

**ELECTROCHEMICAL BIO-SENSING OF CAFFEIC ACID IN REAL
SAMPLES USING Ag@g-C₃N₄ COMPOSITE MATERIAL
FOR HIGHLY SELECTIVE AND SENSITIVE APPLICATIONS**

**A DISSERTATION
SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
AWARD OF THE DEGREE**

**OF
MASTERS OF SCIENCE
IN
(CHEMISTRY)**

**Submitted by
MD Shoaib Akhter
(2K23/MSCCHE/69)**

**Under the Supervision of
Prof. D. Kumar & Prof. Rajinder Gupta**



**DEPARTMENT OF APPLIED CHEMISTRY
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur Bawana Road, Delhi -110042**

JUNE, 2025



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

CANDIDATE'S DECLARATION

I, MD SHOAIB AKHTER, Roll No. 2K23/MSCCHE/69 student of MSc (Chemistry) hereby declare that the project Dissertation titled **“ELECTROCHEMICAL BIO-SENSING OF CAFFEIC ACID IN REAL SAMPLES USING Ag@g-C₃N₄ COMPOSITE MATERIAL FOR HIGHLY SELECTIVE AND SENSITIVE APPLICATIONS”** in partial fulfillment of the requirements for the award of the Degree of Master of Science in Chemistry submitted in the Department of Applied Chemistry, Delhi Technological University is an authentic record of my own work carried out during the period from 2024 to 2025 under the supervision of Prof. D. Kumar and Prof. Rajender K. Gupta.

Place: Delhi

MD SHOAIB AKHTER

Date: 20/06/2025



**DEPARTMENT OF APPLIED CHEMISTRY
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Bawana Road, Delhi-110042**

CERTIFICATE

Certified that MD Shoaib Akhter (2K23/MSCCHE/69) has carried out his research work presented in this thesis entitled **“ELECTROCHEMICAL BIO-SENSING OF CAFFEIC ACID IN REAL SAMPLES USING Ag@g-C₃N₄ COMPOSITE MATERIAL FOR HIGHLY SELECTIVE AND SENSITIVE APPLICATIONS”** for the award of Degree of Master of Science in Chemistry from Department of Applied Chemistry, Delhi Technological University, Delhi under our supervision. The dissertation embodies the results of original work and studies are carried out by the student himself and the content of the thesis does not form the basis of the award of any other degree to the candidate or to any other body else from this or any other University/Institution.

(PROF. RAJENDER K. GUPTA)

SUPERVISOR

(PROF. D. KUMAR)

JOINT SUPERVISOR

ABSTRACT

Caffeic acid (CA) is recognized as a phenolic antioxidant with anti-inflammatory, antibacterial, anticarcinogenic and immunomodulatory effects, positioning it as a key representative of hydroxycinnamic acids found in wine. Caffeic acid is detectable in various products including fruits, vegetables, wine, olive oil and coffee. Numerous studies suggest that eating foods rich in CA may provide protection against cancer by inhibiting the production of nitro compounds (including nitrosamines and nitroamides) that are primary contributors to this disease. The goal of this research is to create an enzyme-based biosensor for the detection of caffeic acid utilising silver nanoparticles on a graphitic carbon nitride (Ag@g-C₃N₄) nanocomposite. The Ag@g-C₃N₄ nanocomposite was synthesised through chemical methods and applied to an indium tin oxide-coated glass substrate via electrophoretic deposition. The resulting biosensor demonstrates a favourable linear range from 1.0 pM to 1.0 μ M with a detection limit of 0.75 pM and displays no significant interference from glucose, ascorbic acid, urea and mixtures. Additional efforts were made to validate the biosensor using real samples from coffee, green tea and black tea. The electrochemical findings indicated that the proposed biosensor is a dependable analytical tool for assessing the quality of caffeic acid in food samples.

ACKNOWLEDGEMENT

At the outset, I would like to extend my profound sense of gratitude and indebtedness to my supervisors, **Prof. D. Kumar and Prof. Rajinder K. Gupta**, Department of Applied Chemistry, Delhi Technological University, Delhi for their constant guidance, valuable suggestions and continuous motivation for carrying out this work. Working under a kind, motivated and supportive supervisor has been a matter of privilege for me.

I am deeply indebted to **Prof. Ram Singh, Prof. Roli Purwar, Prof. Anil Kumar and Dr. D. Santhiya** for their constant guidance and facilities to carry out my project work.

I was lucky to have a positive work atmosphere in the laboratory, and I'm thankful to all the laboratory staff and research scholars for their cooperation. I must thank **Ms. Divya Hudda, ma'am**, for her massive help in making me understand the basics of techniques and all the literature part, and **Ms Ritu Sharma, ma'am**, for her constant help and patiently responding to my incessant doubts during my research work. It gives me great pleasure to offer my heartfelt gratitude to **all the faculty members of the Department of Applied Chemistry at DTU for their constant cooperation and support**. I am also thankful to the **technical and non-technical staff** for their help whenever needed. Finally, I would like to thank my family and friends for their encouragement, support, and motivation throughout.

TABLE OF CONTENTS

CANDIDATE’S DECLARATION.....	ii
CERTIFICATE.....	iii
ABSTRACT.....	iv
ACKNOWLEDGEMENT.....	v
TABLE OF CONTENTS.....	vi
LIST OF FIGURES.....	ix
LIST OF TABLES.....	xi
ABBREVIATIONS.....	xii
CHAPTER 1: INTRODUCTION.....	1-26
1. Introduction of Biosensors	1
1.1. Components of Biosensors.....	3
1.1.1. Biomolecular Recognition Element.....	3
1.1.2. Immobilization of Biomolecules.....	4
1.1.3. Transducer.....	5
1.2. Methods of Immobilization.....	6
1.3. Configuration of Biosensors.....	11
1.3.1. Biosensors Based on the Biological Recognition Element.....	11
1.3.2 Types of Biosensors Organized by Transducer Technology	16
1.4. Future of Biosensors.....	23
1.4.1. Emerging Technological Developments Miniaturised and Wearable Monitoring Systems.....	24
1.4.2. Multi-target Detection & Integration of Systems.....	24
1.4.3. Diagnostic Tools at the Point of Care.....	25
1.4.4. Effectively Connecting and Digitally Integrating.....	25
1.4.5. Improved Detection Capability.....	25
1.4.6. Non-Invasive Monitoring Solutions.....	26
1.4.7. Applications of Biosensors for Therapy.....	26
1.4.8. Transformative Impact.....	26

CHAPTER 2: LITERATURE REVIEW	27-34
2.1. Introduction to Caffeic Acid.....	27
2.2. Importance of Analytical Detection.....	29
2.3. Literature Review.....	30
2.4. Properties of Material.....	31
2.4.1. Graphitic Carbon Nitride (g-C ₃ N ₄).....	31
2.4.2 Silver Nanoparticles.....	33
CHAPTER 3: MATERIALS & METHODS	35-44
3.1. Introduction.....	35
3.2. Materials.....	35
3.2.1. Chemical Reagents.....	35
3.2.2. Buffer Solutions.....	36
3.3. Analytical Techniques.....	36
3.3.1. X-Ray Diffraction (XRD).....	37
3.3.2. Fourier Transform Infrared (FTIR) Spectroscopy.....	38
3.3.3. UV-Vis spectroscopy (UV-Vis).....	39
3.3.4. Field Emission Scanning Electron Microscopy (FESEM).....	41
3.3.5. Electrochemical Methods.....	42
3.3.5.1. Cyclic Voltammetry (CV) Measurements.....	44
3.3.5.2. Chronoamperometry.....	44
CHAPTER 4: Ag@g-C₃N₄ MODIFIED ELECTROCHEMICAL BIOSENSOR FOR THE DETECTION OF CAFFEIC ACID	46-61
4.1. Introduction.....	46
4.2. Methodology.....	46
4.2.1. Synthesis of g-C ₃ N ₄	46
4.2.2 Synthesis of Nanocomposite.....	47
4.2.3. Electrophoretic deposition (EPD) of Ag@g-C ₃ N ₄ on ITO electrode.....	47
4.2.4. Fabrication of Ag@g-C ₃ N ₄ Nanocomposite-Based Biosensor.....	48
4.3. Result and Discussion.....	48
4.3.1. Structural Studies.....	48
4.3.2. Morphological Studies.....	52

4.3.3. Electrochemical Characterization.....	54
4.3.4. Optimization of Experimental Parameters.....	57
4.3.5. Electrochemical Biosensing Studies.....	59
4.3.6. Interference, Reproducibility, and Stability Studies.....	60
4.3.6. Validation of the Biosensor with Real Sample.....	60
CHAPTER 4: CONCLUSION.....	62
References.....	63
APPENDIX-I.....	68
APPENDIX-II.....	69

LIST OF FIGURES

Fig. 1.1	Components of Biosensor	2
Fig. 2.1	Structure of Caffeic acid	28
Fig. 3.1	(a) Schematic diagram of X-Ray Diffraction, (b) Instrument diagram of X-Ray Diffractometer	38
Fig. 3.2	Diagram illustrating the optical configuration of the Michelson Interferometer	39
Fig. 3.3	Pictorial representation of UV-Visible Spectrophotometer	40
Fig. 3.4	Pictorial representation of FESEM	41
Fig. 3.5	Pictorial view of Potentiostat	43
Fig. 4.1	Illustration for the detection of CA using Ag@g-C ₃ N ₄ /ITO	47
Fig. 4.2	XRD of (a) g-C ₃ N ₄ (red) and (b) Ag@g-C ₃ N ₄ (black)	48
Fig. 4.3	FTIR of (a) g-C ₃ N ₄ (red), and (b) Ag@g-C ₃ N ₄ (blue)	50
Fig. 4.4	UV-visible spectra of absorption as (a) G: g-C ₃ N ₄ and (b) GA: Ag@g-C ₃ N ₄	51
Fig. 4.5	FESEM images of (a) &(b) g-C ₃ N ₄ ; (c)&(d) Ag@g-C ₃ N ₄ (ii) Elemental mapping for compounds (e) g-C ₃ N ₄ , (f) Ag@g-C ₃ N ₄ and EDAX (Energy Dispersive X-ray Analysis) spectra of (g) g-C ₃ N ₄ and (h) Ag@g-C ₃ N ₄	52
Fig. 4.6	Nyquist plot for the electrodes (a) GCN3: g-C ₃ N ₄ /ITO and (b) GA4: Ag@g-C ₃ N ₄ /ITO	55
Fig. 4.7	(a) CV analysis of different fabricated electrodes: ITO (Black), g-C ₃ N ₄ (Red) and Ag@g-C ₃ N ₄ /ITO (Blue) (b) CV response of electrode Ag@g-C ₃ N ₄ /ITO in a solution of 0.1 M PBS containing 5 mM [Fe(CN) ₆] ^{3-/4-} at various scan rates (10-120 mVs ⁻¹) (c) Plot of peak current versus square root of scan rate	56

and (d) Plot between peak potential versus log scan rate for Ag@g-C₃N₄/ITO

- Fig. 4.8** (a) Study of the effect of pH (6.0 to 8.0), (b) Different enzyme concentration of laccase (0.5 - 2.5 mgmL⁻¹), and (c) Incubation time period analysis of biosensor for CA via chronoamperometry method of Lac/Ag@g-C₃N₄/ITO fabricated electrode 57
- Fig. 4.9** (a) Chronoamperometry method for electrochemical response of Lac/Ag@C₃N₄/ITO electrode as a function of CA concentration (from top to bottom 1 pM to 1μM) at PBS (pH = 7.5) (b) Linear relationship between the magnitudes of saturation of current vs. logarithm of concentration of Lac/Ag@g-C₃N₄ /ITO (c) Interference study of different analytes with respect to CA and (d) Reproducibility study of the fabricated Lac/Ag@C₃N₄/ITO electrode 59

LIST OF TABLES

Table 1:	Working principles of different types of biosensors	5
Table 2:	Advantages and Disadvantages of various methods of immobilization	10
Table 3:	Comparison of the electrochemical sensing performance of different modified electrodes towards CA detection	31
Table 4:	Detection of CA in three real samples using Ag@g-C ₃ N ₄ electrode	60

ABBREVIATIONS

AgNO ₃	Silver Nitrate
AgNPs	Silver Nanoparticles
g-C ₃ N ₄	Graphitic Carbon Nitride
CV	Cyclic Voltammetry
UV-Vis	Ultraviolet-Visible Spectroscopy
XRD	X-Ray Diffraction
FTIR	Fourier Transform Infrared Spectroscopy
EDC	N-Ethyl-N'-(3-Dimethyl Aminopropyl) Carbodiimide
NHS	N-Hydroxy Succinimide
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
CA	Caffeic Acid
Lac	Laccase

CHAPTER 1

INTRODUCTION

1. Introduction of Biosensor

A biosensor is a device that interfaces a biological component with a physicochemical sensor to detect and analyse a specific organic or chemical substance. It is shaped to facilitate a natural reaction into a quantifiable signal allowing for the discovery, evaluation and observation of various organic and biochemical forms. The natural component of a biosensor can be a protein, counteracting agent, microorganism, cell or tissue depending on the target analyte. This natural component interacts specifically with the analyte or target atom, starting a particular organic response. The physicochemical sensor at that point interprets this natural reaction into a quantifiable signal such as an electrical, optical or electrochemical signal. The operation of a biosensor typically involves three primary steps: recognition, transduction and signal handling. During the acknowledgement step, the natural component interacts with the target particle, resulting in a specific response or action. Within the transduction step, the natural response is converted into a distinguishable signal by the physicochemical mediator. At long last, within the signal handling step, the obtained signal is measured, handled and analysed to provide quantitative or subjective data regarding the target analyte.

Biosensors have a wide range of applications in various areas including healthcare, environmental monitoring, food security and bioprocessing. They offer various advantages including high accuracy, specificity, transportability, rapid response and real-time observation capabilities [1]. Biosensors have been utilised for recognising biomarkers in therapeutic diagnostics and observing glucose levels in diabetes administration,

recognising pathogens in food and water and analysing natural toxins among numerous other applications. Progressions in nanotechnology, microfabrication procedures and biotechnology have led to the improvement of miniaturised, exceedingly delicate and flexible biosensors [2,3]. These progressions have extended the conceivable outcomes for point-of-care testing, personalised pharmaceuticals and further observation of well-being conditions. It can be concluded that biosensors are effective analytical tools that harness the specificity and sensitivity of biological components to distinguish and analyse a wide range of organic and chemical substances. Their applications continue to develop, offering promising solutions in various areas to move forward in healthcare, ensure food security and protect the environment.

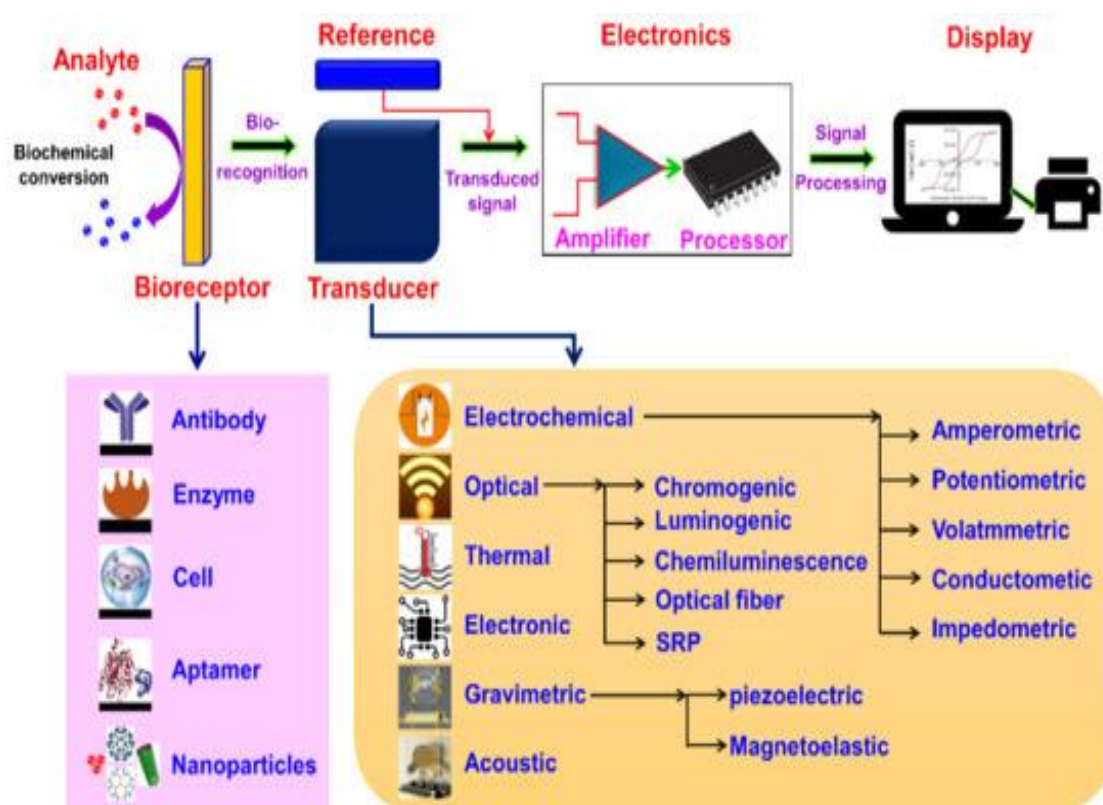


Figure2 : Components of Biosensor

1.1. Components of Biosensor

Essentially, there are three components that have been recognised as constituting a biosensor. The three components are biomolecular acknowledgement components, anchoring of biological compounds and sensing components.

