

Graphene Based Nanocomposites for Electrochemical Sensing of Ascorbic Acid



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No part of this thesis has been submitted anywhere else for any other degree. This thesis is submitted to the Faculty of Sciences, The Superior University, Lahore in partial fulfillment of the requirements for the degree of Master of Science/Master of Philosophy in the field of “Chemistry” in Faculty of Sciences at The Superior University, Lahore.

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Dedication

I wholeheartedly dedicate this thesis to my beloved parents, whose endless prayers, unconditional love, and constant encouragement have been my greatest source of strength throughout this journey. Their unwavering support and sacrifices have shaped my path and made this achievement possible. I also extend this dedication to my teachers, mentors, and dear friends, whose guidance, motivation, and companionship enriched this experience. Above all, I am deeply grateful to Almighty Allah for granting me the courage, patience, and wisdom to complete this work.

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List of Abbreviations

AA	Ascorbic acid
GO	Graphene oxide
CV	Cyclic voltammetry
LOD	Limit of detection
NiCo-O	Nickel Cobalt Oxide
SEM	Scanning electron microscopy
MWCNTs	Multi-walled carbon nanotubes
SWCNTs	Single-walled carbon nanotubes
EDX	Energy dispersive X-ray spectroscopy
UV	Ultraviolet
pH	Potential of Hydrogen
DNA	Deoxyribonucleic acid
Ag/AgCl	Silver/ Silver chloride
AC	Alternating current
DC	Direct current
DPV	Differential pulse voltammetry
LSV	Linear Sweep Voltammetry
EIS	Electrochemical impedance spectroscopy
PBS	Phosphate Buffer Solution
FIA	Flow injection analysis
rGO	Reduced graphene oxide
GCE	Glassy carbon electrode
XRD	X ray diffraction
CA	Chronoamperometry
PTSD	Post-Traumatic Stress Disorder
GCE	Glassy carbon electrode
NFB	Nitrogen fixing bacteria
TNF	Trinitrofluorenone
HIF	Hypoxia inducible factor

ABSTRACT

A composite of reduced graphene oxide (rGO) and nickel cobalt oxide (NiCoO) was created by hydrothermal method and employed in electrochemical detection of ascorbic acid (AA). The conductive matrix was graphene oxide (GO) and NiCoO nanoparticle was added to the surface to increase the activity of the electrode and its surface reactivity. The successful integration of NiCoO was confirmed by the scanning electron microscope (SEM) where the NiCoO was seen to have a uniform distribution and fibrous morphology on the surface of GO. The crystalline and the phase purity of NiCoO@GO nanocomposites was confirmed by X-ray diffraction (XRD) analysis. The reaction between NiCoO and GO was previously identified to be successful which was confirmed by Fourier Transform Infrared (FTIR) spectroscopy as to the successful chemical interaction between functional groups. A systematic study was conducted regarding the effect of pH, scan rate and interfering chemicals on the electrochemical reaction with AA. In terms of the electrochemical performance evaluation, a high sensitivity of $0.53 \text{ mA mM}^{-1} \text{ cm}^{-2}$ and a strong linear response in large concentration range was observed to the rGO/NiCoO modified GCE exhibiting good electrocatalytic activity toward AA. The detection limit under ideal circumstances was found to be 0.056 mM, allowing for precise single-point injection measurement of low AA concentrations. The superior flexibility, electrical conductivity, and electrochemical stability of the composite electrode suggest its strong potential for next-generation biosensing platforms, especially in real-time detection systems for wearable, implantable, or portable electrochemical devices. Additionally, the nanocomposite exhibited favorable magnetic properties, facilitating easy separation and recovery.

1.1 Historical Perspective

Multiple physiological systems are impacted by ascorbic acid deficiency illnesses, which cause progressive degradation of connective tissue, immunological response, scurvy, wound healing and anemia. The most common of these disorders along with Parkinson and Huntington disease is Alzheimer with multiple sclerosis and amyotrophic lateral sclerosis (ALS). The numerous pathologies that cause diseases aggravation in these disorders cause toxicity due to over stimulation of glutamate receptors that stimulate death of the neurons. Neurotoxicity in biological tissue can be brought about by high levels of glutamate which is the main or most pronounced stimulating neurotransmitter related to the functioning of within the central nervous system. When the glutamate receptors, and especially N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors of the cell become over-stimulated, glutamate produces an overburden of calcium into the cell. Elevated calcium levels damage intracellular calcium equilibrium which then compromises mitochondria so that toxic chain reactions trigger energy exhaustion and oxidative damage generation while producing harmful reactive oxygen species (ROS) like H_2O_2 and O_2^- . The ROS damage neurons through mechanisms which cause lipid damage while inducing DNA breakdown and apoptosis which results in cell death that advances neurodegeneration [1].

Excitotoxicity involving glutamate is one of the common causes of clinical manifestation of neurodegenerative diseases, which have many similarities even though they possess different genetic basis. This excitotoxicity that is known to kill the neurons using convergent routes and also leads to the onset of various neurodegenerative diseases has numerous causes. The diseases afflict millions of people on earth and pose a significant threat to the health of the world given the high morbidity and mortality rates attributed to the diseases. Ascorbic acid (AA) has emerged as an important biochemical constituent imparted to these disorders due to the protective and antioxidant effects. An imbalance or lack of AA has been considered as the main threat of developing and developing many neuropsychiatric and neurodegenerative conditions. These are Parkinson diseases and other related conditions like post-traumatic stress disorder and long-term depression,

where AA will be required to maintain the redox balance in brain tissues and decrease increased oxidative stress, which contributes to the bulk of the damage to the neurons in such circumstances. To prevent oxidative and excitotoxic destruction which is typical of neurodegenerative diseases, it is necessary to ensure the preservation of about adequate levels of AA [2].

The primary symptom of scurvy proves a lack of vitamin C because the disease results in exhaustion along with inflammation of gums and bruise marks and infallibility of wounds. The synthesis of collagen suffers when its essential component ascorbic acid becomes inadequate thus leading to these symptoms. Prolonged vitamin C deficiency results in both anemia and weak bones as well as making the body more prone to illnesses. The main factors that raise vitamin C deficiency risk consist of tobacco use and inadequate dietary patterns and certain medical circumstances [3].

The commercially available enzyme-based biosensors that detect ascorbic acid undergo denaturation problems which reduces stability and result in low sensitivity along with high manufacturing expenses for enzyme extraction and stabilization while maintaining single-use functionality. Developments of non-enzymatic electrochemical sensors gained momentum because of the drawbacks with enzyme-based sensors that depend on direct analyte detection. Non-enzymatic sensors demonstrate promising usage as diagnostic ingredients since they cost-effectively integrate rapidly into practical high-end diagnostic applications because they require simple production methods while maintaining operational reliability and resisting chemicals and heat exposure [4].

1.2 Sensors

The analytical device known as a sensor identifies specific substances within samples so that the device both detects their presence and determines their concentration through quantitative measurements. Three fundamental operational components work in unison within its system [5]. The receptor functions as the initial component by selectively binding to analytes through lock and key mechanism which facilitates specific recognition of substances. After detecting an analyte, the transducer modifies the biochemical or chemical recognition event into a detectable physical or chemical signal like electricity, light or heat changes. The readout system receives the signal from the transducer leading

to data processing and visualization display of analytical information as numerical values or graphical outputs. A sensor becomes precise and reliable for industrial processes and clinical diagnostics and environmental monitoring applications through the integration of various operational components [6].

1.3 Classification of Sensors

Different sensor groups exist based on their measurement criteria and their operational principles. Sensor systems operate as contact or non-contact devices which indicates their need for physical sample engagement or not. The first classification of sensors varies based on the presence or lack of an external power source because passive sensors have no such requirement while active sensors do need a power supply. Sensor measurement references determine their classification as absolute or relative sensors which respectively measure specific values independently of any reference or track changes against baseline values. Sensors differ from one another through the output signal format where continuous analog sensors output and digital sensors generate discrete values. Detection mechanisms used to classify sensors include physical detection in combination with chemical detection and thermal detection and biological detection. Specific sensor selection requires these classifications because they determine the most appropriate type for measuring pressure and temperature as well as chemicals or biological processes as depicted in the diagram 1.1 [7].

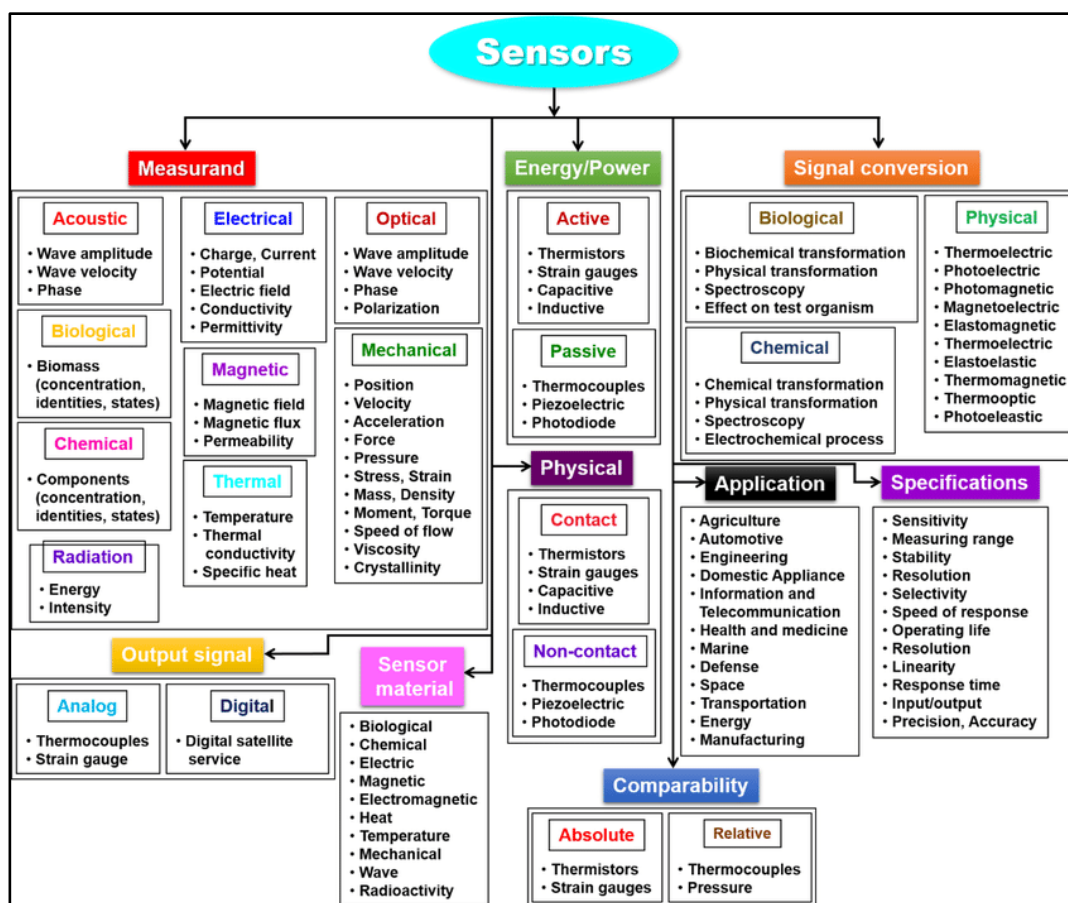


Figure 1.1 Illustrated classification of Sensors on the basis of different properties

1.4 Biosensors

Biosensors make use of analytical tools that identify certain analytes through biological recognition components. These recognition elements typically consist of enzymes or biomolecular receptors form part of these sensors while responding to environmental changes that include detecting small amounts of toxins and pollutants and pH level alterations [8]. A system within the sensor includes electronic components which convert the biological reaction into quantifiable output signals. A standard biosensor contains fundamental elements including an analyte along with a bioreceptor that detects this substance and implements a transducer to convert biological detection signals into electronic signals which leads to result presentation through the display system (Figure 1.2). Each biosensor device shows specific configurations because they adapt to the requirements of their biological monitoring purposes [9].

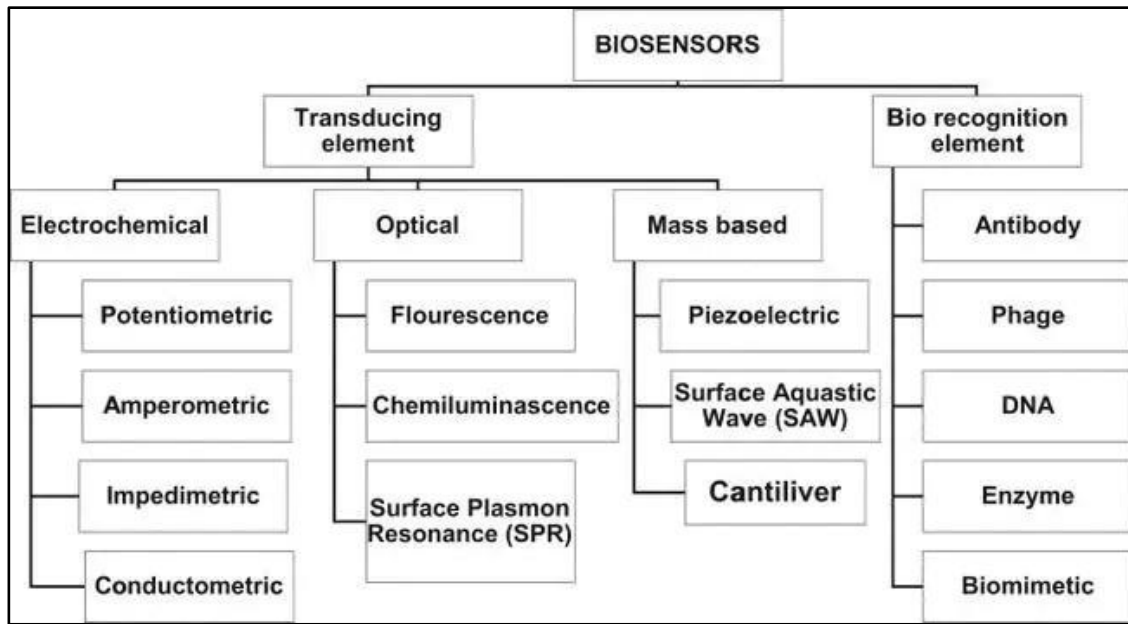


Figure 1.2 Schematic classification of Biosensors

The target material undergoing quantitative evaluation is called an analyte when it includes substances such as glucose and lactose along with ammonia and ethanol. Bioreceptors operate as biological molecular interfaces with enzymes or antibodies among other biological components to detect specific molecules by binding them in cells or nucleic acids [10].

