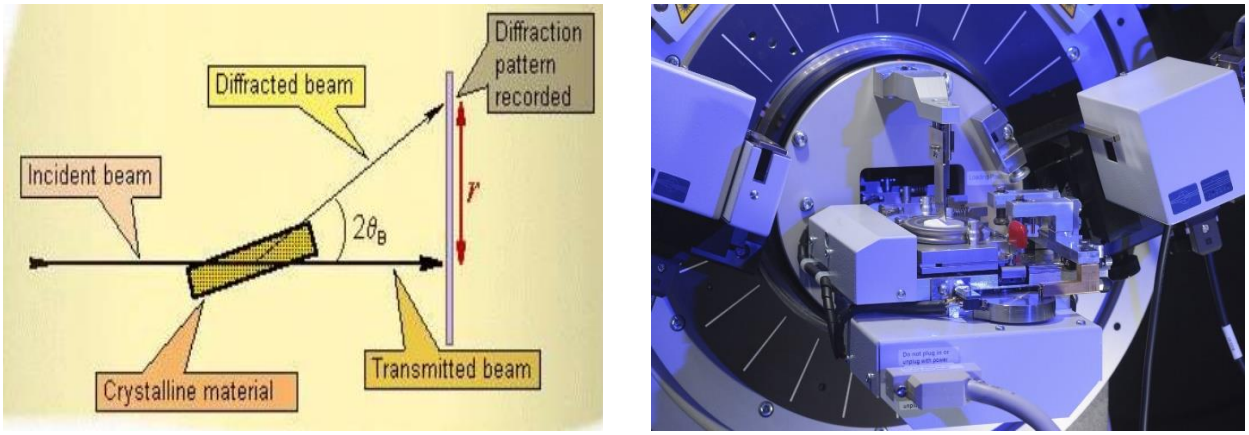


Figure 3.3 (a) Schematic representation of XRD (b) Instrument representation of X-ray diffractometer

3.3.4 Field emission scanning electron microscopy (FESEM)

Field emission scanning electron microscopy (FESEM) is a type of electron microscopy that uses a field emission source to produce a highly focused electron beam. This allows for high-resolution imaging of the surface of a sample, with spatial resolutions down to a few nanometers.

In FESEM, the sample is positioned in a high vacuum chamber. A sharp metal tip, typically made of tungsten, is placed near the sample. To the tip, a high voltage is applied, which creates a strong electric field. This field causes electrons to be emitted from the tip, forming a beam. The beam is then scanned along the surface of the sample, and the electrons that are emitted from the surface are collected by a detector. The detector converts the electrons into an electrical signal, which is then displayed on a screen. The images produced by FESEM are used to study the surface morphology of a sample. This can be used to identify features such as grains, pores, and defects.

The elemental composition of a sample can also be obtained with the help of FESEM. This is done by using an energy-dispersive X-ray spectrometer (EDS) to detect the X-rays that are emitted when the electrons interact with the sample.

3.3.5 Electrochemical techniques

An analytical method called electrochemistry is used to detect potential, charge, or current in order to determine the concentration of an analyte or chemical reactivity. The processes that occur in a system when an electric potential is applied are the focus of electrochemical techniques. Three electrodes, including a working electrode, a reference electrode, and a counter electrode, are included in an electrochemical cell. A potentiostat, which controls the potential of the working electrode and monitors the corresponding current response, is attached to these electrodes. In this kind of electrochemical investigation, the working electrode is given potential, and the response of the current is plotted against time. While in another scenario, a linear change in potential is made and the resulting current is plotted against the applied potential. The Nernst equation, which connects the potential of an electrochemical cell to the activity of the analyte, links the equilibrium concentrations of both the reduced and oxidized forms of a redox system to the potential.

$$E = E_O \pm \left(\frac{RT}{nF}\right) ln \frac{C_{oxi}}{C_{red}} \quad (3.1)$$

In this equation, E is the measured potential, E_0 is the equilibrium, C_{Oxi} is the concentrations of oxidation and C_{Red} is the concentration of reduction domains. R is the gas constant, T is the absolute temperature, F is the Faraday constant, and n is the number of charges on the ion. In accordance with Nernst's equation, the redox system present at the electrode adjusts their concentration ratios, if the voltage is applied to the working electrode (WE). The signal transduction and electrochemical performance (CV and Chrono) of sensors have been studied using potentiostat/galvanostat with platinum as the counter electrode (CE) and Ag/AgCl as the reference electrode (RE).

3.3.5.1 Cyclic voltammetry (CV) measurements

CV is one of the most often used electro-analytical methods for characterizing the electrochemical behavior of electrochemically active species. The potential of the working electrode is swept with time in CV studies. After reaching the set point, the potential of the working

electrode is swept in the opposite direction and returned to its starting value. These potential cycles can be swept as required to generate a cyclic voltammetry graph. Cyclic voltammetry is applied to get insights into the electrochemical reaction between the electroactive species bearing a known reduction-oxidation potential. Since the counter electrode transports the electricity from the signal source to the working electrode, the current at the working electrode is measured during the potential scans against a fixed reference electrode potential. In order to provide ions to the electrodes, the electrolytic solution is used during the reaction.