1.1.1. Biomolecular Recognition Element

The biomolecules that can recognise the fabric are utilised in a biosensor to detect a target chemical. This component may be a biomolecule or an atomic assembly such as a protein, an antagonist, a fragment of the film or an entire cell. A suitable environment is vital for these biomolecules to keep their structure and acknowledgment capacities. They deliver a biochemical change that's recognised by a transducer when they contact a target inside the biosensor. Chemicals have customarily been the foremost commonly utilized biomolecules in biosensors but antibodies and protein receptor atoms are presently progressively being utilized [4]. The specificity of a biosensor depends on the particular functional properties of the biomolecule utilised. For example, in spite of the fact that antibodies contain Y-shaped atoms with two indistinguishable antigen binding sites, the proteins have a particular spatial configuration that essentially binds to one specific target molecule. Immunoglobulins bind to antigens with specificity but not like proteins; they do not work as catalysts. Their work involves tying and disposing of external materials from the body. Compared to this, receptor proteins have an unmistakable affinity for numerous naturally occurring substances including hormones, antibodies and other proteins. These proteins are frequently arranged in layers and are responsible for controlling how ion channels open and close in a coordinated manner to facilitate the transport of specific metabolites.

1.1.2. Immobilization of Biomolecules

Immobilisation is the method of settling biomolecules onto a strong support to stabilise their activity and prolong their stability. This preparation is known as immobilisation and it involves connecting biomolecules to a framework that can be made of various materials such as inorganic substances (e.g., glass), natural materials (e.g., polymer films), self-assembled monolayers, Langmuir-Blodgett films, nanomaterials or screen-printed anodes. This could be done for an assortment of reasons such as to improve soundness, reusability or selectivity. There are several different methods for immobilizing enzymes and other biological materials, each with its advantages and disadvantages. The selection of the appropriate method will depend on the mechanical and chemical characteristics required for the application as well as the stability of the biosensor. Properties of the matrix such as pH and the charged microenvironment at its surface can influence the ideal pH for a particular biomolecule. Immobilization restricts biomolecules to a defined location in space thereby enhancing the stability and reusability of that biomolecule. Some of the immobilization methods include physically trapping the biomolecule, adsorbing the biomolecule to the matrix surface, covalently bonding the biomolecule to the matrix and linking the biomolecule to the matrix with cross-linking agents. The choice of method to use will depend on the type of result desired as well as how well the active site and reactive groups of the biomolecule retain their activity after immobilisation. Chemicals and other organic materials combined with microelectronics have become a promising field in biotechnology. Immobilized chemicals are utilised as biocatalysts in broad biotransformation and as biosensors which enable mechanical forms and make it possible to utilise sensitive discovery frameworks. It is essential to select a connection technique that preserves the enzyme's activity by avoiding modifications to its chemical properties or the presence of reactive groups in order to ensure effective immobilisation. To achieve this objective, a satisfactory understanding of the enzyme's dynamic location is vital.

1.1.3. Transducer

A transducer is a device that converts a natural signal into an electrical signal. A biochemical response between an organic component and an analyte produces the natural *signal*. The transducer at that point passes the electronic signal to a locator framework which can be utilized to determine the proportion of the test material [5,6]. Essentially, the measurement device serves as an interpreter, converting the natural flag into another flag that the processor can comprehend. The biochemical transducer or bio-component confers selectivity or specificity to the biosensor. It is capable of recognising and associating with the target analyte. The transducer on the other hand, reacts to this interaction in a way that permits the signal to be electronically intensified and shown. The sort of transducer utilized depends on the nature of the signal obtained and it incorporates four sorts of transducers: electrochemical, optical, thermal and piezoelectric. Modifications in current or voltage are measured by electrochemical transducers. Optical transducers track varieties in reflectance, absorbance or fluorescence. Warm transducers track temperature varieties. Piezoelectric transducers track recurrence varieties and acoustic transducers track recurrence varieties brought on by modest changes in mass joined to their surface.

Table 1: Working principles of different types of biosensors

Type of Transducer	Working principle
Amperometric	This biosensor type operates by monitoring current variations at a fixed voltage as electrons move between biological components and the electrode surface during biochemical reactions
Conductive	These sensors function by detecting alterations in electrical conductivity that occur between electrode pairs when biological interactions take place
Optical	This category utilizes mass or concentration variations to produce direct modifications in light properties through fluorescent or luminescent mechanisms
Piezoelectric	These devices respond to mass fluctuations that occur throughout the reaction process by generating measurable electrical signals
Potentiometric	This sensor type functions by detecting potential differences while maintaining constant current flow during biological interactions
Thermal	These biosensors operate by measuring temperature variations that arise as biological reactions proceed

1.2. Methods of Immobilization

(i) Physical Adsorption

Immobilizing biomolecules on a matrix's surface through physical adsorption. One of the most punctual and least complex methods, it barely modifies the protein's structure or disrupts its dynamic centre. In any case, the framework might permit the adsorbed protein to elude due to the weak interaction between the framework and the biomolecule during use [7]. A major advantage of physical adsorption is that it can be a straightforward and economical strategy that requires no reagents or activation steps. The powerless authoritative strengths are involved in physical adsorption. They are too profitable as they permit the biomolecule to be effortlessly

discharged from the lattice when craved. In any case, the frail official strengths moreover make physical adsorption a less steady strategy of immobilization than other strategies. As a result of variations in heat levels, acid-base balance, salt content or the closeness of the substrate, the adsorbed protein may desorb from the network. Generally, physical adsorption is a simple and reasonable method for immobilizing biomolecules, suitable for a wide range of applications. Be that as it may, its soundness is restricted by the powerless official strengths included.

Here are a few extra points of interest around physical adsorption.

- The frail official powers included in physical adsorption are ordinarily van der Waals strengths, hydrogen bonds and electrostatic intuitive
- The rate of physical adsorption is ordinarily controlled by the diffusion of the biomolecule to the surface of the lattice
- The degree of physical adsorption is ordinarily restricted by the accessibility of surface locales on the lattice
- Physical adsorption is reversible and the biomolecule can be effectively discharged from the network by changing the conditions such as by expanding the temperature or pH

(ii) Covalent Binding

Covalent immobilisation could be a strategy for immobilizing biomolecules to a back framework by forming covalent bonds between the two. This can be the most widely used immobilisation method as it is suitable for a wide range of biomolecules and backbones. The most common useful groups utilised for covalent bonding are amino, carboxylic, sulfhydryl, imidazole, thiol, hydroxyl, phenolic, threonine and indole [7]. Covalent binding can be more complicated and unforgiving than physical adsorption because it requires particular conditions to make the covalent bonds. This will lead to changes within the adaptation and movement of

the biomolecule. In any case, the functional drive between the biomolecule and the support lattice is exceptionally strong which prevents the biomolecule from leaking even in the presence of any substrate or arrangement of high ionic strength.

Here are a few of the points of interest of covalent authoritative.

- It could be a flexible strategy that can be utilised to immobilize a wide range of biomolecules.

- The bonds are exceptionally solid which anticipates spillage of the biomolecule.

- The biomolecule can be reused numerous times.

Here are a few of the impediments to covalent binding.

- It can be more complicated and extreme than physical adsorption.

- The bonds can modify the adaptation and action of the biomolecule.

By and large, covalent immobilisation may be a capable immobilisation procedure that provides several key advantages. In any case, it is essential to be mindful of the potential impediments associated with utilising this technique.

(iii) Cross-linking

The strategy of cross-linking includes joining biomolecules to a steady backbone network. Compounds with a few utilitarian groups that can bind different materials under various circumstances can be utilised to achieve this by employing multifunctional or Chemical reagents possessing two reactive sites such as glutaraldehyde, 1,5-difluoro-2,4-dinitrobenzene, 4-azido-1-fluoro-2-nitrobenzene and bis-diazobenzidine-2,2'-disulfonic acid. Intermolecular cross-linking of biomolecules has been achieved. There's a small to no desorption due to the covalent association between the biomolecule and the bolster lattice. The most widely used

reagent for cross-linking is glutaraldehyde. Cross-linking can be utilised to improve the properties of biomolecules such as their stability, solubility and reactivity. Analysts are seeking modern cross-linkers that can bind diverse biomolecules under gentle conditions influence to anticipate filtration and loss of activity during the handling.

(iv) Entrapment

The capture strategy of immobilizing proteins includes keeping them inside a framework or layer. This preparation enables the specified biomolecules to be retained within the lattice while still allowing for the infiltration of the substrate. One way to achieve this is by planning a polymeric gel with a structure containing biomolecules which occurs through their entanglement within the gel lattice (e.g., polyacrylamide, sol-gel films). Nevertheless, this procedure has a downside in the form of a critical dissemination obstruction that prevents the transport of substrate and product thereby increasing the reaction time compared to other strategies. Unlike covalent or cross-linking procedures, the biomolecules themselves don't bind to the framework or layer amid entanglement. The particles within the lattice and the immobilised biomolecules are compatible, either through capture or physical entrapment, resulting from the ionic interactions within the biomolecules. Ionic compounds are often more stable than covalent compounds which causes the biomolecule's shape and dynamic location to change only slightly. As a result, immobilized proteins delivered utilising this approach as often as possible have higher mobility. The connection of biomolecules to the framework is more grounded with ionic interactions than with physical adsorption; however, it is still weaker than with covalent interactions. In any case, in arrangements with high ionic quality or when there are variations in pH, there may be a risk of biomolecule leakage from the system.

There are a few key points to consider when utilizing the entanglement strategy of chemical immobilisation. To begin with, it may be a relatively straightforward and reasonable approach.

It does not require any covalent alteration of the chemical which can, in some cases, harm the chemical, the entanglement strategy can be utilised to immobilise a wide assortment of chemicals.

Be that as it may, there are also several drawbacks to the entanglement strategy. To begin with, the dissemination of the substrate to the protein can be moderate which can decrease the rate of the response. Moment, the chemical can sometimes spill out of the lattice or layer which can diminish the proficiency of the immobilised protein. In general, the capture strategy is a flexible and successful approach to protein immobilization. It could be a great choice for applications where basic, inexpensive and non-covalent immobilization is required.

Here are a few additional points of interest for the entanglement strategy.

- The framework or film can be made from a wide range of materials including polymers, hydrogels and silica.
- The measure of the pores within the lattice or layer can be controlled to permit the specified substrate to diffuse to the chemical while avoiding the protein from diffusing out.
- The capture strategy can be utilized to immobilize a wide assortment of chemicals including hydrolases, oxidases and transferases.
- The capture strategy could be a flexible and successful strategy of protein immobilization. It could be a great choice for applications where basic, reasonable and non-covalent immobilization is required.

Table 2: Positive and negative aspects of different immobilization procedures

Method	Advantages	Disadvantages
Physical adsorption	No modification of biocatalyst. Low cost and re-generable matrix	The stability of molecular connections varies with changes in proton concentration, kinetic

		energy of particles, and dissolved salt content
Entrapment	Mechanical confinement of biocatalyst at sensor surface	Diffusion barrier is high.
Cross-linking	Minimum Enzyme inactivation	Harsh treatment of biocatalysts by toxic chemicals
Covalent binding	Stable under adverse conditions	Matrix not re-generable

1.3. Configuration of Biosensors

Generally, biosensors have been categorised into several groups based on the biorecognition component and the methods of detection, i.e., transducers.

1.3.1 Biosensors based on the biological recognition element

(i) Bio-affinity sensors

Bio-affinity sensors employ receptors with limited attachment strength to determinant analogues fixed onto transducer surfaces. These analytical devices utilize selective binding mechanisms between biological molecules for target analyte detection. Their applications span medical diagnostics, environmental surveillance and food quality assessment [6,8]. Antibody-based detection systems represent the predominant bio-affinity sensor category. These protein-based recognition elements demonstrate exceptional specificity and binding strength toward particular antigens. The sensor configuration involves antibody immobilization onto the transducer interface where target molecule introduction triggers binding events that generate detectable responses. These biosensing platforms are often employed in environmental monitoring due to the abundance of antibodies and their ability to identify a wide range of environmental contaminants. An example of this type of platform is the avidin-biotin system coupled with an oxygen electrode. Hormones, pharmaceutical compounds and other substances including metabolites can be detected using these sensors. For instance, lectins can also detect

carbohydrates using an antigen/antibody approach. Each of these sensors has physical and chemical properties that are altered (e.g., decreasing thickness, shift in refractive index, change in optical absorption, change in electrical charge) when a complex forms. The measurement cycle includes dissociation to return to baseline measurements. Consequently, the sensors are not suitable for continuous monitoring or tracking analytes with decreasing concentrations. A biocompatible technology for creating polypyrrole nanowire biosensors was developed by Ramanathan and others by utilizing a simple method that allows for the custom size parameters, material combinations, size ratios and placement of the nanowires used in creating the sensors. The new technology enables label-free detection through bioaffinity interactions. Pei and their co-authors also developed a quartz crystal microbalance (QCM) platform to facilitate the rapid screening of glycosyl-disulfide inhibitors of lectins from dynamic combinatorial libraries [9]. The QCM format enabled the study of carbohydrate-lectin interactions using mixtures of thiol-containing components and those that had been chemically modified for use with QCM. This work led to the identification of 1-thio and 6-thiomannoside-derived dimeric, potent inhibitors of lectins, importantly, the difference in properties between the 6-thio and its oxo analogue was found to be significant. Other bio-affinity sensing devices have utilized various combinations of enzymatic systems, nucleic acid recognition elements and other types of biomolecular components as a means to enhance the analytical capabilities compared to currently available antibody-based approaches. Ongoing research remains focused on developing novel bio-affinity sensing devices through the utilization of other innovative techniques.

(ii) Immunosensors

Immunosensors are highly advanced analytical instruments that use the high degree of selectivity provided by antibody-antigen binding to detect target molecules in solution. The basic mechanism through which immunosensors work is that the target molecules are captured by biological recognition elements (antibodies), producing a detectable signal. An antibody is

immobilised onto the surface of the sensor to act as a molecular recognition element. When the target binds to the immobilised antibody, a molecular complex is formed between the two. The binding event is transduced into a measurement by various means of transduction. The combination of immunosensor technology with the ELISA method creates highly capable analytical platforms. ELISA amplifies the immuno-recognition events by enzymatically amplifying the amount of target detected. The quantification of the targets is dependent on the enzyme-substrate interaction that produces a measurable product and the sensitivity of the detection system. Chemiluminescent methods of detection are used to detect chloramphenicol through a competitive binding assay where the target molecule competes with an enzyme-labelled tracer for a limited number of antibody binding sites. The intensity of the signal generated is inversely proportional to the concentration of the target present, allowing for the generation of a calibration curve based on the logarithmic relationship. The limits of detection for this type of immunosensor are in the nanomolar (10^{-8}M) range. Immunosensors have several advantages over traditional analytical methods. They are faster than traditional analytical methods. Constructs created via genetic engineering can introduce genes encoding proteins into a user's cell. These proteins include fluorescent markers and/or enzymes capable of causing an observable pH change (catalytic enzymatic function). Genetic constructs take advantage of the ability of cells to recognize and respond to environmental signals. A correctly executed construction will allow for viable cells and the ability to differentiate one or more signals from many different cellular processes. Cells are composed of many different classes of biochemical material (proteins, nucleic acids, metabolites, etc.) and the presence of multiple signals makes isolating individual signals from the many presents in a cell very complex. When evaluating how to construct a sensor, the selection of the organism being used will have a large impact on how well the sensor will function. The higher-level organisms will have difficulties with preservation and will require complicated maintenance, therefore, they would be

impractical choices for the development of sensors. Lower-level organisms such as bacteria, yeast and algae demonstrate a higher level of environmental adaptability and stability, therefore, they are the more desirable organisms to use when constructing sensors.

(iii) Operational Mechanisms

Whole cell biosensor functions require several coordinate processes.

Cellular Platform Selection

Researchers select the appropriate organism (Dickinsonia, yeast, etc.) according to the organism's natural abilities and ability to be modified to achieve the proper target detection.

Recognition Element Engineering

Cells can be genetically modified to contain binding proteins that have a high affinity for certain target molecules. These binding proteins may be naturally occurring receptors found in cells or custom-made recognition elements created in the laboratory.

Signal Processing

When the target molecule binds, it constitutes as the first signal of a cellular response that triggers a cascade of intracellular signalling events (for example, the activation of various proteins and the expression of various genes) leading to the activation of the target and ultimately the production of the reporter molecules.

Detection Systems

When the target and reporter molecules bind together, a signal is generated that can be measured using several reporter mechanisms such as fluorescent proteins, enzymatic indicators and genetically encoded sensors. The intensity of the generated signal is indicative of the presence or concentration of the targeted molecule.

Applications and Considerations

Using whole-cell biosensing technology plays a groundbreaking role in the progression of technology and systems to represent the biological viability of living cells, with applications found in a variety of industries such as environmental monitoring, medicine, food safety and drug development. Biosensors can be used to detect pollutants, pathogens and toxic agents while at the same time providing a way to monitor an analysis in real-time. To create an effective biosensor requires the maintenance of the health of the cell from which the biosensor was constructed, the optimization of the signal's quality and the enhancement of the sensitivity of the sensor. Future research efforts will continue to focus on developing better performance characteristics and expanding the types of biosensors available using new biosensor designs and cellular engineering methods.