A biosensor contains the transducer as its primary essential component. The detection of chemical or biological targets by the transducer leads to the creation of an electrical signal that can be easily measured. Energy signals through different forms when it performs signaling operations. The electronic system takes the transducer output to create a detectable signal [11]. The process includes signal amplification while digital data is generated through signal conversion for output preparation. Processed data appears on the user-read ready format through display units which consist of computer displays or printers. The results display either numerical values or graphical and tabular formats that present clear information for analysis and decision-making purposes as diagram 1.3 shows [12].

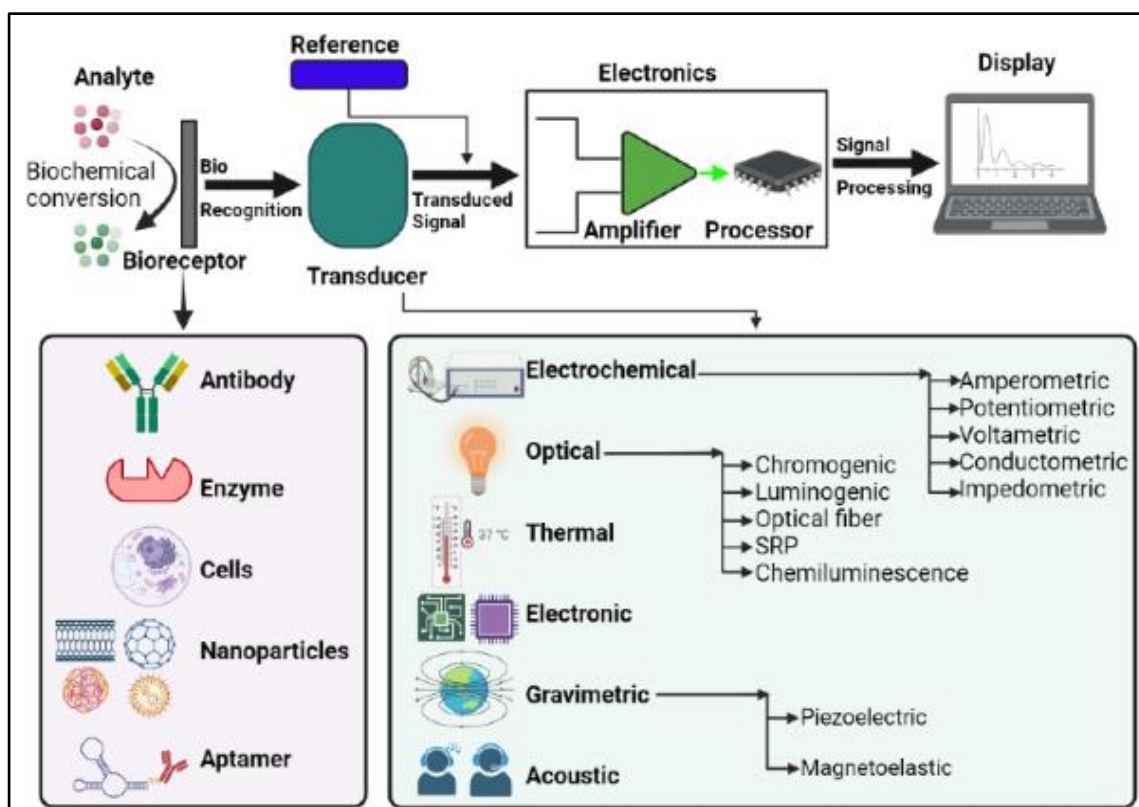


Figure 1.3: A typical biosensor structure comprises a bioreceptor, a transducer, an electronic unit (including processor and amplifier), and a data display system (such as a PC or printer)

1.5 Electrochemical Sensing

Electrochemistry has developed into a well-established field throughout its 200-year history because it serves essential functions in fuel cells and supercapacitors along with battery technologies solar cells as well as glucose monitoring systems for diabetes patients and pH detectors. These electrochemical systems offer multiple advantageous traits including both fast measurements beneath one minute and affordable costs alongside detection precision measured in the nm range per 1000 targets. The systems also support point of care usage [13].

The movement of electric charge across interfaces represents the fundamental topic in electrochemistry since researchers mostly analyze redox reactions. A redox reaction takes place between substance "r" which occurs in two states as reduced " r^n " and oxidized " o^{n+1} ". The electrochemical cell includes the substance in solution as well as water serving as the solvent while the 0.1 M KNO_3 functions as background electrolyte to

improve solution conductivity. An electric potential applied on the working electrode with sustained positive voltage allows "r" species to transform into "o" while transmitting one electron toward the electrode surface. The functional electrode reduction process returns the target species "o" to its original state as "r" under a negatively applied potential [14, 15]. The electron transfer process obeys the OIL RIG principle that stands for Oxidation Is Loss and Reduction Is Gain. The implementation of amperometry depends on measuring current during Potentiostat-controlled electric power distribution with simultaneous working electrode voltage production. The supply of steady current in potentiometry allows researchers to measure the produced voltage measurements. The working electrode receives its applied potential of 1.0V from a reference electrode that also ensures steady voltage conditions. Reference electrodes operate by keeping their potential stable even when test solution pH and temperature change. A silver wire with an AgCl coating is used in many reference electrodes. It is placed inside a 3.0 M KCl solution and is isolated from the testing solution by a tiny divider. Since the Ag/AgCl reference electrode is insensitive to the pH and other characteristics of the target solution, its potential stability is entirely dependent on the amount of chloride present in its reference solution [16].

Operation of the Potentiostat finishes the circuit by watching the working electrode current and creating equal opposing currents for the counter electrode (figure 1.4). The counter electrode requires a stable large surface metal like platinum gauze because of its conductivity properties [17].

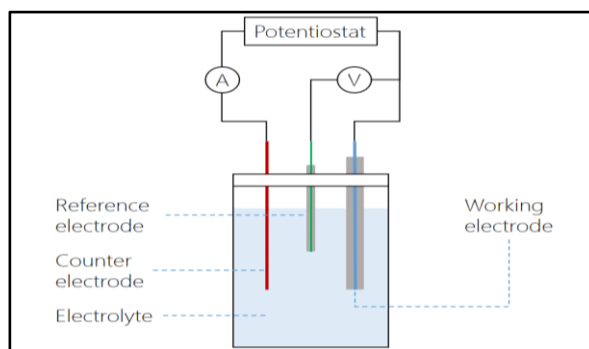


Figure 1.4 Illustration of typical three-electrode electrolytic cell

1.6 Sensing Techniques

The techniques used for electrochemical analysis operate through different mechanisms which produce specific advantages and constraints. These below methods present a summary of standard approaches for electrochemical sensing delivery. All available electrochemical analysis methods are not included in this overview yet the list provides several fundamental techniques utilized for sensing purposes [18].

1.6.1 Chronoamperometry

Chronoamperometry operates as an uncomplicated Amperometric approach which tracks electrical current evolution during a specified time interval (10 seconds) when applying a constant voltage. The operation of this technique requires a one-step process that shifts the potential either positively or negatively for target species transformation (such as changing from 0V to +0.5V). Chronoamperometry operates at the second scale as a budget-friendly technique yet suffers from excessive apparatus interference that prevents identifying low-level analyte concentrations [19].

The flow injection analysis (FIA) technology uses a standard 1 mL/min stream to continuously conduct measurements on 5 to 50 μL sample volumes. Real-time analytics of the electroanalytical system becomes possible by using this monitoring approach. Through flow injection analysis scientists achieve superior analytical sensitivity together with better throughput which allows each minute for testing up to six samples. Higher detection efficiency results from enhanced sample and fluid mixing capability in FIA because the system improves liquid transport behavior. A single-step method changes the potential (from 0V to +0.500V) or negative range (from 0V to -0.500V) in order to initiate target species transformations. Chronoamperometry provides fast cost-effective detection that operates on second durations but researchers face challenges from excessive background noise during small analyte monitoring [20, 21].

Flow injection analysis (FIA) uses this method by applying 5-50 μL sample volumes to a moving solution that keeps at a rate of 1 mL/min. By applying this method continuous monitoring occurs for the electrode's response to target substances. Flow injection analysis performs better than conventional chronoamperometry through superior sensitivity and throughput thereby permitting the analysis of six test samples each minute.

The upgraded performance in FIA takes place because better fluid transport along with mixing increases the efficiency of detection (Figure 1.5) [22].

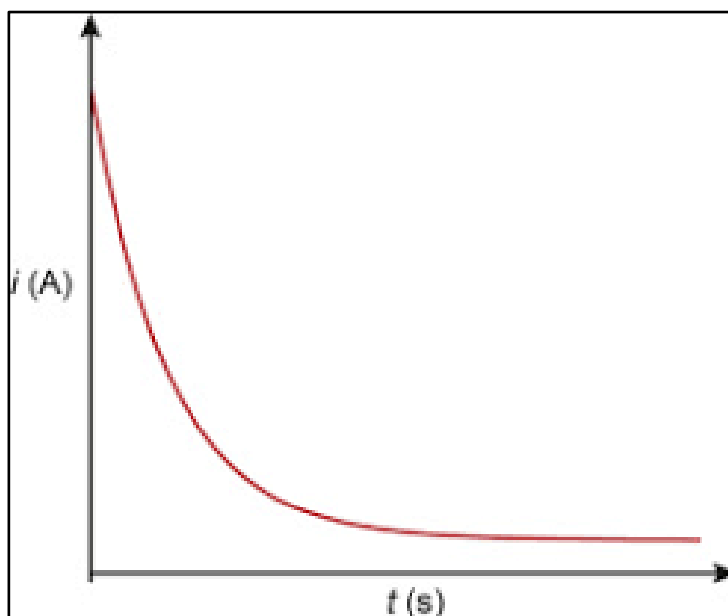


Figure 1.5 current-time plot from a chronoamperometry experiment

1.6.2 Cyclic Voltammetry

Serial changes in applied electrical potential through time enable detection of current response during voltammetric techniques. Among all fundamental methods cyclic electrochemical analysis stands as the most commonly utilized method. The working electrode undergoes linear potential scan from starting potential (E_s) to final potential (E_f) in multiple repetitive cycles displayed as stair-step waveforms as depicted in Figure 4. The working electrode enables increased current output because it performs oxidation of the target analyte during the forward sweep phase [23].

The measurement output depends on both the working electrode shape as well as its physical dimensions. The planar mass transport behavior results from using a 1 mm diameter disk electrode whereas the 10 μ m diameter disk electrode generates a hemispherical diffusion profile because of the quick radial diffusion of analyte. Diffusion programming produces cyclic voltammograms with different profiles because of variations in its geometric dimensions. A microelectrode maintains a stable current at

strong oxidative potentials because analytical movement is efficient allowing redox kinetic processes to become the limiting factor (Figure 1.6) [24].

The current profile observed for bigger electrodes shows peak-shaped voltammograms while the mass transport controls most diffusion by linear diffusion and triggers current decline from its maximum until establishing a steady-level. The steady-state current receives its limiting value from the rate which analytes arrival at the electrode surface. The method becomes linear sweep voltammetry (LSV) when the voltage sweep ends after the forward scan without any reversal phase [25, 26].

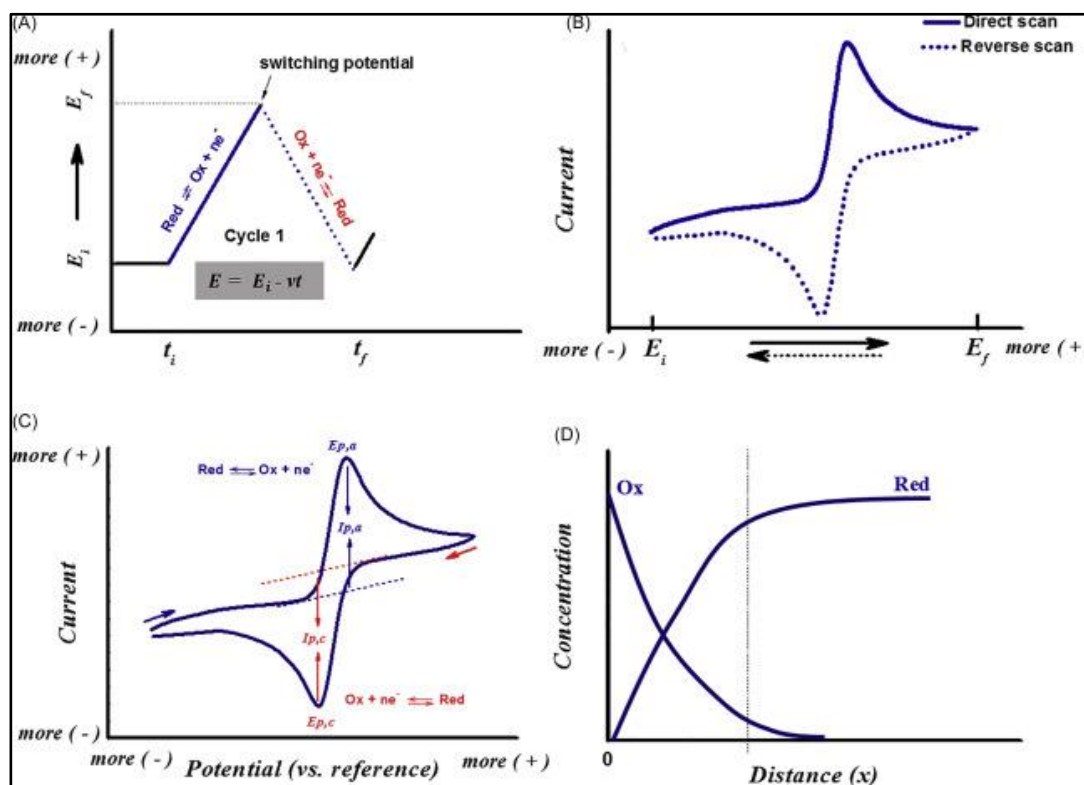


Figure 1.6 The oxidation process is illustrated with two main features: (A) a waveform with long-term potential and (B) a cyclic voltammogram, showing how the current changes with potential. This measurement is shown for two types of electrodes (B) micrometer-sized electrode (C) macro-scale electrode and thickness (D) showing concentration and distance respectively.

1.6.3 Pulsed Voltammetry

In Figure 1.7 you can see how series of voltage pulses overlap the staircase structure which defines cyclic voltammetry. DPV stands as a variant of traditional cyclic

voltammetry which records current measurements at two separate times both before and after each applied pulse instead of tracking steady-state current behavior [27, 28].