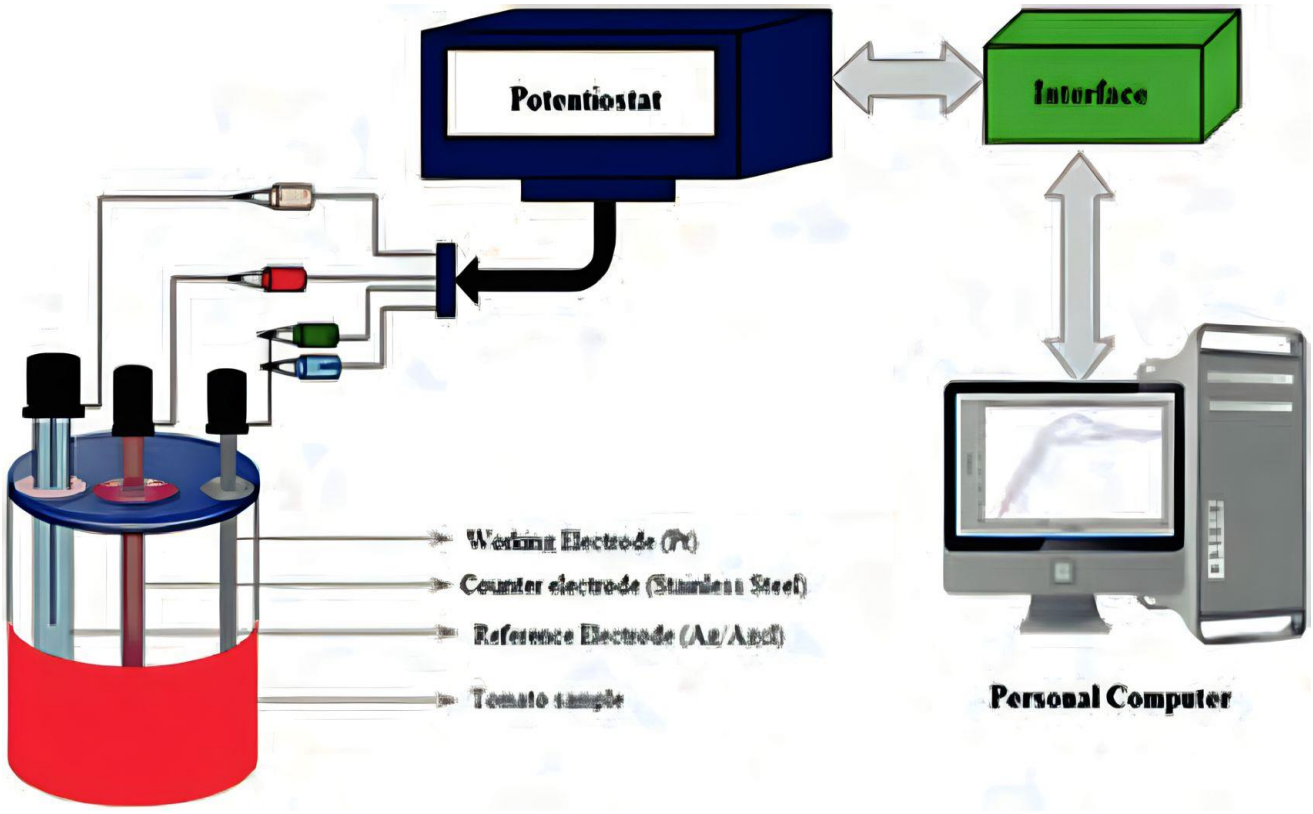


Figure 3.4 Working scheme of a potentiostat

3.3.5.2 Chronoamperometry

Chronoamperometry is an electrochemical method used to study the behavior of an electrode/electrolyte interface. It involves monitoring the current response of an electrode at a

constant potential or voltage over a period of time. Chronoamperometry is often used to investigate various electrochemical processes, including electrode reactions, electron transfer kinetics, and diffusion-controlled processes. Chronoamperometry's basic concept is that the amount of electroactive species present at the electrode surface is proportional to the current that runs through the electrode. A significant current flow when a potential step is applied because the concentration of electroactive species at the electrode surface increases rapidly. The concentration and flow of current decrease when the electroactive species diffuse away from the electrode surface. The resulting current-time curve, also known as a chronoamperogram, provides information about the electrochemical system under study. The current vs. time curve can be used to determine a number of parameters, including the rate constant of the chemical reaction, the diffusion coefficient of the electroactive species, and the surface area of the electrode. Chronoamperometry is a flexible method that can be used to investigate many different kinds of systems. It is frequently employed to investigate the rate of ion diffusion in solution, the kinetics of electrochemical reactions, and the molecular adsorption on electrodes.

It is used for investigating the behavior of electrochemical systems and understanding the underlying processes at the interface of electrode-electrolyte, such as the kinetics of electrochemical reactions, the rate of diffusion of ions in solution, and the adsorption of molecules onto electrodes.

Some of the applications of Chronoamperometry:

- Determination of the rate constant of a chemical reaction
- Determination of the diffusion coefficient of an electroactive species
- Determination of the surface area of an electrode
- Study of electrochemical reactions
- Study of the rate of diffusion of ions in solution
- Study of the adsorption of molecules onto electrodes

CHAPTER 4

CuS decorated g-C_3N_4 nanosheets based novel electrochemical sensor for sensitive detection of caffeic acid in green tea

4.1 Introduction

In this chapter, a sensitive electrochemical biosensor for the detection of caffeic acid has been described. In order to fabricate the biosensor, copper sulfide (CuS) nanoparticles and CuS@g-C_3N_4 nanocomposite were used. The CuS@g-C_3N_4 nanocomposite was hydrothermally synthesized before being electrophoretically deposited on a glass plate with an ITO coating. With the help of microscopic and electrochemical methods, the deposition was validated. The fabricated biosensor demonstrated good selectivity and reproducibility and can potentially be used to identify caffeic acid.

4.2 Experimental section

4.2.1 Synthesis of g-C_3N_4 nanoparticles

Graphitic carbon nitride (g-C_3N_4) was synthesized by thermally condensing melamine. Typically, 5g of melamine was added to a crucible with a lid. Following that, the crucible was put in a muffle furnace and heated for 4 hours at a rate of 5 °C min^{-1}, eventually reaching a temperature of 550°C. The substance was then washed many times with distilled water and ethanol before being vacuum dried in an oven and the collected product was crushed into powder.

4.2.2 Synthesis of nanocomposite

Using $CuCl_2.2H_2O$, thiourea, and graphitic carbon-nitride as starting materials, a one-pot hydrothermal reaction was used to synthesize the hierarchical CuS@g-C_3N_4 nanocomposite. In a typical reaction, 100mg graphitic carbon-nitride is dissolved in 40ml deionized water and the solution was sonicated for 10 min. Then, 100mg $CuCl_2 \cdot 2H_2O$ was dissolved sufficiently in a 100 mL beaker containing 40 mL aqueous solution and added to the above g-C_3N_4 solution, under sonication treatment. Then the above mixture was magnetically stirred for about half an hour. Then, 40 mL of an aqueous solution containing 80mg thiourea was added dropwise to the above mixture along with continuous magnetic stirring. Then, a teflon-lined stainless-steel autoclave with a 200 mL capacity was filled with the 120 mL mixture solution. An autoclave sealed with the mixture solution was treated at 180 °C for 18 h in an oven. After the reaction, the stainless-steel

autoclave was cooled to room temperature naturally. The resulting black precipitate was obtained by centrifugation and washed with ethanol and deionized water several times, respectively. Next, the obtained black precipitate was dried at 50 °C in a vacuum oven for several hours until the sample was dried thoroughly.