(iv) DNA Biosensors

DNA biosensors are devices that identify target molecules based on the interaction of complementary DNA strands - one strand attaches to the target molecule and subsequently creates a double helix with it. The completion of genome projects has led to several new approaches to the identification of genes. One common application of genomic sequencing is the DNA hybridization array which enables simultaneous identification of 100 to 1,000 genes across a number of different samples. Month-to-Month expenses associated with the operation of traditional microarrays and the need for sophisticated optical imaging systems to identify and quantify the number of DNA copies produced make them impractical for usage in a clinical setting. As a result, scientists have developed new approaches that incorporate electrochemical sensor technology for providing sequence-specific readouts from these expanded scale DNA microarrays. In addition to providing sequence-specific information regarding DNA sequences of interest, the electrochemical sensors also enable researchers to use polymerase chain reaction

(PCR) techniques to extract viral targets from human sample specimens and increase their overall number using amplification methodologies while at the same time utilizing the electrochemical sensor to monitor the outcome of the hybridization reaction. Various methods have been tested as potential means of detecting hybrids such as attaching either labelled proteins (enzyme) or electroactive tags (metal ions or organic dyes) to oligonucleotides for hybrid identification. All of the various methods used will increase the potential for targeted nucleotide sequences to be detected with increased specificity.

1.3.2 Types of Biosensors Organized by Transducer Technology

(i) Amperometric Biosensors

Electrochemical devices used to measure current produced by oxidation and reduction of analytes at the surface of electrodes are called amperometric biosensors. In amperometric biosensor systems, there are biological recognition elements that selectively binds to their target analytes which cause the current generated from this interaction to be measured.

Amperometric biosensors detect and quantify electrons during the interactions between enzymes and the electrode material. The amperometric biosensor is a widely used analytical tool for identifying and measuring a variety of analytes in biological matrices. Target analytes do not participate in the electrochemical reaction but when they bind to an enzyme, they become electroactive and participate in an electrochemical reduction/oxidation process occurring at the electrode surface.

While the electrodes have a fixed potential relative to a reference electrode during the actual measurements, the amperometric transduction system allows for electrochemical reactions to take place at the interface of the electrode where the quantity of current generated is proportional to the concentration of analyte as the electrochemical reactions proceed.

Amperometric biosensor technologies have evolved through several generations based on different ways of transferring electrons. The first-generation devices utilized oxygen or hydrogen peroxide as the electron acceptor or donor, respectively, when detecting analytes, while the second generation introduced mediator molecules that transport electrons between the enzymatic components and the electrodes for electrochemical reactions to occur.

The third generation of amperometric biosensors builds on this concept of electrochemical detection using chemically modified electrochemical electrode architectures to improve performance. Many of these types of sensors incorporate the use of conducting polymeric materials for two purposes both as platforms to immobilize enzymes onto and as an electron transfer facilitator. The polymeric matrices provide a pathway for the efficient transfer of electrons from the electrodes to the biological components, thus allowing for greater sensitivity and stability of the biosensor.

Advantages of Amperometric Biosensors

1. They can be constructed and manufactured cheaply.
2. They can detect a wide range of analytes in samples from various sources.
3. They are very sensitive, allowing them to detect very low levels of compounds.
4. They are very quick, usually returning results in seconds to minutes.

Drawbacks of Amperometric Biosensors

1. They can have interference from other substances present in the same sample.
2. Their performance can change dramatically depending on the temperature and pH levels they are being used in.

3. They can be prevented from working properly or drift in accuracy over time due to buildup on their surfaces which can occur with constant use or even after just one use on a dirty or contaminated surface.

(ii) Potentiometric Biosensors

The potentiometric biosensor is part of the family of devices that measure an electrical potential or voltage produced by an electrochemical reaction as a result of a biological process. These biosensors are traditionally designed to be able to determine both presence (what) and quantity (how much) of certain amounts (ion, gas, small molecule, protein or enzyme) of analytes in various types of biological materials. The main difference between a potentiometric biosensor and an amperometric biosensor is that potentiometric biosensors measure electrical potential while amperometric biosensors measure electrical current. Additionally, while amperometric biosensors measure a current flow during a reaction, the potentiometric biosensors operate in a state of equilibrium, meaning that during an analysis, there is no current flowing. The Nernst equation forms the foundation for the theory behind the measurement of the electrical potential and provides a relationship between electrical potential and activity/amount of an analyte in a solution.

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

Expression of voltage detected with a sequence of values (electric) has a relationship to target chemical substance reference potential for a chemical compound primarily found in a concentration of one molar by the universal constants of gas law (gas law constant (R_g), temperature (K) and faraday constant (F)) and ionic charge number (i.e., number of charges) and activity level (concentration of analyte) associated with a detection electrode and reference electrode of all potentiometric biosensors. The reference electrode serves as the reference point for measuring the electric voltage (E) for the detection electrode to determine the concentration

of analyte present by using special design features in the detection electrode, the electric potential of the detection electrode can change in relation to the concentration of analyte, thereby allowing direct electrical measurements to occur. Enzymatic proteins and immunoglobulins, nucleic acid sequence binding affinity for specific target molecules and enzyme and protein-based recognition element interactions all create electrical potential changes at the interface of the electrode and solution (biochemical criteria) and enable electrical potentials (E) to be measured as voltage differential between sensing and reference electrodes using voltmeter devices and/or potentiostat instrumentation. The voltage difference measured between the electrodes, electromotive force (EMF) corresponds directly to the target analyte concentration levels present in the sample matrix. Response curves obtained from systematic calibration procedures performed with known analyte concentrations allow for the quantitative determination of unknown sample concentrations based on measured responses to the analyte being tested. The voltage response generated by the sensing electrode is linearly proportional to the logarithmic concentrations of all electroactive compounds present in the reaction. This logarithmic relationship provides extensive ranges of detection, allowing for the measurement of both high and low levels of analyte concentration. Many biochemical reactions involve either the absorption or release of thermal energy providing opportunity to detect the occurrence and progression of reactions as they occur. Systems utilizing thermistor detection utilize temperature-dependent resistive devices as temperature sensors. This type of sensor detects thermal energy generated during the specific biochemical reaction being studied. Thermistors are resistive devices with a negative temperature coefficient that exhibit decreasing resistance values as temperatures increase. During enzymatic catalysis, heat is generated when thermal energy is released through the reaction. This heat generated causes the thermistor to decrease in resistance value. Changes in resistance correspond linearly to the amount of thermal energy released and can be used to determine the concentration of the

analyte. Temperature-dependent resistors exhibit variations in electrical resistance in response to fluctuations in ambient temperature. Thus, the incorporation of thermistors into biosensor designs enables the determination of temperature changes due to biochemical reactions and/or molecular interactions.

Systems utilizing thermistors offer users many advantages in terms of ease of operation, fast response times and high sensitivity levels via sensor design. Thermistor sensors can be used in applications in various industries such as clinical laboratories, environmental monitoring and food safety. Because different biochemical processes have unique thermal signatures associated with them, each individual reaction system requires a specially designed thermistor sensor and calibration procedure tailored to that specific system. Piezoelectric biosensors utilize the piezoelectric effect to detect specific types of molecules. The piezoelectric effect is defined as the ability of some materials to produce electric charge when mechanical pressure is applied. The piezoelectric biosensor utilizes the properties of crystal materials to determine the oscillation frequency (or amplitude) change that occurs after a target molecule has bound to the crystal surface. The basic operating principle of the piezoelectric biosensor is predicated on the binding (attachment) of either a biomolecule or analyte to the surface of piezoelectric crystals. Once this occurs, the overall mass of the crystal increases due to additional molecules binding to the crystal surface. The mechanical stress applied to the crystal, therefore, will change, affecting the oscillatory frequency as well. Piezoelectric crystals are often made from quartz-based materials because of their piezoelectric properties and usually have a specific geometric cut, i.e., an AT-cut configuration (to provide maximum sensitivity and stability).

(v) Optoelectronic Biosensors (OEBs)

OEBs consist of two key components (1) an optical signal conversion device that detects biological molecular interactions and transforms them into an optical measurement and (2) a

photo detection system that detects and quantifies the resulting optical signal changes. The optical signal conversion device serves as an interface between the biological interactions and the optical signal generated by the detection system using various photophysical mechanisms such as a decrease in light transmission, the measurement of the emitted luminescence from an interacting target molecule or an increase in the scattering of light from the target molecule. The choice of material used to produce the optical signal conversion devices is based on several factors including (i) the type of target molecule, (ii) the level of sensitivity required for detection, (iii) the level of operational stability required for long term operation and (iv) the level of compatibility required with the OEB system as a whole. The selection of the optical signal conversion device materials will be unique to the application. A few common optical signal conversion device materials used in OEBs include semiconductor-based materials, organic polymers and oxide materials (metallic oxides). Each has its own advantages for their intended applications. OEBs utilize various technologies to produce photo detection signals from optical signals generated by biological interactions. Some examples of photo detection technology used in OEBs include photodiodes made from silicon, photomultipliers and spectroscopic instruments. All of these technologies convert optical signals into an electrical signal that can be processed digitally and quantitatively analysed.

Optical Biosensor Technology

Optical biosensors are analytical devices that utilise the mechanisms of light and biomolecule interactions to measure the concentration of a substance or the presence of a specific biomolecule in a sample. There are many applications for this type of structure including medical research and diagnosis, food safety, environmental monitoring etc.

High Responsiveness and Efficiency

Optical biosensors are highly sensitive analytical instruments that allow the detection of small amounts of specific molecules. They can measure very low levels of substances and do so with high accuracy and fast response times.

Simple and Efficient Use

The optical biosensors are designed to operate without the need for any reference signals which simplifies the procedure for obtaining measurements compared to other types of analytical sensors.

Remote Monitoring and Detection Proficiency

Optical biosensors that are based on fibre-optic technology can transmit optical signals for long distances (hundreds of metres). Consequently, the optical signal can be transferred to multiple points without degradation, this enables the development of distributed sensing networks and allows for remote monitoring of processes.

Operational Limitations

The effect of non-direct light on environmental conditions

Ambient light may affect how accurately measurement can occur, therefore, shields must be in place to protect against this interference or additional technology will need to be utilized (e.g., filters) to facilitate accurate results.

Long-Term & Stability Issues

The stability of optical biosensor technology over long periods continues to create difficulty for manufacturers because of light source modulation, component wear-out and the effects of surface contamination. Consistent calibration and maintenance of an optical biosensor are required to maintain the sensor's long-term performance.

Response Kinetics

Optical technology typically has a longer response time compared to other technologies, especially when it comes to immunoassay-type systems where the binding process takes time before the optical signal can be measured. Improving sensor designs and advances in signal processing are now eliminating some of this delay.

Total Internal Reflection Spectroscopy (TIRS) Applications

Optical biosensors also similar to TIRS products use light propagated down an optical fiber or waveguide to introduce an evanescent field that is sensitive to weak non-covalent interactions (Ligand-receptor interactions) via their evanescent wavefronts through an area where they are predominately absorbed onto the waveguides. The area between the waveguide and the surrounding medium which contains the low-energy evanescent wavefront/s extends to a depth of 100 nm or more into the surrounding solution forming an interrogation area on which attachment of molecules to the recognition elements occurs. Since, target molecules can be only found at the surface of the termination of the optical waveguide, actual attachment events can only happen if the target concentration level exceeds the total binding capacity of the immobilized recognition elements. Therefore, for accurate quantitative detection of target molecules in a wide dynamic range, the amount of total binding events needs to be decreased and thus the signal has to be calculated by measuring the change in either the intensity or phase shift of the transmitting light. In addition, although TIRS-based products require minimal interference from components contained in the bulk solution, they are more sensitive compared to other methods, e.g., spectroscopy. Thus, when quantifying the presence of molecules in a real sample using TIRS-based products as the analytical tool of choice is ideal.

1.4. Future of Biosensors

Biosensor development is one of the most exciting potential areas of advancement in science as biosensors are highly advanced analytical devices that detect and quantify various biological molecules such as nucleic acids, enzymatic proteins and live cell structures. Many factors influence the positive future of biosensor innovations.

Biosensors technical foundation continues to improve as a result of breakthroughs made in research. New materials and techniques are continuously being discovered and developed by scientists resulting in greater accuracy of detection, reliability of measurement and increased portability of devices. Also, biosensors are experiencing rapid growth in sales as the demand for the various uses of these analytical devices continues to increase in the areas of medical diagnostics, environmental monitoring, food safety and the detection of threats to security.

1.4.1 Emerging Technological Developments Miniaturised and Wearable Monitoring Systems

The increasing trend towards miniaturisation of devices will result in the development of more sophisticated wearable monitoring systems. These systems can be made into fabrics, jewellery and implanted surgically into the body to provide continuous monitoring of health. These new systems will change the delivery of medical care to the individual by providing instantaneous feedback on physiological indicators, molecular markers of disease pathology.

1.4.2. Multi-target Detection & Integration of Systems

Next-generation biosensors will be able to identify multiple biological components simultaneously. The use of numerous analytes will help streamline testing processes, lower costs and create complete diagnostic databases. Such biosensors will also benefit from the convergence of other technological advancements such as complementary devices, e.g., microfluidics, nanotechnology and artificial intelligence (AI) which will make them more effective and expand their utility.

1.4.3. Diagnostic Tools at the Point of Care

The move to use advanced testing devices to deliver diagnostic services at the point of care will give health providers the tools to make quick diagnoses right where patients are located - whether that be in a doctor's office at home or in remote areas with limited resources available. This creates opportunities for health providers to detect disease processes earlier, manage chronic disease or start treatment right away thereby improving patient outcomes and lowering the overall cost of care.

1.4.4. Effectively Connecting and Digitally Integrating

Modern biosensors will become part of an integrated digital network with many other technologies. They will allow effortless transfer of information between them and improve the speed at which information is processed remotely. These biosensors will communicate with consumer electronics (smartphones and tablets), devices worn by users (neckbands, fitness trackers, etc.) and the internet via cloud computing systems to enable health monitoring at a distance and real-time analysis. This entire digital network will also support the creation of personalized health and wellness programs, remote supervision of patients and continuous monitoring of the health and well-being of the environment surrounding them.

1.4.5. Improved Detection Capability

Future sensor technologies are expected to provide the ability to detect smaller amounts of a particular item within a larger volume of biological material better than current equipment. There has been recent progress in a variety of areas, including advances in nanotechnology, improvements in signal amplification, and advances in bioengineering. These advances will be particularly valuable when it comes to early detection of diseases, detection of environmental contaminants and verification of food quality.

1.4.6. Non-Invasive Monitoring Solutions

As technology continues to advance, future sensor technologies will focus on developing solutions for non-invasive monitoring, allowing for the monitoring of patients without the need for surgical intervention or blood collection. New technologies will be developed that will be able to collect and analyse alternative biological specimens to obtain diagnostic information. In addition, continuous real-time monitoring will enable long-term physiologic monitoring of patients for better disease management and prevention strategies.

1.4.7. Applications of Biosensors for Therapy

The combination of sensing technology with therapeutic modalities will create new opportunities in the field of bioelectronic medicine. Through direct interfacing with the neural and/or targeted organ systems, biosensor/therapeutic devices can alter the course of biological processes provide effective management for chronic disease conditions and/or restore normal physiologic function. This new area of study has great potential for developing more personalised approaches to therapeutic treatment and precision medicine.

1.4.8. Transformative Impact

These innovations indicate the significant potential of biosensor technologies to change various industries and scientific fields. Continued collaboration between scientists and engineers through synergy will lead to more efficient, effective and flexible analytical devices able to be utilized for numerous areas of healthcare, environmental research etc.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction to Caffeic Acid

Caffeic acid is a phenolic compound that naturally occurs in many foods such as coffee, tea, fruits and vegetables and olive oil. Caffeic acid is one of several classes of molecules called hydroxycinnamic acids which belong to a large group of secondary plant compounds known as flavonoids. Flavonoids have been shown to provide a wide variety of health benefits. Caffeic acid is chemically classified as a hydroxylated form of cinnamic acid with a molecular formula of $C_9H_8O_4$. The structure of caffeic acid includes a hydroxyl (-OH) group attached to the phenyl ring of the cinnamate backbone. This hydroxyl substitution imparts to caffeic acid some of its unique chemical properties and it plays an important role in how caffeic acid interacts with other biochemicals. Caffeic acid's antioxidant abilities are the most notable reason for its increasing importance. Caffeic acid works as an antioxidant and thus protects the human body from the harmful effects of reactive oxygen species. Reactive oxygen species are unstable and can cause damage to a person's cell, causing an individual to develop diseases. Reactive oxygen species are especially involved in the development of malignancies, cardiovascular diseases and other medical problems. Caffeic acid effectively protects the integrity of the person's cells through its ability to scavenge reactive oxygen species. In addition to its ability to scavenge reactive oxygen species, caffeic acid also has powerful anti-inflammatory properties which increases its therapeutic capabilities. Caffeic acid's anti-inflammatory properties add to the overall therapeutic potential of caffeic acid, although inflammation is an important part of an individual's natural immune response to injury and infection. However, excessive inflammation is associated with increased risk of developing chronic diseases including malignancies, cardiovascular diseases and joint diseases. Caffeic acid's anti-inflammatory

effects are achieved by inhibiting the synthesis of pro-inflammatory mediators and therefore make caffeic acid a valuable resource in both natural therapies and scientific studies. Caffeic acid has also attracted significant interest for its potential anti-cancer properties. Cancerous diseases are characterized by uncontrolled cell division and caffeic acid may exert protective effects against cancer through several means including causing direct cell death to cancer cells and inhibiting their growth. In addition, caffeic acid influences the regulation of enzymes and genetic expression, thus affecting fundamental cellular mechanisms and how cellular networks communicate.