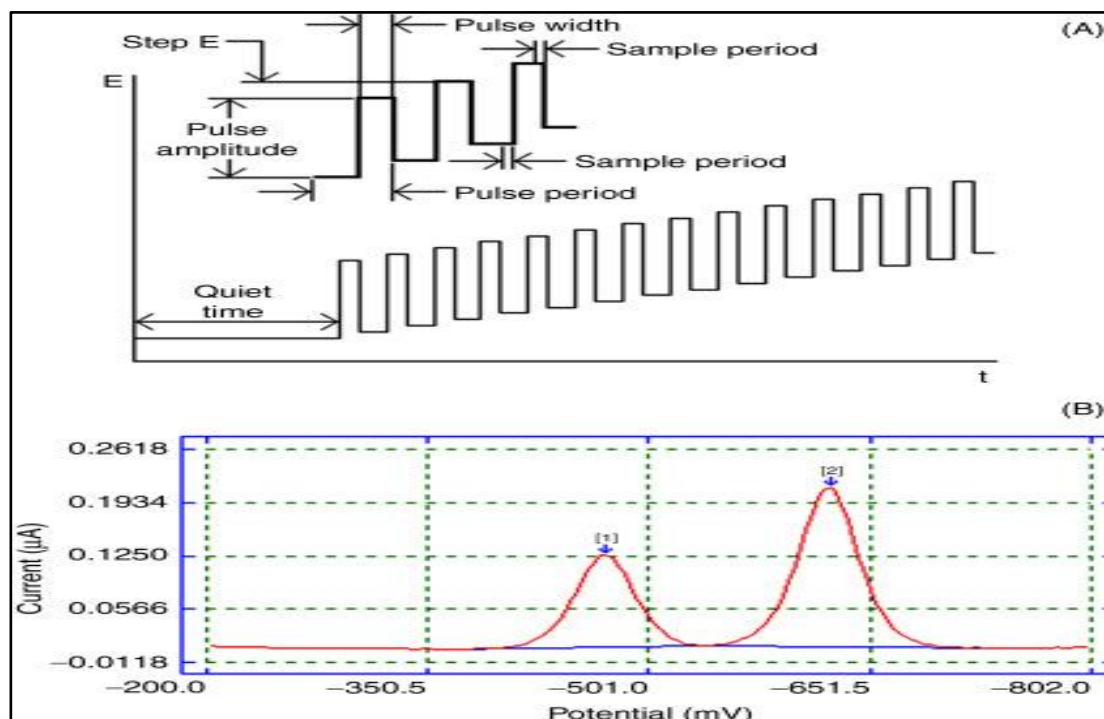


Figure 1.7 A potential staircase in (A) and a current-potential plot using differential pulse voltammetry (solid line) superimposed on top of a linear sweep voltammogram (dashed line) are used to illustrate the oxidation process. In this case, the current at time t_0 is denoted by i_{t0} , the differential current by Δi , and the current at time τ by i_τ .

The signal-to-noise ratio becomes hugely enhanced through this method enabling lower detection limits than those achieved by other electrochemical techniques. The signal becomes more sensitive because non-faradaic background currents caused by fast electrode potential fluctuations between electrolytes and electrodes get minimized. A background capacitive current emerges from the electrode potential that experiences brief duration shifts versus solution potential during pulse events. Taking measurements right after capacitive decay allows DPV to measure the faradaic current specifically associated with analyte redox processes. The targeted analytical approach of differential pulse voltammetry selects specific signals while filtering background noise which makes it an optimal technique for detecting low concentration analytical targets in highly complex solutions [29].

1.7 Nanotechnology in biosensing

The field of nanotechnology pursues developments that enable specific and rapid diagnostic tests and medical devices at affordable costs through heightened performance levels. Biosensors derived from nanomaterials unite scientific fields that include materials science and both chemistry and molecular engineering and biotechnology. The highest sensitivity levels found in nanoscale biosensors let the detection of single parasites per microliter blood volume as shown in figure 1.8 [30]. Early-stage disease detection becomes achievable through biomarker recognition because this system detects minimal abundances effectively. Through its exceptional capabilities' nanotechnology presents strong opportunities to improve standard medical practices especially when conducting routine observations and tracking diseases and performing diagnostic follow ups thus enhancing overall patient health management.

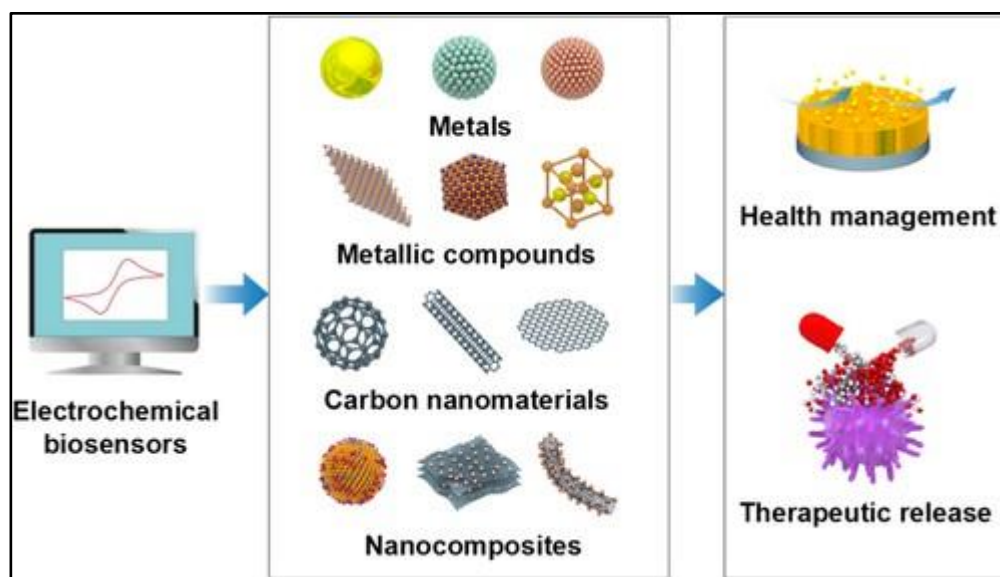


Figure 1.8 Diagrams showing how nanomaterial-based electrochemical sensors work, are made, and are used in medicine.

Nanotechnology integrated with biosensing operations provides essential support to healthcare settings without established pharmaceutical facilities. The testing of biomarkers using inorganic nanoparticles demonstrates as shown in figure 1.9, particular usefulness in this scenario [31]. The schematic model depicts the step-by-step operations of biosensing that start with analyte identification followed by binding events then leads

to signal conversion before quantifying output data. This study provides a comprehensive analysis of medical diagnostic applications employing nanomaterial-based biosensing technologies to enhance condition diagnosis in under resourced areas [32].

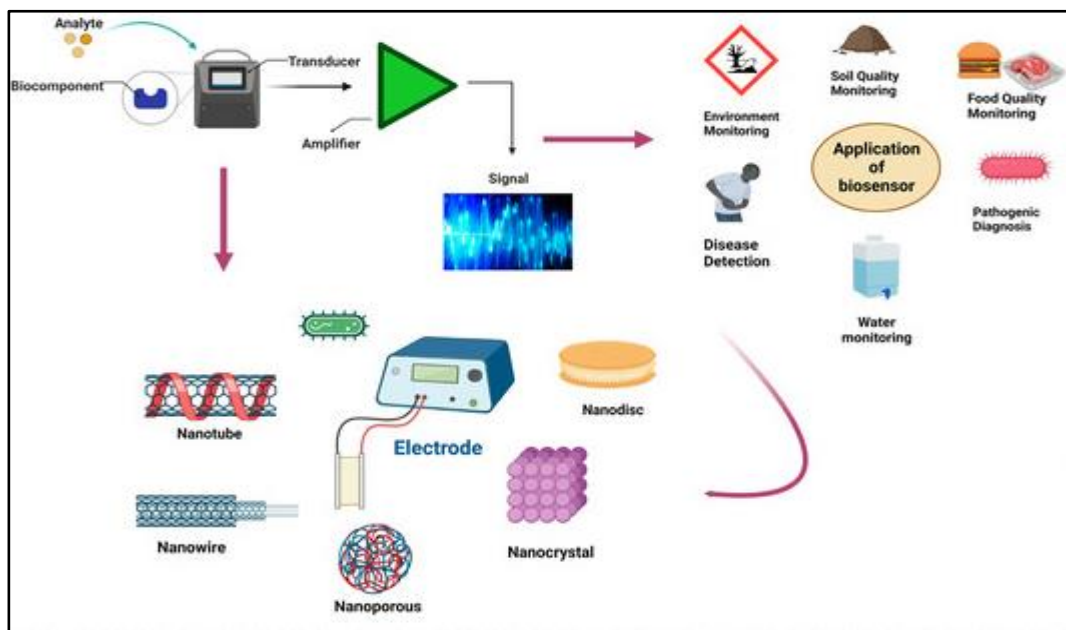


Figure 1.9 Role of different nanomaterials in biosensing

1.8 Transition Metal

Several researchers currently investigate new electrochemical sensor technologies that use nanomaterials unique attributes. Nanostructured materials surpass bulk materials in optical properties and physical and electrical and chemical characteristics to make them highly beneficial for sensor applications. Multiple synthesis methods exist for producing transition metal oxide nanoparticles across different morphological variations. Nanoparticles retain their effectiveness because they have enlarged surface area combined with higher stability and adjustable dimensions and shape which allow them to execute exceptional electrochemical and photocatalytic functions (figure 1.10) [33].

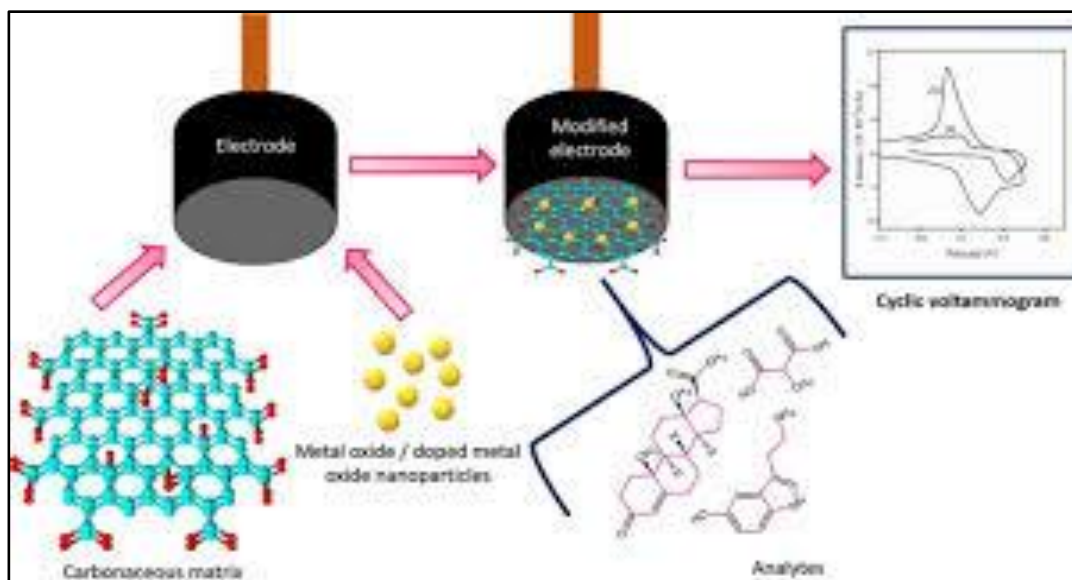


Figure 1.10 Schematic illustration how transition metal oxide nanoparticles are used in analyte electrochemical determination

Electrochemical research widely uses semiconducting metal oxide nanoparticles as signal indicators because of their advantageous electronic properties. Metal oxide nanoparticles that show compatibility with biological materials serve multiple roles in creating biosensors for medical detections and substance measurement systems. Widely accepted techniques for surface functionalization of electrodes with metal oxide nanoparticles include physical adsorption together with electro polymerization and covalent attachment and electrodeposition. The data depicts how transition metal oxide nanoparticles serve as essential elements for signal transmission and detection improvements of multiple target compounds in electrochemical detection systems [34].

1.9 Nickel Cobalt Oxides

Nickel cobalt oxides (NiCo_2O_4) are the mixed transition metal oxides which have received a lot of interest because of their superior chemical stability, electrochemical activity, and electrical conductivity. A material inherits the characteristics of both the nickel and cobalt oxides thus they are very suitable to be used in the energy storage devices, supercapacitor, catalysis, and sensors. The structure of NiCo_2O_4 is of spinel type, in which both nickel and cobalt ions are divided into tetrahedral and octahedral positions. Se device properties NiCo_2O_4 falls between Nickel oxide (NiO) and cobalt oxide (Co_3O_4) in their pure forms

[35]. Oxide of Nickel is generally a band gap insulator with a 3.6–4.0 eV energy band gap, whereas cobalt oxide is either metalloid, having an energy band gap 1.5 to 2.1 eV, or metallic, energy band gap 0 eV. NiCo_2O_4 is a mixed oxide and its band gap is narrower than that of the oxides with band gaps varying between 1.7 to 2.2 eV thus making NiCo_2O_4 superior in electricity conductivity as compared to oxides. This is because the band gap of the nickel cobalt oxides is tunable and also the materials exhibit combined electrochemical properties, which are valuable properties in a variety of advanced functional materials (figure 1.11) [36].

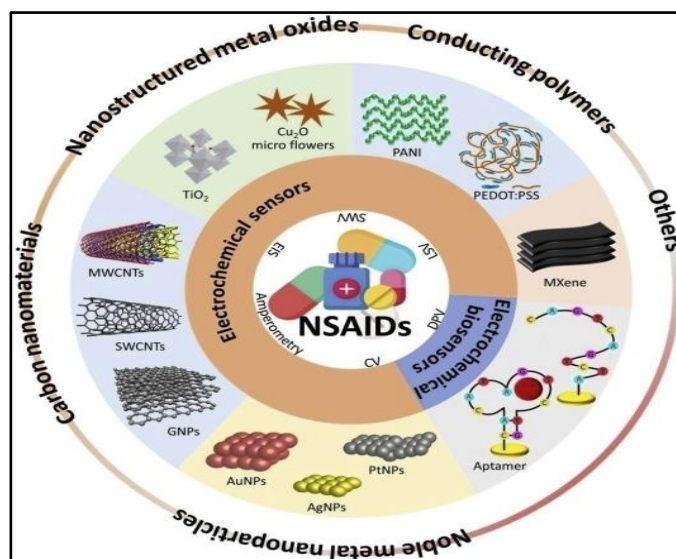


Figure 1.11 Recent applications of nanomaterials in biosensing

Active research is being done on Nickel-Cobalt, a substance that shows promise for use in biological systems. Wet chemical routes, solvothermal synthesis, reverse micelle procedures, sol-gel processes, thermal evaporation, hydrothermal techniques, and microemulsion approaches are some of the synthesis methods used to create Ni-Co nanostructures. The hexagonal wurtzite phase and the cubic zinc blende (sphalerite) phase are the two structural forms in which Ni-Co crystallizes; the latter is thermodynamically stable in ambient circumstances. Synthesis parameters including temperature, reducing agents, and surfactants or capping agents have a major impact on the physicochemical and morphological features of Ni-Co nanoparticles. These factors can affect particle size, distribution, and surface shape, which can then have an effect on how well the particles function in processes like environmental cleanup, chemical sensing, and catalysis. While

surfactants and polymers can be utilized as protective coating to modify the morphology of nanoparticles, higher synthesis temperatures often produce smaller, more evenly sized nanoparticles as can be seen in figure 1.12. Furthermore, a smaller particle size has been linked to raising the pH during synthesis. Ni-Co nanostructures may be produced economically and ecologically, which increases their applicability for a variety of uses [37].