The copper sulfide nanoparticles have been synthesized in the same manner as the above-mentioned nanocomposite with $CuCl_2.2H_2O$ and thiourea as precursors.

4.2.3 Electrophoretic deposition (EPD) of CuS@g-C_3N_4 nanocomposite:

Using a two-electrode system where platinum was acting as a counter-electrode, the synthesized CuS@g-C_3N_4 nanocomposite was deposited using the electrophoretic deposition (EPD) technique (Genetix, Model GX300C) onto a pre-hydrolyzed ITO electrode. The spacing between both electrodes was 0.5 cm. To obtain a clear solution, the CuS@g-C_3N_4 nanocomposite was sonicated in deionized water. 10 mL of the sonicated solution was taken for the electrophoretic deposition. The film was deposited at various voltages, and at 10 V and an 8-second time interval, a smooth film formation was observed. The prepared electrode was kept for drying, after the deposition.

4.2.4 Fabrication of CuS@g-C_3N_4 nanocomposite-based biosensor

The fabrication of the biosensor has been carried out by incubating the CuS@g-C_3N_4/ITO electrode with Lac (1 mg mL^{-1}) using EDC-NHS as a cross-linker. The fabricated electrodes were stored at 4°C overnight. The fabricated electrodes were washed with phosphate buffer saline (PBS; 0.1M, pH 7) prior to being used for bio-sensing applications.

4.3 Results and discussion

4.3.1 Characterization

The structural features of synthesized materials were confirmed by X-ray diffraction (XRD) studies. The broadening of the diffraction peaks and high intensity indicates the good crystalline nature of CuS nanoparticles. Fig 4.1(B) shows the XRD pattern of the as-synthesized CuS. The diffraction peaks at 2θ values of 21.0, 27.8, 29.3, 31.9, 32.9, 38.8, 47.9, 52.7, and 59.4 were indexed to the (100), (101), (102), (103), (006), (105), (110), (108), and (116) planes of the hexagonal covellite CuS crystal phase, indicating the crystalline nature of CuS particles. The

diffraction pattern of the synthesized nanoparticles matches well with the standard diffraction pattern of hexagonal-phase CuS as found in the literature. The XRD pattern in Fig 4.1(B) confirms that impurities such as Cu, Cu_2S, Cu_2O, and CuO are not observed. These results confirm that the CuS nanoparticles are synthesized with high chemical purity. The XRD pattern of as-synthesized g-C_3N_4 shows two diffraction peaks, the primary intense peak (002) at 27.6° is a characteristic reflection peak of graphitic carbon formed by inter-layer stacking of structural tri-s-triazine rings. The second weak peak at 13.1° is attributed to the reflection at (100), corresponding to the in-plane structural repeating units of tri-s-triazine. These two specific diffraction peaks confirm the successful formation of g-C_3N_4, and these results are in agreement with the literature. The characteristic peaks at 27.8, 29.3, 32.9, 47.9, 52.7, and 59.2 correspond to the (101), (102), (006), (110), (108), and (116) planes, respectively confirming the presence of CuS nanoparticles in the synthesized CuS@g-C_3N_4 nanocomposite, however the pattern of synthesized CuS@g-C_3N_4 nanocomposite was found to be identical with CuS (Figure 4.1(B)) and does not reflect any specific peak of g-C_3N_4 pattern. This may be due to the weak crystallinity and small adding content of g-C_3N_4 or may be due to highly crystalline behavior of CuS which overpower the intensity of the characteristic peaks of g-C_3N_4.

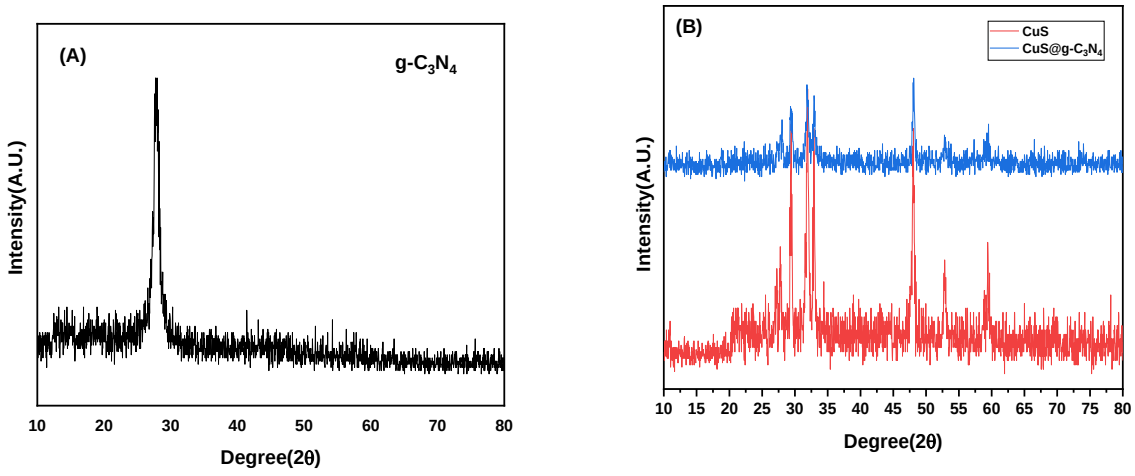


Figure 4.1 XRD of (A) g-C_3N_4, (B) CuS (Red), and CuS@g-C_3N_4 (Blue)

The bonding information of the as-synthesized CuS, g-C_3N_4, and nanocomposite was investigated by Fourier transform IR (FTIR) spectroscopy, as shown in Fig 4.2. The FTIR spectra of the g-C_3N_4 nano-sheet show a broad peak at 3250cm^{-1} for stretching vibrations of the terminal ─NH_2 or ═NH groups and C═N stretching peaks at 1575 and 1642 cm^{-1}, stretching peaks at 1245, 1324, and 1404 cm^{-1} were observed for aromatic (heterocyclic tri-s-triazine ring) C−N stretching. The sharp peak at 808 cm^{-1} is due to tri-s-triazine bending vibration modes in the g-C_3N_4 structure. The FTIR spectra of CuS@g-C_3N_4 nanocomposite, show the peaks are slightly shifted to higher or lower wavenumber when compared to CuS. Few characteristic vibrational peaks of pure g-C_3N_4 are observed in CuS@g-C_3N_4, indicating the presence of the g-C_3N_4 nano sheet in the composite.