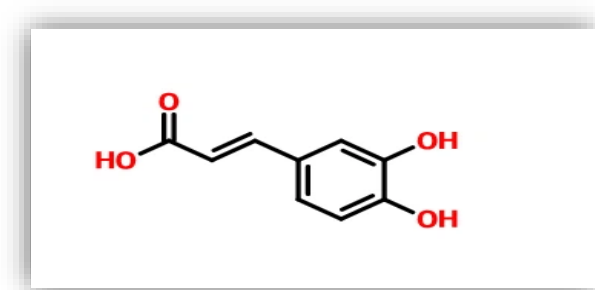


Fig. 2.1 Structure of Caffeic acid

Caffeic acid is a nutritional component widely found in many common food sources. However, the amount of caffeic acid present will vary depending on what type of food and how it is prepared [10]. As such, this compound can be found scattered amongst many food categories and due to its unique biological activity including antioxidant and anti-inflammatory properties, it continues to be a focus for nutritional and health-related research.

Indicative of the negative effects associated with caffeic acid consumption are mentioned below.

Sleep Disturbances: Caffeic acid is mildly stimulating and may interfere with a person's ability to obtain restful sleep.

Gastrointestinal Irritation: Certain individuals may experience gastrointestinal irritation, manifested as nausea, vomiting and diarrhea due to irritation of the gastric lining.

Hypersensitivity Reactions: For some individuals, caffeic acid may stimulate an immune-mediated reaction, producing skin rash, itching and swelling.

Drug Interactions: Caffeic acid may alter or interfere with the action of certain therapeutic medications, particularly anticoagulants (anti-blood clotting agents), through changing their efficacy or safety profile.

2.2. Importance of Analytical Detection

Analytical determination of caffeic acid provides several very important aspects in all areas.

Assurance of the Quality of Products for the Food & Beverage Industry

Caffeic acid, a naturally occurring phenolic compound is found in a wide range of food and beverage products originating from medicinal plants such as coffee, tea, fresh fruit and vegetables and many others. The analytical measurement of this compound indicates the quality of these products. The correct measurement of caffeic acid guarantees that the correct concentration of this compound was produced thus maintaining the flavour, aroma and health benefits associated with these products. Concerns related to the public are discussed below.

Health and Safety

The antioxidant and anti-inflammatory properties of caffeic acid, for example, have created much interest in the possible therapeutic use of this compound. Therefore, analytical measurement of caffeic acid at accurate concentrations provides valuable data allowing researchers to better define what role caffeic acid plays in the human body and establish guidelines and recommendations for the appropriate use of this compound as a nutritional food or a therapeutic product. Additionally, confirmation of the presence of caffeic acid in botanical

extracts, dietary supplements or any medicinal product provides consumer safety and reduces the potential for product adulteration and contamination.

Environmental Assessment and Monitoring

Caffeic acid is a phenolic metabolite that naturally occurs in the environment as well as being produced through human actions. Caffeic acid can be used to measure how much pollution is present in the environment and identify the type of wild plant that is growing.

By regularly analysing the concentration of caffeic acid, we have valuable information on how much humans are affecting different ecosystems. The level of caffeic acid also helps us protect our environment.

Quantitative analysis of caffeic acid is also extremely important when assessing nutrition and dietary guidance as well as monitoring food safety. In addition, as the development of accurate, rapid and inexpensive analytical techniques for determining caffeic acid is a breakthrough for food analysis and texture assessment methodology. The analytical detection of caffeic acid is thus essential for all areas of environmental protection (monitoring) as well as for the development of human pharmaceuticals.

2.3. Literature Review

There are a number of modern studies that have studied the use of nano-catalyst modifications to electrodes for detection of caffeic acid using many different types of analytical techniques. A review of the research works that have been done previously on this area of the field has provided a comprehensive look at how the characteristics of modified electrodes from these studies meet and compare to newly developed modified electrodes reported in the current literature. A comparison of the electrochemical sensing performance of different modified electrodes towards caffeic acid detection has been presented in Table 3.

Table 3: Comparison of the electrochemical sensing performance of different modified electrodes towards caffeic acid detection

S.No.	Immobilization matrix	Detection technique	Linear range (μM)	LOD (μM)
1.	Ag/g- C_3N_4 /ITO	CA	1.0 pM to 1.0 μM	0.75 pM
2.	MWCNT@Au-g-PMAEFc/GCE	DPV	50nM–10 μM	27 nM
3.	CHIT/Au electrode SGGT	DPV	300–3000nM	10 nM
4.	Lac/rGO-MoS ₂ /ITO	CA	1.0–100	0.1 μM
5.	AChE/ g- C_3N_4 @MoS ₂ /ITO	DPV	5–100nM	1nM
6.	Nafion-AChE/PB-DSPE	CA	0.1–5 $\mu\text{g/mL}$	0.1 μM
7.	MHPBC/GCE	DPV	0.1nM–10 μM	35nM

2.4. Properties of Material

The selection of appropriate electrode material is essential for electrochemical procedures. In order to detect caffeic acid, 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 caffeic acid. Graphitic carbon nitride (g- C_3N_4) and silver nanoparticle (AgNps) are the two major materials that have received a lot of attention.

2.4.1. Graphitic Carbon Nitride (g- C_3N_4)

Graphitic carbon nitride is a versatile two-dimensional material in its graphitic form (g- C_3N_4) represents one of the most thermodynamically stable configurations of carbon-nitrogen compounds at ambient conditions. The material exhibits structural similarities to graphene, featuring stacked layers interconnected through weak intermolecular van der Waals interactions [10]. By chemical exfoliation, the layered material can be separated into layers (individual nanosheets) to make a true 2D material of atomic thickness. While many of the properties of g- C_3N_4 can be derived from its nanosheet form (that is, nanosheet will have its own unique set of properties), g- C_3N_4 can also be synthesized into other

dimensional structures such as 1D rods and tubules or 0 D quantum dots. The properties of all these various shapes differ based on what function/management or use the materials are intended for.

The g-C₃N₄ nanosheets represent a unique type of material (non-metal, organic polymer) with some of the most desirable properties for use in a variety of scientific and engineering fields. Some of these properties include environmental compatibility through biodegradability, the ability to be produced sustainably, the tunability of its electronic bandgap (the ability to change its electronic properties) and a high nitrogen content which gives g-C₃N₄ a high availability of electrons.

Because of these beneficial properties, g-C₃N₄ has opened up many new and innovative applications in various fields of technology. Photocatalytic ways to make chemical products from solar energy can be provided with the help of the photocatalytic action of g-C₃N₄. Electric battery storage systems are also able to take advantage of g-C₃N₄'s electrochemical properties. Photovoltaic devices take advantage of the semiconductor properties of g-C₃N₄. Additionally, g-C₃N₄ is an important material for sensors and has recently received a lot of interest.

Sensing Applications and Advantages

The use of g-C₃N₄ as sensing devices has demonstrated their remarkable ability to provide high performance characteristics. As the result of g-C₃N₄'s surface chemistry, physiochemical properties and other features, g-C₃N₄ based devices exhibit a unique and exceptional sensitivity and selectivity to the target analytes [11,12]. g-C₃N₄ offers a high surface area consent for effective molecular adsorption resulting in the formation of strong interactions with the detection targets. Furthermore, the chemical stability of g-C₃N₄ ensures consistent performance over the long-term use which is essential in numerous environmental applications where g-C₃N₄ based sensors can be used for long-term environmental monitoring, pollutant monitoring, biological threats and toxic chemical analysis.

Significant Material Properties

- Atomic thickness in the form of sheets
- No metallic components in a polymer structure
- Electronically tuneable properties

- High structural stability
- Environmentally friendly and biodegradable

Prominent Areas of Applications

- Photocatalytic processes for remediation and production of hydrogen and chemicals,
- Development of sensors to monitor environmental conditions, detect pathogens and analyse chemicals

With its extensive structural flexibility, electronic characteristics and environmental properties, graphitic carbon nitride material is a highly consequential technological material. Because of the combination of high reactivity, stability and selective molecular interactions, graphitic carbon nitride is well positioned to provide the foundation on which many next generation technologies in sensing systems are built.

2.4.2. Silver Nanoparticles

Silver nanoparticles (AgNPs) represent ultrafine metallic particles with dimensions typically ranging from 1 to 100 nm [13]. These microscopic structures exhibit unique physicochemical properties that differ significantly from their bulk counterparts. Their high surface-to-volume ratio enhances the reactivity and biological activity.

These nanoparticles exhibit remarkable antimicrobial properties making them valuable in various medical applications including wound dressings and antimicrobial coatings. They effectively combat bacteria, viruses and fungi through multiple mechanisms including the disruption of cell membranes and interference with cellular processes.

Manufacturing methods include chemical reduction, biological synthesis using plant extracts or microorganisms and physical approaches like laser ablation. The synthesis method influences particle size, shape and stability which directly affects their performance characteristics.

Applications span across healthcare, textiles, electronics, water treatment and food packaging industries. In healthcare, they're incorporated into bandages, medical devices and diagnostic tools [14]. The textile industry utilises them for creating antibacterial fabrics and odour-resistant materials.

However, the environmental and health concerns regarding their widespread use continue to be studied, particularly their potential toxicity and bioaccumulation in ecosystems. Proper risk assessment and regulatory frameworks are essential for their safe implementation across various applications.

CHAPTER 3

MATERIALS & METHODS

3.1. Introduction

The fabrication of a biosensor system employing silver nanoparticles as well as a composite structure of graphitic carbon nitride with silver nanoparticles for the electrochemical detection of caffeic acid is covered in this section and describes various approaches and methods to characterise the materials used in designing the nanomaterials and composite materials. Detailed descriptions of the materials in terms of specifications and methods used to make the nanomaterials are being reported in this chapter.

Comprehensive descriptions and specifications of the chemical reagents and materials used in the investigation of the electrochemical detection of caffeic acid are provided in the sections below.

3.2. Materials

Below is a thorough description of all the materials used.

3.2.1. Chemical Reagents

All research materials and chemical reagents used in this project are being described in different subsections. Silver nitrate, as AgNO_3 , has a purity of 99.8% and is manufactured by Sigma-Aldrich. Graphitic carbon nitride was synthesized through thermal decomposition of melamine (5.0 g) at 550°C for a duration of 4 h [15]. Essential buffer components including sodium chloride (NaCl), monosodium phosphate (NaH_2PO_4) and disodium hydrogen phosphate hydrate (Na_2HPO_4) were acquired from Fisher Scientific India. Fresh *Phyllanthus emblica* (Indian gooseberry) samples were purchased from regional suppliers.

Working solutions of caffeic acid at various concentrations were prepared by serial dilution from a primary stock solution (1 mM) using phosphate buffer saline (PBS, 150 mM, pH 7.5). The electrochemical studies utilised PBS buffer (pH 7.5) containing $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as the electroactive probe, formulated using standardised concentrations of NaH_2PO_4 and Na_2HPO_4 with a 10% sodium chloride addition.

Indium tin oxide (ITO) substrate with 90% optical transparency, a sheet resistance of $25 \Omega/\text{sq}$, and a thickness of 1.1 mm was obtained from Blazers, UK. All reagents were of analytical grade and used without further purification. Deionized water was employed throughout all the experimental procedures.

3.2.2. Buffer Solutions

The following solutions were prepared for electrochemical measurements.

1. Phosphate buffer saline (0.1 M PBS) adjusted to pH = 7.5
2. The 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ was prepared in a phosphate-buffered saline (PBS) solution for use as the electrochemical redox probe.

3.3. Analytical Techniques

The nanomaterials synthesised composites built with nanomaterials and the bioelectrodes have been extensively characterised at various stages of their development using multiple techniques. Crystallinity and lattice parameters have been evaluated for both materials using X-ray diffraction (XRD). Bonds between atoms and identification of functional groups have been evaluated using Fourier-transform infrared spectroscopy (FTIR). The field-emission scanning electron microscope (FESEM) was used to observe the surface shape and size as well as the microstructure features. The electrochemical performance of the materials (redox

reactions) has been quantified using cyclic voltammetry (CV) and chronoamperometry (for measuring the current measured over time).

3.3.1. X-ray Diffraction (XRD)

The analytical technique of X-ray diffraction is an effective method for the study of how crystals are arranged atomically and molecularly. The basic function of this technique is based on the way that an electromagnetic (X-ray) waveform interacts with the orderly arrangement of atoms in a crystal lattice [16]. The result of this interaction is a diffraction pattern which represents the amplitude of the scattered RF signals.

When RF (X-ray) waves interact with a crystalline material, there are scattering events caused by the interaction of the electromagnetic waves with the systematic arrangement of the atoms in a crystal lattice. After the RF waves are reflected, they are then modulated by other RF waves that have also interacted with the same crystal. These multiple modulations produce a diffraction profile which once captured and analysed through suitable means allows researchers to ascertain the atomic distribution of the three-dimensional structure of the crystal's atomic framework as well as to derive. X-ray diffraction data provide important crystallographic information including unit cell parameters, the types of atomic symmetries and accurate atomic positions. This instrument (**Fig. 3.1**) is widely used in various fields to characterise the structure of minerals, metals, polymers, proteins and other crystalline materials. X-ray diffraction data are critical to better understanding how materials function, developing new materials with specific properties and conducting research on pharmaceuticals as well as for understanding the structure of biomolecules.

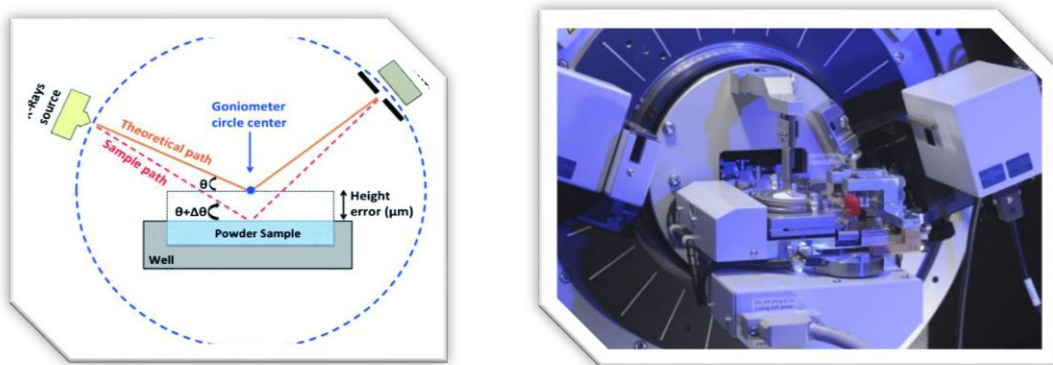


Fig. 3.1 (a) Schematic diagram of X-ray diffraction (b) Instrument diagram of X-ray diffractometer

3.3.2. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy is an analytical technique to measure how materials interact with infrared (IR) radiation. The FTIR process will expose various materials to a continuous view of the IR spectrum enabling them to selectively absorb only those IR wavelengths matching the vibrational states of their respective molecules. After being exposed to the IR spectrum, transmitted light is detected to yield an absorption profile (i.e., IR absorption spectrum). Through FTIR analysis, the chemical structure and composition of molecular materials can be determined by quantifying the IR energy sources absorbed, emitted or reflected which creates a unique signature profile for that substance. The absorption profile essentially encompasses every IR energy source and they may present identifying features associated with vibrations or rotations (or combinations thereof) of molecules; these movements occur at frequencies in the IR range of the electromagnetic spectrum (i.e., $100\text{-}10,000\text{ cm}^{-1}$).

Thus, IR spectroscopy provides a means of investigating many types of molecular characteristics including chemical bonding arrangements, functional groups, stoichiometric relationships and some basic molecular behaviour (i.e., a unique spectral signature). FTIR spectroscopy has been developed as a non-destructive method for analysing various types of samples, delivering rapid, precise and efficient results while allowing for both qualitative identification and quantitative evaluation of spectral information. The Michelson interferometer is the basis for the design of FTIR instruments (**Fig. 3.2**) which use a beam splitter to split the incoming radiation into two different paths of optical radiation.