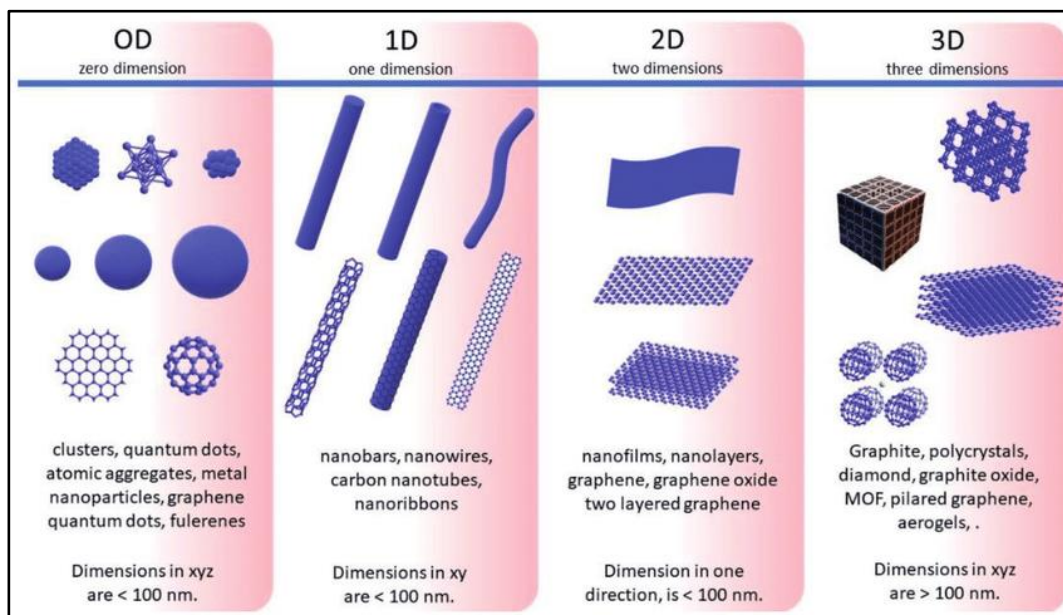


Figure 1.12 The Ni-Co oxide nanostructures come in four distinct sizes, each with distinct advantages [38]. Large surface areas are provided by 0-D nanostructures. The electron transport in 1-D nanostructures is straightforward and stable. Specific planes for the immobilization process are provided by 2-D nanostructures, enabling simultaneous detection of multiple analytes. Three-dimensional nanostructures provide more stabilizing sites due to their increased external and internal surface areas.

1.10 Nickel Cobalt Nanoparticles

1.10.1 Synthesis of Nickel Cobalt Oxide

Nickel-Cobalt nanostructures with exact morphologies, sizes, and topologies are created using a variety of synthetic processes. Of these, wet chemical techniques, such as molecular beam epitaxy (MBE) and chemical vapor deposition (CVD), are often used. An accessible and successful method for creating Ni-Co nanostructures, wet chemical synthesis is especially beneficial because of its ease of use, affordability, speed of processing, and low equipment needs.

Wet chemical processes include a variety of methods such as sol-gel it allows for better mixing of the materials. Many reports show how to make Ni-Co nanostructures using hydrothermal and solvothermal methods, and they explain how these structures can be used in different areas. Ni-Co nanoparticles are prepared and placed located on the exterior of a bulk material called tungsten carbide using a simple method. The results indicate that Ni-Co is a possible material to aid in the conversion of water into hydrogen gas. They usually only need a single step in the synthesis process; (b) high temperatures and pressures make it easier for a variety of precursors to dissolve; (c) using liquid-phase environments improves mass transport and reaction kinetics; and (d) these conditions help form well-defined nanostructures with regulated morphology and crystallinity [39].

1.11 Sensing of Bio-Analyte: Ascorbic Acid

1.11.1 Ascorbic Acid

A common component of human blood, ascorbic acid (AA) is essential for many physiological processes. It is an important nutrient and antioxidant, and 100 mg should be consumed daily to stay healthy. Scurvy, which is caused by a lack of AA, commonly referred to as vitamin C, might affect a woman's ability to conceive and interfere with the body's production of collagen. Moreover, AA levels may be used as a biomarker to identify diseases. Research has shown that the degree of oxidative stress in metabolic processes is correlated with changes in AA concentrations in body fluids. Numerous illnesses, including as diabetes, cancer, and liver problems, have been connected to elevated oxidative stress. Thus, it is essential to use sensitive, dependable, and repeatable testing techniques to monitor AA levels. A number of approaches, such as liquid chromatography, spectrophotometry, fluorescence, and electrochemical techniques, have been used to precisely identify AA in bodily fluids [40].

Because of their ease of use, high sensitivity, and accuracy, electrochemical techniques are preferred among them. Glassy carbon electrodes (GCE) and other traditional electrodes, however, have little electrochemical activity for AA detection. Highly active electrocatalysts are crucial for improving the GCE's performance since they increase its sensitivity and selectivity for precise AA detection in blood samples. Numerous

substances, including metal oxides and carbon-based materials have been extensively investigated and used to alter GCEs, greatly improving their electrochemical characteristics and detecting powers according to figure 1.13 [41].

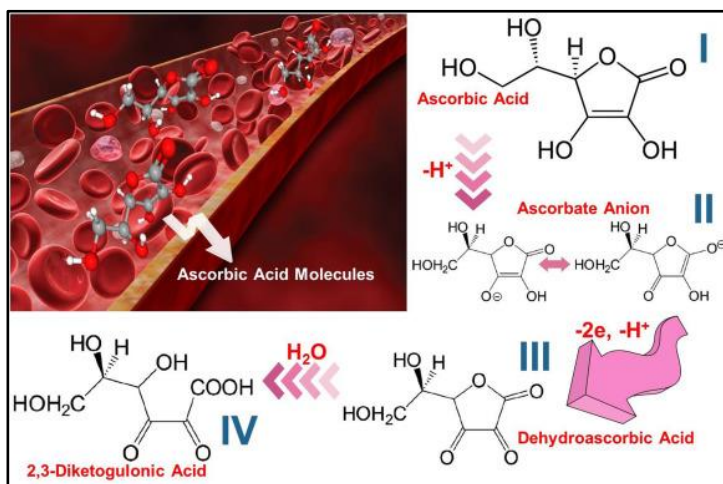


Figure 1.13 Mechanism of oxidation of AA that leads to its electrochemical sensing [42]

1.12 Problem Statement

Because of the human physiological level of ascorbic acid (AA) both hypervitaminosis and hypovitaminosis are observed. Numerous epidemiological studies demonstrated a correlation between the levels of AA and several health conditions such as diarrhea, gastrointestinal discomfort, nausea, scurvy, fatigue and impaired wound healing. Several analytical methods have been developed to quantify AA in biological matrices, but some of these are time consuming, expensive, and labor intensive. However, due to their many advantages such as high sensitivity, ease of operation and fast response, electrochemical techniques have been widely adopted for neurotransmitter monitoring. Hence, electrochemical sensors are a suitable candidate for real time screening of ascorbic acid which can be accomplished quickly and easily with low cost, and precise as a routine analysis. Sensing technique is widely used in the routine analysis because it is very simple, sensitive and specific among all techniques.

1.13 Aim and Objectives

- To develop and assess a new electrochemical sensor that uses nickel-functionalized graphene to detect ascorbic acid (AA) in biological matrices in a sensitive, reliable, and selective manner.
- Analysis of the morphological, structural and elemental properties of the produced electrode using X-ray diffraction, scanning electron microscopy (SEM) and UV-visible spectroscopy.
- Evaluate the manufactured sensor's analytical qualities, including its sensitivity, selectivity, limit of detection, and response time.

CHAPTER 2 LITERATURE REVIEW

The usage of graphene is common in form of composite, where it is mixed with other nanomaterials. Attaching iron (Fe) nanoparticles on graphene sheets is one of the outstanding approaches to surface functionalization. Here, Fe nanoparticle-decorated Graphene oxide nanohybrids that were synthesized through a one-pot solvothermal method, hydrothermal and post-thermal treatment under an argon (Ar) environment to form an electrochemical sensing platform. The synthesized composites were characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), infrared spectroscopy and voltammetric technique. In the monitoring of ascorbic acid (AA), composite Fe/reduced graphene oxide (Fe/rGO) showed excellent electrocatalytic activity. Fe nanoparticles of a size of 10 nm were evenly dispersed over the rGO surface in a post-calcination procedure done at 450 °C in an Ar environment. A high correlation was observed in electrochemical measurement on the detection of AA resulting in a linear regression correlation giving variables $\Delta \text{SET} = 0.61 \text{ (U)M}$ with standard deviation of 0.00 (U)M; the detection limit at signal-to-noise ratio (S/N) 3, and concentration range of 0.01 mM to 1.15 mM ($r = 0.99919$)[43].

Ascorbic acid operates inside human bodies as ascorbate while functioning as one of the basic exogenous vitamins which displays strong antioxidant combined with anti-inflammatory capacities. The review demonstrates how ascorbate functions to eliminate free radicals while explaining its antioxidant effects through various pathways including intracellular antioxidant activation and NFB/TNF pathway regulation and apoptosis control. The antioxidant properties of ascorbate enable it to work with small molecule antioxidants such as tocopherol and glutathione and thioredoxin and simultaneously enhances the expression and activation to antioxidant enzymes, consisting glutathione peroxidase (GPX), catalase (CAT), and superoxide dismutase (SOD). Ascorbate enhances target proteins AP-1, Ref-1, Nrf-2 transcription factors in order to activate genes that encode antioxidant enzymes. The substance boosts the performance of external polyphenolic antioxidants. Both DNA as well as proteins and lipids experience protection from oxidation under these circumstances which produces an inflammatory state that may

result in cell demise. When ascorbate interacts with free transition metals its antioxidant capabilities decrease and it starts producing oxidative reactions. The effects of ascorbate on DNA mutation along with inflammation and cell apoptosis particularly in cancer cells remain debatable within scientific circles [44].

The primary clinical signs of aging skin include reduced firmness, decreased dermal density, irregular pigmentation, and an increased tendency toward erythema. A growing number of individuals are seeking aesthetic interventions aimed at preserving youthful skin appearance. However, the administration of appropriate therapies can be particularly challenging in individuals with sensitive skin. In such cases, symptoms such as stinging, erythema, burning, and pruritus may occur in response to various stimuli manifestations that are typically absent in normal skin without external insult. Approximately 50% of the European population exhibits varying degrees of skin sensitivity. Using sonophoresis and micro needling techniques, this study has to approach the disadvantage of ascorbic acid on photodamaged skin. Ascorbic acid, a well-established antioxidant, was delivered via sonophoresis and micro needling to target signs of photoaging in reactive and erythema-prone skin. Repeated testing indicated a significant enhancement in skin elasticity. The combined application of both treatment modalities resulted in marked reductions in skin redness. The most pronounced improvement, particularly in cheek area appearance, was observed with the integration of vitamin C and micro needling. Furthermore, the treatments demonstrated excellent tolerability, even in sensitive skin, while producing significant therapeutic benefits [45].

Organic functional groups with high binding affinities, such as thiol, hydroxyl, carboxyl, and amine, are frequently found in compounds based on nanomaterials. However, some materials' intrinsic low electrical conductivity restricts how effective they may be in electrochemical applications. As a result, scientists are creating plans to use sophisticated fabrication techniques to increase the conductivity of metal oxides in order to reach particular detection thresholds. In the current experiment, reduced graphene oxide (rGO)/nickel oxide (NiO) nanoparticles and silver nanoparticles (AgNPs), were synthesized by simultaneous operation of simple electrochemical deposition of the reduced graphene oxide (rGO) and nickel oxide (NiO) employing nickel oxide (NiO)

nanoparticles (interfaced with silver nanoparticles on bare glassy carbon electrodes (GCE). Once the nanocomposite materials were deposited, they were removed off the GCE surface to further analyze it. The characterization of synthesized materials was made by Raman spectroscopy, scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX) and electrochemical impedance spectroscopy and by X-ray photoelectron spectroscopy (XPS). The electrochemical abilities of the materials were evaluated after deposition regarding ascorbic acid detection. For AA, the sensor's limit of quantification (LOQ) was 0.457 μM , and the recovery rates for commercial vitamin C tablets were 100.61% and 99.40%, indicating high analytical accuracy and reliability of the developed system [46].

A sensor system for ascorbic acid (AA) detection operates to monitor pharmaceutical and human samples. The synthesis of Au NPs and GO composites successfully took place through Hydrothermal method. Predominantly the Au NP/GO composites functioned to build essential components for practical AA monitoring. The sensor electrode demonstrates excellent analytical characteristics through its 10.15 V low working potential which enables 101.86 mA good specificity, a low limit detection of 100 nm, great sensitivity, and dependable repeatability. This technique was used both in order to precise quantification related to AA in the human body serum as well as clinical vitamin C tablets even when food additives were present in the samples. We believe the Cu NPs and GO sheets function synergistically to produce better electrochemical properties toward AA at the modified electrode due to increased Au NP electroactive area. The Au NP/GO/GCE system enables enhanced possibilities for sensitive and selective AA determination during medical patient examinations [47].