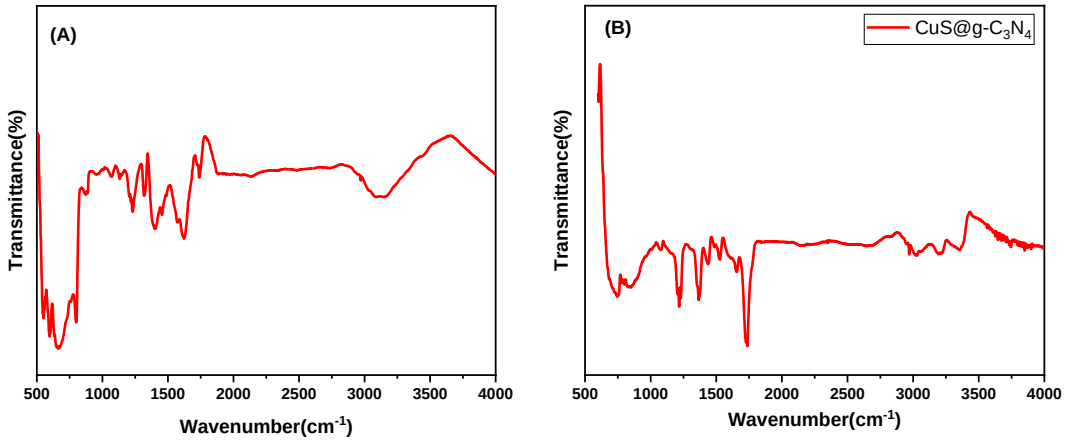


Figure 4.2 FT-IR of (A) g-C_3N_4, (B) CuS@ g-C_3N_4

The thermal stability of the nanocomposite has been analyzed using TGA. Figure 4.3 shows the mass losses of CuS@g-C_3N_4 nanocomposite, heated in an N_2 atmosphere from room temperature to 800℃ at a heating rate of 10 °C min^{-1}. Initially, in CuS@g-C_3N_4 nanocomposite there is no weight loss of the sample from room temperature to 340 °C. At 340°C there is rapid and major weight loss of the sample till 460°C which is attributed to the water adsorbed in the active material and because of the removal of oxygen-containing functional groups like ester. There is weight gain of the sample at about 520°C to 570 °C, which is mainly owing to the oxidation of CuS to Cu_2S and $Cu_{1.8}S$. The sample weight finally remained stable at 720 °C to 900℃. As the heating prohibits any nitrogen deposition, weight gain is only possible by the presence of materials that undergo oxidation like copper.

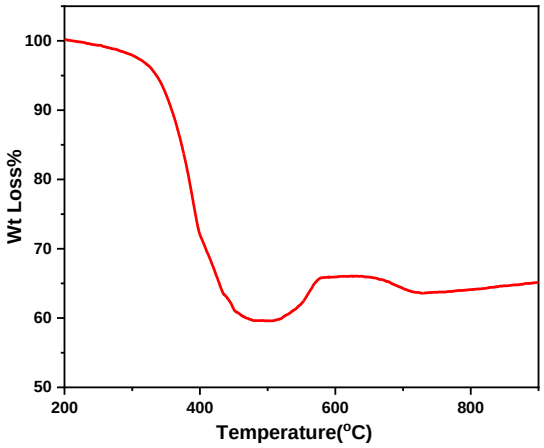


Figure 4.3 TGA Curve of CuS@g-C_3N_4

4.3.2 Microscopic studies

The surface morphology of CuS, g-C_3N_4, and CuS@g-C_3N_4 nanocomposite was investigated by FESEM, as shown in Fig 4.4(B). A low-magnification image of CuS shows that the samples are almost monodispersed. FESEM images with medium and high magnifications clearly depict the structure of the prepared CuS nanostructures that bear hexagonal nano flakes. The low magnification images of the as-prepared CuS appeared to be flower-like structures, composed of rough and uneven flakes, which may attribute to the agglomeration of nano flakes due to uncontrolled growth. The FESEM images of CuS@g-C_3N_4 nanocomposite indicate the layered and sheet-like morphology of g-C_3N_4 and flakes-like morphology of CuS nanoparticles which confirms that the CuS particles are randomly spread over the g-C_3N_4 sheets, which is further in evidence with the successful formation of the CuS@g-C_3N_4 nanocomposite.