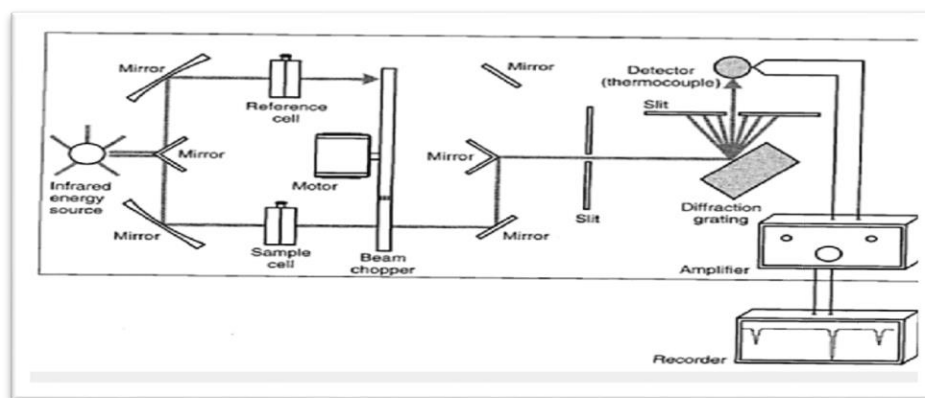


Fig. 3.2 Diagram illustrating the optical configuration of the Michelson Interferometer

3.3.3. UV-Vis spectroscopy (UV-Vis)

UV-Vis spectroscopy is a type of analytical procedure for analysing the absorption/transmission of certain wavelengths from ultraviolet and visible light through comparable samples with respective blank or reference samples. The nature, composition and composition characteristics of a sample have a direct effect on its optical behaviour which can then be used to identify the type and concentration of the sample's components. Since UV-Vis Spectroscopy is dependent on the interaction of electromagnetic wave energy, it is essential to understand the characteristics of light as a form of electromagnetic energy. In general, electromagnetic radiation is made up of two types of energy, known as potential energy (E)

and kinetic energy (K) and can therefore be defined using only those two forms of energy. Shorter wavelengths of electromagnetic radiation are associated with higher energy potentials and longer wavelengths of electromagnetic radiation are associated with lower energy potentials [17]. The process of raising the ground-state electron to an "excited" energy level also requires a set energy threshold in order to create an absorptive phenomenon detectable at that point. Different bonding environments create varying amounts of kinetic energy that must be present to excite electrons into the orbitals of the molecules resulting in the absorptive phenomenon. There are also different bonding arrangements and therefore some substances have been identified as having specific wavelengths at which they absorb electromagnetic radiation. In general, human-designed vision can be viewed as detecting electromagnetic radiation from approximately 380 to 780 nm (violet to red). Ultraviolet radiation comprises wavelengths that are even shorter than the detectably visible portion of light (ultraviolet radiation has a wavelength of around 100 nm).

Due to their specific properties related to the way different forms of electromagnetic waves absorb at certain wavelengths, UV-Visible Spectrophotometers (**Fig. 3.3**) have a very broad application potential within many analytical laboratories for identifying and quantifying unknown materials. When working with UV-VIS Spectrophotometers, samples are placed in cuvettes that allow direct light to pass through the sample, allowing for the determination of maximum absorbance wavelengths at each wavelength for each sample

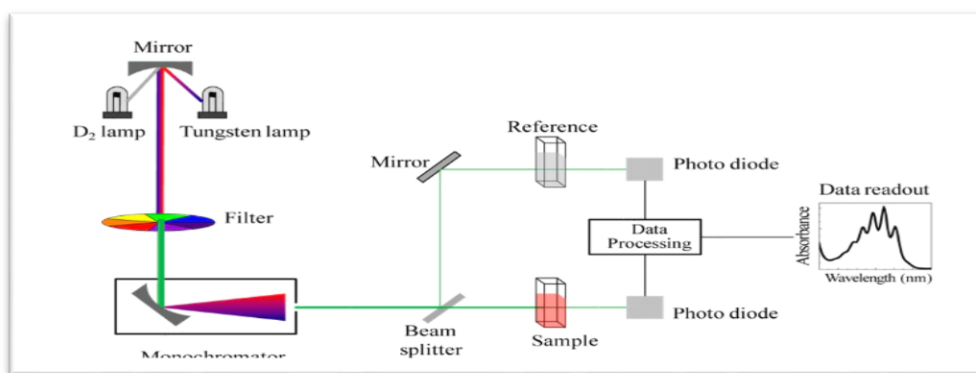


Fig. 3.3 Pictorial representation of UV-Visible Spectrophotometer

3.3.4. Field Emission Scanning Electron Microscopy (FESEM)

This is a specific type of scanning electron microscopy that utilises a field-emission source for the creation of a concentrated electron beam. FESEM provides a high level of spatial resolution on the surface of a given specimen (i.e., down to less than 1 nm) [18]. In FESEM, an electron microscope (**Fig. 3.4**) is housed inside a vacuum chamber where the sample is placed. The high-voltage source of the FESEM typically employs a sharp metal (usually tungsten) tip to direct a high-voltage electric field to the specimen surface [19,20]. The electric field produced by the applied high voltage causes electrons to be emitted from the metallic tip and directed toward the sample surface where they interact with and acquire energy from the specimen resulting in the production of secondary electrons. Secondary electrons emitted from the surface are collected by a detector and displayed on a computer. The device converts electrons into an electrical signal which is then displayed on a monitor. FESEM images are used for analysing a sample's surface morphology such as identifying grain boundaries, porosity or defects present on the surface of a material [16,21,22].

In addition, information about the chemical composition of a sample can be provided through FESEM by utilizing energy dispersive X-ray spectroscopy (EDS) to measure the X-rays generated when the electron beam strikes the sample.

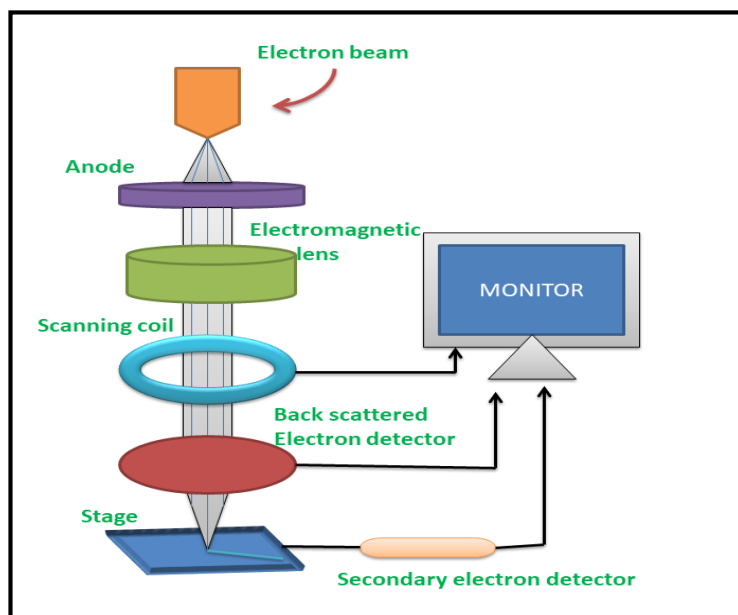


Fig. 3.4 Pictorial representation of FESEM

3.3.5. Electrochemical Methods

Electrochemistry is a key analytical approach for determining potential, charge or current as indicated by analyte concentrations and/or chemical reaction parameters. Electrochemical techniques focus on examining the interactions that occur within an electrochemical system when a voltage is applied.

Electrochemical cells contain three necessary electrodes (working, reference and counter). All three electrodes are used in conjunction with a controller (a potentiostat) which controls the voltage on the working electrode while recording the current response. In most electrochemical experiments, a set voltage is applied to the working electrode and the resulting current is recorded over time. When a linear voltage is applied, a graph of current versus applied voltage is produced. The interaction between voltage and electrochemical reaction in an electrochemical system is defined by the Nernst equation; it also defines how the concentration of oxidized and reduced species in an electrochemical system at equilibrium is related to the electrochemical cell potential and given in equation (1).

$$E = E_0 \pm \left(\frac{RT}{nF}\right) \ln \frac{C_{oxi}}{C_{red}} \quad (1)$$

The parameters E , E_0 , C_{oxi} and C_{red} represent measured potential, standard equilibrium potential, concentration oxidized species and reduced species respectively for this out-of-equilibrium redox system. The parameters R , T , F and n represent universal gas constant, absolute temperature, Faraday constant and number of electron charges associated with the ionic species respectively [23]. The Nernst equation states that when a potential is applied to the working electrode, the concentrations of the oxidized and reduced forms at the electrode surface will change due to the effect of the redox system on the ratio of these two concentrations. The evaluation of the mechanisms of signal transduction and the electrochemical performance of sensing devices (CV and chronoamperometry) has been performed using potentiostat/galvanostat instruments (**Fig. 3.5**) with platinum as the counter electrode and Ag/AgCl reference electrode.

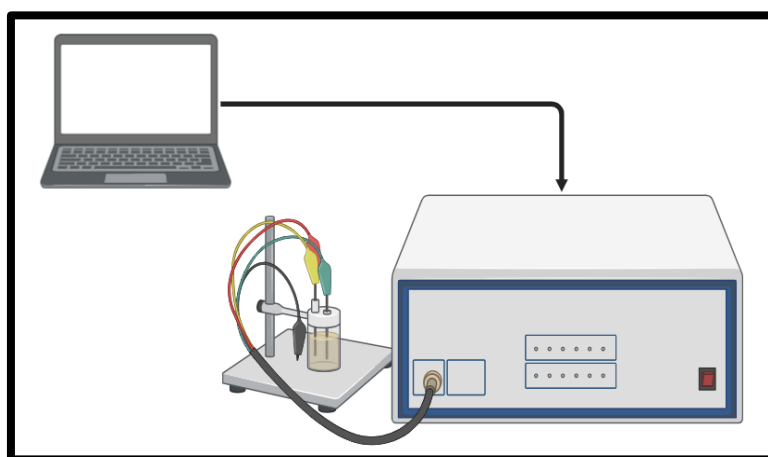


Fig. 3.5 Pictorial view of Potentiostat

3.3.5.1. Cyclic Voltammetry (CV) Measurements

Cyclic voltammetry is widely used to understand the electrochemical properties of different materials. In cyclic voltammetry experiments, the working electrode potential changes in a controlled manner as a function of time [24]. A predetermined potential has been reached at which point the working electrode potential is reversed back to its original value. The series of potential cycles will continue until sufficient information is available to build complete cyclic voltammetry profiles.

Through cyclic voltammetry, an understanding of electrochemical species that have established oxidation/reduction potentials based on their electroactivity can be acquired. During the initial stage of potential scanning, the current at the working electrode is recorded against the predetermined reference electrode potential while the corresponding counter electrode allows current to flow from the source to the working electrode. The ionic solution is required to provide the necessary ions to the surface of the electrodes as the electrochemical reaction proceeds.

3.3.5.2. Chronoamperometry

Chronoamperometry is a technique used in electrochemistry to study the interaction between an electrode and electrolyte. This technique measures the current flowing through an electrode over time while maintaining a constant potential/voltage across the electrode. Chronoamperometry can be used to study various aspects of an electrochemical process including electrochemical reactions, electron transfer rates and diffusion-controlled systems. The basic idea behind chronological amperometry is that the current flowing through an electrode is directly proportional to the quantity of electroactive species located on the electrode surface. When a potential step is applied to the electrode, a large amount of current flows through the electrode because a significant increase in electroactive species occurs at the electrode's surface causing the current to flow through the electrode. Once the electroactive

species leave the electrode surface and begin to diffuse away, the concentration of electroactive species at the electrode surface decreases and the associated current decreases as well. Chronoamperograms which are the resulting current vs time plots created using chronoamperometry include information on the electrochemical phenomena occurring within the electrochemical system being studied. For example, the plot can be used to find out the rate constant of the chemical reaction, the flow rate of the electroactive species and the surface area of the electrode being studied.

Often used to investigate the rate at which ions diffuse through solutions, it is commonly used to determine how quickly atoms and molecules move through the electrolyte and how quickly they are adsorbed by the electrodes.

Below is a list of some ways in which chronoamperometry can be used.

- To determine the rate constant for a specific chemical reaction
- To determine the diffusion coefficient of an electroactive substance
- To determine the surface area of an electrode
- Determine the characteristics of electrochemical reactions
- Find out how fast ions diffuse through a solution

CHAPTER 4

Ag@g-C₃N₄ MODIFIED ELECTROCHEMICAL BIOSENSOR FOR THE DETECTION OF CAFFEIC ACID

4.1. Introduction

This chapter outlines the development of a sensitive biosensor for detecting caffeic acid using an electrochemical sensor to identify caffeic acid. This was accomplished by employing silver nanoparticles (AgNps) and Ag@g-C₃N₄ as a nanocomposite material to develop the analytical device by using hydrothermal synthetic methodology to prepare Ag@g-C₃N₄ to construct the sensor and subsequently, the hydrothermally prepared nanocomposite (Ag@g-C₃N₄) was deposited electrophoretically on the glass plate with an ITO coating to form the sensor. The hydrothermally deposited material was characterised using both microscopy and electrochemical techniques. The performance of the biosensor prepared in this manner is reproducible and selective for the determination of caffeic acid.

4.2. Methodology

4.2.1. Synthesis of g-C₃N₄

Graphitic carbon nitride (g-C₃N₄) was obtained by the thermal polymerisation of melamine. The g-C₃N₄ was synthesised by the addition of 5 g of melamine into a closed crucible and thermally cycling it at 5°C per minute for 4 h, finally reaching 550°C in temperature inside a muffle furnace and allowing the melamine sample to cool and wash in several consecutive steps in distilled water followed by washing in ethanol, vacuum drying and then grinding into a powder form.

4.2.2. Synthesis of Nanocomposite

An electrodeposition technique was employed by using a g-C₃N₄ composite with varying amounts of Ag nanoparticles. The following is a description of the Ag@g-C₃N₄ composites preparation method. First, 50 mg of g-C₃N₄ powder and 50 ml of distilled water were combined in a 250 ml beaker. At 30°C, the resultant mixture was sonicated for 60 min. Next, 25 ml of distilled water was added to a 100 ml conical flask containing 0.2 g of dried amla mucilage and the previously produced amla filtrate.

Additionally, 25 mL of distilled water was added to a second 100 mL conical flask containing 25 mM AgNO₃ (0.109 g). To stop photolysis, the two solutions were combined in a 250 ml conical flask and then covered with foil paper. Afterwards, 1.5 M of NaOH was introduced to the mixture which had a pH ranging from 8 to 11. The mixture was then heated for 40 min at temperatures between 40 and 50°C while being stirred, followed by centrifugation. Through the processes of centrifugation, washing with alcohol and deionised water and drying at 60°C, the Ag@g-C₃N₄ composite was formed.

In this synthesis, various loadings of AgNPs 1%, 3%, 5%, 8%, and 10% (w/w based on the weight of g-C₃N₄) were utilised to create the corresponding Ag@g-C₃N₄ composites which were labelled as Ag@g-C₃N₄-1, Ag@g-C₃N₄-3, Ag@g-C₃N₄-5, Ag@g-C₃N₄-8, and Ag@g-C₃N₄-10, respectively.

4.2.3. Electrophoretic deposition (EPD) of Ag@g-C₃N₄ on ITO electrode

The synthesised g-C₃N₄ hybrid was electrophoretically deposited onto the pre-hydrolysed ITO (1.4×0.7 cm) covered glass substrate using a three-electrode setup that included RE (reference electrode), CE (counter electrode) and WE (working electrode). The platinum wire, counter electrode and ITO are all separated by one cm. Before deposition, 0.5 mg of Ag@g-C₃N₄ is ultrasonically dissolved in 15 mL of deionised water to create a colloidal suspension. Then, at a perfect DC potential of 10V, the deposition is performed for 10 s.

4.2.4. Fabrication of Ag@g-C₃N₄ Nanocomposite-Based Biosensor

The fabrication of the biosensor was carried out by incubating the Ag@g-C₃N₄ electrode with Lac (1 mg/mL) using EDC-NHS as a cross-linker. The fabricated electrodes were stored at 4°C overnight. The fabricated electrodes were washed with phosphate-buffered saline (PBS; 0.1 M, pH 7) prior to use in bio-sensing applications.

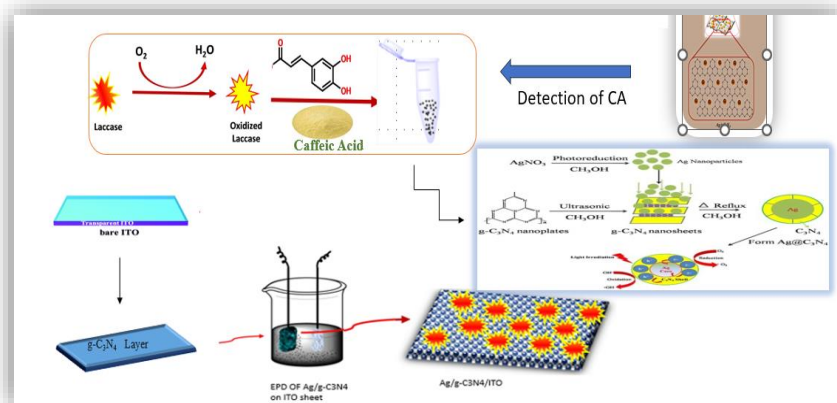


Fig. 4.1 Illustration for the detection of CA using Ag@g-C₃N₄/ITO

4.3. Results and Discussion

4.3.1. Structural Studies

The structural features of synthesized materials were confirmed by X-ray diffraction (XRD) studies. The broadening of the diffraction peaks and high intensity indicate the good crystalline nature of AgNPs. In the study of X-ray diffraction (XRD), the crystalline structure was appraised to identify and phases of nanomaterials. Fig.4.2 illustrates the XRD patterns for g-C₃N₄ and Ag@g-C₃N₄ nanosheets. The distinct peaks observed for both g-C₃N₄ and Ag@g-C₃N₄ suggest that the synthesised nanomaterials are devoid of impurities. The peaks for g-C₃N₄ were located at 12.8° and 27.6° aligning with values documented in earlier research concerning g-C₃N₄ nanomaterials. The peak intensity at 27.6° is attributed to the layer packing of

conjugated aromatic systems, corresponding to the (002) plane in g-C₃N₄. A less prominent peak was also noted at 12.8°[25].