The dietary compound ascorbic acid known as vitamin C represents a vital vitamin possessed by humans the diet due to its critical role in many biological processes. Although the human body cannot firm bodily structures require vitamin C because it functions as many enzymes' cofactor and serves as an essential component that maintains healthy bodily structure. Enzymes that produce collagen along with an effective immune response depend on ascorbic acid at the same time, AA is Cosmetic labs utilize AA because it demonstrates antioxidant behavior while preventing pigment development and

food producers utilize it because it functions as an additive. The chemical synthesis field also consumes this substance due to its reducing properties. additive, and in chemical synthesis as reducing agent. AA exists as a commercially synthesized compound available through the production process. Bacteria use the combustion process for fermentation to generate this molecule while natural sources also provide available substances. This review addresses Modern techniques and procedures used to extract vitamin C from living organisms represent the current developments in its production process. Research centers on extracting Vitamin C from various plant materials and fruits and vegetables and algae and leaves Using the most prevalent environmentally-friendly extraction procedures. Ultrasound-assisted extraction, microwave-assisted extraction, pressurized liquid extraction, and supercritical fluid extraction are among the methods used in these circumstances. These extraction techniques are thought to be environmentally sustainable and use modest operating conditions. The system uses friendly solvents alongside low time and cost together with minimal energy input. In contrast to conventional methods, the methods' extraction results are on par with or even better than those of conventional methods [48].

Ascorbic acid represents an essential micronutrient required by human beings for its multiple biological functions. This substance exhibits antioxidant capabilities to directly neutralize reactive oxygen species and secondarily restore other defense mechanisms in the body. Vitamin C functions as a pro-oxidant agent at low concentrations in vitro solution and when contacted by metal ions but mainly acts as an antioxidant agent. A thorough evaluation of vitamin C's effects on DNA damage and gene expression patterns and protein oxidation exists both inside laboratory environments and human bodies. The current era marks the end of basic scavenger theories regarding ascorbate as recent bioinformatics highlight its ability to directly or indirectly affect genetic information [49].

Supporting the catalytic activity of collagen hydroxylases. These enzymes operate optimally when their iron cofactors are preserved in the reduced state by vitamin C. Additionally, vitamin C contributes to the epigenetic regulation of cells and tissues and influences gene expression through the hypoxia-inducible factor (HIF) pathway, among other responses that control a range of 2-oxoglutarate-dependent dioxygenases. Ascorbate's redox capacity more especially, its function as an electron donor is

responsible for all of its physiological and metabolic actions. Ascorbate easily experiences autoxidation that is dependent on pH, leading to the generation of hydrogen peroxide (H_2O_2), a process that accelerates in the presence of catalytic transition metals. This dual behavior enables ascorbate to function as both an antioxidant and a prooxidant, depending on the context and concentration. According to pharmacokinetic research, ascorbate administered intravenously (IV) circumvents the strictly controlled intestinal absorption processes, leading to noticeably higher plasma concentrations. At pharmaceutical dosages, ascorbate functions as a prodrug, specifically raising H_2O_2 levels in tumor cells while passing through the intestinal barrier without any problems. The clinical potential of pharmacological ascorbate, especially in oncology, has sparked increased scientific attention as a result of these findings. [50].

Since fresh evidence supports the use of high-dose intravenous vitamin C as an adjuvant therapy in the treatment of cancer, research on its processes and therapeutic benefits is still ongoing. This work employed a two-step electrochemical reduction approach to produce a glassy carbon electrode (GCE) modified with copper iron nanoparticles and reduced graphene oxide (Cu-Fe NPs/rGO/GCE). The homogeneous distribution of spherical Cu-Fe nanoparticles on the rGO-modified electrode surface was confirmed by field emission scanning electron microscopy (FESEM) analysis without compromising the intrinsic morphology of rGO. Electrochemical impedance spectroscopy (EIS) showed that the Cu-Fe NPs/ rGO/GCE possessed significantly high electrical conductivity as compared to the naked GCE and the rGO-modified GCE. The electrochemical sensing performance of the Cu-Fe NPs/rGO/GCE has been evaluated by using ascorbic acid (AA) as the analyte through cyclic voltammetry (CV), differential pulse voltammetry (DPV) as well as chronoamperometry. The sensor showed the wide range of AA detection under ideal experimental conditions, which indicated 0.5-3.5 mM, 3-15 mM, and 15-42 mM in ternary mixture experiments. Particularly impressive long-term stability, high repeatability, fast reaction time (probably falls into the range of 1-5 s), and very selective behavior of the sensor demonstrated the potential of exact AA identification in a complicated sample matrix. [51].

In this paper, the authors have introduced Ni-Co/graphene oxide (GO) nanocomposite to modify glassy carbon electrodes (GCE) in order to be able to electrochemically detect ascorbic acid (AA) simultaneously. Individual hydrothermal synthesis of cobalt selenide (Co-Se) and carbon nanotubes (CNTs) was performed. A complete characterization of the synthesized nanocomposite was carried out, with Fourier-transform infrared spectroscopy (FTIR), powder X-ray diffraction (XRD), energy-dispersive X-ray spectroscopy (EDX) and transmission electron microscopy (TEM), respectively. The prepared modified GCE was electrochemically analyzed by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Co-Se electron mediation characteristics combined with high-electrical conductivity properties of GO allow subsequent efficient transfer of electrons that can be attributed to the superior performance of the modified electrode. The composite CNT/Co-Se/GCE showed three easily resolved oxidation peaks at 0.05 V versus SCE, which was a better peak separation as compared to bare GCE, CNT/GCE and Co-Se/GO/GCE. Sensor exerted a linear response detection to ascorbic acid in a 0.1–1.0 mM range of mM concentration [52].

A notable scientific need is a comparison of an effective synthesis of graphene oxide compounds, doping with silver. Here the scientists have managed to synthesize silver doped reduced graphene oxide (Ag/rGO). Some of the methods employed in combination during structure investigation were scanning electron microscopy (SEM), Raman spectroscopy and infrared (IR) spectrometry. In the same experiment Ag/rGO has high sensitivity to detect ascorbic acid (AA) among other chemicals. Some of the methods included in combination with structure investigation were scanning electron microscopy (SEM), Raman spectroscopy, and infrared (IR) spectrometry. It is evident that Ag/rGO has high recognition capability of ascorbic acid (AA) in a similar experiment with other chemical compounds. Sensing range of the AA detection at operating point is at 0.1 mM. The AA detection method offers a 9.6 mM limit. It presents a convenient way of developing graphene-based materials that have outstanding electrochemical sensing capability. Functionally, the AA detection sensing range is 0.1 mM. There is a limit of 9.6 mM technique of detecting AA. The specified approach would provide an effective way to generate graphene-derived materials with impressive outcomes regarding the electrochemical sensing tasks [53].

In this study, a sensitive, in fact very sensitive Amperometric biosensor has been developed in the detection of ascorbic acid (AA) that is done with a Nano bio composite that consist of zinc (Zn) modified graphene oxide (GO). The fabrication of Zn/GO composite electrodes entailed the use of one-step electrochemical deposition technique that involved placing Zn on a glassy carbon electrode that was modified with GO (GO/GCE). Fourier-transform infrared spectroscopy (FTIR) ultraviolet / visible (UV-Vis) spectroscopy and the scanning electron microscope (SEM) methods have been used to determine the optical and structural characteristics of the Zn/GO nanocomposites. Differential pulse voltammetry (DPV) was used in phosphate-buffered saline (PBS) (pH= 7) to determine the electrochemical activity of the compounds in 0.2 M concentration. Electrochemical experiments proved the enhanced Zn/GO electrode had superior electrocatalytic properties to the oxidation of AA with a clear anodic peak observed at 0.09 V versus the SCE. The Zn/GO modified electrode had a 2.9-fold increase in peak current responsiveness, when compared to GO/GCE. The sensor displayed a linear range of detection of AA between 0.5 and 5 mM and a detection limit at 242mM. Also, Zn/GO-based electrochemical sensor showed high stability, low detection limit, good selectivity, reasonable repeatability, an expanded detection range, indicating that it potentially could be used in practical AA sensing applications [54].

To achieve an ascorbic acid (AA) selective electrochemical sensing, Cu₂O/graphene oxide (GO) nanoparticles supported on carbon-based structures were adopted on a glassy carbon electrode (GCE). The electrochemical impedance spectroscopy (EIS) result showed that the modified electrode had a much larger reduced charge transfer resistance, which meant that the electron transfer items were much more efficient. The electrochemical characteristics of the modified electrode under preparation were scrupulously analyzed under AA oxidation. After the alteration, the AA sensitivity increased by approximately five folds, and the oxidation potential shifted drastically to - 0.20 V. The sensor had a wide linear detection range of 1.0×10^{-5} M to 2.663×10^{-3} M ($R^2 = 0.9929$) with a low detection sensitivity of 0.5 mM with signal to noise ratio 3. The Cu₂O/GO nanocomposite exhibited excellent electrocatalytic properties for AA detection, characterized by rapid response, high stability, and reproducibility. These features enabled accurate quantification of AA in real sample matrices. Furthermore, the sensor

showed strong selectivity, effectively distinguishing AA from potential interfering substances, confirming its utility as a reliable electrochemical platform for AA-specific sensing application [55].

High-efficiency electroactive materials that are inexpensive, safe for the environment, and simple to synthesize are thought to be the best options for energy conversion, energy storage, and non-enzymatic sensing applications. Bioactive chemicals that were isolated from the biomass of garlic green leaves were used in this study to improve the electrochemical characteristics of FeCo_2O_4 nanostructures while also acting as capping, stabilizing, and reducing agents. The hydrothermal process was used to create FeCo_2O_4 nanostructured materials using different amounts of garlic leaf extract (5 mL, 10 mL, and 15 mL). The final materials were described in terms of their electrochemical behavior, crystallinity, and surface chemical composition. Without the assistance of enzymes, ascorbic acid (AA) was detected electrochemically in phosphate-buffered saline (PBS) at pH 7.4. Pure FeCo_2O_4 underwent a morphological change from nanorods to nanowires made of aggregated nanoparticles when garlic extract was present. Enzyme-free biosensors showed better stability, repeatability, and easier manufacturing procedures than enzyme-based biosensors. The goal of current study is to employ 10 mL of garlic leaf extract to create FeCo_2O_4 nanostructures as non-enzymatic AA sensors. With a detection limit of 0.01 mM, the manufactured sensor demonstrated a linear detection range for AA from 0.5 to 8.5 mM. Together with dependable performance in the detection of AA in actual samples, the sensor also showed great selectivity, strong operating stability, and reproducibility [56].

Researchers developed Fe-Ni/CNTs/GCE-Co/GO/GCE bimetallic supported GO modified electrode for sensitive and electroactive along with stable detection of ascorbic acid. The electrocatalytic properties and high electrical and thermal conductance arise from Fe and Ni transition metals through their surface-to-volume ratio and d-band electron presence. The presence of GO in the system enables it to maintain high surface area alongside a stable framework and outstanding conductivity. The synthesized material undergoes SEM, EDS, XRD and FTIR analysis to determine its structural and morphological composition. The combined use of these materials enables the analysis of ascorbic acid through electrochemical measurements where their operational parameters

receive thorough optimization. The Fe-Ni/CNTs/GCE-Co/GO/GCE operates optimally for analytical purposes when the electrolytic solution reaches pH 7.4. Under optimized conditions the modified electrode demonstrates both high sensitivity and good reproducibility along with thirty cycles of reusability without loss of efficiency. Good linearity combined with reproducibility and detection limit ensures this material demonstrates potent prospects as a non-enzymatic biosensor for diverse types of analytes [57].

Identifying a natural, non-enzymatic electrode catalyst capable of sensitively and selectively detecting biomolecules has posed considerable challenges. Current research has increasingly focused on the application of metal-based nanocomposites for the development of ascorbic acid (AA) sensing electrodes, owing to their outstanding redox activity, high electrical conductivity, low porosity, and robust mechanical properties. Nevertheless, a major obstacle to their extensive commercial use is still their poor electrochemical stability. These constraints have led to the successful synthesis and use of a novel nitrogen-doped graphene-encapsulated nickel–cobalt nitride (Ni–xN/NG) core shell nanostructure with a tunable Ni/Cu molar ratio as a highly sensitive and selective electrode for the detection of glucose and hydrogen peroxide (H₂O₂). Additionally, because of the significant synergistic interaction between the Ni, Cu, and GO components, the Ni–Cu/graphene oxide (GO) modified electrode showed outstanding sensitivity and selectivity towards ascorbic acid, improving the overall electrocatalytic performance for AA detection [58].

The current paper includes the steps and procedure that will be used to prepare a partially reduced glassy carbon electrode (RGO-GCE). Graphite oxide is obtained through the process of a modified Hummer and further exfoliated under ultrasonic conditions to produce its graphite oxide (GO). RGO-GCE is then prepared by running two potentiodynamic cycles (decreasing to bubbling of the working electrode) to electrochemically decrease GO in a 0.1 M buffer answer at pH 3.0. The RGO-GCE was tested electrochemically by the means of differential pulse voltammetry (DPV) to measure ascorbic acid (AA), dopamine (DA) and uric acid (UA) simultaneously after performing a 3-minute preconcentration process. The modified electrode in acidic medium (in acidic pH 3.0) demonstrated outstanding resolution and reproducibility with a relative standard

deviation of 1.42, 1.92 and 2.20 per cent of AA, DA and UA, respectively. The sensor demonstrated broad linear ranges of detection 4×10^{-5} to 1×10^{-3} M of AA, 1×10^{-7} to 1×10^{-4} M of DA and 8×10^{-7} to 8×10^{-4} M of UA. The respective limit of detections was 4.2:10⁶ M 8:10⁹ M and 6:10⁷ M when three times the standard deviation of blank signal was used. One can also observe that minimal fouling on the sensor shown can cause a lot of difference. This material was well characterized by various electrochemical methods, transmission electron microscope (TEM), scanning electron microscope (SEM), infrared (IR) and Raman spectroscopy among others. The designed RGO-GCE sensor has been able to effectively sense numbers of uric acid in the sample matrices and measure AA in the human serum with high recovery percentages [59].

The hydrothermal synthesis technique was used to prepare a RGO-GCE. The following methods were used to complete the description of the structural and morphological characteristics of nanocomposites obtained: X-ray photoelectron spectroscopy (XPS), transmission electron microscopy (TEM), scanning electron microscopy (SEM) and X-ray diffraction (XRD). To assess the electrochemical analysis in ascorbic acid (AA) detection a Ni-Co/rGO nanocomposite-modified screen-printed carbon electrodes (SPCEs) were used to perform cyclic voltammetry (CV). In comparison with electrodes exchanged solely with low quantity graphene oxide, the Ni-Co/rGO-cored SPCEs showed better electrocatalytic action in oxidizing AA. In simultaneous analyte sensing this was illustrated by distinct and strongly resolved oxidation peaks, cathodic shift in peak potential and an increase in peak current response. The linear response of AA at 45-1550 μ M, together with low detection limit (0.81 μ M) indicated that the Co/rGO-modified electrode is extremely sensitive so that it can be used in analytical studies on AA measurement [60].