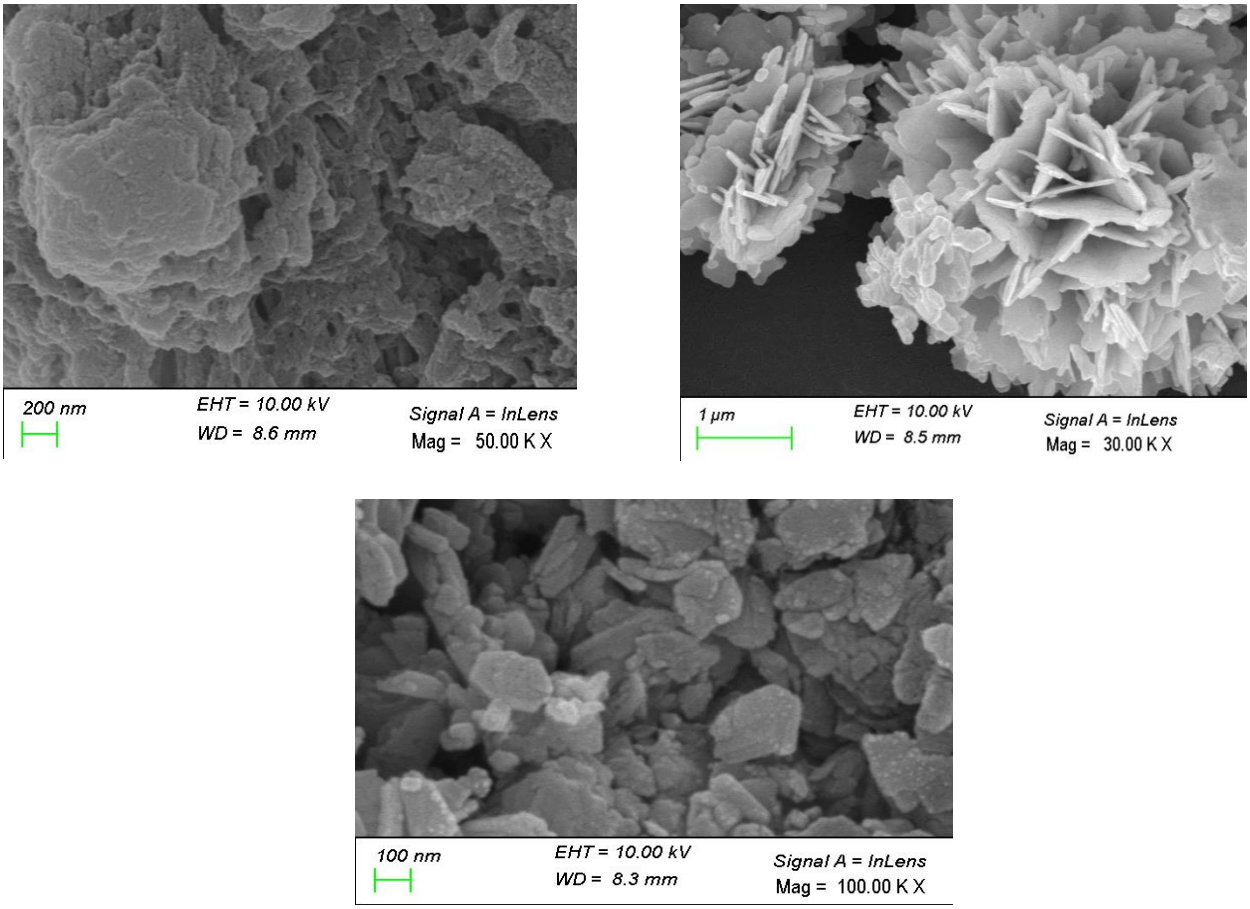


Figure 4.4 FESEM of (A) g-C_3N_4, (B) CuS, (C) CuS@g-C_3N_4

The elemental composition and purity of CuS, g-C_3N_4, and CuS@g-C_3N_4 nanocomposite were investigated by EDX analysis. EDX data of CuS shows the elemental composition of copper (63%) and Sulphur (37%). EDX data of g-C_3N_4 shows the elemental composition of carbon (46%) and Nitrogen (54%). The EDX confirmed the presence of carbon (14%), nitrogen (18%), copper (42%), and sulfur (25%) on the CuS@g-C_3N_4 nanocomposite. Also, the elemental mapping of nanocomposite and the elemental distribution of carbon, nitrogen, sulfur, and copper is shown in Figure. These results confirm the uniform distribution of carbon, oxygen, sulfur, and copper in the structure of CuS@g-C_3N_4 nanocomposite.