According to the JCPDS 04-0783 card, the packing arrangement of tri-s-triazine units, as reflected in the (100) plane, appears to be well-defined. In the XRD pattern of Ag@g-C₃N₄, the peaks associated with silver (111), (200), (220) and (311) indicate its cubic structure. The presence of the (002) plane at 27.6° further confirms its existence within the graphitic structure. One reason the (100) peak was not visible in the Ag@g-C₃N₄ pattern might be that a broad, weaker peak at 12.8° is concealed by the overlapping sharp and intense peaks of silver [26]. The plasmonic properties of silver along with its uniform distribution in the Ag@g-C₃N₄ samples likely contribute to the heightened intensities of Ag peaks seen in the Ag-doped g-C₃N₄ samples. After synthesising Ag@g-C₃N₄, the crystal phase structures of both silver and g-C₃N₄ remained unchanged as demonstrated in Fig. 4.2.

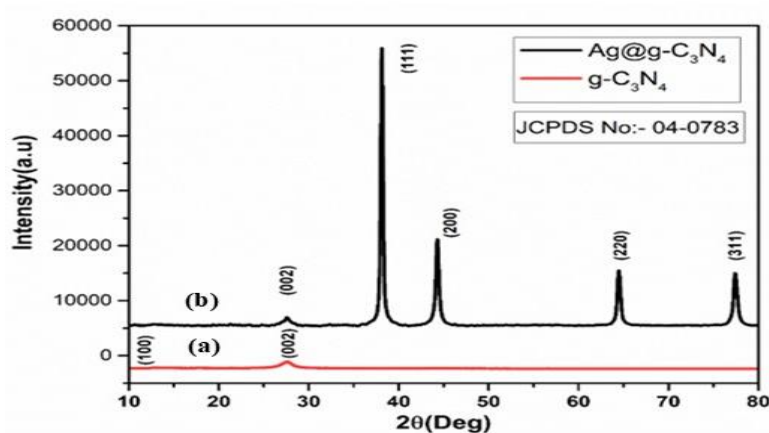


Fig.4.2 XRD of (a) g-C₃N₄ (red) and (b) Ag@g-C₃N₄ (black)

Ag@g-C₃N₄ composites with varying Ag loading levels and pure g-C₃N₄ nanosheets had comparable FTIR spectra (Fig. 4.3). The aromatic C-N is brought about by the peaks at 1244, 1569 and 1640 cm⁻¹ whilst the stretching vibration of C-N groups is responsible for the peak at 1569 cm⁻¹ [27]. For g-C₃N₄'s FTIR spectrum, a number of distinctive peaks typically appear, representing the material's various vibrational modes. These include peaks associated with the

breathing mode of the heptazine or s-triazine ring as well as C-N and C=N stretching. At around 1640 cm^{-1} (C=N), 1569 cm^{-1} (C-N), 1244 cm^{-1} (C-N) and 809 cm^{-1} (heptazine ring) peaks are commonly observed [28]. N defects and g-C₃N₄ precursor doped with Ag [29,30].

FTIR spectroscopy enhanced our understanding of the molecular structures of N-H and O-H stretching vibrations. The N-H and O-H stretched maxima have shifted to 3171 and 3435.65 cm^{-1} , respectively as shown in Fig. 4.3a broadened due to terminal N-H groups, adsorbed water or surface hydroxyls. Additionally, a clear peak appears to reflect the asymmetrically growing vibrations of the cyano sites. Modest changes to C-N or C=N peaks indicate an electrical contact between the Ag and N atoms in g-C₃N₄ which is frequently associated with partial charge transfer or coordination.

The breathing mode of triazine units is represented by the peak at 809 cm^{-1} . All of these distinctive FTIR peaks imply that following AgNPs loading the general structure of g-C₃N₄ retains its original form.

The existence of g-C₃N₄ and its interaction with silver are indicated by a number of distinctive peaks in the Ag@g-C₃N₄ FTIR spectrum (Fig.4.3b). Here, g-C₃N₄ is frequently seen to exhibit the peaks at 808 cm^{-1} (triazine ring breathing), $1220\text{-}1650\text{ cm}^{-1}$ (C-N stretching) and 1629 cm^{-1} (C=N stretching) [31]. Additional peaks associated with Ag-O or Ag-N bonds may emerge with the incorporation of silver perhaps expanding or displacing pre-existing peaks. The particular synthesis technique and silver loading determine the precise number of peaks.

Bands Ag-N or Ag-O bonding indicate that silver nanoparticles have been successfully decorated on the g-C₃N₄ framework below 600 cm^{-1} [32,33]. Ag may decrease surface-adsorbed water or alter the hydrogen-bonding environment as indicated by the broad N-H or O-H stretching region. With minor changes in functional vibrations and additional low-wavenumber

properties, Ag@g-C₃N₄ indicates that the Ag-N bonding was successful without causing damage to the g-C₃N₄ structure. The combined effect of Ag and g-C₃N₄ suggests enhanced plasmonic and photocatalytic capabilities.

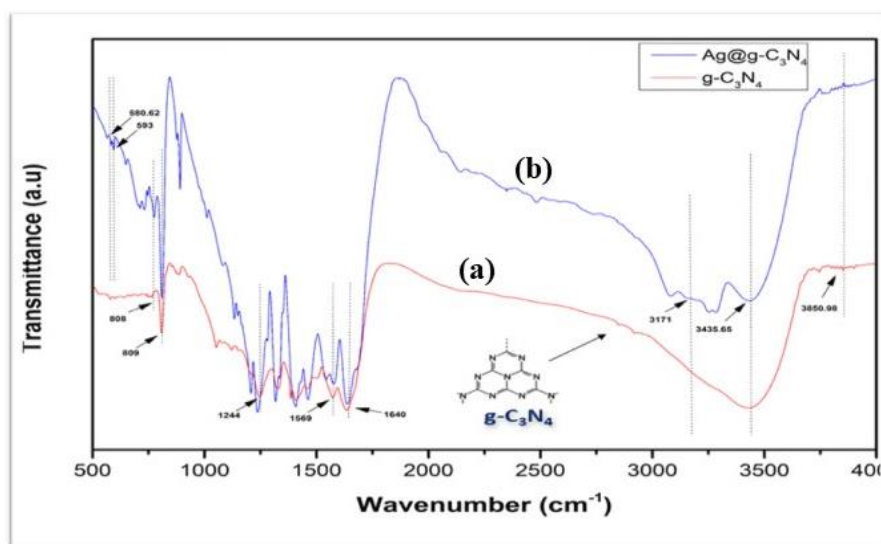


Fig. 4.3 FTIR of (a) g-C₃N₄ (red) and (b) Ag@g-C₃N₄ (blue)

A Cary 500 UV-vis-DRS spectrometer was used to record UV-vis diffuse reflectance spectra (UV-vis/DRS). UV-vis-DRS is used to examine the light absorption characteristics of the g-C₃N₄-based materials; the outcome is displayed in Fig. 4.4 [34]. Pure g-C₃N₄ shows a typical semiconductor absorption in the region of 200 - 450 nm, originating from the charge transfer response of g-C₃N₄ from the VB populated by N of 2p orbitals to the CB formed by C of 2p orbitals. The characteristic absorption edge of G (g-C₃N₄) is located between ~360 and 400 nm which corresponds to its intrinsic bandgap transition ($n \rightarrow \pi^*$) which is normally approximately 2.7 eV [35]. The surface plasmon resonance (SPR) of silver nanoparticles is characterised by a markedly increased feature near ~270 nm in GA (Ag@g-C₃N₄).

UV-visible spectroscopy analysis is used to examine the optical adsorption characteristics of pure, unaltered g-C₃N₄ nanosheets and Ag@g-C₃N₄ composite materials (Fig.4.4b). In my sample the ~269.63 nm peak aligns well with an AgNPs SPR band [13,36] indicating that Ag

is present as nanoparticles on the $g\text{-C}_3\text{N}_4$ surface. The peak around 270 nm in GA (black) in Fig.4.4 corresponds to the SPR absorption of silver nanoparticles decorating the $g\text{-C}_3\text{N}_4$ confirming a successful Ag coating. In comparison to $g\text{-C}_3\text{N}_4$, $\text{Ag}@g\text{-C}_3\text{N}_4$ exhibits red-shifted and enhanced bandgap absorption (311-361 nm) as a result of AgNPs' enhancement of light absorption and promotion of charge separation.

From the inherent band gap of bulk $g\text{-C}_3\text{N}_4$, pure $g\text{-C}_3\text{N}_4$ contains an absorption edge of light at 269.63 nm. Even though the $\text{Ag}@g\text{-C}_3\text{N}_4$ composite has a spectrum of light absorption that is comparable to that of pure $g\text{-C}_3\text{N}_4$, Fig.4.4 (b) shows that visible light absorption has increased. As the ratio of silver loading increases, the samples' hue shifts from yellow to dark grey.

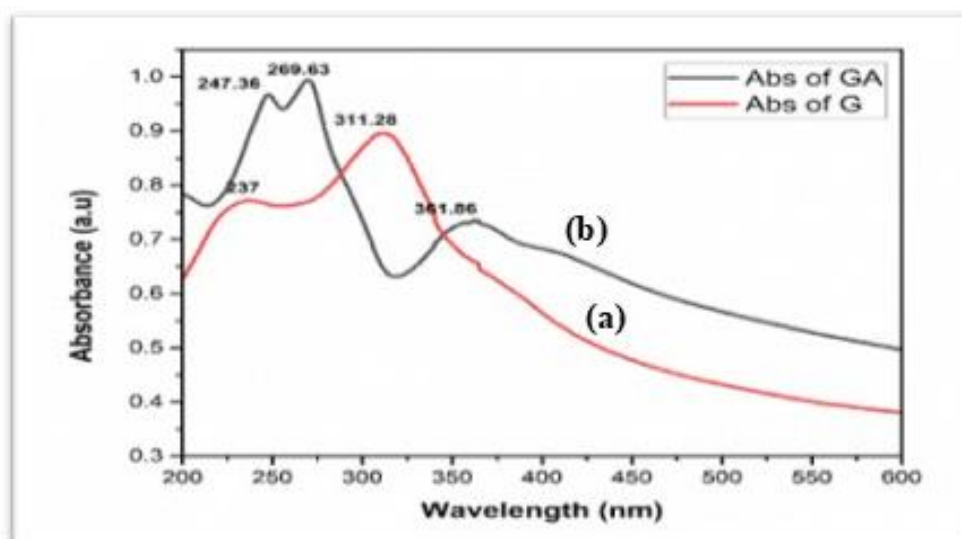


Fig. 4.4 UV-visible spectra of absorption as (a) G: $g\text{-C}_3\text{N}_4$ and (b) GA: $\text{Ag}@g\text{-C}_3\text{N}_4$

4.3.2. Morphological Studies

In contrast to pristine $g\text{-C}_3\text{N}_4$ which is composed of clumps that are several micrometres in size (Fig. 4.5), the typical $g\text{-C}_3\text{N}_4$ appears to be loose. FESEM at a voltage of 5 KV was used to examine the surface morphology of $g\text{-C}_3\text{N}_4$, Ag nanoparticles and Ag-doped $g\text{-C}_3\text{N}_4$. As shown in Figures 4.5 (a) (i) and (ii), the 100 nm FESEM shows that porous sheet-like topologies

confirm that the electron beam separated the secondary electrons that were expelled from the graphitic carbon nitride as g-C₃N₄[37]. Consequently, it agglomerates readily at a size of 1 micrometre. All of the samples had a tremella-like structure. But the fluffiness of g-C₃N₄ decreased as Ag-NPs were doped [38,39].

Ag nanoparticles with a spherical form covered the g-C₃N₄ sheets' surface (Fig. 4.5). The Ag@ g-C₃N₄ retains its morphology which displays a texture characteristic of flaking, exfoliated and wrinkled nanosheets (Fig.4.5). With Ag nanoparticles evenly distributed among nanosheets, Ag@g-C₃N₄'s appearance is further confirmed to be rather comparable to graphene by the high-magnification FESEM image shown in Fig.4.5 [40]. Ag nanoparticles which are black specks about 10 nm in size and evenly distributed across the thin surface of the g-C₃N₄ nanosheet are readily visible.

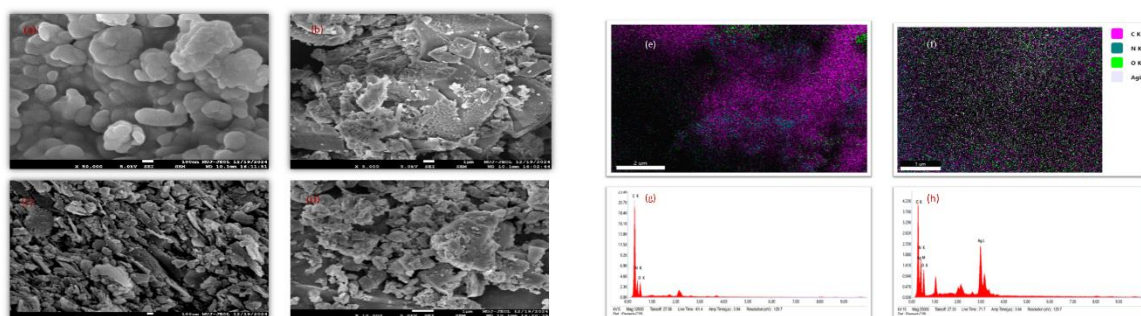


Fig.4.5 FESEM images of (a) &(b) g-C₃N₄ (c) & (d) Ag@g-C₃N₄ (ii) Elemental mapping for compounds (e) g-C₃N₄, (f) Ag@g-C₃N₄ and EDAX (Energy Dispersive X-ray Analysis) spectra of (g) g-C₃N₄ and (h) Ag@g-C₃N₄

EDX analysis of the Ag@g-C₃N₄ sample revealed atomic % ratios of Ag, N, O and C to be 2.6, 31.1, 14.8 and 51.5%, respectively. While the weight per cent ratios of Ag, N, O and C were 17.7, 27.8, 15.1 and 67.72%, as shown in Figs. 4.5 (e) and (f). Additionally, four elements (Ag, N, O and C) were found in Fig.4.5 (g) [36,41] from the elemental mapping of the Ag-doped g-C₃N₄ (1.5 mM) samples indicating that the Ag-doped g-C₃N₄ 1.5 mM nanocomposites were

successfully generated. The presence of carbon, nitrogen and oxygen in the sample has been verified.

Additionally, we can see that Ag and g-C₃N₄ formed a very close link to form the heterostructure, ensuring smooth charge transfer in the space. According to the FESEM, the EDS with its elemental analysis data, a picture of Ag@g-C₃N₄ is inset in Fig. 4.5 showing that the (111) lattice planes of metal Ag have a lattice spacing of 0.236 nm.

4.3.3. Electrochemical Characterization

As illustrated in Fig. 4.6 (a), electrochemical impedance spectroscopy (EIS) was used to study the electrochemical properties to investigate the electron transfer resistance of various fabricated electrodes including ITO, g-C₃N₄/ITO, Ag@g-C₃N₄/ITO and Lac/Ag@g-C₃N₄/ITO in pH=7.5 of PBS (containing 5 mM [Fe(CN)₆]^{3-/4-}) in the 0.01 to 10⁵ Hz frequency range (set potential 0.01 V). The outcome shows that g-C₃N₄ can transmit electrons more effectively than the modified bare electrode [42]. The Ag@g-C₃N₄/ITO is the composite-modified electrode.

Electrochemical impedance spectroscopy (EIS) was performed in the 0.01–10⁵ Hz frequency range with a set potential of 0.01 V under PBS containing 5 mM of [Fe(CN)₆]^{3-/4-}. The interfacial behaviour of the electrode surface upon change has been studied using it. The Nyquist plot is displayed for each of the several manufactured electrodes in Fig.10(a). Fig. inset 4.6 (a) represents an equivalent circuit comprising charge transfer resistance (R_{ct}), solution resistance (R_s), a constant phase element (CPE) and Warburg impedance (Z_w). GCN₃ (g-C₃N₄/ITO) and GA4 (Ag@g-C₃N₄/ITO) electrodes. R_{ct} was found to be 3.04 kΩ and 1.15 kΩ, respectively.

$$i_o = nRT / R_{ct} F \quad (2)$$

$$K_{app} = RT / n_2 F_2 A R_{ct} C \quad (3)$$

The decrease in the R_{ct} value of the Ag@g-C₃N₄/ITO electrode compared to the g-C₃N₄/ITO electrode is attributed to the strong cohesion between the Ag and g-C₃N₄ interfaces.