It is critical to detect ascorbic acid (AA) simultaneously used in clinical diagnosis and monitoring treatment processes. A polypyrrole/graphene oxide composite decorated with gold nanoparticle on carbon fiber paper (CFP), (Au NPs@GO/PPy), was easily prepared in two steps. Graphene oxide (GO) was instead integrated with polypyrrole (PPy) to retain as many possible oxygen-containing functional groups that are useful in the improvement of the electrochemical sensing activity than when the graphene is reduced to rGO. The electrochemical performance of utilized gold nanoparticle GO/PPy/CFP electrode was

tested with regard to the appreciable determination of AA. Three different analytes were observed as low overpotentials in three oxidation peaks set at 0.02, 0.16, and 0.28 V on the modified electrode. Having a linear response range of 1021600 mM, 0.260 mM, and 2360 mM, and detection limits of 2.43 mM, 0.115 mM, and 1.68 mM ($S/N = 3$), the sensor exhibited extraordinary sensitivity towards detection of AA. The proposed sensor had a high electrocatalytic performance and sensing selectivity to AA. The practical feasibility of the Au NPs@GO/PPy/CFP modified electrode in the precise and reliable determination in challenging biological conditions was also demonstrated by operating it in the analysis of real samples successfully [61].

This paper outlines the creation of the ultra-sensitive electrochemical sensor of voltammetric detection of ascorbic acid (AA). A hybrid nanocomposite (Graphene oxide (GO), multi-walled carbon nanotube (MWCNTs), gold nanorod (AuNRs)) was used to modify the glassy carbon electrode (GCE). In order to avoid aggregation of MWCNTs, GO was incorporated in the composite so that its extremely large surface area and conductive paths could be maintained. The AuNRs showed greater positive charge which led to high electrocatalytic activity because the oxidation peak current of AA was raised and the oxidation overpotential was decreased. The sensor that arose had great analytical capability with a very low detection limit of 0.85 nM and high sensitivity of $7.61 \mu A \mu M^{-1} cm^{-2}$. Moreover, it has two different linear ranges of responses 1 nM-0.5 wider M and 1 wider M-8 mM which made it capable of detecting a wide range of concentration. The sensitivity of the sensor was also tested at a low applied potential of 0.036 V (vs. Ag/AgCl) to prove its feasibility in terms of efficient and selective AA monitoring in complex types of matrices. [62].

In this work, an electrochemical sensor using nitrogen doped graphene (NG)/gold nanorods (AuNRs) has been designed to carry out the electrocatalytic detection of ascorbic acid (AA). AuNRs in combination with NG, possess a high surface area with faster kinetic electron-transfers, NG owing to its high surface area, a high number of AuNR anchoring sites, higher electrical conductivity and intrinsic electrocatalytic activity makes a very sensitive AA sensing platform. The NG and AuNRs were synthesized by following a standard previous method, and the synthesized NG and AuNRs were well characterized with a variety of morphological and spectroscopy measures. The

electrochemical analysis was carried out with the help of differential pulse voltammetry (DPV). At optimal pH conditions, the sensor displayed two broad linear dynamic ranges (5×10^{-9} to 1×10^{-6} and 1×10^{-6} to 8×10^{-3}), at which to measure AA. The sensor limit of detection was really low and stood at 2.6 nM. Moreover, the AuNR/NG- modified electrode revealed very high degree of repeatability, long term stability as well as higher degree of reliability in the determination of AA in dilute human serum samples. These findings have outstanding future prospects of AuNR/NG interface as a stable and viable product in terms of electrochemical biosensing [63].

The article has eloquently offered the description of the synthesis of a three- dimensional, networked, edge rich graphene Nano structure that would be used in advanced electrochemical sensing equipment. The framework of this architecture was built with a template of network of nanofiber γ -alumina, having diameters approximating 7nm. The one step chemical vapor deposition (CVD) uniformly covered this network with highly exfoliated graphene. The unique and variable foliate densities of the resulting graphene-coated nanofibers allowed the creation of a very sensitive platform through which the simultaneous electrochemical detection of dopamine (DA), uric acid (UA), and ascorbic acid (AA) were possible. The main aim was to find AA with high sensitivity and selectivity over a wide range of concentrations 5 nM to 1 mM. Evaluation of electrochemical performance using hexacyanoferrate (redox probe) was made by running differential pulse voltammetry (DPV), cyclic voltammetry (CV), and chronoamperometry. Optimized sensor showed a linear resolution in the range of AA (1uM - 60uM) with a high sensitivity of (1.06 micro A/ micro M/ cm²) and a limit of detection of (117 nM) at a foliate density of (50 foliates/ micro m)¹. The effectiveness of sensor was also confirmed cause it was able to measure the AA in manipulated samples of human urine. The findings indicate a possibility of formulating highly stable and responsive electrochemical sensors having the ability of analyzing complex biological samples, using morphology-engineered graphene nanostructures. [64].

Direct heat oxidation was a low-cost method of manufacturing two-dimensional porous graphitic carbon nitride (g- C₃N₄) nanosheets. These nanosheets were further conjugated with the graphene oxide (GO) yielding a porous g-C₃N₄/GO composite making it a highly efficient electrochemical sensor to simultaneously detect several common ascorbic acid

(AA). The next measure was to immobilize this compound on a glassy carbon electrode (GCE). Analyses of cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used to investigate the electrochemical and electrocatalytic of the enhanced electrode. The special hierarchical composition of pores, and big specific surface area contributed to improve the indirect electrochemical signals response to AA at the sensor. The peak separation was observed at observable oxidation potential of 0.15V when AA was used. AA could be detected by the sensor at the limit of 3.7 μ M. It also showed very high recovery efficiency in actual sample estimation, with a range of 95.1-105.5 percent mean recoveries as well as relative standard deviations (RSDs) in human serums which were tampered that fell within the region of very less than 5 percent. These performances evince the appropriateness of the sensor in precise and reliable, AA measurement in complex biological matrices [65].

A one step co-deposition method was used to apply carbon quantum dots reduced graphene oxide (CQDs C rGO) nanocomposites on the surface of a glassy carbon electrode (GCE) to create an effective electrochemical detector of both ascorbic acid (AA) with minimal changes in the structure of the original nanocomposite. Combination with synergistic effect between CQDs and rGO provided an improved electrocatalytic activity and AA selectivity of the sensor. A maximum potential difference of 0.147 V was measured by the sensor in the presence of detection of AA. It gave two linear ranges of responding in AA: 20-90 mM and 0.2-0.9 mM having correlation value since 0.99. The synthesized CQDs-rGO-based sensor is very promising to use in the real life since the sensitivity of the sensor in combination with its stability and capacity to accurately measure AA in the realistic samples [66].

Polyaniline/graphene oxide (PANI-GO) fibrous nanocomposites have been widely studied in the aspect of electrochemical oxidation of ascorbic acid (AA), through the electrocatalytic performance. Those nanocomposites were assessed in terms of their morphologic, structural, thermal, and electrochemical qualities using scanning electron microscopy (SEM), X-ray diffraction (XRD), Raman spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, thermogravimetric analysis (TGA), and cyclic voltammetry (CV). The nanocomposites were in situ chemically polymerized. Electrochemical experiment on a PANI-GO-modified glassy carbon electrode (GCE) in

the presence of AA by applying differential pulse voltammetry (DPV) and CV techniques reveals that the modified electrode has much higher catalytic capacity with regard to the oxidation of AA in relation to bare GCE. Whereas the maximum possible separation of AA oxidation in the study of CV was found to be 343 mV, the study of DPV exhibited a separation of 320 mV. Best DPV conditions showed that maximum current response to AA was found to be directly proportional to the concentration with high R^2 of 0.9955 with a concentration (25-200 mM). The demonstrated efficiencies of PANI GO nanocomposites as effective electrocatalyst in the detection of AA and the proposed attractive synergistic effects on simultaneous electrochemical sensing open new opportunities in analytical applications in the fields of bioanalysis and diagnostics [67].

There was a development of a three-dimensional porous nanocomposite material (3DCu₂O--ZnO NPs/PPy/rGO) in combination with reduced graphene oxide (rGO) and polypyrrole (PPy) nanofibers which were used to simultaneously detect ascorbic acid (AA) through a voltammetry experiment. Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS) and field emission scanning electron microscopy (FE-SEM) were used to wholly detail the morphological and structural characteristics of the nanocomposite. The electrochemical characteristics were determined based on a differential pulse voltammetry (DPV) and a cyclic voltammetry (CV). The 3DCu₂O 3DCu₂O-ZnO NPs/PPy/rGO-modified GCE showed high selectivity and excellent electrocatalytic behavior since the broad flat-sensing ranges were 0.08- 475 mM and the low limit of detections were 0.024 mM, 0.012 mM, 0.010 mM, and 0.016 mM (S/N equal to 3), respectively, 0.04- 420 mM Its synergetic interaction, high conductivity and large surface area of the heterostructure nanocomposite lead to the enhanced performance. Moreover, the observed high selectivity and good application in the quantitative determination [68].

This work presented a very effective and simple nanohybrid material with which ascorbic acid (AA), dopamine (DA), and uric acid (UA) were electrochemically detected. The CRGO/Fc-ac-PPP composite is composed of chemically reduced graphene oxide (CRGO) and redox active polymer (para-phenylene) which contains ferrocene moieties. Adding ferrocene units provided the hybrid with greater redox activity, a synergistic effect greatly enhancing the electrical conductivity of the hybrid, electroactive surface area, and

catalytic activity. The resulting electrode had good resolved redox peaks of AA, DA, UA, and therefore it was possible to determine each separately and in any complex mixture. The sensor also exhibited large linear response windows of 0.1-10000 μM of AA with comparable detection limits ($S/N = 3$) of 0.046 μM , 0.2 nM and 0.013 μM . tested on human urine samples, which could be tampered on, the modified electrode was able to identify differences in physiological and pathological levels of the target analytes thus, suggesting the possibility of gainful biological and clinical diagnostic uses [69].

The paper looks into the electrochemical sensing potential of graphene (GR)/glass electrodes (GCEs), multi-walled carbon nanotubes (MWCNTs)/GCEs and their composite (GR-MWCNT)/GCEs to sense ascorbic acid (AA), dopamine (DA) and uric acid (UA) simultaneously. Among the three, the GR-MWCNT-modified GCE attained the best sensitivity and the least detection limits. GR-MWCNT/GCE recorded a simultaneous signal at low negative potentials (-0.032, 0.206 and 0.34 V vs Ag/AgCl against Fe/AgCl), respectively, of AA, DA, and UA using differential pulse voltammetry (DPV). The detection limits of the sensor were 6.71 and 0.58 in terms of AA and DA, respectively, and 7.30 in UA whereas its sensitivity was 0.076, 1.38, and 0.181 according to AA, DA, and UA, respectively. The GR-MWCNT/GCE demonstrated remarkable anti-interference, reproducibility of the measurement, and stability of the operation, as well. The effectiveness of the designed sensor was demonstrated by the possibility of the simultaneous recognition of AA, DA, and UA in treated samples of human blood and urine, which shows its significant implications in clinical and diagnostic activities. [70].

The electrochemical reaction of the produced RGO-GCE to uric acid (UA), dopamine (DA), and ascorbic acid (AA) was measured by checking the electrochemical behavior of the made RGO-GCE after three minutes of accumulation through differential pulse voltammetry (DPV). All three analytes presented resolved oxidation peaks at a pH of 3.0 and the sensor was capable of individual as well as simultaneous detection, with relative standard deviations of 1.42%, 1.92% and 2.20% of AA, DA and UA, respectively. Ideally, the sensor showed large linear detection limits such as 4×10^{-5} to 1×10^{-3} M in AA, 1×10^{-7} to 1×10^{-4} M in DA, 8×10^{-7} to 8×10^{-4} M in UA. The related detection limits which are dependent on the minimum surface fouling and three times standard deviation of the blank reads were calculated as 4.2×10^{-6} M, 8×10^{-9} M and 6×10^{-7} M. The nature of the

modified electrode was thoroughly studied by scanning and the transmission electron microscopy (SEM and TEM), Raman spectroscopy, infrared (IR) spectroscopy and electrochemical procedures. Last but not least, when a series of experiments were already able to secure the appropriateness of the RGO-GCE sensor to the quantitative analysis and determination recovery of AA, DA, and UA in human serum samples, its applicability in the biomedical and clinical diagnostics was confirmed [71].

3.1 Chemicals Required

Hexahydrates of nickel nitrate ($\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) and cobalt nitrate ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), ethylene glycol, Graphene powder, sulfuric acid (H_2SO_4), hydrochloric acid (HCl , 37%), ascorbic acid (AA), ethanol, Deionized (DI) water, sodium hydroxide (NaOH).

3.2 Apparatus Used

Potentiostat, beakers, conical flask, measuring cylinder, pipette, Centrifuge machine centrifuge tube, Vacuum oven or desiccator, pH meter or pH indicator strips, Teflon-lined stainless-steel autoclave (50–100 mL capacity), Magnetic stirrer and sonicator.

3.3 Functionalization of Graphene

The functionalization of graphene begins with the selection of the solvent, which in this case was sulfuric acid. In the process, 50% solution of sulfuric acid was prepared. 50 ml of sulfuric acid was taken in a beaker and then added 50 ml of water in it in little amounts. Graphene was mixed in the above prepared solution and then this solution was then subjected to stirring for a duration of 3 hours using magnetic stirring to ensure proper mixing and interaction. After mixing, the washing of graphene was done by distilled water and ethanol. After proper washing the drying process was done and stored the graphene oxide for further use.

3.4 Preparation of Solutions

3.4.1 Preparation of Nickel Precursor

20 mL of deionized water was used to dissolve 290 mg of precisely measured nickel (II) nitrate hexahydrate $[\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}]$. To enable full dissolution, the resultant mixture was sonicated for ten minutes, producing a transparent and uniform nickel precursor solution.

3.4.2 Preparation of Cobalt Precursor

290 milligrams of properly weighed cobalt (II) nitrate hexahydrate $[\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}]$ were dissolved in 20 milliliters of deionized water. Sonication of the mixture was done in ten minutes to make sure cobalt salt is completely dissolved and a clean and equal precursor cobalt solution was obtained.

3.5 Dispersion of Graphene Oxide

Once 20 mg of highly inspected graphene oxide (GO) powder (distilled) was fixed in 10 mL of deionized water, the blend was sonicated in 10 minutes to obtain a reliable and uniform GO suspension.

3.6 Preparation of the Homogeneous Precursor Mixture

The mixture of the three prepared solutions of nickel precursor, cobalt precursor and dispersed GO was later prepared and 17 milliliters of ethylene glycol which acted as a co-solvent and reducing agent was added to this mixture. It was then permitted to simmer half an hour. The final mixture was sonicated for an additional 15 to 20 minutes to ensure uniform dispersion of all constituents to create a homogenous precursor solution.