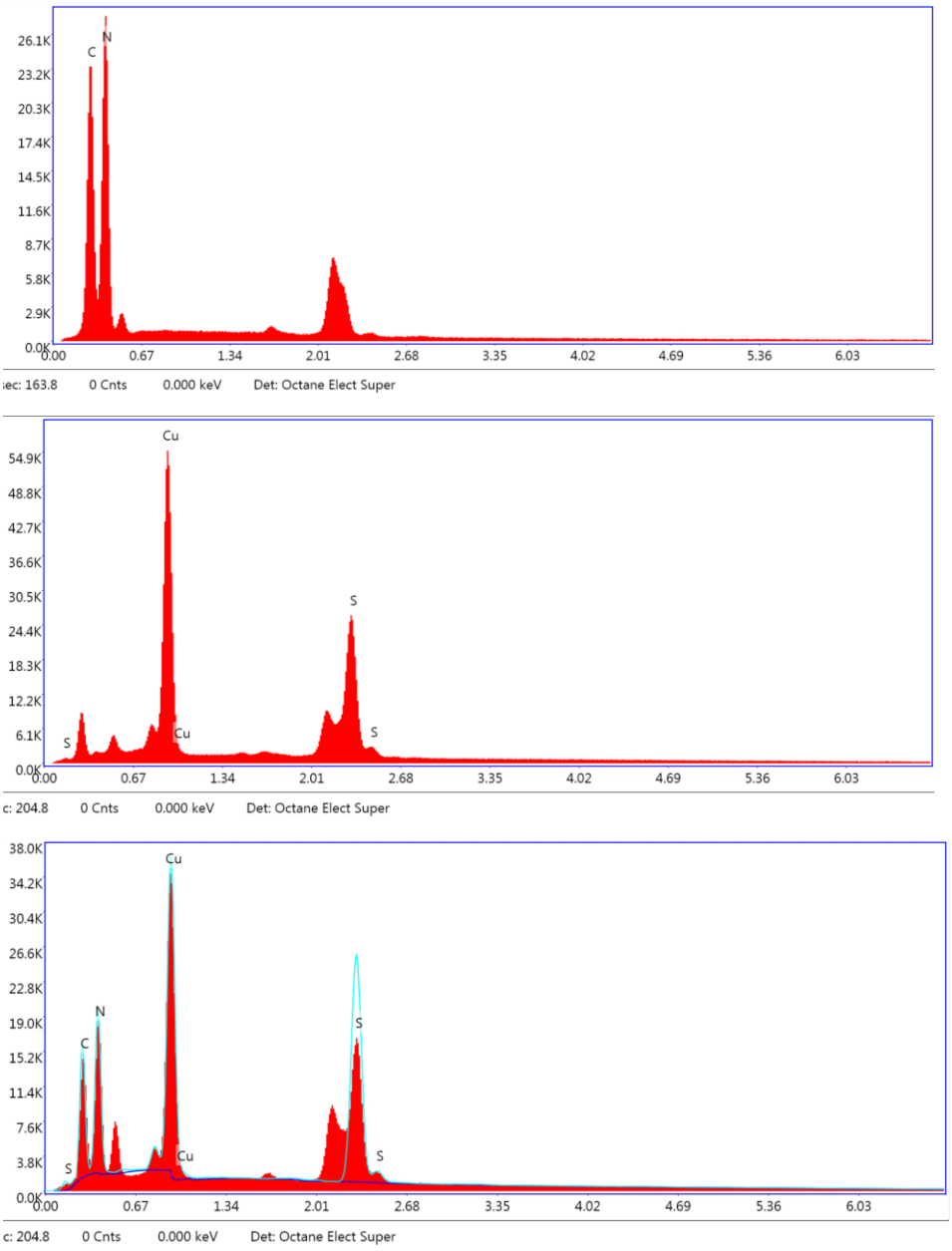


Figure 4.5 EDX analysis of (A) g-C_3N_4, (B) CuS, (C) CuS@g-C_3N_4

4.3.3 Electrochemical characterization

Cyclic voltammetry studies have been carried out for bare ITO electrode, g-C_3N_4/ITO, CuS/ITO, CuS@g-C_3N_4/ITO, and Lac/CuS@g-C_3N_4/ITO electrodes in PBS buffer. It has been seen that the redox peak current of the CuS@g-C_3N_4/ITO electrode (curve (iv); 0.47mA) (Figure

4.6(A)) is higher compared to bare ITO(i), g-C_3N_4/ITO (ii), CuS/ITO (iii) electrode. It can be inferred that CuS improves the conductivity of the CuS@g-C_3N_4/ITO electrode when compared to the g-C_3N_4/ITO electrode.

The peak current decreases ((curve v); 0.39 mA) after the immobilization of Lac on the CuS@g-C_3N_4/ITO electrodes may be due to the fact that Lac is a non-conducting protein. The peak current value shifts to the positive side as the enzyme creates a non-conducting layer on the composite-modified electrode and decreases the peak current value thus indicating a decrease in the oxidation signal.

The effect of changing the scan rate on the CuS@g-C_3N_4/ITO electrode was explored, and an increase in the scan rate from 10 to 300 mV/s was observed which resulted in a linear increase in oxidation peak current and a positive shift in the oxidation peak potential. Both the peak anodic (I_{pa}) and cathodic (I_{pc}) currents increases linearly with increasing scan rate and follow equations (4.1) and (4.2), showing that the transfer of electron is a surface (adsorption) controlled process (Fig)

$$I_{pa}(\mu A) = 1.3730 \times 10^{-4} + 1.7013 \times 10^{-5}\sqrt{v}\left(\frac{mV}{s}\right) ; R^2 = 0.98996 \quad (4.1)$$

$$I_{pc}(\mu A) = -1.5584 \times 10^{-4} - 1.2965 \times 10^{-5}\sqrt{v}\left(\frac{mV}{s}\right) ; R^2 = 0.98677 \quad (4.2)$$

Similarly, for anodic and cathodic peak potentials (E_{pa} and E_{pc}) a linear correlation was observed w.r.t the natural logarithm of scan rate (lnv) for the fabricated electrodes. The linear regression for the same has been shown in the equation below,

$$E_{pa}(V) = 0.1583\ln(v) + 0.0052; R^2 = 0.97688 \quad (4.3)$$

$$E_{pc}(V) = 0.1945\ln(v) + 0.0068; R^2 = 0.9759 \quad (4.4)$$

Using the Laviron equation and the linearity curve, α and K_α have been calculated and the charge transfer coefficient (α) and the heterogeneous electron transfer rate constant (K_α) for surface-confined electroactive species have been found to be 0.901 and 0.1230 at a scan rate (v) of 50mV/s

for the CuS@g-C_3N_4/ITO electrode using the expressions for anodic and cathodic slope utilizing peak to peak separation.

$$E_{pa} = RT/(1-\alpha)nF \quad (4.5)$$

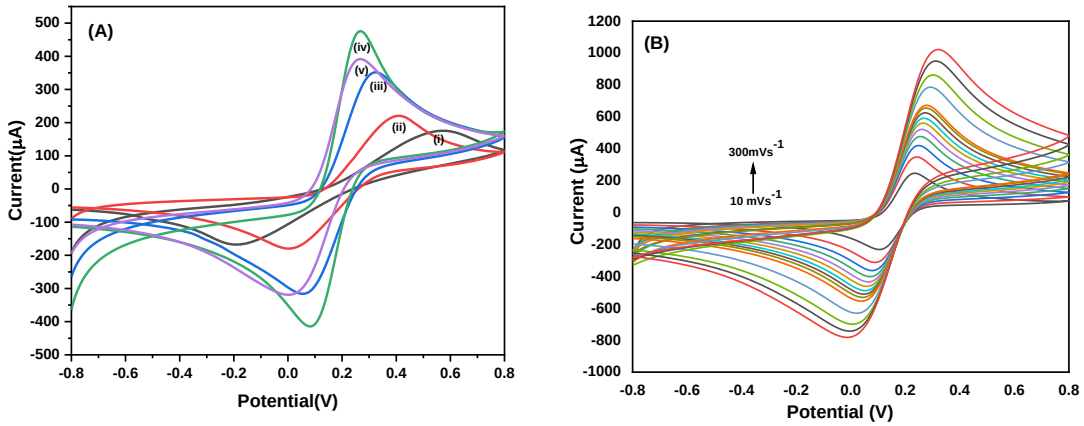
$$E_{ca} = -2.303RT/\alpha nF \quad (4.6)$$

$$\ln K = \alpha \ln(1-\alpha) + (1-\alpha)\ln\alpha - \ln(\frac{RT}{nFV}) - \alpha(1-\alpha)nF\Delta E_p/RT \quad (4.7)$$

The electrochemically active surface area was calculated for the CuS@g-C_3N_4/ITO electrode and it was found to be 0.5400 cm^2. Using linearity curves and Randle-Sevick equation, Eq. (4.8) D was calculated and hence the diffusion coefficient (D) for the CuS@g-C_3N_4/ITO electrode was found to be $10.11 \times 10^{-12}\ cm^2 s^{-1}$.

$$I_p = (2.99 \times 10^5)\alpha^{1/2} n^{3/2} A C D^{1/2} v^{1/2} \quad (4.8)$$

Where C is the molar concentration of $[Fe(CN)6]^{3-/4-}$ in mol $cm^{-3}$and A is the effective electrode area. I_p is the peak current, v= scan rate, F = Faraday's constant, D = diffusion current and R = universal gas constant.