Additionally, the exchange current per geometric unit area (i_0) and heterogeneous electron transfer rate constant (k_0) of the various electrodes have been assessed using the R_{ct} values in conjunction with equations (2) and (3) [8,43]. Where F is Faraday's constant, R is the universal gas constant, n is the number of electrons transferred, A is the geometrical area of electrodes, T is the Kelvin temperature and C is the electroactive redox probe's concentration. The exchange current per geometric unit area " i_0 " for g-C₃N₄/ITO electrode and Ag@g-C₃N₄/ITO electrode was found to be 8.487 and 22.434 μAcm^{-2} , respectively [43]. Furthermore, the value of apparent electron transfer rate constant " k_{app} " for $[\text{Fe}(\text{CN})_6]^{3-/4-}$ using the respective g-C₃N₄/ITO electrode and Ag@g-C₃N₄/ITO electrodes was found to be 1.143×10^{-2} and $3.017 \times 10^{-2} \text{ cm.s}^{-1}$, respectively. Because of the synergistic interaction between AgNPs and g-C₃N₄, the Ag@g-C₃N₄/ITO electrode has greater values of " i_0 " and " k_{app} ".

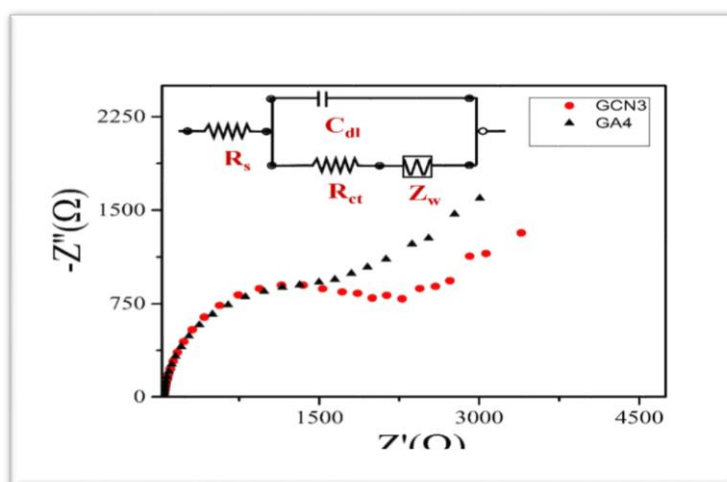


Fig. 4.6 Nyquist plot for the electrodes (a) GCN3: g-C₃N₄/ITO and (b) GA4: Ag@g-C₃N₄/ITO

In PBS containing 5 mM of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ the Ag@g-C₃N₄/ITO electrode (480.11 μA) exhibits the maximum current response in comparison to g-C₃N₄/ITO (428.79 μA) and bare ITO electrode (358.12 μA), at a scan rate of 50 mV/s based on cyclic-voltametric investigations carried out in the potential window of -0.6 to +0.6 V (Fig. 4.7a).

The increased surface area that facilitates electron transfer may be the cause of the Ag@g-C₃N₄/ITO electrode's improved conductivity which is expressed as:

$$I_{pa}(\mu A) = 1.10764 \times 10^{-4} + 4.80024 \times 10^{-5} \sqrt{v} \text{ (mV/s)}, R^2 = 0.99475 \quad (4)$$

$$I_{pc}(\mu A) = -1.11675 \times 10^{-4} - 3.39613 \times 10^{-5} \sqrt{v} \text{ (mV/s)}, R^2 = 0.99613 \quad (5)$$

The scan rate studies (10 - 120 mV/s) using CV have been performed for Ag@g-C₃N₄/ITO electrode (Fig.4.7b). It has been observed that redox potential plotted against log (scan rate) increases linearly for modified Ag@g-C₃N₄/ITO electrodes (Fig.4.7d).

$$E_{pa}(V) = 0.1328 \ln (v) + 0.0792; R = 0.9755 \quad (6)$$

$$E_{pc}(V) = 0.1742 \ln (v) - 0.0928; R = 0.9868 \quad (7)$$

The charge transfer coefficient (α) and heterogeneous electron transfer rate constant (K_s) are linked to the oxidation/reduction of [Fe(CN)₆]^{3-/4-} according to Laviron's equation [44]. Using the Ag@g-C₃N₄/ITO electrode, these values were found to be 0.927 and 0.080 s⁻¹, respectively.

$$\ln (K_s) = \alpha \ln (1 - \alpha) + (1 - \alpha) \ln(\alpha) - \ln (RT/ nFv) - \alpha (1 - \alpha) nF\Delta E_p/RT \quad (8)$$

Likewise, a linear relationship can be observed in a plot of the anodic peak current of CV versus the square root of the scan rate (Fig. 4.7c). A diffusion control method that complies with Equations 4 to 9 is indicated when the oxidation peak gradually rises to positive values with increasing scan rates.

The diffusion coefficient (D) for the Ag@g-C₃N₄/ITO electrode was found to be 1.298× 10⁻⁵ cm² s⁻¹ utilising linearity curves and the Randles-Sevick equation.

$$I_p = (2.69 \times 10^{-5}) n^{3/2} ACD^{1/2} v^{1/2} \quad (9)$$

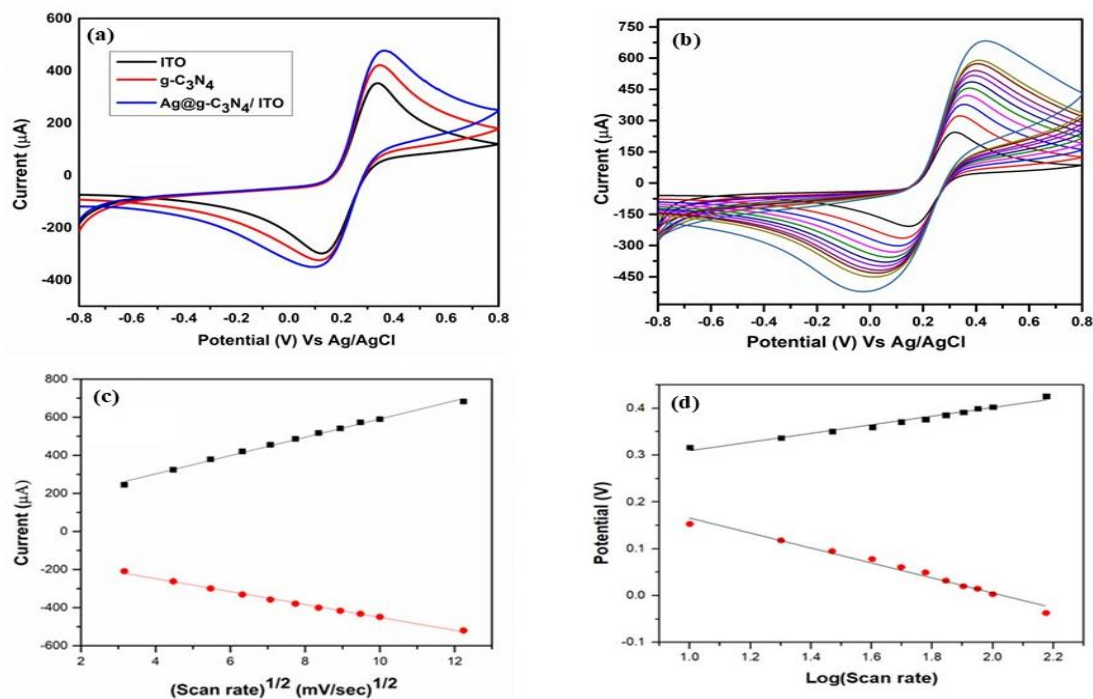


Fig. 4.7 (a) CV analysis of different fabricated electrodes: ITO (Black), g-C₃N₄ (Red) and Ag@g-C₃N₄/ITO (Blue) (b) CV response of electrode Ag@g-C₃N₄/ITO in a solution of 0.1 M PBS containing 5 mM [Fe(CN)₆]^{3-/4-} at various scan rates (10-120 mVs⁻¹) (c) Plot of peak current versus square root of scan rate and (d) Plot between peak potential versus log scan rate for Ag@g-C₃N₄/ITO

4.3.4 Optimization of Experimental Parameters

A pH range of 6.0 to 8.0 has been employed to investigate the effect of pH on the electrochemical performance of g-C₃N₄/Ag/ITO electrodes (Fig.4.9a). It has been observed that the highest current response was obtained at pH 7.5.[36,45] The concentration of the Lac enzyme has also been optimised by varying the enzyme concentration from 0.5 to 2.5 mg mL⁻¹ clearly indicating the presence of redox peaks for CA detection using a Lac/Ag@g-C₃N₄/ITO electrode in PBS (pH 7.5).

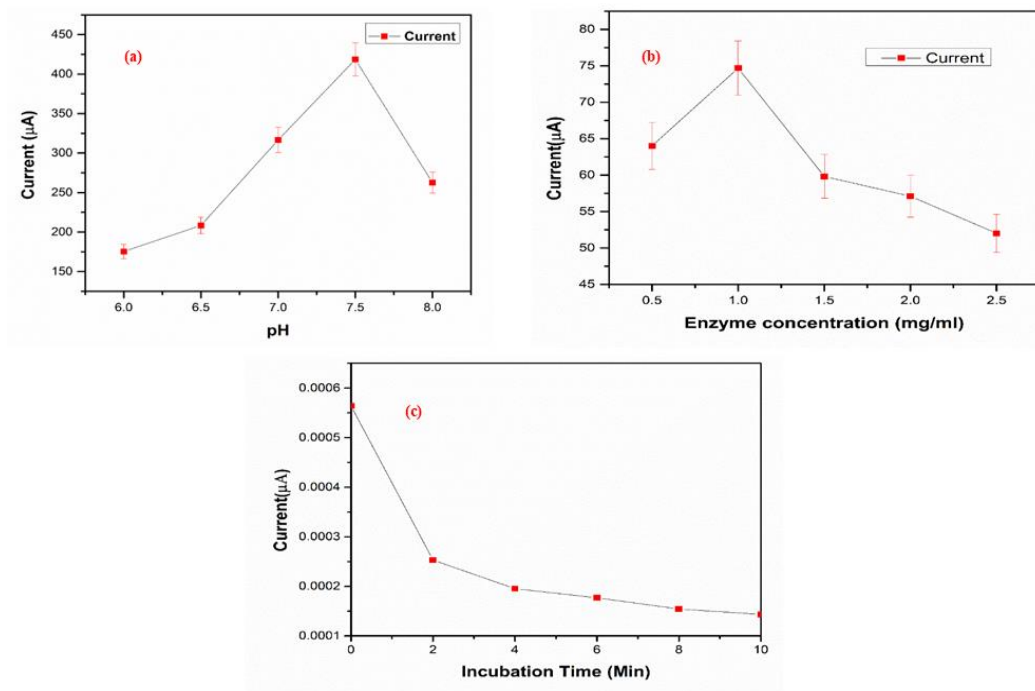


Fig. 4.8 (a) Study of the effect of pH (6.0 to 8.0), (b) Different enzyme concentration of laccase (0.5 - 2.5 mg mL⁻¹) and (c) Incubation time period analysis of biosensor for CA via chronoamperometry method of Lac/Ag@ g-C₃N₄/ITO fabricated electrode

Another crucial factor was the quantity of the enzyme laccase that was immobilised on the surface of the ITO electrode. It shows a rise in the concentration of laccase (0.5 - 2.5 mg mL⁻¹) [51,52]. The current response initially increases and reaches a maximum of 1.0 mg mL⁻¹. Subsequently, a decrease in current was observed because of the high amount of mass transfer resistance. Thus, 1.0 mg mL⁻¹ concentration was selected for further studies.

Incubation time has a significant impact on the performance of biosensors. The peak diminishes dramatically as the incubation duration increases from 2 to 12 min. The interaction between CA and laccase is complete when it reaches saturation within 6 min. Thus, 6 min was chosen as the best incubation duration for the following biosensing research.

4.3.5. Electrochemical Biosensing Studies

Chronoamperometry is a time-dependent method that records the current in relation to time intervals by stepping a constant electric potential. The fabricated Lac/Ag@g-C₃N₄/ITO electrode has been used to study the electrochemical response towards different concentrations of CA (1.0 pM to 100.0 μ M) in PBS (pH 7.5) using the chronoamperometric technique (Interval time > 0.1 s) [46].

It has been observed that with a successive increase in caffeic acid concentration, there was a decrease in current ($n = 5$). The decrease in current response with the successive addition of caffeic acid concentration may be due to a decrease in the availability of electrons on the electrode surface. Linearity was achieved when saturated current responses were plotted against different concentrations of caffeic acid yielding a linear regression equation.

$$\text{Current } (\mu\text{A}) = 233.94 \mu\text{A} + 15.7707 \mu\text{A} / \mu\text{M} \times C_{\text{CA}}(\mu\text{M}), R^2 = 0.83724 \quad (11)$$

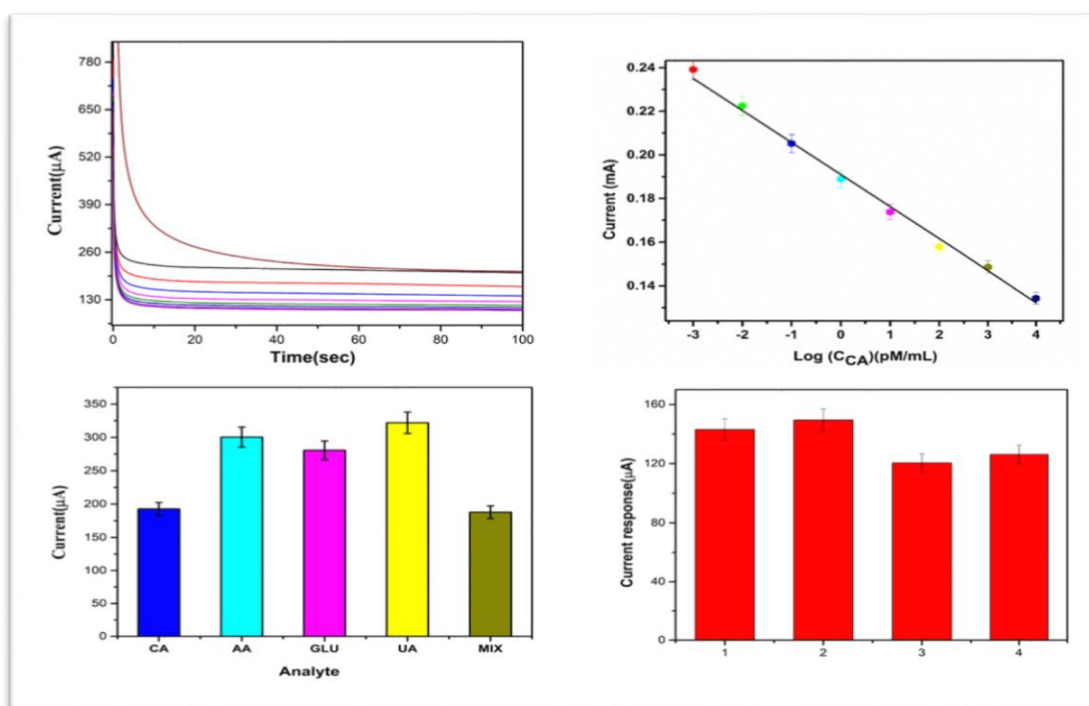


Fig. 4.9 (a) Chronoamperometry method for electrochemical response of Lac/Ag@g-C₃N₄/ITO electrode as a function of caffeic acid concentration (from top to bottom 1 pM to 1 μ M) at PBS (pH = 7.5) (b) Linear relationship between the magnitudes of saturation of current vs. logarithm of concentration of Lac/Ag@g-C₃N₄ /ITO (c) Interference study of different analytes with

respect to caffeic acid and (d) Reproducibility study of the fabricated Lac/Ag@C₃N₄/ITO electrode

4.3.6. Interference, Reproducibility and Stability Studies

The interference of fabricated biosensors was tested in the presence of common interfering analytes including glucose, acetic acid, uric acid, sodium chloride, potassium chloride and all mixtures. The Ag@g-C₃N₄/ITO electrode has been used to test seven concentrations of each interfering analyte for the investigation (100 μ L of 1 nM CA + 100 μ L of 100 nM analyte) [49,50]. When compared to the CA, the lac/Ag@g-C₃N₄/ITO electrode exhibited a small deviation (± 5 %) in the presence of other interfering analytes, demonstrating the electrode's selectivity for caffeic acid detection. The reproducibility of the Lac/Ag@g-C₃N₄/ITO electrode was investigated by creating five identical electrodes with 5 nM caffeic acid present. The produced biosensor's good repeatability is confirmed by its response, which has an RSD (Relative Standard Deviation) value of 2.45% ($n = 5$). When the stability of the manufactured biosensor was evaluated seven days later, it was discovered that the current response had decreased by $1.3 \pm 0.31\%$ after twenty days [51]. To explore the reproducibility of the lac/Ag@g-C₃N₄/ITO electrode, three different electrodes were used to detect caffeic acid and the current response was compared to exhibit a relatively low RSD (Relative Standard Deviation) of 3.21%.

4.3.7. Validation of the Biosensor with Real Sample

The practicality and accuracy of the (Ag/g-C₃N₄/ITO) biosensor were evaluated by detecting caffeic acid in three different real samples (Coffee, Black Tea & Green Tea) with different concentrations (1 to 100 nM) by the chronoamperometry method. Before the measurement, these samples were treated with DI water for washing, and each sample was mixed with 7.5 pH buffer followed by centrifugation at 5500 rpm for 15 min. The supernatant was subsequently filtered and spiked with known concentrations of caffeic acid (1nM) [48]. The outcomes displayed in Table 4.1 confirm satisfactory recovery (97 to 108%) and further

illustrate the accuracy and dependability of the created biosensor for each of the actual samples examined.