3.7 Synthesis of NiCoO@GO

In the beginning, graphene oxide (GO) was created using a modified version of Hummer's method. Then, 0.02 g of GO that had been dissolved in 15 mL of ethylene glycol was mixed with 0.29 g of nickel (II) nitrate hexahydrate $[\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}]$ and 0.29 g of cobalt (II) nitrate hexahydrate $[\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}]$. The mixture was stirred for ten minutes to guarantee even dispersion. The final solution was then placed in a 50 mL Teflon-lined stainless-steel autoclave and heated to 180 °C for 12 hours. After the reaction was

complete, the autoclave was allowed to naturally cool to ambient temperature. The resulting precipitate was collected by centrifugation, washed several times with ethanol and deionized water, and then dried in an oven set at 60°C for 24 hours. The final NiCoO@GO composite was created by calcining the dry precursor for two hours at 550°C in an argon (Ar) atmosphere. The product was then stored in an airtight container for subsequent use.

3.8 Formation of Slurry

To prepare the slurry, 5 milligrams of nickel-cobalt oxide with graphene oxide (NiCoO@GO) was taken and mixed with a 1:1 solution of ethanol and water. To this mixture, a few drops of Nafion binder were added. The reaction mixture was then thoroughly mixed by using sonicator to form a uniform slurry, which was further used for coating or electrode preparation in various electrochemical applications.

3.9 Fabrication of Electrode

To get rid of surface contaminants, the glassy carbon electrode (GCE) was first ultrasonically sonicated in ethanol for around ten minutes. The electrode was then thoroughly rinsed with distilled water after being polished with alumina slurry until it had a mirror-like appearance. After that, a volume of 8 μ L of NiCoO@GO suspension (10 mg/mL) was drop-cast onto the GCE's polished surface and let to dry naturally. For electrochemical sensing applications, the modified electrode was later used as the working electrode.

3.10 Electrochemical Measurements

A standard three-electrode electrochemical cell setup was used for all voltammetric experiments. An Ag/AgCl electrode (saturated KCl) was used as the reference electrode, a platinum wire as the counter electrode, and a glassy carbon electrode modified with NiCoO@GO as the working electrode. The monobasic and dibasic versions of the phosphate buffer solution were used as the electrolyte. A constant rate will be used to perform the amphoteric measurements, and the potential window was set at -0.4_0.8. The scan speeds were varied.

4.1 Characterization of NiCoO

4.1.1 Scanning Electron Microscopy (SEM)

To study surface morphology and to verify the homogenous dispersion of structure integrity of NiCoO@GO nanocomposites, SEM analysis is used.

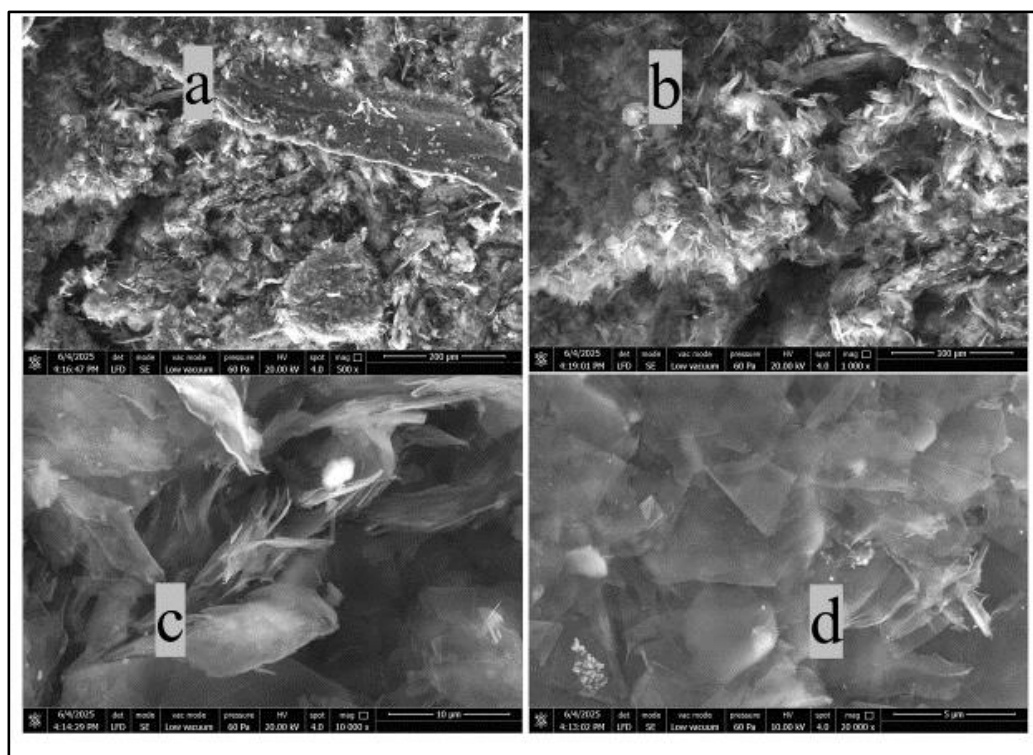


Figure 4.1 SEM images of NiCoO@GO at different magnification

4.1.2 X-Ray Diffraction (XRD)

The crystalline structure and the effective synthesis of NiCoO@GO is proved by XRD analysis by recognizing the typical diffraction peaks of both NiCoO and graphene oxide.

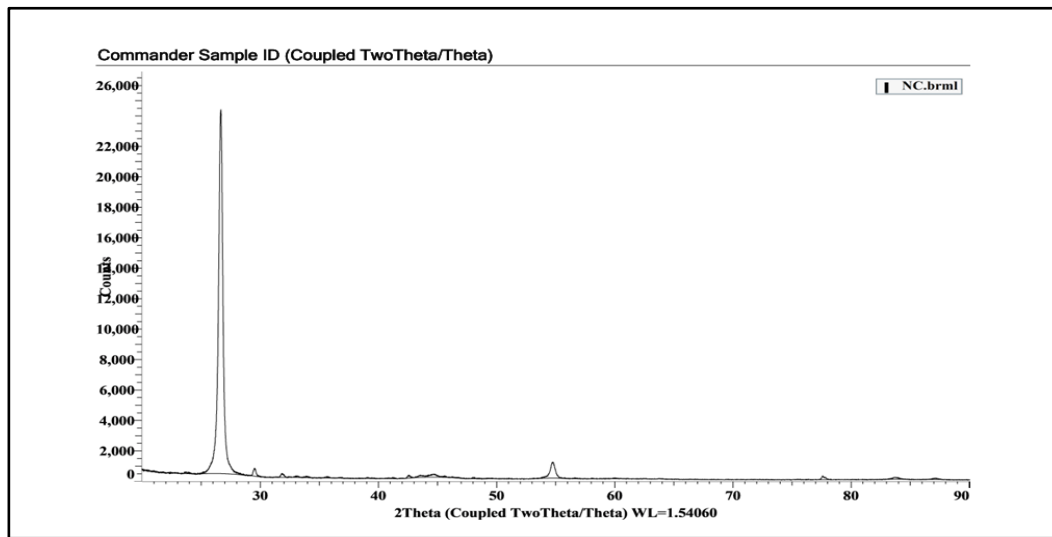


Figure 4.2 XRD image of NiCoO@GO

4.1.3 Ultra-Violet Visible Spectroscopy (UV-VIS)

The optical properties of the NiCoO@GO nanocomposites are studied using UV-Vis spectroscopy and their electronic transitions confirmed.

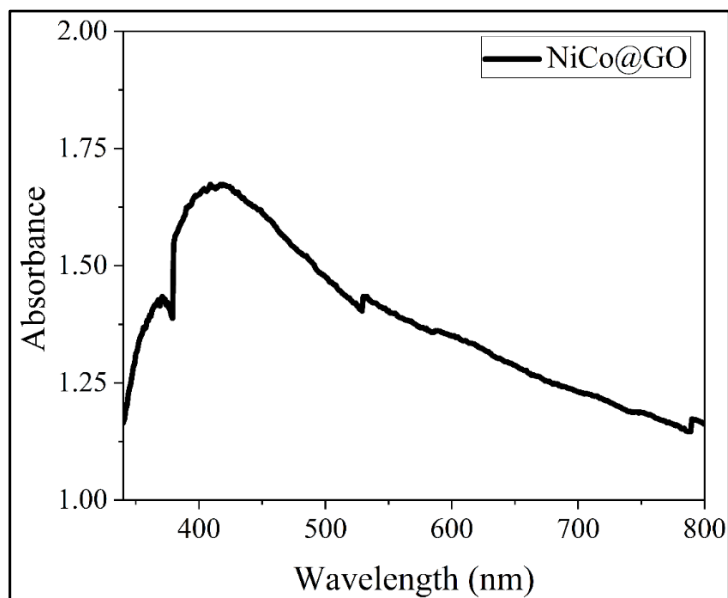


Figure 4.3 UV-VIS spectra of NiCoO@GO

4.2 Electrochemical Oxidation of Ascorbic Acid

4.2.1 Cyclic Voltammetry (CV)

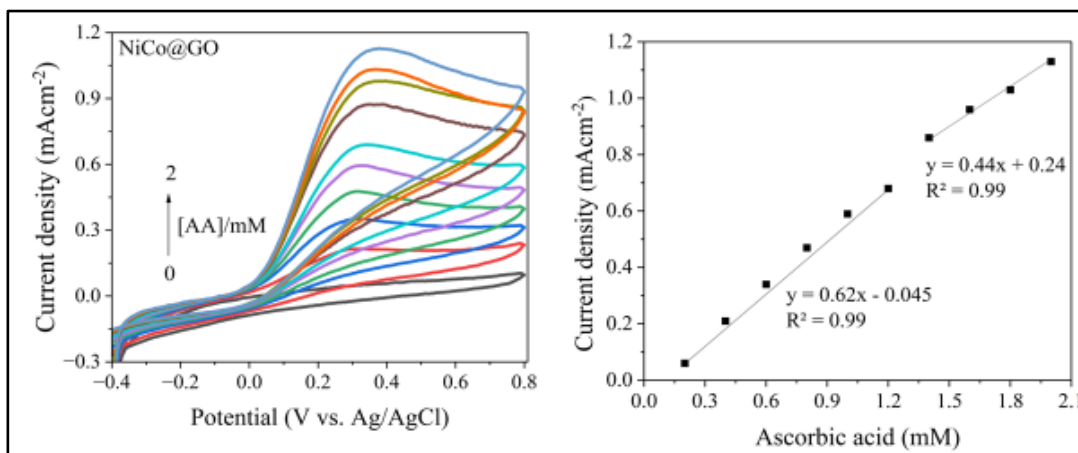


Figure 4.4 (a) cyclic voltammogram of Ascorbic Acid (b) representing respective calibration curve

4.2.2 Influence of Scan Rate

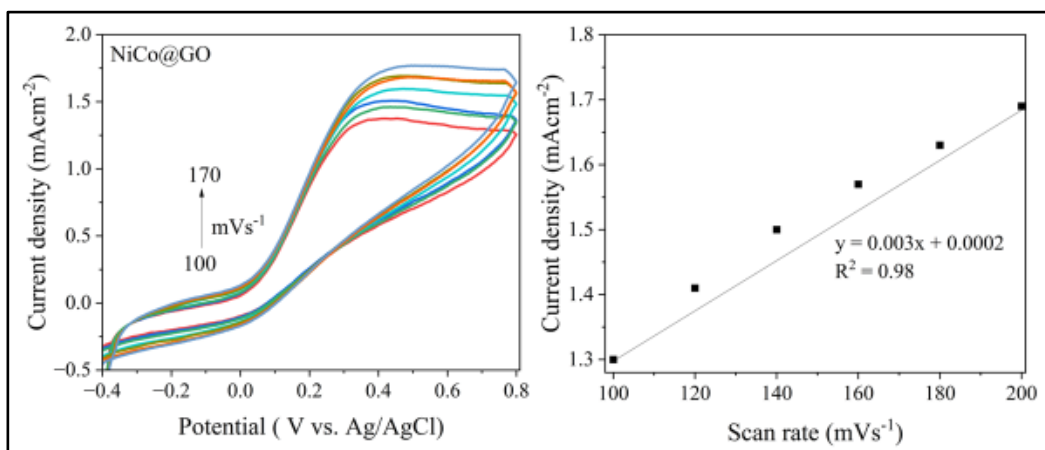


Figure 4.5 (a) Cyclic voltammogram at different scan rates (b) representing respective calibration curve

4.2.3 Square Wave Voltammetry

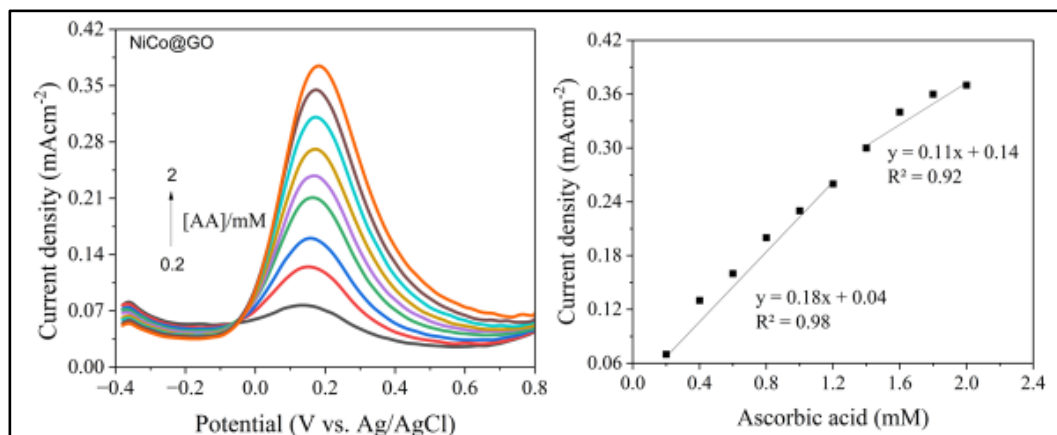


Figure 4.6 (a) Square Wave Voltammogram of Ascorbic Acid (b) representing respective calibration Curve