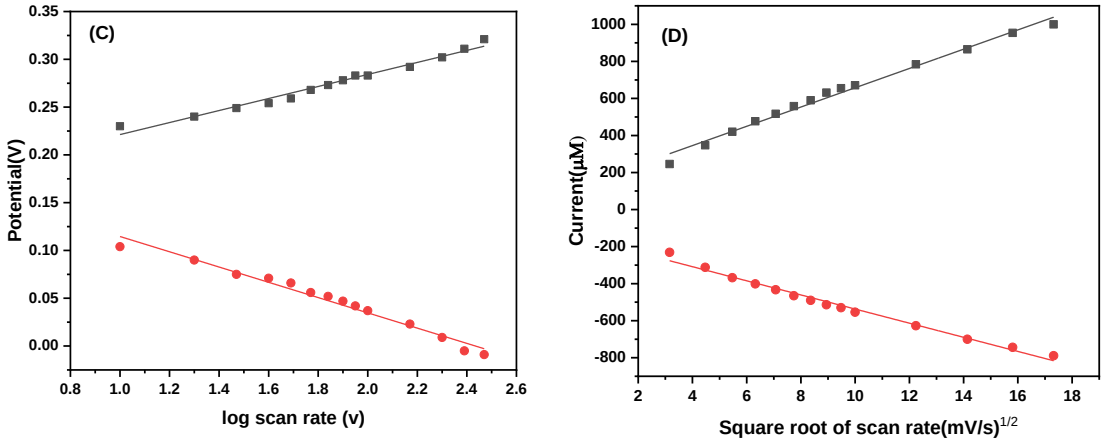


Figure 4.6 (A) CV studies of (i) Bare ITO (ii) g-C_3N_4/ITO (iii) CuS/ITO (iv) CuS@g-C_3N_4/ITO (v) Lac/CuS@g-C_3N_4/ITO electrode; **(B)** CV analysis of Lac/CuS@g-C_3N_4/ITO electrode with varying scan rate (10-300mV). **(C)** Peak potential with log scan rate for Lac/CuS@g-C_3N_4/ITO electrode in PBS (100mM, pH 7.0) consisting of 5mM $[Fe(CN)_6]^{3-/4-}$ and **(D)** Peak current vs square root of scan rate for Lac/CuS@g-C_3N_4/ITO electrode

4.3.4 Optimization studies

The ideal concentration for Lac binding on the CuS-g-C_3N_4/ITO electrode was determined using chronoamperometry. Using EDC-NHS as a cross-linker, various Lac concentrations were covalently immobilized on the CuS@g-C_3N_4/ITO electrode. The current increased following each subsequent addition of Lac in the concentration range of 0.5–3 mg mL^{-1}, reaching a plateau at 1 mg mL^{-1}. So, 1 mg mL^{-1} of Lac concentration was used to fabricate the biosensor. The performance of the biosensor is influenced by the electrolytic solution's pH. The pH of the Lac/CuS@g-C_3N_4/ITO electrode was thus varied from 5.0 to 8.0, and a change in current was recorded. The maximum current response was achieved at pH 7.0, indicating that this is the ideal pH for biosensing studies.

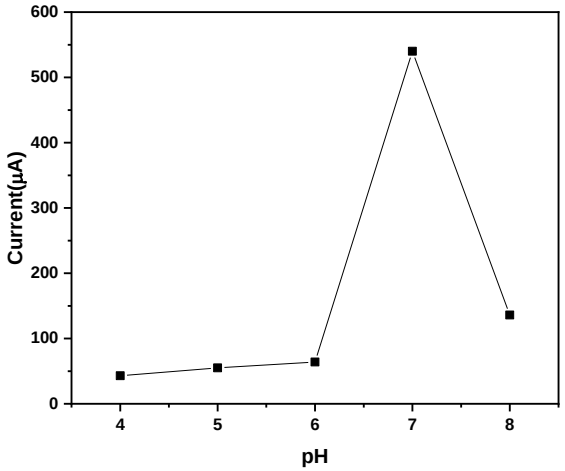


Figure.4.7 Optimization of Lac/CuS@g-C_3N_4/ITO electrode at different pH (5-8).

4.3.5 Electrochemical biosensing studies

Chronoamperometry is an electrochemical method that yields a current response as a function of time when the voltage of the working electrode is stepped. Chronoamperometry was used to analyze the Lac/CuS@g-C_3N_4/ITO electrode's biosensing capabilities for caffeic acid detection. Caffeic acid was applied to the Lac/CuS@g-C_3N_4/ITO electrode at varying concentrations (from 1 to 100 µM), and it was found that as the concentration of caffeic acid increased, the saturation current decreased (Fig. 4.8). The calibration curve demonstrates a linear dependence between the slope of the saturated current vs. caffeic acid concentrations plot (Fig. 4.8). Using the regression equation: y=1.7704x+0.4156 (y=peak current and x=concentration of CA), the sensitivity (S) of the biosensing electrode (S = slope/active surface area of the electrode) was calculated. It was found to be 0.720 µA M^{-1} with R^2= 0.9965. The limit of detection (LOD) was calculated using Eq. 3σ/S. The LOD was found to be 0.37 µM, where σ is the standard deviation and S is the sensitivity obtained from the slope of the calibration curve.