Table 4.1: Detection of caffeic acid in three real samples using Ag@g-C₃N₄ electrode

S.No.	Real Sample	Added (nM)	Found (nM)	Recovery (%)	RSD (%)
1.	Coffee	1	1.04	104.90	3.426
		10	9.55	95.50	3.230
		100	96.98	96.98	2.163
2.	Green Tea	1	1.03	103.50	2.461
		10	10.38	103.80	2.630
		100	92.90	92.90	5.200
3.	Black Tea	1	1.03	103.9	2.730
		10	10.11	101.1	0.790
		100	107.4	107.4	5.040

CHAPTER 5

CONCLUSION

The research work presented here describes how a single-step approach using ultra-sonification led to the successful production of a composite material of Ag@g-C₃N₄. The hydrothermal method was found to be superior to traditional methods of making Ag@g-C₃N₄ because it produced more homogeneous distributions of silver particles provided better interactions between the metal and substrate surfaces and enhanced the functionality of the resulting composite.

The physical characteristics of the composite have been fully characterised using XRD, FESEM, EDS and FTIR. The silver nanoparticles were deposited on the g-C₃N₄ surface using cyclic voltammetry and chronoamperometry to prepare an electrochemical sensor based on biosensor technology. This supported the notion that the biosensor had a higher level of electrocatalytic activity for detecting caffeic acid than Ag/g-C₃N₄ composites prepared with other methods.

The results obtained during the electrochemical evaluation of Lac/Ag@g-C₃N₄/ITO biosensors demonstrated a high level of analytical performance with a wide linear detection range, low detection limit and highly reproducible results. Notably, it maintained high selectivity in the presence of structurally similar interferents. Real-sample analysis using coffee, green tea and black tea yielded satisfactory recovery rates confirming the biosensor's practical applicability. These results establish the Lac/Ag@g-C₃N₄/ITO nanocomposite as a viable electrode material for simple, accurate and selective caffeic acid detection in food and beverage samples. The Ag@g-C₃N₄ nanocomposite therefore shows significant promise for electrochemical sensing applications

References

1. Y. Yang, Y. Guo, F. Liu, X. Yaun, Y. Guo, S Zhang, W Guo, and M Huo, Preparation and enhanced visible-light photocatalytic activity of silver deposited graphitic carbon nitride plasmonic photocatalyst. *Applied Catalysis B: Environmental* 142 (2013) 828-837.
2. V.A. Kothandan, S. Mani, S. Chen, and SH. Chen, Ultrasonic-assisted synthesis of nickel tungstate nanoparticles on poly(3,4-ethylene dioxythiophene):poly(4-styrene sulfonate) for the effective electrochemical detection of caffeic acid. *Materials Today Communications* 26 (2021) 101833.
3. M. Mohammadzadeh, Z. Bagheri, and J. Jaafari, Enhanced catalytic degradation performance of tetracycline by NbCuO₃-ZrO₂ in the presence of hydrogen peroxide and visible light radiation. *Journal of Hazardous Materials Advances* 19 (2025) 100767.
4. L. Lema, R. Oliveira, I. Amorim, AP. Bettencourt, F. Bento, Facile preparation of a Pt-ERGO composite modified screen-printed electrode for the sensitive determination of phenolic compounds. *Heliyon* 9 (2023).
5. G. Kesavan, T. Subramaniam, and HV. Manemaran, Development of Promising Flower-like Ag/SrFeO₃ Nanosheet Electrode Materials: An Efficient and Selective Electrocatalytic Detection of Caffeic Acid in Coffee and Green Tea. *ACS Omega* 8 (2023) 46414-46424.
6. AT. Lawal, Recent developments in electrochemical sensors based on graphene for bioanalytical applications. *Sensing and Bio-Sensing Research* 41 (2023) 100571.
7. K. Starzec, C. Cristea, M. Tertis, B. Feier, M Wieczorek, P. Koscielniak, and J. Kochana, Employment of electrostriction phenomenon for label-free electrochemical immunosensing of tetracycline. *Bioelectrochemistry* 132 (2020) 107405.
8. D. Thakur, C.M. Pandey, and D. Kumar, Graphitic Carbon Nitride-Wrapped Metal-free PoPD-Based Biosensor for Xanthine Detection. *ACS Omega* 8 (2023) 2328-2336.
9. F.R. Simões, and M.G. Xavier. Electrochemical sensors. *Nanoscience and its Applications* 1 (2017) 155-178.
10. D. Kadam, B. Momin, S. Palamthodi, and S.S. Lele, Physicochemical and functional properties of chitosan-based nano-composite films incorporated with biogenic silver nanoparticles. *Carbohydrate Polymers* 211 (2019) 124-132.
11. S. Sekar, J. Huijun, Z. Liuzhu, C Jin, S. Lee, D.Y. Kim, and R. Manikadan, Copper phthalocyanine conjugated graphitic carbon nitride nanosheets as an efficient electrocatalyst for simultaneous detection of natural antioxidants. *Electrochimica Acta* 413 (2022) 140150.
12. S. Chatterjee, H. Singh, D. Hudda, Sweetey, and D. Kumar, A Novel Acetylcholinesterase-Based Electrochemical Biosensor Using g-C₃N₄@MoS₂

- Nanohybrid for the Detection of Trichlorfon. *Applied Organometallic Chemistry* 38 (2024) e7721.
13. G. Alberti, C. Zanoni, LR. Magnaghi, and R. Biesuz, Gold and silver nanoparticle-based colorimetric sensors: New trends and applications. *Chemosensors* 9 (2021) 305.
 14. A. Salayová, Z. Bedlovičová, N. Daneu, M. Balaz, Z. Lukáčová, Bujňáková, and Ľ Balážová Green synthesis of silver nanoparticles with antibacterial activity using various medicinal plant extracts: Morphology and antibacterial efficacy. *Nanomaterials* 11 (2021) 1005.
 15. NE. Sunny, A. Kaviya, and S.V. Kumar, Mechanistic approach on the synthesis of metallic nanoparticles from microbes. In: *Agri-Waste and Microbes for Production of Sustainable Nanomaterials*. Elsevier, 2022: 577-602.
 16. P. Veerakumar, C. Rajkumar, SM. Chen, B. Thirumalraj, and KC. Lin, Ultrathin 2D graphitic carbon nitride nanosheets decorated with silver nanoparticles for electrochemical sensing of quercetin. *Journal of Electroanalytical Chemistry* 826 (2018) 207-216.
 17. MT. Ameen, A. Haider, I. Shahzadi, A. Shahbaz, A. UI-Hamid, H. Ullah, S. Khan, and M. Ikram, Exploring catalytic efficacy and anti-bacterial performance with molecular docking analysis of g-C₃N₄-grafted-Ag doped SnO₂ QDs. *Research on Chemical Intermediates* 50 (2024) 1661-1678.
 18. TS. Priya, N. Nataraj, TW Chen, SM Chen, E. Tamilalagan, BS. Lou, AM. Al-Mohaimeed, M.A. Binobead, and M.S. Elshikh, Non-enzymatic electrochemical detection of caffeic acid in food samples with spinel magnesium cobalt oxide incorporated graphene nanohybrid electrocatalyst. *Journal of Alloys and Compounds* 1002 (2024) 175335.
 19. AE. Pap, K. Kordás, R. Peura, and S. Leppävuori, Simultaneous chemical silver and palladium deposition on porous silicon; FESEM, TEM, EDX and XRD investigation. *Applied Surface Science* 201 (2002) 56-60.
 20. D. Sumanth, and JG. Manjunatha, Detection of caffeic acid using a nanotube paste-based electrochemical sensor. *Monatshefte für Chemie-Chemical Monthly* 155 (2024) 937-946.
 21. X. Lü, J. Shen, Z. Wu, J. Wang, and J. Xie, Deposition of Ag nanoparticles on g-C₃N₄ nanosheet by N,N-dimethylformamide: Soft synthesis and enhanced photocatalytic activity. *Journal of Materials Research* 29 (2014) 2170-2178.
 22. PC. Nagajyothi, M. Pandurangan, SVP. Vattikuti, CO. Tettey, TVM. Sreekanth, and J. Shim, Enhanced photocatalytic activity of Ag/g-C₃N₄ composite. *Separation and purification technology* 188 (2017) 228-237.
 23. A. Azevedo, AF. Prado, JPM. Issa, and RF. Gerlach, Matrix metalloproteinase 2 fused to GFP, expressed in *E. coli*, successfully tracked MMP-2 distribution in vivo. *International Journal of Biological Macromolecules* 89 (2016) 737-745.

24. N. Elgrishi, KJ. Rountree, BD McCarthy, ES. Rountree, TT. Eisenhart, and JL. Dempsey, A Practical Beginner's Guide to Cyclic Voltammetry. *Journal of Chemical Education* 95 (2018) 197-206.
25. H.A.-Toupanloo, M.K. -Nazarabad, M. Shakeri, and M. Eftekhari, Photocatalytic mineralization of hard-degradable morphine by visible light-driven Ag@g-C₃N₄ nanostructures. *Environmental Science and Pollution Research* 26 (2019) 30941-30953.
26. MT. Ameen, A. Haider, I. Shahzadi, A. Shahbaz, A.UI-Hamid, H. Ullah, S. Khan, and M. Ikram, Exploring catalytic efficacy and anti-bacterial performance with molecular docking analysis of g-C₃N₄-grafted-Ag doped SnO₂ QDs. *Research on Chemical Intermediates* 50 (2024) 1661-1678.
27. O. Iqbal, H. Ali, N. Li, Al Al-Sulami, KF. Alshammari, HSM Abd-Rabbooh, Y. Al-Hadeethi, I. Ud Din, AI. Alharthi, R. Altamimi, A. Zada, Z. Wang, A. Hayat, and MZ. Ansari, A review on the synthesis, properties, and characterizations of graphitic carbon nitride (g-C₃N₄) for energy conversion and storage applications. *Materials Today Physics* 34 (2023) 101080.
28. H. Li, Y. Jing, X. Ma, T. Liu, L. Yang, B. Liu, S.Yin, Y.We, and Y. Wang, Construction of a well-dispersed Ag/graphene-like g-C₃N₄ photocatalyst and enhanced visible light photocatalytic activity. *RSC Advances* 7 (2017) 8688-8693.
29. K. Qi, Y. Li, Y.Xie, S. Liu, K. Zheng, Z. Chen, and R. Wang, Ag loading enhanced photocatalytic activity of g-C₃N₄ porous nanosheets for decomposition of organic pollutants. *Frontiers in Chemistry* 7 (2019) 91.
30. O. Iqbal, H. Ali, N. Li, Al Al-Sulami, KF. Alshammari, HSM Abd-Rabbooh, Y. Al-Hadeethi, I. Ud Din, AI. Alharthi, R. Altamimi, A. Zada, Z. Wang, A. Hayat, and MZ. Ansari, A review on the synthesis, properties, and characterizations of graphitic carbon nitride (g-C₃N₄) for energy conversion and storage applications. *Materials Today Physics* 34 (2023) 101080.
31. D. Das, BD. Kumar, R. Sarkar, S. Mukherjee, and K. Chattopadhyay, Copper and nickel decorated g-C₃N₄ as superior catalysts for reduction of toxic pollutants: A combined experimental and theoretical approach. *Applied Surface Science* 580 (2022) 152137.
32. K. Qi, Y. Li, Y. Xie, S. Liu, K. Zheng, Z. Chen, and R. Wang, Ag loading enhanced photocatalytic activity of g-C₃N₄ porous nanosheets for decomposition of organic pollutants. *Frontiers in Chemistry* 7 (2019) 91.
33. Li H, Jing Y, Ma X *et al.* Construction of a well-dispersed Ag/graphene-like g-C₃N₄ photocatalyst and enhanced visible light photocatalytic activity. *RSC Adv* 2017; 7: 8688–8693.
34. M. Roškaric, G. Žerjav, J. Zavašnik, and A. Pintar, The influence of synthesis conditions on the visible-light triggered photocatalytic activity of g-C₃N₄/TiO₂ composites used in AOPs. *Journal of Environmental Chemical Engineering* 10 (2022) 107656.
35. Y. Yang, Y. Guo, F. Liu, X Yuan, Y Guo, S Zhang, W Guo, and M Huo, Preparation and enhanced visible-light photocatalytic activity of silver deposited graphitic carbon

- nitride plasmonic photocatalyst. *Applied Catalysis B: Environmental* 142 (2013) 828-837.
36. AH. Alshammari, K. Alshammari, M. Alshammari, and TAM. Taha, Structural and Optical Characterization of g-C₃N₄ Nanosheet Integrated PVC/PVP Polymer Nanocomposites. *Polymers* 15 (2023) 871.
 37. PT. Lan, NH. Hao, NT. Hieu, NTT. Ha, CT. Brown, and LM. Cam, Graphitic carbon nitride supported silver nanoparticles (AgNPs/g-C₃N₄): synthesis and photocatalytic behavior in the degradation of 2,4-dichlorophenoxyacetic acid. *RSC Advances* 14 (2024) 19014-19028.
 38. S. Dahiya, A. Sharma, and S. Chaudhary, Synthesis of phytoextract-mediated Ag-doped graphitic carbon nitride (Ag@GCN) for photocatalytic degradation of dyes. *Environmental Science and Pollution Research* 30 (2023) 25650-25662.
 39. M. Ye, R. Wang, Y. Shao, C. Tian, Z. Zheng, X. Gu, W. Wei, and A. Wei, Silver nanoparticles/graphitic carbon nitride nanosheets for improved visible-light-driven photocatalytic performance. *Journal of Photochemistry and Photobiology A: Chemistry* 351 (2018) 145-153.
 40. AM. Idris, An In-Situ Anion Exchange Method Synthesized of Ag₃PO₄ Functionalized with Fe₃O₄ and AgI for Photocatalytic Degradation of Methyl Orange under Visible Light Irradiation. *International Journal of Materials Science and Applications* 3 (2014) 303.
 41. J. Kalaiyarasi, K. Pandian, S. Ramanathan, and SCB. Gopinath, Graphitic carbon nitride/graphene nanoflakes hybrid system for electrochemical sensing of DNA bases in meat samples. *Scientific Reports* 10 (2020) 12860.
 42. B. Kanthappa, JG. Manjunatha, SM. Osman, and N. Ataollahi, Electrochemical polymerized DL-phenylalanine modified carbon nanotube sensor for the selective and sensitive determination of caffeic acid with riboflavin. *Scientific Reports* 14 (2024) 1-13.
 43. BA. Prabowo, PD. Cabral, P. Freitas, and E. Fernandes, The challenges of developing biosensors for clinical assessment: A review. *Chemosensors* 9 (2021) 299.
 44. F. Saman, CHS. Ling, A. Ayub, NHB Rafeny, AH. Mahadi, R. Subagyo, RE. Nugraha, D. Prasetyoko, and H. Bahruji, Review on synthesis and modification of g-C₃N₄ for photocatalytic H₂ production. *International Journal of Hydrogen Energy* 77(2024) 1090-1116.
 45. MA. Qamar, M. Javed, S. Shahid, M Shariq, MM. Fadhali, SK. Ali, and MS. Khan Synthesis and applications of graphitic carbon nitride (g-C₃N₄) based membranes for wastewater treatment: A critical review. *Heliyon* 9 (2023).
 46. S. Verma, D. Thakur, C.M. Pandey, and D. Kumar, Recent Prospects of Carbonaceous Nanomaterials-Based Laccase Biosensor for Electrochemical Detection of Phenolic Compounds. *Biosensors* 13 (2023) 305.

47. Y. Xu, W. Lei, J. Su, J Hu, X Yu, T Zhou, Y Yang, D. Mandler, and Q. Hao, A high-performance electrochemical sensor based on g-C₃N₄-E-PEDOT for the determination of acetaminophen. *Electrochimica Acta* 259 (2018) 994-1003.
48. S. Verma, C.M. Pandey, D. Kumar, An enzymatic biosensor based on MgO nanoparticles grafted on reduced graphene oxide nanoflakes for the ultrasensitive detection of phenolic compounds from wastewater. *ChemistrySelect* 8 (2023) e202302420.
49. D. Hudda, and D. Kumar, Molecularly imprinted polypyrrole decorated Ti₃C₂T_x electrochemical sensor for highly selective and sensitive detection of levofloxacin. *Journal of Materials Science* 59 (2024) 21684-21695.
50. T. Alizadeh, S. Nayeri, and A. Habibi-Yangjeh. Graphitic carbon nitride (g-C₃N₄/Fe₃O₄/BiOI)-carbon composite electrode as a highly sensitive and selective citric acid sensor: Three-component nanocomposite as a definitive factor for selectivity in catalysis. *Sensors and Actuators B: Chemical* 279 (2019) 245-254.
51. H. Zhang, SF. Hua, and L. Zhang, Co-immobilization of cellulase and glucose oxidase on graphene oxide by covalent bonds: a biocatalytic system for one-pot conversion of gluconic acid from carboxymethyl cellulose. *Journal of Chemical Technology and Biotechnology* 95 (2020) 1116–1125.
52. S. Verma, C.M. Pandey, and D. Kumar, Non-enzymatic electrochemical biosensor based on MgO@rGO-MoS₂ nanohybrid for the Detection of phenolic compounds detection. *Applied Organometallic Chemistry* 38 (2024) e7325.