4.2.4 Chronoamperometry

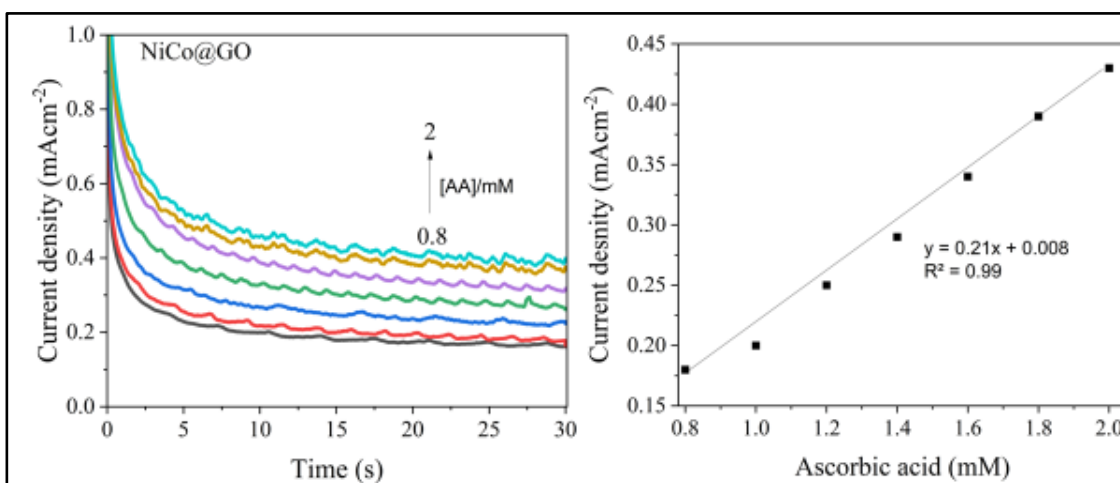


Figure 4.7 (a) Chronoamperometry at different applied potentials (b) representing respective calibration curve

Table 4.1 Electrochemical performance parameters of NiCoO@GO for ascorbic acid sensing, including sensitivity, linear range and limit of detection

Electrode	Analyte	Sensitivity mAmm ⁻¹ cm ⁻²	Linear range mM	LOD mM
NiCoO@GO	AA	0.53	0-2	0.056

5.1 SEM Images of NiCoO@GO

The scanning electron microscopy (SEM) in figure 4.1 shows the 10 x 10 and the 200 x 200x enlargements of the surface morphology of the NiCoO@GO. Image (a) shows at the magnification of 500x, a very rough, porous, and irregular substrate surface, which is evidence that loosely packed and interconnected structures are present throughout the matrix. In image (b), with 1000x magnification, the fibrous and sheet-like morphology are shown in higher details, showing the formation of an entangled network of thin flakes and layered structures, which also lead to an increased amount of surface area. Arriving at image (c), acquired at 10,000x magnification, one can observe unambiguously thin, crumpled, and even wrinkled NiCoO, a characteristic trait of graphene oxide materials that provides plenty of active spots and enhances surface roughness. Lastly, image (d) demonstrates, at a magnification of 20,000X, very transparent and well-formed and stacked nanosheets with insolvency at overlapping layers showing that the layered structure of graphene oxide has been indeed produced successfully. All these lead to the realization that upon deposition, a high-surface-area, connected, multiple layered NiCoO@GO is as formed, which is rather valuable to potential uses in electrochemical sensing.

5.2 XRD Pattern of NiCoO@GO

The image 4.2 represents the intensity (au) is plotted in the graph on the y-axis and the 2 theta (theta) in degrees on the x-axis, which is the conventional manner of presenting XRD data. The curve shows a number of sharp spikes at certain 2 theta angles, each of those spikes is related to the X-rays diffraction by the crystalline planes of the material under analysis in this situation, the Nickel Cobalt Graphene Oxide (NiCoO@GO) nanocomposite. The material is crystalline as there are sharp well-defined peaks. The respective peak positions (2 value) can be calibrated with accepted diffraction data sources in order to determine the individual crystalline phases that are present. In this sample, there should be characteristic peaks of NiCo₂O₄ spinel structure and probably some broader, weaker peaks which would be associated with graphene oxide (GO).

Normally, GO has a wide peak in 1013 the area whereas NiCo_2O_4 could have very sharp peaks at the locations such as 26, 27, 44.8, 54, 55, respectively signifying the (220), (311), (400), (511), and (440) planes respectively. The relative strengths of these peaks provide information on the crystallinity and phase purity.

5.3 UV-VIS Spectra of NiCoO@GO

The visible ultraviolet absorption spectrum of NiCoO@GO nanocomposite in Figure 4.3 indicates that the characteristic feature of UV-vis absorption spectrum shows a broad absorption band at a range of about 300 nm to 800 nm, with a sharp peak at around 380-410nm. This maximum is caused by charge transfer transitions between the metal ions (Ni^{2+} / Co^{2+}) and ligands oxygen in the metal oxide structure. The high absorption in the visible domain means the improved light-harvesting capacity making transition metal oxides as such and combined with graphene oxide. The gradual decline of absorbance above 450 nm indicates less absorption of light at high wavelength which implies that the material is a semiconductor. It also shows spectral evidence of successful addition of NiCoO nanoparticle on the GO sheets thus improving the optical functions as there are synergistic interactions between the metal oxides and the pi-conjugated structure of graphene oxide.

5.4 FTIR Spectra of NiCoO@GO

As can be seen in Figure 4.4, the FTIR spectrum of Nickel Cobalt Oxide (NiCoO@GO) graphene-based fabricates a number of sharp, notched vibration peaks, which prove that it has multiple functional groups and bonds between metals and oxygens. There is a wide and very strong peak noticed at 3420 cm^{-1} that can be ascribed to OH stretching vibration of the hydroxyl groups showing that the composite is a good absorber of water molecules, or that it has surface OH groups. The peak around 1620 cm^{-1} is attributed to the bending vibration of H-O-H reinforcing the existence of water molecules. The corresponding characteristic peak at 1380 cm^{-1} can be attributed to corresponding C-O stretching vibration of carboxyl or epoxy groups that is characteristic of graphene oxide. Moreover, there are also sharp peaks located at the frequency range of $600\text{--}500\text{ cm}^{-1}$, which are assigned to the metal oxygen (MO) stretch vibrations, i.e., NiO and CoO bond corresponding to the fact of successful synthesis of the mixed metal oxide. These findings

verify a successful synthesis of the anchored NiCoO nanoparticles on a graphene oxide substrate with the occurrence of the graphene oxide functional groups and metal oxide binding connections.

5.5 Cyclic Voltammetry

The image 4.5 shows 2 graphs that seem to be connected to some electrochemical measurement, namely cyclic voltammetry (CV) and calibration curve.

The left graph 4.5 (a) is a cyclic voltammogram and it plots the potential (in V vs. Ag/AgCl) on the x-axis against the current density (in mA/cm²) on the y-axis. The graph depicts several curves with different concentrations of the ascorbic acid 0-2 mM, as it is specified in the label "[AA] mM." The curves assume a distinctive pattern, as an anodic peak appears at 0.4V - 0.5V indicating oxidation of ascorbic acid. Ascorbic acid density also increases with the concentration and the trend reveals direct correlation between the two.

The right graph 4.5 (b) on the right is a calibration graph, which is a graph of current density (mA/cm²) Vs concentration of ascorbic acid (mM). The graph indicates that there is a linear association between two variables where the two linear regression takes the form $y = 0.44x + 0.24$ and $y = 0.62x - 0.045$. The coefficient of determination (R^2) in both lines is high (0.99) proving that the current density is highly dependent upon the concentration of ascorbic acid. The existence of two linear regression lines can be indicative of the fact that data has been compared of using various methods or there are two separate linear areas in the calibration curve. With the aid of the graph, it is possible to obtain quantitative correlation between the current density and the amount of ascorbic acid, therefore enabling one to get an ascorbic acid concentration unknown using the measured current density. On the whole, the two diagrams represent useful information regarding the electrochemical properties of ascorbic acid at NiCoO@GO electrode and show the possibility of utilizing this electrode with regard to quantitative analysis of ascorbic acid.

5.6 Scan Rate

The image 4.6 reveal the electrochemical behavior of a NiCoO@GO-modified electrode experimentally determined by cyclic voltammetry (CV) over a range of scan rate and give information on the reaction kinetics and scan rate correlations with current response.

On the left-hand plot in figure 4.6(a), the x-axis corresponds to the applied potential as measured against an Ag/AgCl reference electrode i.e. -0.4 V to 0.8 V, whereas the y-axis corresponds to the current density in milliamperes per square centimeter (mA/cm^2) - 0.50 - 2.0 mA/cm^2 . A set of cyclic voltammograms is taken at various scan rates as 100 mV/s, 150 mV/s, 170 mV/s etc. with the scan frequency written above the voltammogram. Because of the increase in scan rate there is proportionate increase in current density, and this can be reflected by the gradually increasing anodic and cathodic peak currents. The voltammograms indicate a quasi-reversible electrochemical behavior that is typical of a relatively distinct anodic and cathodic peaks granting validity to the redox activity of the NiCoO@GO-modified electrode.

The linear calibration plot (graph 4.6 b), on the right was extracted based on the CV data and indicated the plot between the anodic peak current density and scan rate. In this case, the x-axis shows the scan rate in millivolts per second (mV/s) where the variance of the x-axis is 100 to 190 mV/s and the y-axis represents the anodic peak current density in mA/cm^2 of the same run where the range of the y-axis is approximately 1.3 to 1.8 mA/cm^2 . The data points present a good linear relationship whose regression is $y = 0.003x + 0.0002$, with the slope value (0.003) depicting increase in current density with respect to scan rate unit. The value of coefficient determination (R^2) is 0.98, and this shows that there is good correlation between the current density and scan rate and there is a likelihood that the electrochemical process is not diffusion controlled; instead, the process is surface controlled. The positive linear dependency affirms the reproducible electrochemical response of the electrode to the different scan rates and proves the high electron transfer rate characteristics of the NiCoO@GO modified electrode to be very appropriate in terms of sensitive, as well as reliable sensing measurements.

5.7 Square Wave Voltammetry

The two figures 4.7 depict the electrochemical sensing pattern of a NiCoO@GO nanocomposite electrode to the detection of ascorbic acid by square wave voltammetry modeling and calibration curve to determine the efficiency of sensors.

On the left graph 4.7(a), the x- axis shows the applied potential against Ag/AgCl reference electrode which varies between -0.4 volt to 0.8 volt, and the y- axis, on the left represents the current density in terms of milliamperes per square centimeters (mA/cm^2) which ranged between $0.00 \text{ mA}/\text{cm}^2$ and $0.42 \text{ mA}/\text{cm}^2$. In this voltammogram, a number of curves aligned according to varying concentrations of ascorbic acid are recorded ranging between 0.2 mM and 2.0 mM. When more ascorbic acid is added, the peak current density also increases and the oxidation peak occurs at around, say, 0.2 V versus Ag/AgCl when more ascorbic acid is added. Both of the curves indicate a pronounced rise in current density as the potential is neared to the oxidation end of ascorbic acid after which the point exhibits a sharp peak and a gradual downfall. The increased height of peaks with the increasing concentration is a direct indicator of more dominant rate of electron transfer facilitated by the higher availability of the electroactive component, and therefore, proves the effective use of NiCoO@GO in modifying an electrode as electrocatalyst.

The right graph 4.7 (b) is a calibration curve of square wave voltammetry. The horizontal and vertical axis are vitamin C concentration that is expressed in millimolar (mM) which varies between zero of about 0.0 mM to the maximum of 2.2 mM of ascorbic acid, and the current density that is expressed in mA/cm^2 which varies between $0.06 \text{ mA}/\text{cm}^2$ and close to $0.42 \text{ mA}/\text{cm}^2$, respectively. We see this calibration graph is broken into the two regions that are linear and each is characterized by a different linear regression equation. The former which is the lower-concentration area has the equation $y = 0.18x + 0.04$ with a coefficient of determination (R^2) value of 0.98, which means that an excellent linear association exists between current density and the concentration in this scope. The second area involves greater concentrations which has an equation $y = 0.11x + 0.14$ whose R value is 0.92 still indicating that it is fairly linear with a bit lower sensitivity, which can be indicated by the value of slope. These two straight lines represent that the sensor will perform in the same way in a very broad concentration range and that it should be reliable

and predictable but that at higher concentrations the sensitivity decreases slightly which is presumably due to the saturation of the active sites on the electrode surface.

5.8 Chronoamperometry

The image 4.8 shows two graphs which might be connected to an electrochemical experiment in which the chronoamperometry, or the relation of current in the electrode and time at a set potential to detect ascorbic acid (AA) with NiCoO@GO electrode is measured.

In the first graph 4.8 (a), the current density (mAcm^{-2}) is plotted against time (s) of various concentrations of ascorbic acid across the range of 0.8 to 2 mM. The graph indicates a number of chronoamperometric curves (plots of current versus time with a fixed potential). The curves show a sharp drop in the current density at the that time stages, but afterwards you see a fairly stabilized current density approximately after 10-15 seconds. With the rise in concentration of ascorbic acid, the values of current density at the plateau region rise.

Graph 4.8 (b) is the calibration curve which is a plot of current density (mAcm^{-2}) and ascorbic acid (mM). A straight-line connection appears on the graph that shows dependence of current density on the concentration of ascorbic acid with an excellent correlation coefficient ($R^2 = 0.99$). The straight-line equation is $y = 0.21x + 0.008$, y denotes current density and x denotes ascorbic acid concentration. This shows that quantitative quantification of ascorbic acid in NiCoO@GO electrode is possible.

CONCLUSION

In this study, bimetallic nickel–cobalt (Ni–Co) Nano catalysts were successfully synthesized using a hydrothermal approach and uniformly immobilized onto reduced graphene oxide (rGO) nanosheets. Electrochemical analysis revealed that the rGO/NiCo composite exhibits superior electrocatalytic activity and electrical conductivity, demonstrating high sensitivity and selectivity toward ascorbic acid (AA) detection. These results establish a strong theoretical basis for the development of advanced AA sensing platforms. The incorporation of graphene as a support material plays a pivotal role in preventing nanoparticle agglomeration, enhancing electron transfer kinetics, and promoting synergistic interactions between the graphene matrix and the bimetallic nanoparticles, thereby significantly improving overall catalytic performance. The evolution of graphene oxide-based nanocomposites further highlights their potential in catalytic applications. Due to their outstanding electrical conductivity, high surface area, and excellent chemical stability, graphene-based nanomaterials have a lot of potential for use in AA electrochemical sensing in the future. The sensitivity, selectivity, and over time operating durability of AA expected to be significantly improved through integration with metal nanoparticles, metal oxides, and conductive polymers as nanotechnology and materials science continue to progress. These nanocomposites are ideal for real-time monitoring in clinical diagnostics, pharmaceutical quality control, and food safety analysis because of their quick response times and low detection limits. Furthermore, non-invasive, on-site, and customized monitoring of essential biomolecules like ascorbic acid is anticipated to be made possible by the incorporation of graphene-based electrochemical sensors into wearable and portable platforms.

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