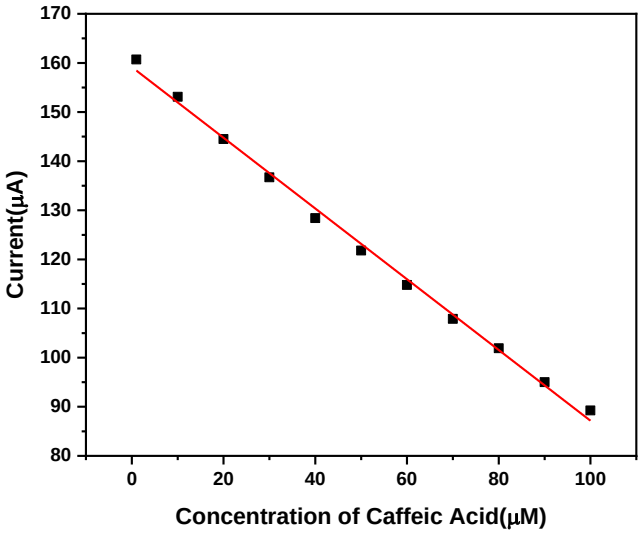


Figure 4.8 Calibration plot between response current and concentration of caffeic acid for Lac/CuS@g-C_3N_4/ITO electrode.

4.3.6 Validation of the biosensor with real sample

The practicality of the fabricated Lac/CuS@g-C_3N_4/ITO biosensor for analyzing CA in green tea samples. Through the use of the conventional addition method, the recovery of CA in samples was examined. The outcomes demonstrate that the substance has a high rate of recovery and can be used in practice without sample purification.

Table 4.1 Recovery data of CA in green tea using a Lac/CuS–g-C_3N_4/ITO electrode using chronoamperometry

Sample	Added amount (μM)	Found amount (μM)	Recovery (%)
Green Tea	10	1.63	105
	20	1.45	96
	30	1.34	97
	40	1.28	95

4.3.7 Interference, reproducibility, and stability studies

The biosensor's specificity was tested in the presence of other analytes, such as catechol, hydroquinone, ascorbic acid, and tartaric acid. The Lac/CuS@g-C_3N_4/ITO electrode was

incubated with an equal amount (10 µM) of the interfering analytes, and it was found that there was no significant change in current with respect to the Lac/CuS@g-C_3N_4/ITO electrode (Fig. 4.9(A)). Furthermore, the fabricated biosensor was tested with a combination of various interfering analytes containing CA, and it demonstrated a positive response in detecting CA. Based on the obtained results, it can be assumed that the biosensor is selective for finding CA even when other analytes are present. Five distinct electrodes were made using the same procedure and were used to assess the biosensor's repeatability. The outcome shows an RSD of less than 5.0%, showing that the fabricated biosensor has acceptable reproducibility (Fig 4.9(B)). Furthermore, using the same electrode maintained at 4°C, the long-term stability of the biosensor was also tested. After 25 days, the electrode retains 95% of its initial response; thereafter, there was a drop in response that might have been due to the leaching of the Lac from the CuS@g-C_3N_4/ITO electrode (Fig 4.9(C)).

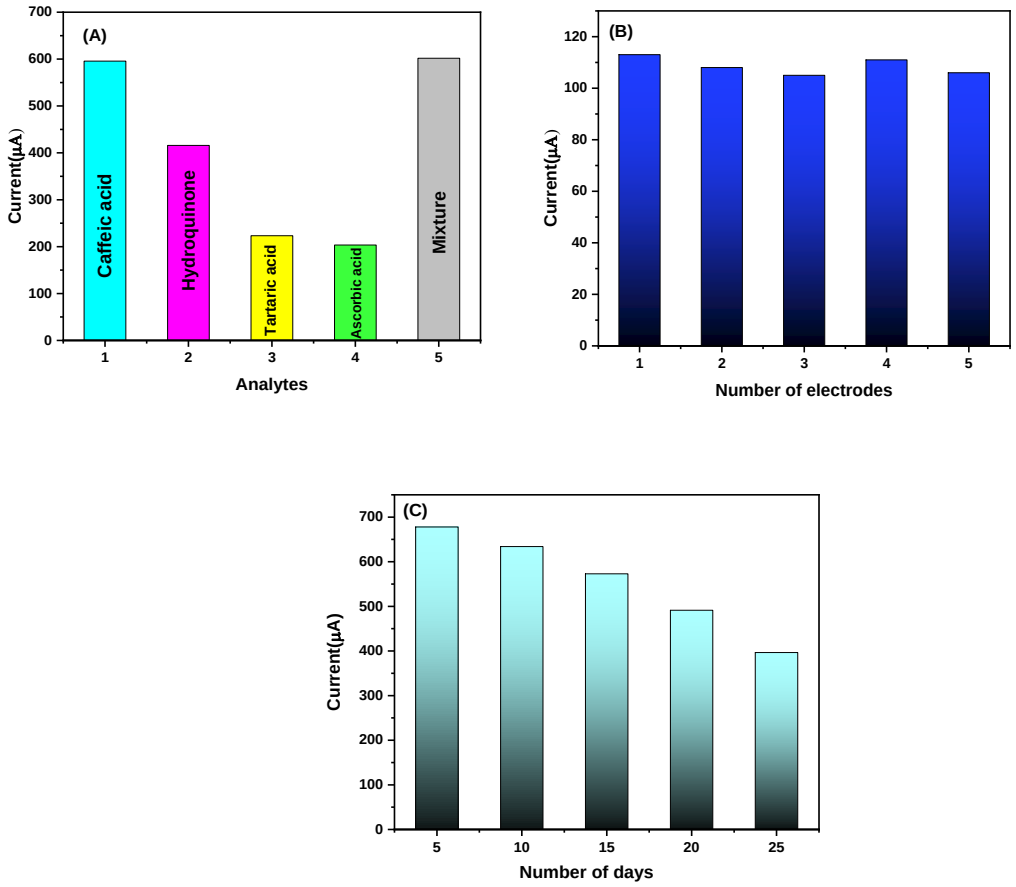


Figure 4.9 (A) Interference study at 10 µM CA **(B)** Reproducibility study at five modified electrodes and **(C)** stability of biosensor for CA detection checked for 25 days.

5. CONCLUSION

An electrochemical biosensor has been developed for CA detection using CuS@g-C_3N_4/ITO nanocomposite. The fabricated biosensor shows improved electrochemical properties, and a reasonable detection limit (0.37 µM). The biosensor's potential for field applications for the detection of CA in green tea is demonstrated by its validation with real samples. The fabricated biosensor was found to be specific, stable, and reproducible. The developed CuS@g-C_3N_4 nanocomposite–based platform has been successfully validated with the green tea sample, which shows its applicability and reliability for caffeic acid detection.

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